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Supplementation of squalene attenuates experimentally induced myocardial infarction in rats

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Abstract

We have investigated the preventive effects of squalene against isoprenaline-induced myocardial infarction in male albino rats. Supplementation with squalene significantly prevented the isoprenaline-induced adverse changes in the levels of protein and glycoprotein components in plasma and heart tissue of experimental groups of rats. It exerted an antioxidant effect by inhibiting the isoprenaline-induced lipid peroxidation and by maintaining the level of non-enzymatic free radical-scavenger, reduced glutathione at near normalcy. Histopathological observations also confirmed the possible cardioprotective action of squalene by maintaining the normal architecture of the heart tissue. The results of the present investigation demonstrate that supplementation with squalene offers cardioprotection in experimental rats by its antioxidant and membrane- stabilizing properties.

Keywords: Squalene; Isoprenaline; Myocardial infarction; Protein; Glycoprotein components; Lipid peroxidation

1. Introduction

It is well known that a low mortality rate of coronary heart disease (CHD) prevails among native Alaskan and Greenland Eskimos who consume a large amount of fish (Newman, Middaugh, Propst, & Rogers, 1993). The same phenomenon was also seen among Japanese people (Yano et al., 1988). On the basis of these ecological data, it has been hypothesized that dietary fish intake may reduce CHD mortality rates. Epidemiological studies indicate that the cardioprotective effect of fish consumption is mainly ascribable to the presence of the omega 3 fatty acids in fish oil (Hu et al., 2002; Hughes et al., 2003). Apart from PUFA, a number of important biologically active compounds, such as taurine, glutamine, betaine, vitamin E and squalene, which can also render protective action in ameliorating coronary heart disease, are present in fish in large quantities. However, studies to prove the efficacy of these compounds in attenuating myocardial disorders are relatively scanty.

Squalene is an isoprenoid molecule present in deep-sea shark liver oil in high concentrations. Squalene has been reported to possess antioxidant (Ko, Weng, & Chiou, 2002) and membrane-stabilizing (Ivashkevich, Apukhovskaia, & Vendt, 1981) properties. Studies on squalene and its interaction with anticancerous agents showed that, among the tested agents, squalene is the most effective in potentiating the antitumor activity of bleomycin (Nakagawa, Yamaguchi, & Fukawa, 1985). Squalene functions as a quencher of singlet oxygen and protects human skin surface from lipid peroxidation upon exposure to ultraviolet radiation and other sources of oxidative damage. The rate constant of quenching of singlet oxygen by squalene is much larger than those of other lipids and 3,5-dibutyl-4-hydroxy toluene (BHT) (Kohno, Egawa, & Itoh, 1995). Squalene has been reported to possess an antilipidemic property. When administered to six week old chicks it

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lowers serum total cholesterol and LDL cholesterol without altering the HDL cholesterol levels (Qureshi, Lehmann, & Peterson, 1996). In addition, it has been found to be an efficient chemo-preventive agent against variety of skin disorders, and liver diseases. Its antiaging (Passi, De Pita, Puddu, & Littarru, 2002) and detoxification (Richter & Schafer, 1982) properties have already been well established. Earlier, we have reported the protective effect of squalene on the tissue defence system (Farvin et al., 2004), mineral status (Farvin, Anandan, Sankar, & Nair, 2005) and lipid metabolism (Farvin et al., 2006) in isoprenaline-induced myocardial infarction in rats.

Intraperitoneal administration of isoprenaline, a synthetic catecholamine and β-adrenergic agonist, to adult rats leads to biochemical and morphological alterations in the heart tissue of experimental animals, similar to those observed in human myocardial infarction (Nirmala & Puvanakrishnan, 1996). The administration of isoprenaline produces necrotic lesions and increases lipid peroxidation in the myocardium, which plays a significant part in the pathogenesis of myocardial infarction (Rathore, John, Kale, & Bhatnagar, 1998). It causes fatty changes in the myocardium, together with disorganization of nucleoli, the appearance of abnormally increased smooth endoplasmic reticulum, atypical dense bodies, detached ribosomes and distorted shape changes in mitochondria.

In the present study, an attempt has been made to assess the action of squalene on isoprenaline-induced changes in protein and glycoprotein components in plasma and heart tissue in male albino rats by virtue of its antioxidant, hypolipidemic and membrane- stabilizing properties.

2. Materials and methods

2.1. Chemicals

Isoprenaline, D-galactosamine and bovine serum albumin 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: 240–245 °C) was prepared from the shark liver oil of *Centrophorus* sp. caught in the Andaman waters. All the other chemicals used were of analytical grade.

2.2. Isolation of squalene

The fresh shark liver was chopped into pieces, kept in wire mesh baskets and heated to 80 °C in 2% caustic soda solution for 30–40 min by dipping the liver in alkali, in an open kettle. The floating oil was skimmed off. Water content was removed by adding anhydrous sodium sulfate and the oil filtered was fractionally distilled under vacuum (2 m bar/760 mm Hg) for isolation of squalene. The low boiling fraction that distilled out at 125–140 °C and the major high boiling fraction distilled out at 240–245 °C were separately collected and the residue was discarded. The fractions were analyzed for purity using an Iatroscan analyzer. The major high boiling fraction, which accounted for 95% pure squalene, was stored in inert atmosphere (Thankappan, 2003) and used for the experimental purposes.

2.3. Animals

Twenty four Wistar strain male albino rats, weighing 100–120 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 according to the guidelines of the Committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC).

2.4. Experimental protocol

Seven days after acclimatization, the animals were divided into four groups of 6 rats each. Group I and Group III animals were fed on commercial feed with added coconut oil at 2% level for 45 days and Group II and Group IV animals were fed on commercial feed with added squalene at 2% level for a period of 45 days. After 45 days of feeding, the Group III and Group IV 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 I and Group II) were i.p. injected with physiological saline alone for 2 days.

At the end of the experimental period, i.e. 24 h after the last injection of isoprenaline, the experimental animals were sacrificed. Blood was collected using sodium citrate as anticoagulant and subjected to centrifugation at 3000 rpm for 10 min at 4 °C for the separation of plasma. The heart tissue was dissected out immediately, washed with chilled physiological saline and part of this was used for histopathology. The protein contents in plasma and heart tissue were determined by the method of Lowry, Rosebrough, Farr, and Randall (1951). Hexose was estimated by the method of Niebes (1972) and hexosamine content by the method of Wagner (1979). Lipid peroxide content was determined by the thiobarbituric acid (TBA) reaction in the presence of promoters [ascorbic acid, ferrous sulphate and *tert*-butyl hydroperoxide] as described by Ohkawa, Ohishi, and Yagi (1979). Reduced glutathione was measured by the method of Ellman (1959).

2.5. Statistics

Results were expressed as mean ± SD. One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Duncan's multiple comparison test, using a statistical package programme (SPSS 10.0 for Windows). A p-value <0.05 was considered as statistically significant.

3. Results and discussion

There were significant ($p < 0.05$) reductions observed in the levels of protein, hexose and hexosamine in the heart tissue with concomitant elevations in plasma of Group III isoprenaline-administered rats as compared to those of Group I control animals (Table 1). This is in accordance with an earlier reported study (Dudnakova et al., 2002), which indicated that isoprenaline induced severe disturbance in protein metabolism through disaggregation of polyribosomal profiles. Reduced incorporation of amino acids into tissue proteins may also be responsible for the decline noticed in the level of protein synthesis in the isoprenaline-induced myocardial infarction condition. Hexose and hexosamine are incorporated into the polypeptide chain while they are still attached to ribosomes (Marshall, Bacote, & Traxinger, 1991; Robinson, Weintein, Lindenmayer, & Buse, 1995). The reduction in the hexose and hexosamine contents in isoprenaline -induced myocardial infarction might be due to inhibition of glycoprotein synthesis.

Table 1
Levels of protein, hexose and hexosamine in plasma (mg/dl) and heart tissue (mg/g wet tissue) of normal and experimental groups of rats

Parameters	Group I	Group II	Group III	Group IV
Plasma:				
Protein	5.94 ± 0.41^{a}	6.06 ± 0.38^{a}	7.57 ± 0.44^{b}	6.28 ± 0.47^{a}
Hexose	$106 \pm 8.1^{a,c}$	102 ± 7.41^{a}	124 ± 8.21^{b}	112 ± 7.87^{c}
Hexosamine	45.8 ± 3.42^{a}	48.1 ± 3.52^{a}	62.4 ± 4.05^{b}	52.7 ± 3.35^{c}
Heart:				
Protein	188 ± 12.1^{a}	175 ± 12.8^{b}	143 ± 10.9^{c}	169 ± 10.4^{b}
Hexose	20.3 ± 1.04^{a}	22.4 ± 0.98^{b}	16.8 ± 0.74^{c}	18.9 ± 0.94^{d}
Hexosamine	7.61 ± 0.45^{a}	7.34 ± 0.56^{a}	5.34 ± 0.36^{b}	6.58 ± 0.49^{c}

Group I and Group II, normal control, rats received standard diet mixed with 2% coconut oil and 2% squalene, respectively, for a period of 45 days; Group III and Group IV, myocardial infarctions were induced by intraperitoneal (i.p.) injection of isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1} for 2 days] after 45 days of feeding with standard diet mixed with 2% coconut oil and 2% squalene respectively. Results are means ± SD for 6 animals. Values that have a different superscripts (a, b, c, d, e) differ significantly ($p < 0.05$; Duncan's multiple range test).

The increase noted in the levels of protein and glycoprotein components in plasma might be due to the increased release of these macromolecules from the damaged myocardium into the systemic circulation. In the present study, the pretreatment with squalene, along with feed, resulted in near normal levels of proteins and glycoprotein components as compared with Group III isoprenaline-administered rats. It probably did so by preventing the isoprenaline-induced necrotic damage to the myocardial cell membrane or by inhibiting the disaggregation of polyribosomes.

Histopathological observations showed that injection of isoprenaline induced aberrations, such as mild to diffused cloudy swelling, focal vacuolar degeneration and pericentral infiltration of round cells, hyperemia and sinusoidal distension in the heart tissue of Group III rats (Fig. 3) as compared to that of Group I control rats (Fig. 1). Occasional occurrences of cellular hyperplasia, central necrosis and fibroplasis in portal areas were also seen. This is consistent with earlier reported studies (Banerjee, Sood, Das, & Maulik, 2003). In the present study, supplementation of squalene was found to prevent the isoprenaline induced alterations (except mild hyperemia) in the heart tissue of Group IV rats (Fig. 4), indicating the cytoprotective action of squalene. It probably did so by its membrane-stabilizing property. Squalene, which is lipophilic in nature, could be

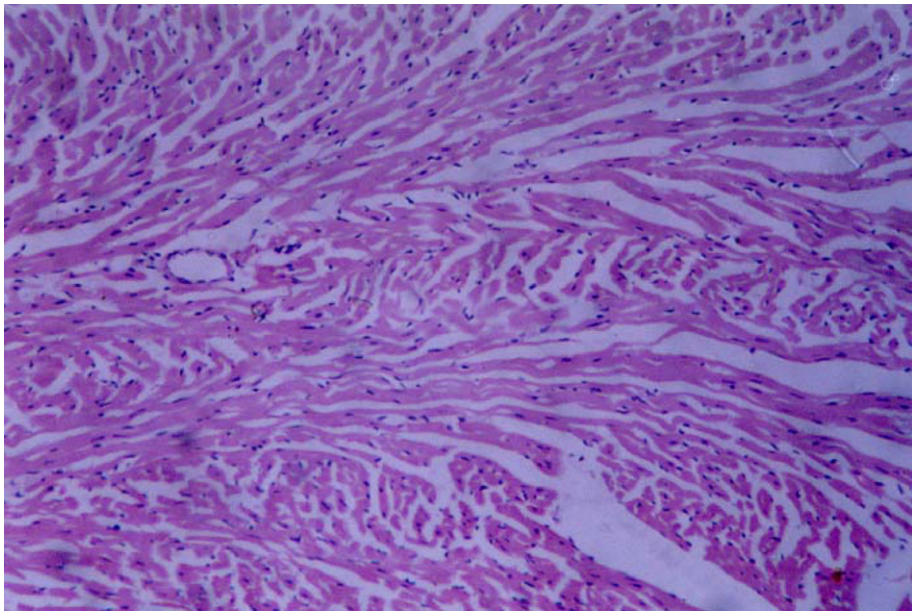

Fig. 1. The architecture of normal cardiac tissue in control rats (Hematoxylin and Eosin 100×).

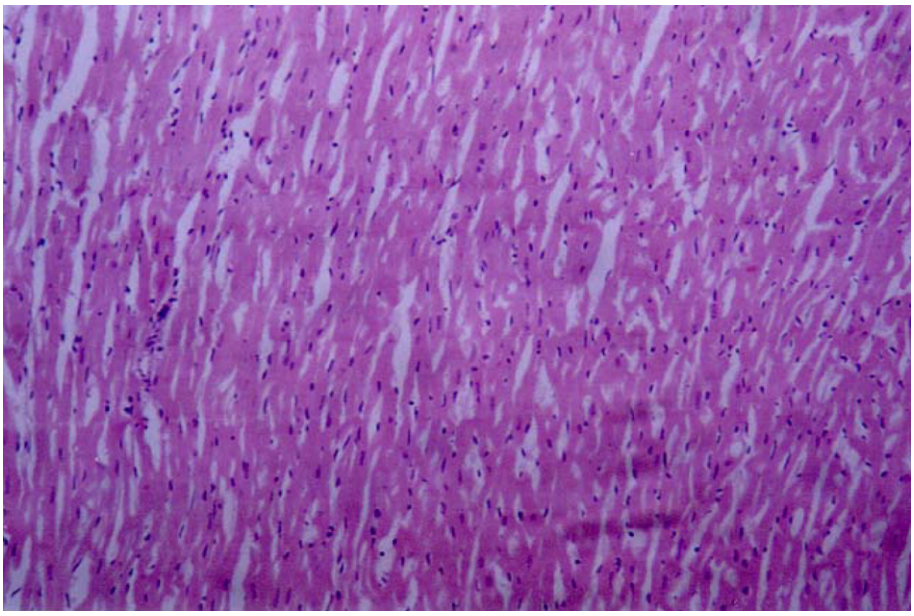

Fig. 2. The cardiac tissue in rats pre-treated with squalene indicating no significant changes in architecture in comparison to the normal condition (Hematoxylin and Eosin 100×).

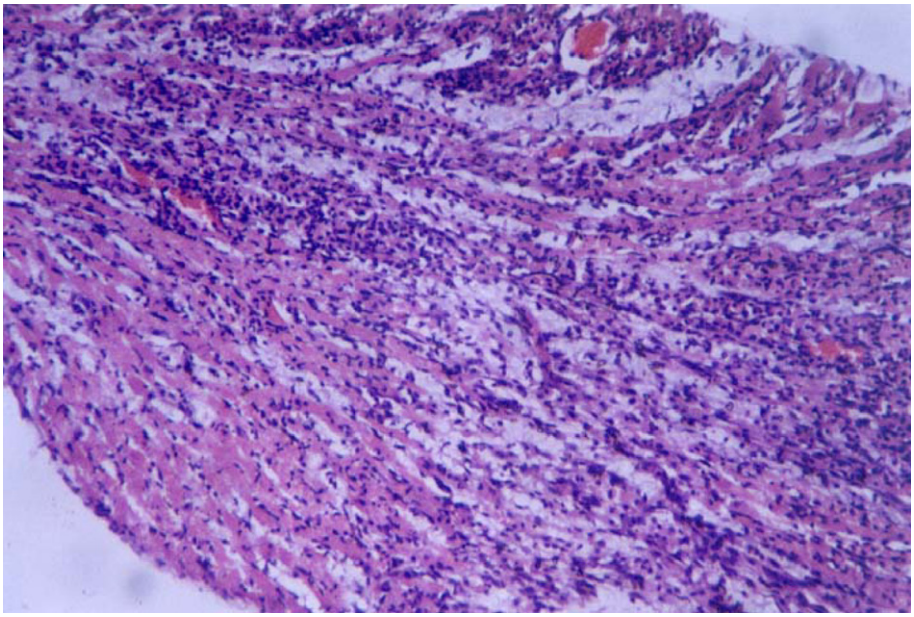

Fig. 3. The architecture of cardiac tissue in the myocardial stress induced rats showing rupture of cardiac muscle fibres with inflammatory cells (Hematoxylin and Eosin 100×).

compared to any other lipophilic agents, such as vitamin E, antipyrin and nifedine. The lipophilic betablocking drugs intercalate into the lipid matrix and impart stabilization to myocardial cell membranes in relation to the degree of their lipophilicity (Cruickshank & Dweyer, 1985). Hence, it is likewise possible that squalene may also protect the viability of myocardial cell membranes from necrotic damage by its membrane- stabilizing action (Ivashkevich et al., 1981). There were no notable changes, either in the form of fatty changes or hydropic degeneration, found in the heart of Group II rats that received squalene alone (Fig. 2), showing that it dose not *per se* have any adverse effects.

Lipid peroxidation of membranes is regulated by the availability of substrate in the form of polyunsaturated fatty acids (PUFA), the availability of inducers such as free radicals and the exited state molecules to initiate propagation, the antioxidant defence status of the environment and the physical status of membrane lipids (Anandan, Devi, Devaki, & Govindaraju, 1998). A significant ($p < 0.05$) increase in the level of lipid peroxides in the presence of

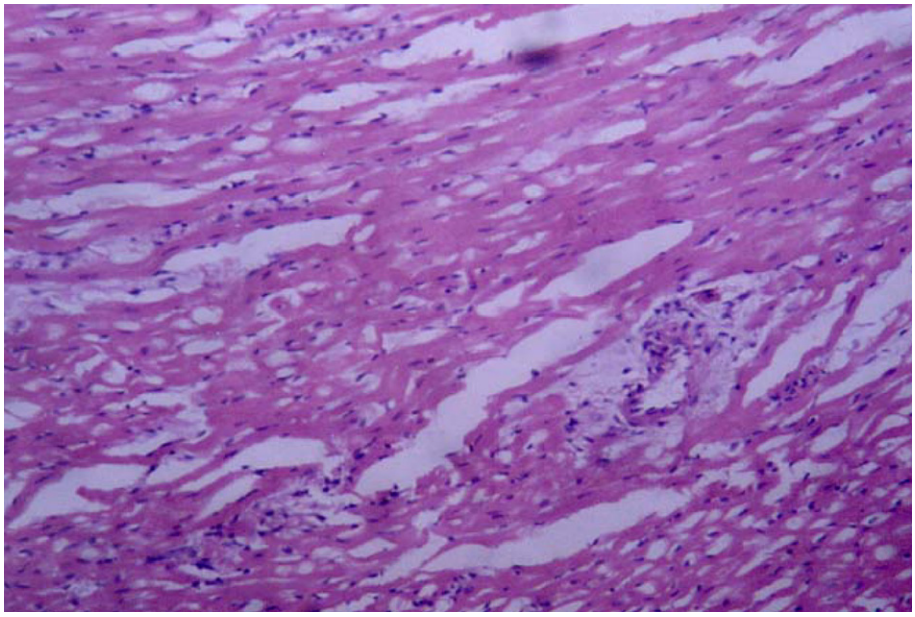

Fig. 4. The architecture of cardiac tissues in rats pre-treated with squalene prior to induction of myocardial stress by isoprenaline, which shows no rupture of cardiac muscle fibres and no presence of inflammatory cells (Hematoxylin and Eosin 100×).

promoters in the cardiac tissue of isoprenaline -treated rats (Table 2) was observed. This was paralleled by a significant ($p < 0.05$) decline in the level of GSH. This is in corroboration with an earlier investigation (Kumar & Anandan, 2007), which suggested that the high vulnerability of myocardium to peroxidative damage is mainly due to a decline in the level of free radical scavengers. The cellular tripeptide, GSH (γ-glutamyl cysteinyl glycine) thwarts peroxidative damage by neutralizing the free radicals.

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. Supplementation with squalene significantly ($p < 0.05$) prevented the isoprenaline induced lipid peroxidation and maintained the level of GSH toward near normalcy, as compared with the levels in the Group III isoprenaline -administered rats. It probably did so by its antioxidant nature by blocking isoprenaline – induced lipid peroxidation. The highly lipophilic squalene can readily pass across the membrane lipid bilayer (Kamimura, Koga, Ogari, & Yoshimura, 1992) and its ability to diffuse into intracellular compartments aids in its capabilities as a potent antioxidant (Kohno et al., 1995). The unpaired electron present in the hydroxyl radical (OH·), generated during isoprenaline -induced myocardial infarction, might have been trapped and dismuted by the free radical-scavenging isoprenoid unit of squalene.

Table 2
Levels of lipid peroxidation (LPO) [in the presence of promoters (2 mM) ascorbic acid, ferrous sulphate ($FeSO_4$) and *tert*-butyl hydroperoxide (*t*-BH)] and reduced glutathione (GSH) in heart tissue of normal and experimental groups of rats

Groups	Group I	Group II	Group III	Group IV
LPO				
Basal	$0.94 \pm 0.08^{a,c}$	0.86 ± 0.08^{a}	1.99 ± 0.11^{b}	1.03 ± 0.08^{c}
Ascorbic acid	3.18 ± 0.25^{a}	2.79 ± 0.12^{b}	5.45 ± 0.29^{c}	3.57 ± 0.21^{d}
$FeSO_4$	4.71 ± 0.39^{a}	4.35 ± 0.41^{a}	6.93 ± 0.52^{b}	4.77 ± 0.35^{a}
t-BH	6.18 ± 0.43^{a}	6.03 ± 0.38^{a}	8.85 ± 0.69^{b}	6.84 ± 0.51^{c}
GSH	5.21 ± 3.16^{a}	5.64 ± 3.43^{a}	2.88 ± 0.21^{b}	4.76 ± 0.25^{a}

Group I and Group II, normal control, rats received standard diet mixed with coconut oil 2% and squalene 2%, respectively for a period of 45 days; Group III and Group IV, myocardial infarction was induced by intraperitoneal (i.p.) injection of isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1} for 2 days] after 45 days of feeding with standard diet mixed with coconut oil 2% and squalene 2% respectively. Results are mean ± SD for 6 animals. LPO, nmol MDA released mg^{-1} protein; GSH, *n* mol g^{-1} wet tissue. Values that have a different superscript letter (a, b, c, d, e) differ significantly with each other ($p < 0.05$; Duncan's multiple range test).

In conclusion, the result of the present study implies that the squalene acts as a potent cardioprotective agent by preventing isoprenaline -induced necrotic damage to the myocardial cell membrane by virtue of its membrane-stabilizing and antioxidant properties.

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