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Antioxidant Defense of Dietary Squalene Supplementation on Sodium Arsenite-Induced Oxidative Stress in Rat Myocardium

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ABSTRACT

Arsenic is a naturally occurring element widely present in the environment, with drinking water accounting for the majority of chronic human arsenic exposure worldwide. Millions of people worldwide are at risk of cancer, heart disease and diabetes because of chronic arsenic exposure. In the present study, we have investigated the antioxidant defense of squalene on myocardial function in sodium arsenite-induced oxidative stress condition in rats. Oral administration of sodium arsenite [10 mg/kg body weight/day for a period of 30 days] caused a significant ($p < 0.05$) increase in the levels of diagnostic markers (troponin T, homocysteine and creatine phosphokinase-MB) in the plasma of rats, indicating necrotic damage to the myocardial membrane. Significant ($p < 0.05$) elevation in the level of lipid peroxidation with a parallel reduction in the level of reduced glutathione and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes in the heart tissue was also noticed. The dietary supplementation of 2% squalene for a period of 30 days significantly ($p < 0.05$) attenuated the sodium arsenite-induced oxidative aberrations in the heart tissue and maintained the levels of diagnostic markers at near normal. The results of the present findings indicate that the cytoprotective potential of squalene is probably related to its ability to counteract free radical formation by its antioxidant nature.

Keywords: antioxidant status, arsenic poisoning, cardiotoxicity, diagnostic markers, isoprenoid, lipid peroxidation

INTRODUCTION

Arsenic is a metalloid element that is widespread in the aquatic environment as a result of both geogenic and anthropogenic processes (Mukherjee and Bhattacharya 2002). Millions of people worldwide are at risk of cancer, heart disease and diabetes because of chronic arsenic exposure (Soucy *et al*. 2004). Although several hypotheses have been proposed, the exact mechanism of arsenic toxicity has not yet been clearly defined. There are, however, studies which suggest that higher concentrations of arsenic cause oxidative stress, increased reactive nitrogen species, and inhibit enzyme and mitochondrial function (Hei *et al.* 1998; Lynn *et al.* 1998). Sodium arsenite induces myocardial dysfunction by a multiple step mechanism. A considerable body of clinical and experimental evidence now exists suggesting the involvement of a free radical-mediated oxidative process in the pathogenesis of arsenic poisoning (Mishra and Flora 2008). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the enzymes catalase, superoxide dismutase, and glutathione peroxidase have been reported in sodium arsenite-induced myocardial toxicity (Maiti and Chatterjee 2001).

Until now the studies regarding treatment of arsenic toxicity are restricted mainly to some sulfhydryl-containing chelating agents [meso 2,3-dimercaptosuccinic acid (DMSA), 2,3-dimercaptopropane-1-sulfonate (DMPS) or British Anti Lewisite (BAL; 2,3-dimercaprol), antioxidants [Vitamin C, Vitamin E and N-acetyl cysteine] and some micronutrients [zinc and selenium] (Aposhian and Aposhian 2006; Modi *et al.* 2006). Most of the conventional metal chelating agents and antioxidants have been reported to possess toxic side effects or disadvantages (Mehta *et al.* 2006). Thus, there has been increased interest in the therapeutic potential of natural products having antioxidant properties in reducing free radical-induced tissue injury (Gupta and Flora 2006).

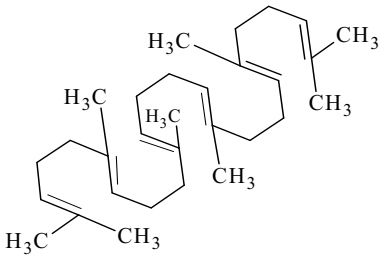

Fig. 1 Structure of squalene.

Interestingly, squalene, an isoprenoid molecule (**Fig. 1**), which is present in large quantities in deep-sea shark liver oil and in smaller amounts (0.1-0.7%) in palm, wheat-germ, olive, and rice-bran oils, has been reported to possess antilipidemic, antioxidant and membrane-stabilizing properties (Qureshi *et al.* 1996; Farvin *et al.* 2006). It plays a role in enhancing health through its part in the building blocks of hormones and cholesterol and as an antioxidant. Squalene is secreted in human sebum, where it protects the skin from ultraviolet radiation (Kohno *et al.* 1995). Studies by Kamimura *et al.* (1992) demonstrated the detoxifying activities of squalene against diverse chemicals such as hexachlorobiphenyl, hexachlorobenzene, theophylline, phenobarbital and strychnine. Squalene has also been found to have a protective activity against several carcinogens, including azoxymethane-induced colon caner and nicotine-derived nitrosaminoketone-(NMK) induced lung carcinogenesis (Rao *et al.* 1998; 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.

Though the beneficial properties of squalene are pro-

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mising and well studied, the protective effects of squalene against arsenic poisoning have not yet been explored. Hence, we thought it was important to study the effect of squalene on myocardial antioxidant system in experimental arsenic poisoning in rats by virtue of its hypolipidemic, antiperoxidative and membrane stabilizing properties

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: 240-245°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.

Isolation of squalene

The fresh shark liver was chopped into pieces, kept in wire mesh baskets, and heated to 80°C in a 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 (25 g/100 ml), and the filtered oil was fractionally distilled under vacuum (2 mbar/760 mm Hg) for isolation of squalene. The low boiling fraction that distilled out at 125–140°C and the major high boiling fraction that distilled out at 240–245°C were separately collected, and the residue was discarded. The fractions were analyzed for purity using an Iatroscan MK-6s analyzer (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The major high boiling fraction, which was 95% pure squalene, was stored in the presence of nitrogen in a dark brown bottle at -4°C and used for the experiment.

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 room temperature. The animals were allowed food and water *ad libitum*. The experiment was carried out according to the guidelines of 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).

Experimental protocol

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% (wt/wt) for 30 days and Group II and Group IV animals were fed on commercial feed with added squalene at 2% (wt/wt) for a period of 30 days. Group III and Group IV animals were orally administered with sodium arsenite [10 mg (dissolved in distilled water)/kg body weight/day] for a period 30 days. Control animals (Group I and Group II) were orally administered with distilled water alone for 30 days.

At the end of the experimental period, the experimental animals were sacrificed by using chloroform anesthesia and blood was collected with ethylenediaminetetraacetate (EDTA) as an anticoagulant for separation of plasma. The heart tissue was excised immediately and washed with chilled isotonic saline. Troponin-T content in plasma was determined by electrochemiluminescence immunoassay (ECLIA) using a Modular Analytics E170 (Elecsys module) immunoassay analyzer. Homocysteine (tHcy) concentration in plasma was assayed by using the Microtiter Plate Assay package (Diazyme Laboratories). Creatine phosphokinase (CPK-MB) activity in plasma was determined by the method of Guzy (1977). 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) (Ohkawa *et al.* 1979), reduced glutathione (GSH) (Ellman 1959), glutathione peroxidase [EC 1.11.1.9] (GPx) (Paglia and Valentaine (1967), glutathione-*S*-transferase [EC 2.5.1.18] (GST) (Habig *et al.* 1974), catalase [EC 1.11.1.6] (CAT) (Takahara *et al.* 1960) and superoxide dismutase [EC 1.15.1.1] (Misra and Fridovich 1972).

Statistical analysis

Results are expressed as mean ± SD. Multiple comparisons of the significant analysis of variance were performed by Duncan's multiple comparison test. A P-value <0.05 was considered as statistically significant. All data were analyzed with the aid of a statistical package program, SPSS 10.0 for Windows.

RESULTS AND DISCUSSION

Arsenic is a naturally occurring element widely present in the environment, with drinking water accounting for the majority of chronic human arsenic exposure worldwide. Millions of people worldwide are at risk of cancer, heart disease and diabetes because of chronic arsenic exposure. Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of sodium arsenite-induced myocardial dysfunction. Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to chemical and pharmacological investigations and general biological screening programs for natural products of all over the world. Squalene is a potent antioxidant molecule abundantly present in shark liver oil. In addition to shark liver oil, squalene is also found in variety of other foods, such as cod liver oil [520.3 µg/g], corn oil [278.9 µg/g], safflower oil [37.2 µg/g], cotton seed oil [27.8 µg/g], lard [22.5 µg/g], chicken meat [31.5 µg/g], duck meat [36.4 µg/g], halibut [96.9 µg/g], flounder [50 µg/g], tuna [14ug/g], butter [61.3 µg/g], almond 13.2 [µg/g] and pistachio [15.1 µg/g] (Liu *et al.* 1976). The focus of the current study was to evaluate the effects of dietary squalene supplementation for its antioxidant and membrane-stabilizing properties during experimentally-induced arsenic poisoning in rats.

Troponins are regulatory proteins essential for contraction and relaxation processes in the myocardium. Myocyte injury results in damage to contractile proteins and is a key mechanism responsible for the release of the structurally bound cardiac troponin T, and once outside the myocyte, these macromolecules are cleared from the interstitium by cardiac lymphatics (Sarko and Pollack 2002). In recent years an increase in the use of myocardial troponins as markers of myocardial injury has been witnessed. O'Brien *et al*. (1997) showed that troponin T is a powerful biomarker in laboratory animals for sensitive and specific detection of cardiac injury. Our findings confirm the same pattern, and show a significant ($p < 0.05$) increase in the level of troponin T in plasma of Group III sodium arsenite-administered rats as compared to that of Group I control animals (**Table 1**). Reports by Carnicer *et al.* (2006) have shown that its detection in systemic circulation could be considered not only a more sensitive but also a specific marker for assessing the severity of necrotic damage to myocardium. Investigations by Ganesan *et al*. (2007) have indicated that betaine intake attenuates the release of this regulatory protein from the ischemic myocardium by modulating the elasticity of the plasma membrane.

In the present study, the dietary supplementation of squalene significantly ($p < 0.05$) reduced the sodium arsenite-induced release of troponin T from myocardium into the blood stream, thereby demonstrating its protective action on the cell membrane. It probably did so by maintaining the delicate balance of tonicity in cells in the myocardium. The presence of squalene in the cell membrane plays a major role in cell volume regulation by modulating the elasticity of the plasma membrane. 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 dysfunction. Reports by Ivashkevich *et al.* (1981) indicate that squalene is capable of overting severe osmolar changes associated with possible cell death.

Homocysteine is a thiol-containing potentially cytotoxic

Table 1 Levels of troponin T, homocysteine and creatine phosphokinase-MB (CPK-MB) in the plasma of normal and experimental groups of rats.

Parameters	Group I	Group II	Group III	Group IV
Troponin T	0.05 ± 0.01 a	0.05 ± 0.01 a	1.85 ± 0.09 b	0.11 ± 0.01 c
Homocysteine	4.82 ± 0.28 a	4.96 ± 0.33 a	14.32 ± 1.27 b	5.48 ± 0.46 a
CPK-MB	94.12 ± 7.42 a	88.54 ± 7.28 a	32.47 ± 2.13 b	106.37 ± 8.45 c

Group I and Group II: Normal control rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days; Group III and Group IV: Experimental rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days and orally administered with sodium arsenite [10 mg/kg body weight/day] for a period 30 days.

Results are mean ± SD for 6 animals. Values expressed: Troponin T, ng ml^{-1}. Homocysteine, µmol l^{-1}. Creatine phosphokinase-MB, µmol creatine liberated h^{-1} l^{-1}. One way ANOVA. Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

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

Parameters	Group I	Group II	Group III	Group IV
Basal	0.85 ± 0.05 a	0.77 ± 0.03 a	2.31 ± 0.18 b	1.18 ± 0.15 c
Ascorbic acid	3.67 ± 0.33 a	3.32 ± 0.26 a	6.02 ± 0.48 b	4.18 ± 0.31 c
$FeSO_4$	4.25 ± 0.22 a	4.11 ± 0.27 a	6.75 ± 0.37 b	4.41 ± 0.31 a
t-BH	5.92 ± 0.39 ac	5.75 ± 0.34 a	7.91 ± 0.52 b	6.27 ± 0.43 c

Group I and Group II: Normal control rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days; Group III and Group IV: Experimental rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days and orally administered with sodium arsenite [10 mg/kg body weight/day] for a period 30 days.

Results are mean ± SD for 6 animals. Values expressed: LPO, nmol malondialdehyde released mg $protein^{-1}$. One way ANOVA. Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

4-carbon α-amino acid formed during methionine metabolism. Recent studies have shown that even mild hyperhomocysteinemia is associated with an increased risk of cardiovascular diseases independently of classical risk factors (Senaratne *et al.* 2000). In the present study, a significant ($p < 0.05$) elevation in the level of homocysteine was noted in the plasma of Group III rats as compared to that of Group I control animals (**Table 1**). This is in accordance with an earlier report study (Gamble *et al.* 2007). Homocysteine has been reported to induce atherosclerosis either by impairing coronary microvascular dilator function (Tawakol *et al.* 2002), or by stimulating smooth muscle proliferation, platelet activation, thrombogenesis, and endothelial dysfunction (Tang *et al.* 1998). Both *in vivo* and *in vitro* studies suggest that homocysteine is a potent inducer of inflammatory processes in endothelial cells at the level of gene expression (Roth *et al.* 2001; Shai *et al.* 2004). An elevated level of homocysteine is associated with increased interleukin production in monocytes and up regulation of vascular cell adhesion molecules (Silverman *et al.* 2002).

In the present study, we observed that the dietary intake of squalene significantly ($p < 0.05$) reduced the level of homocysteine in plasma of Group IV rats as compared to that of Group III sodium arsenite administered rats. It probably did so by inhibiting the production of monocyte/macrophage-derived interleukins, which triggers firm adhesion of rolling monocytes to vascular endothelium, a necessary prelude to the initiation of atherosclerosis (Gerszten *et al.* 1999). The HMG-CoA reductase inhibitors like lipophilic cerivastatin and fluvastatin have been reported to reduce the cardiovascular risk and vulnerability of atherosclerotic plaque through non-lipid mechanisms such as inhibition of interleukin expression (Ito *et al*. 2002). Since squalene is more lipophilic than statins, it is more permeable to vascular smooth muscle cells. Hence, it is possible that likewise the prime HMG-CoA reductase inhibitor squalene may also inhibit both homocysteine and interleukin production.

The plasma concentration of CPK-MB, a cardiac diagnostic marker enzyme was significantly higher ($p < 0.05$) in sodium arsenite administered rats (Group III) compared to the control group (**Table 1**). This is in line with an earlier reported study (Saad *et al.* 2006), which showed that the level of CPK-MB released from the damaged myocardium into the blood stream was directly proportional to the number of necrotic cells present in the heart tissue. The release of CPK-MB reflects non-specific alterations in the plasma membrane integrity and permeability as a response to sodium arsenite intoxication. Supplementation of squalene significantly ($p < 0.05$) prevented the sodium arsenite-induced concentration of CPK-MB in plasma of Group IV animals compared to Group III rats, indicating the cytoprotective activity of squalene. The membrane stabilizing action of squalene is comparable to any other membrane-stabilizing agents like antipyrine and nifedine, which can intercalate into the lipid matrix and impart stabilization to myocardial cell membranes (Farvin *et al.* 2005). It is possible that likewise squalene may also prolong the viability of myocardial cell membranes from necrotic damage by its membrane stabilizing property.

Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. 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 membrane structure. Oxygen-free radicals are implicated as mediators of tissue injury in sodium arsenite-induced cardiovascular pathology (Kekreja and Hess 1992). In the present investigation, a significant ($p < 0.05$) rise in the level of lipid peroxidation was observed in the heart tissue of Group III sodium arsenite-administered rats as compared to controls. Lipid peroxidation has been identified as one of the basic deteriorative reaction in cellular mechanisms of the sodium arsenite-induced myocardial dysfunction (Yáñez *et al.* 1991). A parallel decline ($p < 0.05$) in the level of GSH (**Table 3**) in the heart tissue of Group III animals was also observed. Peroxidation of endogenous lipid might be a major factor involved in the cytotoxic nature of sodium arsenite. Our results also suggested that sodium arsenite-administered rats might be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate, $FeSO_4$ and *t*-BH (**Table 2**).

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 defense status of environment and the physical status of membrane lipids (Dhandapani *et al*. 2007). The unpaired electron present in the hydroxyl free radical reacts with polyunsaturated fatty acids to form reactive lipid radicals harmful to the structural and functional integrity of the myocardial membrane. The results of the present study suggested that the high vulnerability myocardium to peroxidative damage may be due to a decline in the level of free radicals for scavengers.

In the present study, the dietary supplementation of squalene significantly ($p < 0.05$) inhibited the sodium arsenite-mediated lipid peroxidation in the heart tissue of Group IV rats, establishing its antioxidant nature. Squalene is highly lipophilic and, when administered exogenously, it can readily pass across the membrane lipid bilayer. The ability of squalene to diffuse into intracellular compartments

Table 3 Level of reduced glutathione 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.

Parameters	Group I	Group II	Group III	Group IV
GSH	5.68 ± 0.38 a	6.23 ± 0.45 b	2.89 ± 0.18 c	4.75 ± 0.29 d
GPx	2.92 ± 0.21 a	3.18 ± 0.28 b	1.38 ± 0.11 c	2.81 ± 0.19 a
GST	1287.14 ± 97.45 a	1315.34 ± 108.07 a	763.49 ± 62.12 b	1118.26 ± 83.09 c
CAT	9.18 ± 0.88 a	9.63 ± 0.92 a	4.32 ± 0.25 b	7.85 ± 0.63 c
SOD	4.32 ± 0.21 a	4.15 ± 0.18 a	1.28 ± 0.09 b	3.87 ± 0.15 c

Group I and Group II: Normal control rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days; Group III and Group IV: Experimental rats were received standard diet mixed with 2% coconut oil (wt/wt) and 2% squalene (wt/wt) respectively for a period of 30 days and orally administered with sodium arsenite [10 mg/kg body weight/day] for a period 30 days.

Results are mean ± SD for 6 animals. Values expressed: GSH, nmol g^{-1} wet tissue; CAT, nmol H_2O_2 decomposed min^{-1} mg^{-1} protein; GST, µmol 1-chloro-2,4-dinitrobenzene conjugate formed min^{-1} mg^{-1} protein; GPx, nmol GSH oxidized min^{-1} mg^{-1}; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autooxidation. One way ANOVA. Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

helps in the capability of this isoprenoid as a potent antioxidant (Buddhan *et al.* 2007). The unpaired electron present in the hydroxyl radical ($OH^{\bullet}$) generated during sodium arsenite-induced during myocardial toxicity might have been trapped for dismutation by its free radical scavenging isoprenoid unit. Studies by Miyachi *et al.* (1983) have shown that subsequent to oxidative stress, such as sunlight exposure, squalene functions as an efficient quencher of singlet oxygen and it prevents the corresponding lipid peroxidation in human skin surface. An earlier report (Dhandapani *et al.* 2008) indicated that dietary supplementation of squalene modulated PUFA-mediated peroxidative damage to the myocardial membrane by its antioxidant and membrane stabilizing properties. 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). Reports by Kohno *et al.* (1995) have shown that squalene is not particularly susceptible to peroxidation and is stable against attacks by peroxide radicals. Hence, it is postulated that the chain reaction of lipid peroxidation is unlikely to be propagated with the presence of adequate levels of squalene in the cellular and subcellular membranes of the myocardium.

The glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. The cellular tripeptide, GSH (γ-glutamyl cysteinyl glycine) thwarts peroxidative damage by neutralizing the free radicals. Significant ($p < 0.05$) decline in the level of reduced glutathione and the activities of glutathione-dependent antioxidant enzymes (GPx and GST) and the antiperoxidative enzymes (SOD and CAT) was noted in the heart tissue of Group III sodium arsenite-administered rats as compared to Group I control animals, indicating the severity of sodium arsenite-mediated oxidative stress (**Table 3**). This is in accordance with earlier reports (Flora *et al*. 2007). Reduction in the activities of these antioxidant enzymes may lead to the excessive formation of O^{2-} 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. The generation of free radical scavenging enzymes in sodium arsenite-induced myocardial toxicity might have exceeded the ability of these free radical scavenging enzymes to dismute the radicals, resulting in enhanced the susceptibility of myocardial cell membrane to peroxidative damage.

In the present study, supplementation of squalene significantly ($p < 0.05$) prevented the sodium arsenite-induced aberrations in the level of GSH and the activities of antioxidant enzymes in the heart tissue of Group IV rats as compared to that of Group III rats. It probably did so by its antioxidant action against sodium arsenite-induced lipid peroxidation. Squalene occurs in the midplane of the lipid bilayer and stabilizes the layers of cellular and subcellular membranes through the formation of complexes with the fatty acids in the phospholipid bilayer membranes (Hauss *et al.* 2002). Squalene maintains the balance between the hydrophilic and hydrophobic clusters inside the cell membrane and suppresses the effect of hydrolyzed products that affect of membrane stability (Haines 2001). Previous studies by Ivashkevich *et al.* (1981) have shown that lipophilic antioxidant molecules inhibit lipid peroxidation in cellular membranes as a result of distinct biophysical interactions with membrane lipid bilayer. Squalene has already been reported to exert antioxidant action through forcing structural interactions with membrane lipids (Buddhan *et al.* 2007). The lipid-soluble squalene has been reported to have a greater free radical scavenging activity than the known potent non-enzymatic antioxidants GSH, and vitamins C and E. Earlier reports by Das *et al*. (2003) have shown 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.

In conclusion, the results of the present study indicate that the dietary supplementation of squalene prevents sodium arsenite-induced myocardial toxicity in rats. The overall protective effect of squalene is probably due to it membrane stabilizing action, or to a counteraction of free radicals by its antioxidant nature, or to its ability to maintain near to the normal status the activities of the free radical scavenging enzymes and the level of reduced glutathione, which protect myocardial membrane against peroxidative damage by decreasing lipid peroxidation and strengthening the myocardial membrane.

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