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Full Length Research Paper

Synergistic effects of squalene and polyunsaturated fatty acid concentrate on lipid peroxidation and antioxidant status in isoprenaline-induced myocardial infarction in rats

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We have studied the synergistic effects of squalene and polyunsaturated fatty acids (PUFA concentrate) on isoprenaline-induced infarction in rats with respect to changes in the levels of plasma diagnostic marker enzymes and myocardial antioxidant defense system. Intraperitoneal injection of isoprenaline caused a significant elevation in the levels of diagnostic marker enzymes; alanine aminotranferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in plasma of experimental rats. There was a significant rise in the level of lipid peroxidation with concomitant decline in the level of reduced glutathione (GSH) and in the activities of glutathione-dependent antioxidant enzymes; glutathione peroxidase (GPX) and glutathione-S-transferase (GST), and antiperoxidative enzymes; superoxide dismutase (SOD) and catalase (CAT) in heart tissue. Combined supplementation of squalene and PUFA concentrate significantly prevented the isoprenaline-induced elevations in the levels of diagnostic marker enzymes in plasma of experimental groups of rats. A tendency to counteract the isoprenaline induced lipid peroxidation was also noticed. Their combined administration maintained the level of GSH and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes at near normalcy. The results of the present study indicated that the combined administration of squalene and PUFA concentrate exerted significantly better cardio-protection against isoprenaline-intoxication as compared to that of per second supplementation.

Key words: Squalene, PUFA, isoprenaline, myocardial infarction, lipid peroxidation, antioxidant defense system.

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. The developing countries like India are struggling to manage the impact of infectious diseases along with the growing burden on society and health systems caused by non-communicable diseases such as myocardial infarction (Farvin et al., 2004). Moreover, it is very much pain-

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ful and of serious concern to realize that myocardial infarction in India occurs 10 to 15 years earlier as compared to that of the west. It is also important to note that an increasing number of young Indians are falling prey to myocardial infarction, and there are an estimated 45 million patients of coronary heart disease in India (Krishnaswami, 1998). Current projections suggest a highly notorious, but a real fact that by the year 2020 India will have the largest cardiovascular disease burden in the world (Yusuf et al., 2001).

Epidemiological studies (Das, 2000; Okuda et al., 2005) have revealed that Greenland Eskimos and Japanese with diets rich in fish oil show low incidence of ischemic heart disease as compared with European and North American populations. Prospective studies (Holub and Holub, 2004; Mori and Beilin, 2004) show that there is an inverse relation between fish intake and mortality from coronary heart disease. It is hypothesized that the highly unsaturated fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) present in substantial quantities in Mediterra-nean diet are the active components responsible for this beneficial effect. However, it is very much important to realize the fact that PUFA are well known for their peroxidative property, which is highly deleterious to the stabilization of cellular and subcellular membranes. Earlier reports by Anandan et al. (2003) indicate that supplementation of peroxidized PUFA accelerates the experimental induction of myocardial infarction in rats.

Interestingly, squalene, an isoprenoid antioxidant molecule, which also comprises a major portion in Mediterranean diet, has not yet been considered in par with EPA and DHA for its beneficial effects on myocardial function. It is present in large quantities in deep-sea shark liver oil and in smaller amounts (0.1 - 0.7%) in palm oil, wheat-germ oil, olive oil, and rice-bran oil (Liu et al., 1976) and has been reported to possess antilipidemic, antioxidant and membrane-stabilizing properties (Qureshi et al., 1996; Ko et al., 2002; Ivashkevich et al., 1981). Further, it is directly involved in the lipid metabolism not only as a precursor molecule of cholesterol biosynthesis, but also as a feed back inhibitor of HMG CoA reductase, a key enzyme regulating the cholesterol metabolism in living beings (Sawada et al., 2001) . It plays an important role in enhancing health through its part in the building blocks of hormones and cholesterol, and as antioxidant. Squalene is secreted in human serum, where it protects the skin from ultraviolet radiation (Kohno et al., 1995) . Storm et al. (1993) demonstrated the protective activity of squalene against radiation-induced injury in a mouse model. Several experimental models (Fan et al., 1996; Kamim-ura et al., 1992) demonstrated the detoxifying activities of squalene against diverse chemicals such as hexachloro-biphenyl, hexachlorobenzene, arsenic, theophylline, phenobarbital and strychnine. Squalene has also been found to have protective activity against several carcino-gens, including azoxymethane- induced colon cancer (Rao et al., 1998) and nicotine-derived nitrosaminoketone-(NMK) induced lung carcinogenesis (Smith et al., 1998). Since squalene is being one of the most powerful antioxidant and antilipidemic agents, it has to be studied in detail as an important compound for better medicinal values.

Table 1. Fatty acid composition of PUFA concentrates prepared from fish oil.

Fatty acids	Percentage (%)
C16:0	5.74
C18:0	2.91
C16:1 n-7	3.58
C18:1 n-9	5.52
C20:4 n-6	9.74
C20:5 n-3	27.5
C22:6 n-3	38.2
Others	6.81

In the present study, we have investigated the synergistic effects of squalene and PUFA concentrate on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats, an animal model of myocardial infarction of human beings.

MATERIALS AND METHODS

Chemicals

Epinephrine, tetraethoxy propane and reduced glutathione were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. Squalene (Specific gravity: 0.853; refractive index: 1.493; saponification value: 30; iodine value: 344; boiling point: 240 - 245ºC) was prepared from the shark liver oil of *Centrophorus* sp. caught in the Andaman waters (Farvin et al., 2004). PUFA concentrate (Table 1) used in this study was prepared from fish oil in our laboratory by removing the major part of saturated and monounsaturated fractions as urea inclusion complexes using a modified procedure of Ackman et al. (1988). All the other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, weighing 120 – 150 g were selected for the study. The animals were housed individually in polyurethane cages under hygienic conditions and maintained at normal room temperature. The animals were allowed food and water *ad libitum.* The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Induction of myocardial infarction

The myocardial infarction was induced in experimental rats by injecting isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1}], i.p. for 2 days (Anandan et al., 2003).

Table 2. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) in plasma of normal and experimental groups of rats.

Groups	Group 1a	Group 1b	Group 2a	Group 2b	Group 3a	Group 3b	Group 4a	Group 4b
ALT	88.3 ± 6.43	345 ± 29.4[a]	84.2 ± 6.94	132 ± 9.78[b,c]	98.7 ± 7.18	165 ± 12.5[b,d]	85.6 ± 6.21	104 ± 8.62[b,e]
AST	98.1 ± 7.94	383 ± 26.8[a]	87.7 ± 6.86	155 ± 10.4[b,c]	110 ± 8.24	167 ± 11.9[b,d]	89.5 ± 6.76	112 ± 7.11[b,e]
LDH	121 ± 9.52	296 ± 18.9[a]	118 ± 8.76	139 ± 12.4[b,c]	135 ± 14.1	157 ± 9.87[b,d]	115± 8.59	129 ± 9.34[b,e]
CPK	107 ± 8.27	297 ± 23.4[a]	105 ± 7.12	129 ± 9.7[b,c]	118 ± 9.45	143 ± 10.4[b,d]	98.7 ± 7.93	123 ± 8.76[b,e]

Group 1a, 2a, 3a and 4a, were normal control rats, received standard diet mixed with coconut oil, squalene, PUFA concentrate and squalene + PUFA concentrate, respectively, at 2% level for a period of 60 days and then intraperitoneally (i.p.) injected with physiological saline for 2 days.
Group 1b, 2b, 3b and 4b, were experimental rats, received standard diet mixed with coconut oil, squalene, PUFA concentrate and squalene + PUFA concentrate respectively at 2% level for a period of 60 days and then intraperitoneally (i.p.) injected with isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1} for 2 days] for the induction of myocardial infarction.
Results are mean ± SD of six animals. Values expressed: ALT, AST, and LDH, mol pyruvate liberated $h^{-1}l^{-1}$; CPK, mol creatine liberated $h^{-1}l^{-1}$. [a]P<0.001 significantly different compared with Group1a control animals; [b]P<0.001 significantly different compared with Group1b isoprenaline-administered rats; [c]P<0.001 significantly different compared with Group2a squalene fed normal rats; [d]P<0.001 significantly different compared with Group3a PUFA concentrate supplemented control animals; [e]P<0.001 significantly different compared with Group 4a squalene + PUFA concentrate administered normal rats.

Experimental design

Five days after acclimatization, the animals were divided into four groups of 12 rats each. Group 1, 2, 3 and 4 animals were fed on commercial feed with added coconut oil, squalene, PUFA concentrate and squalene + PUFA concentrate, respectively, at 2% level for 60 days. After 60 days, the rats were further subdivided into eight groups [Group 1a and 1b; 2a and 2b; 3a and 3b; and 4a and 4b] of 6 rats each. Group 1b, 2b, 3b, 4b animals were intraperitoneally (i.p.) injected with isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1} for 2 days] for the induction of myocardial infarction. Control animals [Group 1a, 2a, 3a and Group 4a] were i.p. injected with physiological saline alone for 2 days.

At the end of the experimental period, i.e., 24 h after last injection of isoprenaline, the experimental animals were killed, blood was collected 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. The heart tissue homogenates prepared in ice cold 0.1 M Tris-HCl buffer, pH 7.2 were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH) and antioxidant enzymes.

Biochemical assays

The activities of alanine aminotransferase [EC 2.6.1.2] (ALT) and aspartate aminotransferase [EC 2.6.1.1] (AST) in plasma were determined spectrophotometrically by the method of Mohur and Cook (1957). The lactate dehydrogenase (EC 1.1.1.27) (LDH) activity in plasma was assayed according to the method of King (1965). The creatine phosphokinase [EC 2.7.3.2] (CPK) activity in plasma was determined by the method of Okinaka et al. (1961).
Tissue lipid peroxide level was determined as TBA-reactive substances (Ohkawa et al., 1979). GSH was determined by the method of Ellman, (1959). Glutathione peroxidase (EC 1.11.1.9) (GPx) activity was measured by the method of Paglia and Valentine, (1967). Glutathione-S-transferase (EC 2.5.1.18) (GST) activity was determined by the method of Habig et al. (1974). Catalase (EC 1.11.1.6) (CAT) activity was assayed according to the method of Takahara et al. (1960). Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme.

Statistical analysis

Results are expressed as mean ± SD. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Tukey's test using a statistical package program (SPSS 10.0 for Windows).

RESULTS

Table 2 depicts the levels of diagnostic markers (AST, ALT, LDH and CPK) in the plasma of normal and experimental groups of rats. There was a significant ($p<0.001$) increase noticed in the levels of these specific diagnostic markers in plasma of Group 1b myocardial infarction induced rats as compared to that of Group 1a control rats. In Group 4b rats, the combined supplementation of squalene and PUFA concentrate significantly ($p<0.001$) reduced the release of these enzymes from the myocardium into the systemic circulation and maintained the rats at near normal status, indicating the cytoprotective action of squalene and PUFA concentrate. Moreover, it is very much interesting to note that the combination exerted significantly a better protection as compared to that of Group 2b squalene supplemented and Group 3b PUFA concentrate supplemented rats.

Table 3 shows the levels of lipid peroxides and reduced glutathione and the activities of glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes in the heart tissue of normal and experimental groups of rats. There was a significant ($p<0.001$) increase in the level of lipid peroxidation along with a concomitant decline in the level of GSH noted in the heart tissue of Group 1b isoprenaline-administered rats as compared to Group 1a controls. Also a significant ($p<0.001$) reduction in the activities of glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) was observed. The combined administration of squalene and PUFA concentrate in Group 4b rats significantly reduced the isoprenaline induced ad-

Table 3. Levels of lipid peroxides (LPO) and reduced glutathione (GSH) and the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the heart tissue of normal and experimental groups of rats.

Groups	Group Ia	Group Ib	Group IIa	Group IIb	Group IIIa	Group IIIb	Group IVa	Group IVb
LPO	1.15 ±0.08	2.39± 0.16[a]	0.91 ±0.06	1.08 ±0.08[b,c]	1.52 ±0.11	2.21± 0.14[b,d]	1.02 ±0.06	1.12±0.07[b,e]
GSH	4.24 ± 0.31	2.15 ± 0.15[a]	4.53 ± 0.28	3.92 ± 0.35[b,c]	5.12 ± 0.42	3.57 ± 0.22[b,d]	4.76 ± 0.34	4.68 ± 0.31[b,e]
GPX	2.55 ± 0.24	1.32 ± 0.11[a]	2.45 ± 0.21	2.18 ± 0.15[b,c]	2.86 ± 0.22	1.93 ± 0.15[b,d]	2.81 ± 0.24	2.77 ± 0.15[b,e]
GST	1167 ± 79	723 ± 58[a]	1231 ± 87	1124 ± 82[b,c]	1457 ± 118	1098 ± 74[b,d]	1296 ± 102	1231 ± 108[b,e]
SOD	4.76 ± 0.33	2.04 ± 0.17[a]	4.51 ± 0.28	3.97 ± 0.25[b,c]	5.65 ± 0.42	3.78 ± 0.12[b,d]	4.97 ± 0.31	4.85 ± 0.27[b,e]
CAT	7.83 ± 0.49	3.15 ± 0.19[a]	7.68 ± 0.52	6.98 ± 0.44[b,c]	8.72 ± 0.52	5.94 ± 0.43[b,d]	8.31 ± 0.55	8.24 ± 0.52[b,e]

Group 1a, 2a, 3a and 4a, were normal control rats, received standard diet mixed with coconut oil, squalene, PUFA concentrate and squalene + PUFA concentrate, respectively, at 2% level for a period of 60 days and then intraperitoneally (i.p.) injected with physiological saline for 2 days
Group 1b, 2b, 3b and 4b, were experimental rats, received standard diet mixed with coconut oil, squalene, PUFA concentrate and squalene + PUFA concentrate respectively at 2% level for a period of 60 days and then intraperitoneally (i.p.) injected with isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1} for 2 days] for the induction of myocardial infarction.
Results are mean ± SD for 6 animals. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, µmol g^{-1} wet tissue; GPx, nmol GSH oxidized min^{-1} mg^{-1} protein; GST, mol 1-chloro-2, 4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein; CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. [a]P<0.001 significantly different compared with Group1a control animals; [b]P<0.001 significantly different compared with Group1b isoprenaline-administered rats; [c]P<0.001 significantly different compared with Group2a squalene fed normal rats; dP<0.001 significantly different compared with Group 3a PUFA concentrate supplemented control animals; [e]P<0.001 significantly different compared with Group 4a squalene + PUFA concentrate administered normal rats.

verse effects and maintained the levels of evaluated parameters at near normalcy. But in the case of PUFA concentrate supplemented Group 3a rats, significant elevation in the levels of lipid peroxidation and GSH content was noticed. However, slight raise in the activities of antioxidant enzymes was also noticed. Though some amount of protective action on the antioxidant system was noticed with the per second supplementation of squalene and PUFA concentrate in Group 2b and 3b rats respectively, it is well evident from the present observation that the supplementation of both in combination offer comparatively better beneficial effects in ameliorating experimentally induced myocardial infarction.

DISCUSSION

Significant (p<0.001) rise noted in the levels of AST, ALT, LDH and CPK in plasma of Group 1b isoprenaline intoxicated rats, which are well known diagnostic markers of myocardial infarction, is an indicative of the extent of isoprenaline-induced formation of necrotic lesions in the myocardium. This present observation is in line with earlier reported studies (Farvin et al., 2004; Anandan, 2003), which have shown that the amount of diagnostic markers present in plasma is directly proportional to the number of necrotic cells present in the cardiac tissue. Of all the macromolecules that leak from the necrotic myocardium, these markers because of their tissue specificity and catalytic activity are the best indicators of cardiac damage. Increased release of these diagnostic markers from the infarcted myocardium into systemic circulation observed in the present study reflects non-specific alterations in the myocardial membrane integrity and permeability as a response to β-adrenergic stimulation.

Cell membranes are rich sources of PUFA and incurporation of PUFA in membrane phospholipids influence membrane stabilization by modulating the fluidity of the myocardial membrane (Kim et al., 2000). However, it is also important to note that the presence of highly unsaturated PUFA makes the myocardial membrane easily susceptible to oxy radicals-induced necrotic damage, resulting in increased release of diagnostic markers into the blood, as observed in the present study. Earlier Anandan et al. (2003) have reported that peroxidised PUFA intake is highly deleterious to myocardial membrane stability. In the present study, it is observed that the combined supplementation of squalene and PUFA concentrate along with feed significantly (p<0.001) prevented the isoprenaline-induced elevation in the levels of diagnostic markers as compared to that of Group 1b myocardial infarcted rats. The presence of PUFA in cell membrane plays a major role in inhibition of cell volume reduction by modulating the elasticity of plasma membrane (Sanchez-Olea et al., 1995). Cell volume affects the most basic processes of cell function, and as such it exerts an important role in the onset, severity, and outcome of myocardial infarction. An earlier reported study (Ivashkevich et al., 1981) indicates that squalene can overt severe osmolar changes associated with possible cell death. Reports by Farvin et al. (2004) have showed that the highly lipophilic squalene easily intercalates into the lipid matrix and imparts stabilization to myocardial cell membranes.

Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. Lipid peroxidation of membranes is regulated by the availability of substrate in the form of PUFA, the availability of inducers such as free radicals and excited state molecules to initiate propagation, the antioxidant defense status of environment, and the physical status of the membrane lipids (Anandan et al., 1998). In the present study, the level of lipid pero-

xidation was significantly ($p<0.001$) elevated in the heart tissue of Group 1b myocardial infarction induced rats as compared to that of Group 1a control rats. The unpaired electron present in the hydroxyl free radical, which is responsible for isoprenaline-induced lipid peroxidation (Paradies et al., 1999), reacts with polyunsaturated fatty acids to form reactive lipid radicals harmful to the structural and functional integrity of the myocardial membrane. Lipid peroxidation worsens the myocardial injury. The oxidative destruction of PUFA present in cell membrane phospholipids proceeds as a self-perpetual reaction. The significant ($p<0.001$) elevation noted in the level of lipid peroxidation in Group 3a and 3b rats as compared to that of Group 2a and 1a rats suggests the enhanced susceptibility of PUFA rich myocardial membrane for peroxidative damage. This concurs with an earlier reported study (Ando et al., 2000), which indicates that supplementation of PUFA along with feed results in increased level of lipid peroxidation in tissues.

In the present study, the combined administration of squalene and PUFA concentrate resulted in significant ($p<0.001$) reduction in the level of lipid peroxidation in the heart tissue towards near normalcy as compared to Group 1b isoprenaline-administered animals. The antioxidant effect is probably due to the presence of isoprenoid unit in the structure of squalene. It probably did so by counteracting the free radicals produced as a result of both isoprenaline administration and PUFA intake. Squalene is not very susceptible to peroxidation and appears to function as a quencher of singlet oxygen, protecting cells from oxidative damage (Kohno et al., 1995). *In vitro* experimental evidence indicates squalene is a highly effective oxygen-scavenging agent (Saint-Leger et al., 1986). Reports by Miyachi et al. (1983) indicate that subsequent to oxidative stress, such as sunlight exposure, squalene functions as an efficient quencher of singlet oxygen and prevents the corresponding lipid peroxidation in human skin surface. The rate constant of quenching of singlet oxygen by squalene is much larger than those of other lipids, and to be comparable to 3, 5-di-t-butyl-4-hydroxytolene (BHT). Hence it is possible that the chain reaction of lipid peroxidation in PUFA rich myocardial membrane is unlikely to be propagated in the myocardium with the presence of adequate level of squalene.

Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of myocardial infarction (Grieve et al., 2004). The oxidation of unsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of myocardial membrane structure (Anandan et al., 2003). Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase are the first line cellular defense against oxidative injury, decomposing $O_2^{\bullet-}$ and H_2O_2 before interacting to form the more reactive hydroxyl radical ($OH^{\bullet}$). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles.

Glutathione plays an important role in the regulation of variety of cell function and in cell protection from oxidative injury. In the present study, significant ($p<0.001$) reduction in the activities of glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (SOD and CAT) with a concomitant decline in the level of reduced glutathione was observed in the heart tissue of Group 1b myocardial infarcted rats as compared to Group 1a normal control animals, reflecting an increased oxidative stress in isoprenaline-induced myocardial injury. This is in accordance with previous investigations (Sathish et al., 2003; Shiny et al., 2005), which indicated that the tissue antioxidant status was being operated at diminished level in isoprenaline-induced myocardial infarction condition. Depletion of GSH results in enhanced lipid peroxidation, and excessive lipid peroxidation can cause increased GSH consumption (Anandan et al., 2003), as observed in the present study. Lowered activities of these prime antioxidant enzymes may lead to the formation of $O_2^{\bullet-}$ and H_2O_2, which in turn can form hydroxyl radical ($OH^{\bullet}$) and bring about a number of reactions harmful to the cellular and subcellular membranes in the heart tissue. Reduction noticed in the activities of the antiperoxidative enzymes in isoprenaline-induced myocardial infarction might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn lead to the inactivation of these enzyme activities.

It is very much interesting to note that the level of GSH and the activities of these myocardial antioxidant enzymes are slightly higher in Group 3a PUFA concentrate administered rats as compared to that of Group 1b control rats. This may be adoptive mechanism to ameliorate the PUFA-mediated peroxidation in the myocardium. Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins. Several antioxidants, including -tocopherol, GSH and GSH-enhancing agents (i.e. drugs that increase the cellular GSH level) have been found to exert cytoprotective activity in experimentally induced myocardial infarction condition. Earlier reports by Das et al. (2003) indicate that squalene exerts a significant protective action against cisplatin-induced toxicity in neuroblastoma cells similar to that of GSH, which is well known to detoxify platinum compounds by enhancing the GSH-GST detoxification system. It also suggests that squalene has a selective *in vitro* cytoprotective effect on bone marrow derived hemopoietic stem cells that is equipotent to GSH. The highly lipophilic squalene can readily pass across the PUFA rich membrane lipid bilayer and its ability to diffuse into intracellular compartments aids in it's capabilities as a potent antioxidant (Hauβ et al.

, 2002; Haines, 2001).

The Group 4b animals fed with squalene + PUFA concentrate in the present study showed a significant ($p<0.001$) elevation in the level of GSH along with a marked ($p<0.001$) rise in the activities of superoxide dismutase and catalase, thus indicating the antioxidant nature of squalene in experimentally induced oxidative stress condition. There are several molecules that directly scavenge free radicals, including many ingested antioxidants such as vitamin C and E as well as β-carotene. Of particular relevance to this present study, recent research has revealed that squalene, both endo-genous and exogenous, may help to neutralize free radicals before they can exert their destructive activity (O'Sullivan et al., 2002) . Squalene is highly lipophilic and, when administered exogenously, it can readily pass across the cellular and subcellular membranes (Kamimura et al., 1992). The ability of squalene to diffuse into intracellular compartments helps in the capabilities of this isoprenoid as a potent antioxidant (Kohno et al., 1995).

Squalene has a much greater efficiency in neutralizing $^{\bullet}OH$ than the endogenous antioxidant GSH (Das et al., 2003). Previous studies (Saint-Leger et al., 1986; Aioi et al., 1995) have shown that squalene accomplishes free radical scavenging by a non-enzymatic process of electron donation. Catecholamines act themselves by directly generating free radicals from oxygen species that cause an immediate destruction or modification of cellular and subcellular functions (Lin et al., 2005; Shiny et al., 2005). Thus, the ability of exogenous, and partly endogenous, squalene to suppress the development of myocardial infarction processes suggests that squalene, as a strong component of the antioxidant defense system, helps to prevent myocardial infarction.

There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the management of cardiovascular diseases. From the present results, it is evident that the supplementation of PUFA concentrate alone along with feed stimulates the formation of lipid peroxidation products, which are very much harmful to the structural and functional integrity of the myocardium. But in the case of squalene, it is observed that this isoprenoid antioxidant molecule supplementation is highly beneficial in counteracting isoprenaline-induced lipid peroxidation and in stabilizing the myocardial membrane. Hence it is postulated that the combined supplementation of squalene and PUFA concentrate may be an effective therapeutic option for ameliorating the myocardial dysfunction instead of supplementing these components individually.

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