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RESEARCH ARTICLE

EVIDENCE FOR CARDIOPROTECTIVE EFFECT OF MICROBIALLY CONVERTED EICOSAPENTAENOIC ACID ON MYOCARDIAL INFARCTION IN RATS THROUGH CARDIAC BIOMARKERS AND HISTOPATHOLOGY

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ABSTRACT

Background: Eicosapentaenoic acid (EPA) from fish is known to have benefits on cardiovascular system by attenuating ischemic changes. Intending to produce alternative source to fish EPA, we have microbially synthesized EPA (mEPA) from α-linolenic acid from plant oil for pharmacological evaluation in myocardial infarction MI.

Objective: The objective of present study was to evaluate cardioprotective activity of mEPA through effect on cardiac enzyme biomarkers and histopathology in experimentally induced MI.

Methods: Animals were divided into 7 groups of six animals each; control, standard (Losartan 20 mg/kg); fish oil (1 g/ kg); 3 groups with mEPA (5, 10 and 50 mg/kg) for 15 days. MI was induced in all groups except control by two doses of Isoproterenol HCl at 24 hrs interval (50 mg/ 100 g; s. c.) at the end of treatment. Biochemical assays for serum CK-MB, LDH, SGOT, NO, ACE and antioxidant enzymes were performed. Histopathology of ventricular apex region was done to compare pathological changes.

Results: Elevated levels of enzymes CK-MB, SGOT, LDH, NO, ACE and reduced levels of antioxidant enzymes in sham control group were significantly reversed in treatment groups. The effects of mEPA were significant as compared to standard and fish oil. This was evident from the histopathological observations of ventricular apex which indicated necrosis and other pathological changes in MI induced group which were decreased in treatment groups.

Conclusion: mEPA has shown promising cardioprotective effect and can fulfil the need of alternative source of EPA to fish for use in MI patients.

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INTRODUCTION

Myocardial infarction (MI) occurs when blood stops flowing properly to part of the heart and the heart muscle is injured due to ischemia. Usually this is because one of the coronary arteries that supplies blood to the heart develops a blockage due to an unstable buildup of white blood cells, cholesterol and fat. It is the acute condition of the necrosis of the myocardium that occurs as an imbalance between coronary blood supply and myocardial demand (Prabhu *et al*., 2006). The known risk factors for MI are smoking, obesity, hypertension, family history of MI, diabetes mellitus and hyperlipidemia. In addition to the traditional risk factors, enhanced oxidative stress is a novel risk factor of MI. Oxidative stress may increase free oxygen reactive species (ROS) formation and reduce antioxidant defenses. Antioxidant enzymes such as catalase, superoxide dismutase (SOD), and peroxidase are the first line of defense against ROS, and a decrease in their activities contributes to the oxidant attack on cells, especially in individuals suffering from MI (Lee *et al*., 2012). MI is also associated with the changes in cardiac biomarkers such as CK-MB (creatine kinase), LDH (lactic dehydrogenase), SGOT (serum glutamic oxaloacetic transaminase), Myoglobin and Troponin etc. as well as the above stated antioxidant enzymes (Pant *et al*., 2012). Inhibition of generation of nitric oxide (NO) and Angiotensin Converting Enzyme (ACE) are known to be beneficial for the treatment of MI and post-MI complications (Jain *et al*., 2000; Brown and Vaughan, 1998; Victoria *et al*., 2012). Thus, these enzymes can be considered

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as biomarkers for evaluation of cardioprotective activity. It has long been recognized from epidemiological studies that Greenland Eskimos consuming fatty fish have substantially reduced rates of acute MI (Hazra *et al*., 1999). As fish oil contains mainly EPA and DHA, the benefits of fish oil can be related with these LCPUFAs (long chain polyunsaturated fatty acids) which are not synthesized in plants. The benefits of fatty fish consumption have been explored in cell culture and animal studies, as well as randomized controlled trials investigating the cardioprotective effects of omega-3 fatty acids. Dietary omega-3 fatty acids seem to stabilize the myocardium electrically, resulting in reduced susceptibility to ventricular arrhythmias, thereby reducing the risk of sudden death. These fatty acids also have potent anti-inflammatory effects, and may also be antithrombotic and anti-atherogenic (Lee and Lip, 2003). LCPUFAs, especially EPA in fish oil is shown to decrease infarct size, stabilize atherosclerotic plaques and inhibit experimental atherosclerosis (Singh *et al*., 2001). A randomized trial of fish diet in patients after myocardial infarction found a significant reduction in mortality caused by ischemic heart disease in the group assigned to consume fatty fish 2 to 3 times per week (Guallar *et al*., 1999). It has also been shown that dietary supplementation with purified EPA attenuates ischemic myocardial damage through inhibition of neutrophilic infiltration into the infarcted myocardium in dogs (Otsuji *et al*., 1993). Fish oil is the only source of EPA so far which may be unacceptable by the vegetarian population. They have α- linolenic acid (ALA) from plant source as the available source of omega 3 fatty acids. Plant oil rich in omega-3 contents gets converted to EPA *in vivo* but does not provide the sufficient amount of EPA and also forms arachidonic acid (AA) which is proinflammatory.

Hence to avoid these problems, the EPA has been synthesized in our laboratory by our team in 2012 (Deshpande *et al*., 2013) by microbial transformation of ALA isolated from rice bran oil (RBO) and have been confirmed by chromatographic techniques. The objective of present study was to provide the evidence for the cardioprotective effect of mEPA (microbially synthesized EPA)' and elucidate the possible mechanism in experimentally induced MI in rats by evaluating its action on cardiac biomarkers and oxidative stress enzymes along with histopathology.

MATERIALS AND METHODS

Drugs and Chemicals

The chemicals required for the antioxidant enzyme assays like riboflavin, NBT, Methionine, Triton X, Pyrogallol, Hydrogen peroxide, Hippuryl-L-Histidyl-L-Leucine (HHL), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES), Ethyl Acetate, Angiotensin Converting Enzyme, Griess reagent were obtained from Sigma-Aldrich, Germany. The kits for the cardiac biomarker enzymes like CK-MB, LDH and SGOT were obtained from Vital Diagnostics, Biosystems and Autopak Siemens respectively. Isoproterenol was obtained from Unichem Laboratories, Mumbai. Other chemicals used were of analytical grade. mEPA was administered by oral route in Tween 80 as vehicle.

Instruments used

The instruments used were UV Visible double beam spectrophotometer (Systronics AU 2701), Remi's Cooling Centrifuge, Motic plus Camera (2 digital microscope dmwb series, model no. 30512782), Microtitre plate reader (Infinite M 200 PRO TECAN with Megellan Software) and Nanodrop 800 spectrophotometer for protein content estimation.

Experimental animals

Adult male wistar rats weighing around 150-200 g were divided into seven groups of six animals each. They were maintained under normal laboratory conditions of temperature 24 ± 2 ^{0} C and natural light-dark cycle and had free access to drinking water and standard pellet diet. The protocols of animal studies were approved by Institutional Animal Ethical Committee of Sharad Pawar College of Pharmacy, Nagpur (Reg. No. 536/02/CPCSEA, dated 20.01.02).

Oral toxicity studies

Acute and subacute (28 days) oral toxicity study were carried out in rats as per OECD- 420 and 407 guidelines (Organisation for Economic Co-operation and Development guidelines for testing of chemicals, 2001). The dose level was selected from one of the four fixed levels 5, 50, 300, 2000 mg/kg body weight. The rats were continuously observed for their mortality and behavioral response during the study period.

Treatment

The animals were treated as follows:
Group 1: control with vehicle
Group 2: Sham control (myocardial infarcted rats)
Group 3: Treated with standard drug Losartan (20 mg/ kg)
Group 4: Treated with mEPA (5 mg/ kg)
Group 5: Treated with mEPA (10 mg/ kg)
Group 6: Treated with mEPA (50 mg/ kg)
Group 7: Treated with fish oil (1 g/ kg)

Induction of MI

After the 15 days treatment, MI was induced by Isoproterenol HCl (ISPH) (50 mg/ 100 g in 0.1 ml of 0.9 % saline; s. c.) on two consecutive days at the interval of 24 hrs (Saroff and Wexler, 1970).

Blood samples were collected 24 hrs after the induction of MI for biochemical analysis except NO for which blood samples were collected immediately after induction and centrifuged at 1500 rpm for 10 min in Remi's cooling centrifuge and serum thus obtained was used for biochemical analysis. All animals were sacrificed; heart was removed and fixed in 10 % formalin solution for histopathological studies.

Estimation of total protein content

The total protein concentration was determined using Nanodrop 800 in which after washing the channels with water, 2 µl of samples were loaded and directly read at 280 nm to have the total protein content.

Estimation of biomarkers of MI

Estimation of CK-MB, LDH and SGOT in the serum samples was performed using commercial assay kits. CK-MB assay is based on the coupling of reverse CPK reaction with hexokinase and glucose-6-phosphate dehydrogenase yielding NADPH which is assayed at 340 nm and the enzyme activity was calculated as per the formula (Hall and Deluka, 1967).

CK-MB activity (U/L) = Δ A/minute x 6752

LDH catalyzes the reaction of pyruvate by NADH to form lactate and $NAD^{+.}$ The enzyme concentration is determined from the rate of decrease of NADH measured at 340 nm (Neiland, 1955). The enzyme concentration was calculated as U/ L = ((Vt x 106) / (ε x l x Vs)) x ΔA/min.

Assay of SGOT is based on the transfer of an amino group from aspartate to a-ketoglutarate to generate glutamate, resulting in the production of a colorimetric (450 nm) product proportional to the enzymatic activity present. One unit of SGOT is the amount of enzyme that will generate 1.0 mmole of glutamate per minute at pH 8.0 at 37 °C (Mohur and Cooke, 1975).

Estimation of oxidative stress enzymes

Superoxide dismutase in the serum was measured following the method of Marklund and Marklund with some modification (Marklund and Marklund, 1974). Assay is based on the inhibition of nitroblue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of O_2 and electron donor like methionine generates superoxide anions. The reduction of NBT by superoxide radicals to blue coloured product was followed at 560 nm. One unit of SOD activity is defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions (Rukmini *et al*., 2004). Catalase activity in serum was estimated spectrophotometrically at wave length of 240 nm after appropriate dilution following the method of Luck *et al* (Luck, 1995) and the values were expressed in units/mg of protein. Peroxidase activity was estimated spectrophoto metrically based on conversion of hydrogen peroxide, in the presence of the hydrogen donor pyrogallol, to H_2O and O_2 by the enzyme. The absorbance of the oxidation product of pyrogallol was read at 430nm (Reddy *et al*., 1995).

Estimation of nitric oxide

In a microplate of sample capacity of 300 µl per well, 20 µl of Griess Reagent, 150 µl sample and130 µl of deionized water were mixed and incubated for 30 minutes at room temperature. The absorbance was measured at wavelength 548 nm. Calibration curve of standard sodium nitrite solution was prepared by adding 20 µl of Griess Reagent, 150 µl of different concentrations of sodium nitrite solution (1–100 µM) and130 µl of deionized water. A standard curve of nitrite concentration (x-axis) against absorbance (y-axis) was plotted and nitrite concentrations corresponding to the absorbance of experimental samples from the standard plot was read (Ignarro *et al*., 1987).

Estimation of Angiotensin Converting Enzyme

The mixture of 100 µl HHL and 2.5 µl of enzyme solution was incubated for 15 min at 37°C. 1.25 µl of HCl and 1000 µl ethylacetate was added to this, shaken vigorously and centrifuged for 2 min. 1.0 ml of supernatant was pipetted out in vials and kept for boiling in water bath for 15 min. This conventional procedure converts synthetic substrate HHL into Hippuric acid in presence of ACE. Hippuric acid thus formed is extracted with ethylacetate, condensed and redissolved and then read at 228 nm. The enzyme level is calculated as Units/ ml enzyme=((A test – A blank) x 2 x1.5) / (9.8 x 15 x 0.91 x 0.0025) where, 2 = Conversion factor; 1.5 = Total volume of hippuric acid solution; 9.8 = Millimolar extinction coefficient of hippuric acid at 228 nm; 15 = Time (in minutes) of the assay; 0.91 = Extraction efficiency of Ethyl Acetate; 0.025 = Volume (in milliliter) of enzyme used (Cushman and Cheung, 1971).

Histopathology

To carry out histopathological examination the hearts were excised and immediately fixed in 10% buffered formalin. The ventricular mass was sectioned from the apex to the base of the heart, which was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin (Bancroft and Stevens, 1995). The sections were examined under light microscope and photomicrographs were taken.

Statistical analysis

All the data is expressed in Mean ± SD. The statistical significance between more than one groups were tested by one way ANOVA using Graph Pad Prism software Version 6.04. The level of significance used are * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

RESULTS

Effect on cardiac biomarkers

Administration of ISPH caused a marked increase in CK-MB, LDH and SGOT when compared with control group indicating induction of MI in experimental rats. The treatment with mEPA showed significant decrease in the serum levels of CK-MB, LDH and SGOT at 10 and 50 mg/kg dose level as compared with experimentally induced MI in sham control animals. The effects were comparable with standard Losartan and fish oil (Table 1).

Effect on oxidative stress enzymes

Oxidative stress increases with ISPH injection as compared to control group as indicated by the significantly decreased levels of catalase, SOD and peroxidase. The treatment with EPA and Losartan significantly reduced oxidative stress and elevated antioxidant markers. The mEPA treatment has significantly increased SOD, catalase and peroxidase enzyme activities maximum at 50 mg/ kg as compared to ISPH induced group (Table 2).

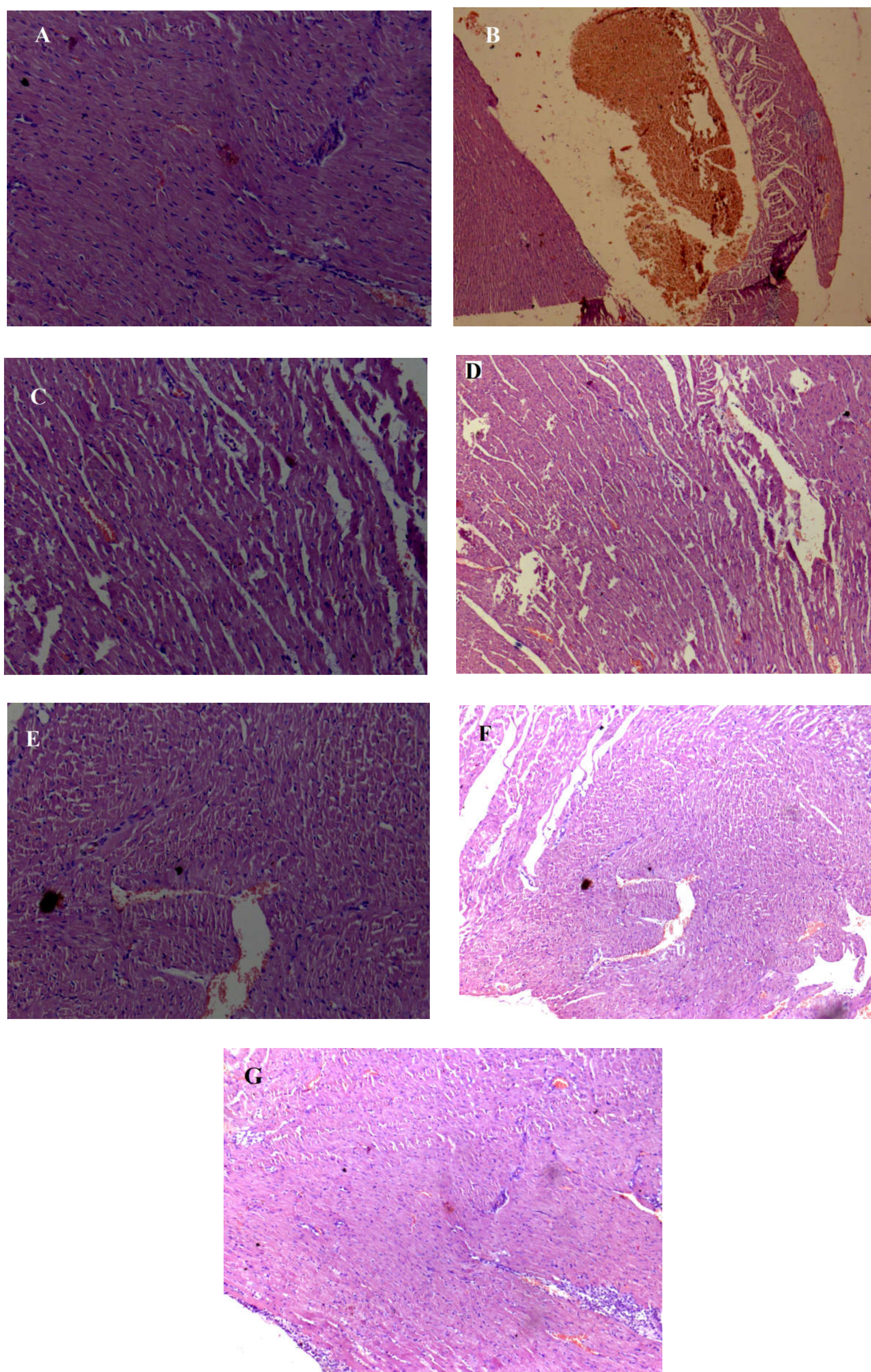

Figure 1. Histopathology of ventricular apex region of rats (A) Group 1: Control, (B) Group 2: Sham Control, (C) Group 3: Standard drug Losartan, (D) Group 4: mEPA 5 mg/ kg, (E) Group 5: mEPA 10 mg/ kg, (F) Group 6: mEPA 50 mg/ kg (G) Group 7: Fish oil 1 g/ kg

Table 1. Effect of mEPA, standard drug Losartan and fish oil on the cardiac enzyme biomarkers in ISPH induced MI in rats

Groups	CK-MB (U / L)	SGOT (U / L)	LDH (U / L)
Control	3126 ± 64.3	464.3 ± 8.7	8531 ± 56.0
Sham Control †	3561 ± 170.1 ****	509.0 ± 10.89 *	8893 ± 64.43 ****
Standard	2819 ± 50.44 ****	447.5 ± 11.56 **	8356 ± 54.78 ****
mEPA (5 mg/ kg)	3705 ± 33.82	448.8 ± 47.74 **	8944 ± 45.74
mEPA (10 mg/ kg)	2169 ± 19.29 ****	280.5 ± 11.62 ****	8742 ± 15.8 **
mEPA (50 mg/ kg)	2031 ± 25.39 ****	206.5 ± 8.7 ****	7582 ± 51.63 ****
Fish oil (1 g/ kg)	2921 ± 42.01 ****	422.3 ± 6.2 ****	8115 ± 18.61 ****

Values are expressed as mean ± SD; n = 6; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. † control group compared with sham control group; treated groups are compared with sham control group. CK-MB: creatine kinase, LDH: lactic dehydrogenase, SGOT: serum glutamic oxaloacetic transaminase

Table 2. Effect of mEPA, standard drug Losartan and fish oil on the antioxidant enzymes in ISPH induced MI in rats

Groups	SOD	Catalase	Peroxidase
	(Units/ mg protein)		
Control	1.326 ± 0.33	11.53 ± 0.74	0.0820 ± 0.023
Sham Control †	1.043 ± 0.11 *	8.120 ± 0.7 **	0.091± 0.005 *
Standard	2.574 ± 0.36	14.52 ± 1.41 **	0.163 ± 0.006 ****
mEPA (5 mg/ kg)	1.055 ± 0.55	10.55 ± 0.82 *	0.1243 ± 0.007
mEPA (10 mg/ kg)	1.219 ± 0.43 ***	11.85 ± 2.07	0.1453 ± 0.04 **
mEPA (50 mg/ kg)	3.652 ± 0.62 ****	20.03 ± 2.05 ***	0.217 ± 0.008 ****
Fish oil (1 g/ kg)	3.383 ± 0.46 ****	23.65 ± 1.8 ***	0.169 ± 0.004 ****

Values are expressed as mean ± SD; n = 6;. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. † control group compared with sham control group; treated groups are compared with sham control group SOD: Superoxide Dismutase

Table 3. Effect of mEPA, standard drug Losartan and fish oil on the NO and ACE in ISPH induced MI in rats

Groups	NO (µM/ ml)	ACE (U/ L)
Control	7.155 ± 0.5	2.305 ± 0.35
Sham Control †	17.24 ± 0.71 ****	10.67 ± 1.37 *
Standard	10.62 ± 0.33 ****	5.460 ± 0.86 *
mEPA (5 mg/ kg)	16.38 ± 0.76	5.886 ± 0.51 *
mEPA (10 mg/ kg)	15.65 ± 0.23 **	3.014 ± 1.02 *
mEPA (50 mg/ kg)	9.709 ± 0.44 ****	2.905 ± 0.51 **
Fish oil (1 g/ kg)	10.10 ± 0.39 ****	3.500 ± 0.64 **

Values are expressed as mean ± SD; n = 6; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. † control group compared with sham control group; treated groups are compared with sham control group. ACE: Angiotensin Converting Enzyme; NO: Nitric oxide

Effect on NO and ACE

In the serum samples withdrawn immediately after the MI induction, NO was found to be increased in the sham control group as compared to control. The treatment with mEPA reduced the release of NO significantly as compared to standard and fish oil treatment. The ACE inhibition in the treatment groups was significant as compared to sham group and the effects were comparable to standard and fish oil treated groups (Table 3).

Histopathology

The architecture of cardiac muscle of ventricular apex region of experimental rats was disturbed with ISPH injection as seen in slide B of Figure 1. It showed muscle edema, splaying of fibres, differential staining characters by infarcted tissue, plump nuclei and haemorrhage within the muscle. These ischemic changes were ameliorated in the treatment groups thus indicating the protection afforded by EPA against isoproterenol induced MI. (Figure 1).

DISCUSSION

The present study has been undertaken to evaluate and confirm the cardioprotection exerted by microbially synthesized EPA (mEPA). As fish oil LCPUFAs are well known to have beneficial effects on heart diseases, it was necessary to prove the similar actions of mEPA synthesized in our laboratory. The experimental model of ISPH induced MI in animals was used for this purpose. ISPH, a synthetic catecholamine and beta adrenergic agonist, has been found to cause a severe stress in myocardium resulting in infarct like necrosis in heart muscle (Prabhu *et al*., 2006).

Mechanism of ISPH induced MI

Generation of NO is increased up to 12 hrs after ISPH administration which is responsible for hypotensive effect of ISPH. The consequent hypoperfusion induces an energy imbalance and increase in energy consumption. This in turn increases demand in ATP production by mitochondria generating oxidative stress and cellular damage (Victoria *et al*., 2012). ISPH is also known to generate free radicals and ROS and stimulates lipid peroxidation which may contribute to the ischemic changes in MI (Prabhu *et al*., 2006). Excessive production of ROS may lead to oxidative stress, loss of cell function, and cell death by apoptosis or necrosis. ISPH infused rats show cardiac remodeling with severe myocardial hypertrophy and myocardial injury, which are resulted from rise in cardiac generation of ROS (Moghaddam *et al*., 2013).

The presence of the cardiac diagnostic marker enzymes like CK- MB, LDH and SGOT in the myocardial tissue homogenate indicates the myocardial integrity whereas their presence in the serum signifies the myocardial injury. Damage to the myocardial membrane induced by ISPH causes release of these enzymes in the serum (Vibha *et al*., 2011). Disruptions of contractile proteins are also seen in hearts failing due to oxidized isoproterenol (Moghaddam *et al*., 2013).

Role of antioxidant enzymes

Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defences. Antioxidant enzymes counteract the activities of ROS. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen and facilitates the removal of hydrogen peroxide. SOD catalyzes the dismutation of the superoxide anion (O_{2-}) into hydrogen peroxide and molecular oxygen. Peroxidase also converts oxidative stressor H_2O_2 to oxygen. Their over expression protects against apoptosis and promote cell differentiation (25).

Role of ACE and NO

ACE inhibition also has shown to lower the free radical oxidation products related to activation of protective antioxidant enzymes and exerts cytoprotective action in MI (Zdionchenko *et al*., 2009). Inhibition of NO release diminishes inflammatory response and reduces hypotensive effect of ISPH, therefore increases perfusion to heart.

Cardiac biomarkers and MI

Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction viz., CK-MB, LDH and SGOT and once metabolically damaged due to ROS, releases its content into the extra cellular fluid (ECF). Hence they have been found to exhibit increased serum levels during the course of an acute MI (Prabhu *et al*., 2006; Moghaddam *et al*., 2013). LDH catalyzes the interconversion of pyruvate and lactate along with interconversion of NADH and NAD+. The enzyme LDH is contained within the tissues and cells, and is released into the bloodstream when cells are damaged or destroyed. SGOT and CK-MB are utilized as a biochemical marker in the diagnosis of acute MI. These enzymes are released into the serum in case of tissue injury, so their levels may increase as a result of myocardial infarction and other diseases. The degree and the duration of elevation in serum enzyme levels approximate the extent of an acute myocardial infarction (Moghaddam *et al*., 2013; Guzy, 1977). The rise in levels of diagnostic cardiac markers is directly proportional to the number of necrotic cells in the myocardial tissue (Gopal *et al*., 2011). This is evident from the observations of the histopathological studies of ventricular apex region which indicated the necrosis and other pathological changes in MI induced group which are reduced in the treatment groups. The sham control group which received only ISPH has shown significant changes in myocardium and serum enzyme levels as evident from the histopathology and biochemical analysis. Pretreatment with mEPA significantly decreased the ISPH induced elevation of serum CK-MB, SGOT and LDH, thus it may be protecting the cell membrane from the destructive effects of free radicals initiated by ISPH. This free radical generation is also affected by mEPA indicative from the increased levels of antioxidant enzymes in the treatment groups as compared to the MI induced group. The mEPA treatment also has shown significant reduction in NO and ACE levels which were increased in sham control MI induced rats.

Possible mechanism of mEPA

ROS formation needs activation of arachidonic acid (AA) cascade via lipooxygenase (LOX) enzyme. PUFAs are known to directly interfere with the AA synthetic pathway by inhibiting LOX. Its incorporation into biological membrane along with inhibition of NO and ACE increases antioxidant status, inactivates ROS formation, normalizes the excited state, controls the physical status of the membrane lipids and prevents rise in intracellular Ca $^{2+}$ in response to oxidative stress (Gopal *et al*., 2011). In addition to this EPA supplementation increases the (Ca^{2+}-Mg^{2+})-ATPase activity within myocardial membranes that is involved in Ca^{2+} metabolism in myocardial cells by increasing the ratio of EPA to AA within cellular membranes. These cellular alterations are likely to reduce the rapid accumulation of intracellular Ca^{2+} following ischemia (Kinoshita *et al*., 1994). mEPA synthesized in the pure form in our study may act by similar mechanism to exert the cardioprotective action.

Conclusion

EPA has been synthesized by our team by microbial transformation of ALA isolated from rice bran oil. This may avoid the fish oil as EPA source and hence fulfil the need of LCPUFAs of vegetarian population. mEPA has also shown the promising effect on cardiovascular system as indicated by amelioration of oxidative stress responsible for MI that is supported by its effect on cardiac biomarkers. Hence it may serve as an alternative source of EPA for the treatment of cardiovascular disorders.

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REFERENCES

Bancroft JD, Stevens A: Theory and Practice of Histological Techniques. 4th Ed., Churchill-Livingstone, New York, 1995.

Brown NJ, Vaughan DE. Angiotensin-Converting Enzyme Inhibitors. *Circulation.* 1998; 97: 1411-1420.

Cushman, D.W., and Cheung, H.S. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharm. 20, 1637-1648.

Deshpande S, Patil T, Alone S. Duragkar N. Microbial Conversion of Plant Based Polyunsaturated Fatty Acid (PUFA) To Long Chain PUFA and Its Identification by Gas Chromatography. J Biotechnol Biomaterial. 2013; S13.

Gopal KM, Mohan M , Meganathan M, Sasikala P, Gowdhaman N, Balamurugan K *et al*. Effect of Dietary Fish Oil (Omega -3- Fatty Acid) Against Oxidative Stress in Isoproterenol Induced Myocardial Injury in Albino wistar Rats. Global Journal of Pharmacology. 2011; 5 (1): 04-06.

Guallar E, Aro A, Jiménez FJ, Martín-Moreno JM, Salminen I, Veer P, *et al.* Omega-3 Fatty Acids in Adipose Tissue and Risk of Myocardial Infarction: The EURAMIC study. Arterioscler Thromb Vasc Biol. 1999;19: 1111-1118.

Guzy P. Creatine phosphokinase-MB (CPK-MB) and the diagnosis of myocardial infarction. West J Med. 1977; 127:455-460.

Hall N, Deluka M. Electrophoretic separation and quantitation of creatine kinase isoenzymes. Anal Biochem. 1967; 76: 567.

Hazra, A., Tripathi, S.K., Ghosh, A., 1999. Pharmacology and therapeutic potential of the n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oils. Indian Journal of Pharmacology 31, 247-264.

Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhari G. 1987. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide
Proc. Natl. Acad. Sci. USA. 1987; 84, 9265-9269.

Jain M, Liao R, Ngoy S, Whittaker P, Apstein CS, *et al*. Angiotensin II receptor blockade attenuates the deleterious effects of exercise training on post-MI ventricular remodelling in rats Cardiovascular Research. 2000; 46: 66–72.

Kinoshita I, Itoh K, Nishida-Nakai M, Hirota H, Otsuji S, Shibata N. Antiarrhythmic effects of eicosapentaenoic acid during myocardial infarction-enhanced cardiac microsomal (Ca(2+)-Mg2+)-ATPase activity. Jpn Circ J. 1994; 58(12):903-12.

Lee B, Lin Y, Huang Y, Ko Y, Hsia S, Lin P. The relationship between coenzyme q10, oxidative stress, and antioxidant enzymes activities and coronary artery disease. The Scientific World Journal. 2012; Article ID 792756, 1-8.

Lee K, Lip G. The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease. Q J Med. 2003; 96:465–480.

Luck H. Estimation of Catalase. In: Methods of enzymatic analysis. (Eds). Luck H. Bergmeyer Academic Press, New York, 1995; 885–898.

Marklund S., Marklund G. Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 1974; 47, 469–474.

Moghaddam R, Akhtar M, Moghaddam A, Najmi A. Evaluation of cardioprotective effects of *Camellia Sinensis* on isoproterenol induced myocardial infarction. IJPSR, 2013; 4(3): 1056-1063.

Mohur A, Cooke I. Simple method of measuring serum levels of glutamate oxaloacetic acid and glutamate pyruvate transaminase in routine laboratories. J Clin Pathol. 1975; 10: 394.

Neiland A. Lactic acid dehydrogenase of heart muscle. In: methods in enzymology, Vol 1, Academic Press, New York & London, 1955; 449.

Organisation for Economic Co-operation and Development guidelines for testing of chemicals. 2001. Acute Oral Toxicity – Acute Toxic Class Method 423, 1-14.

Otsuji S, Shibata N, Hirota H, Akagami H, Wada A. Highly purified eicosapentaenoic acid attenuates tissue damage in experimental myocardial infarction. Jpn Circ J. 1993, 57(4): 335-43.

Pant S, Deshmukh A, Neupane P, Kavin Kumar M, Vijayashankar C. 2012. Cardiac Biomarkers, Novel Strategies in Ischemic Heart Disease, Dr. Umashankar Lakshmanadoss (Ed.), ISBN: 978-953-51-0184-0.

Prabhu S, Jainu M, Sabitha K, Shyamala Devi C. Cardioprotective effect of mangiferin on isoproterenol induced myocardial infarction in rats. Indian J Exptl Biology. 2006; 44: 209-215.

Reddy K, Subhani S, Khan P, Kumar K. Effect of light and benzyl adenine on dark-treated growing rice leaves, II changes in peroxidase activity, Plant cell physiol. 1995; 24; 987-994.

Rukmini M, D'Souza B, D'Souza V. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. Indian Journal of Clinical Biochemistry. 2004; 19 (2): 114-118.

Saroff J, Wexler R. Isoproterenol-Induced Myocardial Infarction in Rats : distribution of corticosterone Circ Res. 1970; 27:1101-1109.

Singh R, Niaz M, Kartik C. Can n-3 fatty acids provide myocardial protection by decreasing infarct size and inhibiting atherothrombosis? European Heart Journal Supplements. 2001; 3: D62–D69.

Vibha L, Asdaq SMB, Nagpal S, Rawri RK. Protective effects of medicinal garlic against isoprenaline induced myocardial infarction in rats. International Journal of Pharmacology. 2011, 7 (4): 510-515.

Victoria CS, Lucía YM, Susana VG, Lidia M, Jorge S, *et al.* Role of Nitric Oxide in Isoproterenol-Induced Myocardial Infarction, Cardiotoxicity of Oncologic Treatments. 2012; 17-32.

Zdionchenko VS, Leksina KS, NIu T, Shekhian GG, Bagatyrova KM, *et al*. Effect of angiotensin converting enzyme inhibitors on oxidative stress, endothelial function in patients with myocardial infarction. Kardiologiia. 2009; 49 (7-8): 32-7.

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