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Protective Effect of Betaine on Changes in Lipid Profile, Lipoproteins and Fatty Acid Composition in Experimentally Induced Myocardial Infarction in Wistar Rats

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ABSTRACT

Myocardial infarction (MI) is one of the most common manifestations of cardiovascular disease. In recent years, accumulating evidence has indicated that the incidence and progression of cardiovascular disease may, to some extent, be modified by dietary means. In the present study, we investigated the protective effect of betaine, a potent lipotropic molecule, on changes in lipid profile and fatty acid composition in isoprenaline-induced myocardial infarction in rats, an animal model of myocardial infarction in man. Oral administration of betaine (250 mg/kg body weight/day for a period of 30 days) significantly reduced the isoprenaline-induced hyperlipidemic abnormalities noted in the levels of lipoprotein, cholesterol, triglycerides and free fatty acids in plasma and heart tissue. Pretreatment with betaine significantly attenuated isoprenaline-induced phospholipid depletion in the heart tissue and preserved the myocardial fatty acid composition at levels comparable to that of control rats. It also significantly counteracted the isoprenaline-mediated lipid peroxidation and maintained the level of non-enzymatic antioxidant, reduced glutathione (GSH) at near normal. The results of the present study indicated that the overall cardioprotective effect of betaine is probably related to an inhibition of lipid accumulation by its hypolipidemic properties or to a counteraction of free radicals by its antioxidant nature.

Keywords: cholesterol, free fatty acids, isoprenaline, phospholipids, triglycerides, trimethylglycine

INTRODUCTION

Myocardial infarction (MI) is emerging as a foremost public health concern in most parts of the world even in developing countries still afflicted by infectious diseases, under nutrition and other illnesses related to poverty. In most Latin American countries and Caribbean countries, myocardial infarction is already the leading cause of death and disability among both men and women (Yach *et al.* 2004). About 17 million people die annually as a consequence of cardiovascular outcomes, particularly heart attacks and stroke (OPAS 2002). In the developing world, demographic and lifestyle changes are resulting in an "epidemiological transition" from perinatal and infectious diseases to non-communicable diseases such as myocardial infarction. It is projected that myocardial infarction will be the leading cause of death in developing countries such as India, Pakistan, Bangladesh, and Nepal by 2020 (Murray and Lopez 1996). Estimates from the Global Burden of Disease Study suggest that by 2020 India will have more individuals with atherothrombotic cardiovascular disease than any other region. It is predicted that economically developing countries such as India will see the greatest increase in cardiovascular deaths over the next few decades (Srinath Reddy *et al*. 2005).

Lipids play an imperative role in cardiovascular disease, not only by way of hyperlipidemia and the development of atherosclerosis leading to myocardial infarction, but also by modifying the composition, structure and firmness of cellular membranes. Hypercholesterolemia, high concentration of low-density lipoprotein cholesterol, hypertriglyceridemia and low high-density lipoprotein are established as independent risk factors for atherosclerotic cardiovascular disease and mortality (Smith *et al*. 2007). During myocardial infarction, the cardiac cells become fibrotic leading to excess accumulation of fat. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism. Alterations in the fatty acid composition of serum triglycerides, cholesterol ester and phospholipids were also reported in acute myocardial infarction condition (Nasa *et al*. 1997).

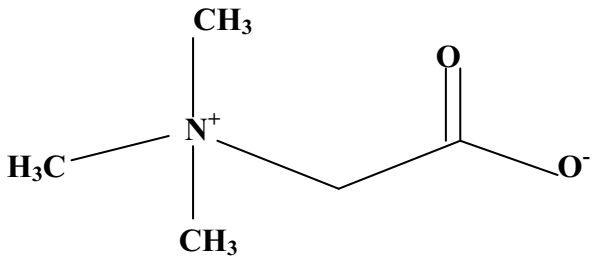

Fig. 1 Chemical structure of betaine.

Betaine (trimethylglycine; **Fig. 1**) is an amino acid derivative distributed widely in nature, such as in microorganism, plants and animals and is a significant component of many foods including wheat, shellfish, spinach and sugar beets (Zeisel *et al*. 2003).

Chemically, betaine is a zwitterionic quaternary ammonium compound and has been characterized as a methylamine because of its three chemically reactive methyl groups (Yancy *et al*. 1982). Betaine is involved in sensitive metabolic pathways, including protein turnover, amino acid and ammonia metabolism, carbohydrate and fatty acid metabolism, plasma membrane transport, bile excretion, pH control, and gene expression (Haussinger 1996). Betaine is also a lipotrope, which may prevent and cure cirrhosis, mobilize tissue cholesterol and phospholipids in rats fed a

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high-cholesterol diet (Sugiyama *et al*. 1986). An earlier report (Barak *et al*. 1993) indicated that the administration of betaine exerted a significant role to protect the liver from ethanol-induced liver injury and non-alcoholic steatohepatitis in rats. It has been shown that betaine may decrease ischemia-reoxygenation injury, presumably by inhibiting Kupffer cell activation (Wettstein and Haussinger 1997). Betaine is now attracting increased research attention not only because of potential effects on protein metabolism, but also as a repartitioning agent and modulator of lipid metabolism. Though the beneficial properties of betaine are promising and well studied in hepatotoxicity, the protective effects of betaine on lipid metabolism in experimentally induced myocardial infarction conditions have not yet been explored.

Myocardial infarction is induced by isoprenaline [L-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride] (ISO), a β-adrenergic agonist. ISO-induced myocardial infarction has been reported to show many metabolic and morphologic aberrations in the heart tissue of experimental rats similar to those observed in human myocardial infarction (Nirmala and Puvanakrishnan 1996). ISO is known to generate free radicals and to accumulate lipid peroxides, and has been recognized as one of the possible biochemical mechanisms for myocardial damage caused by this catecholamine (Sushmakumari *et al*. 1989). It has been previously reported that lipid metabolism plays an important role in myocardial necrosis produced by ISO (Mathew *et al*.1981). ISO mainly increases the low-density lipoproteins (LDL) cholesterol level in the blood, which in turn leads to the build-up of harmful deposits in the arteries, thus favoring coronary heart disease (Goldstein and Brown 1984).

In the present study, an attempt was made to assess the protective effects of betaine on ISO-induced myocardial infarction with respect to changes in the levels of diagnostic marker enzymes, lipid components and lipid peroxidation

MATERIALS AND METHODS

Chemicals

Cholesterol, isoprenaline (isoproterenol) and betaine were obtained from Sigma Chemical Company, St. Louis. MO, USA. All other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, weighing 150–180 g, were selected for the study. The animals were housed individually in polypropylene cages (with stainless steel grill top) under hygienic and standard environmental conditions (28 ± 2°C, humidity 60-70%, 12 h light/dark cycle). The animals were allowed a standard diet [Sai Feeds, Bangalore, India] 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

Myocardial infarction was induced in experimental rats by intraperitoneally (i.p.) injecting ISO [11 mg (dissolved in physiological saline)/100 g body weight/day] for 2 days (Anandan *et al*. 2003).

Experimental protocol

Five days after acclimatization, the experimental animals were divided into four groups, comprising six rats each. Rats in Group I (normal control) received a standard diet for a period of 30 days. Group II animals were orally administered with betaine [250 mg (dissolved in distilled water)/kg body weight/day] by intragastric intubation for a period of 30 days. In Group III, rats were injected with ISO [11 mg (dissolved in physiological saline)/100g body wt/day] i.p. for 2 days. In Group IV, the animals were pretreated with betaine [250 mg/kg body weight/day, for 30 days before the induction of myocardial infarction as described for Group III. Control animals (Groups I and II) were injected with physiological saline alone for 2 days.

At the end of the experiment, i.e. 24 h after the last dose of ISO, the experimental animals were killed by using chloroform anesthesia and blood was collected without any anticoagulant for the separation of serum. HDL-cholesterol and LDL cholesterol in serum were separated according to the method of Burstein and Scholnick (1972). Lipoprotein (a), apolipoprotein AI and apolipoprotein B were determined by using immunoturbidimetric test kits (DiaSys Diagnostic Systems GmbH, Germany).

The heart tissue was excised immediately and washed with ice-cold saline. Lipids were extracted from heart tissue according to the method of Folch *et al*. (1957) using a chloroform–methanol mixture. Cholesterol content was determined by the method of Parekh and Jung (1970), triglycerides by the method of Rice (1970), and free fatty acids by the method of Horn and Menahan (1981). The phospholipid content was estimated by the method of Fiske and Subbarow (1925) as inorganic phosphorous liberated after perchloric acid digestion. Myocardial fatty acid composition was analyzed according to the method of AOAC (1975) using a Perkin Elmer Autosystem XL-Turbomass GC-MS. Lipid peroxidation (LPO) was assayed by the method of Ohkawa *et al*. (1979) in which the malondialdehyde (MDA) released served as the index of LPO. Reduced glutathione was estimated by the method of Ellman (1959).

Statistical analysis

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

RESULTS AND DISCUSSION

Myocardial infarction is the most common cause of morbidity and death worldwide. Hyperlipidemia is one of the mechanisms with a central role involved in the pathogenesis of myocardial infarction. The focus of the current study was to evaluate the effects of betaine for its antilipidemic, antioxidant and membrane-stabilizing properties during experimentally-induced myocardial injury.

Increased levels of circulatory cholesterol and its accumulation are well associated with cardiovascular damage (Maczewski and Maczewska 2006). In the present study, there was a significant ($p < 0.05$) elevation in the levels of cholesterol, triglycerides and free fatty acids in plasma and heart tissue of Group III rats as compared to Group I control rats, which is an indication of the severity of ISO-induced hyperlipidemic condition. The level of LDL cholesterol was significantly ($p < 0.05$) higher in Group III myocardial infarction-induced rats, whereas HDL cholesterol levels were significantly lower compared to Group I animals. Increased mobilization of LDL-cholesterol from the blood into the myocardial membranes might have resulted in abnormal deposition of cholesterol in the myocardium. This present findings are in accordance with previous reports (Yogeeta *et al*. 2006) which showed that plasma concentration of atherogenic LDL-cholesterol is regulated by the production of rate of VLDL and the utilization of LDL-cholesterol by LDL receptors.

In the present study, prior oral treatment with betaine significantly ($p < 0.05$) attenuated the ISO-induced elevation in the level of total cholesterol, triglycerides and free fatty acids in plasma and heart tissue of Group IV rats compared to Group III rats (**Tables 1, 3**). It also maintained the levels of LDL-cholesterol and HDL-cholesterol in plasma at levels concentration comparable to that of Group I rats (**Table 2**). A report by Verreschi *et al*. (2000) indicated that betaine supplementation was effective in lowering plasma total cholesterol, VLDL cholesterol and LDL cholesterol levels in experimental animals. A slight reduction in the level of total cholesterol and LDL-cholesterol were observed in Group II rats compared to Group I rats, showing the anti-

Table 1 Levels of cholesterol, triglycerides, free fatty acids (FFA), phospholipids and lipid peroxides levels in the plasma of normal and experimental groups of rats.

Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
Cholesterol	69.2 ± 4.91 a	67.5 ± 4.86 a	154.00 ± 11.39 b	75.60 ± 5.51 a
Triglycerides	37.4 ± 2.65 a	35.1 ± 2.56 a	68.70 ± 5.08 c	41.50 ± 2.98 b
Free fatty acids	18.9 ± 1.34 b	16.3 ± 1.18 a	39.40 ± 2.91 d	22.20 ± 1.62 c
Phospholipids	98.7 ± 7.10 ab	95.8 ± 6.99 a	152.00 ± 11.24 c	108.00 ± 7.88 b
Lipid peroxides	1.82 ± 0.12 ab	1.67 ± 0.12 a	3.99 ± 0.29 c	1.94 ± 0.14 b

(A): Betaine, 250 mg kg^{-1} body wt day^{-1}, oral administration for 30 days
(B): Isoprenaline, 11 mg 100 g^{-1} body wt day^{-1}, i.p for 2 days.
Results are mean ± SD for 6 animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

Table 2 Levels of LDL-cholesterol and HDL-cholesterol in the plasma of normal and experimental groups of rats

Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
LDL-Cholesterol	38.2 ± 2.82 a	37.5 ± 2.73 a	70.4 ± 5.20 c	42.8 ± 3.08 b
HDL-Cholesterol	20.5 ± 1.51 c	24.9 ± 1.84 d	12.6 ± 0.90 a	18.7 ± 1.38 b

(A): Betaine, 250 mg kg^{-1} body wt day^{-1}, oral administration for 30 days
(B): Isoprenaline, 11 mg 100 g^{-1} body wt day^{-1}, i.p for 2 days.
Results are mean ± SD for 6 animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

Table 3 Levels of cholesterol, triglycerides, free fatty acids (FFA), phospholipids and lipid peroxides levels in the heart of normal and experimental groups of rats

Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
Cholesterol	6.21 ± 0.45 a	5.76 ± 0.41 a	12.3 ± 0.91 c	6.91 ± 0.49 b
Triglycerides	4.34 ± 0.31 a	4.15 ± 0.30 a	7.97 ± 0.58 c	4.88 ± 0.36 b
Free fatty acids	0.18 ± 0.01 b	0.12 ± 0.008 a	0.32 ± 0.02 c	0.17 ± 0.01 b
Phospholipids	24.2 ± 1.76 c	26.5 ± 1.96 d	14.9 ± 1.11 a	21.3 ± 1.57 b
Lipid peroxides	0.59 ± 0.03 a	0.65 ± 0.04 b	1.95 ± 0.01 c	0.64 ± 0.04 b

(A): Betaine, 250 mg kg^{-1} body wt day^{-1}, oral administration for 30 days
(B): Isoprenaline, 11 mg 100g^{-1} body wt day^{-1}, i.p for 2 days.
Results are mean±SD for 6 animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

cholesterolemic property of betaine. The cardioprotective effect of betaine is related to its ability to inhibit the increased accumulation of lipids both in systemic circulation and in myocardium by its antilipidemic property. The hypolipidemic property of betaine was already reported in high-fat diet fed experimental rats (Brent and Neuschwander-Teri 2001). Previous studies (Verreschi *et al*. 2000) indicated that supplementation of betaine suppressed VLDL secretion and enhanced plasma HDL concentration in a dose-dependent manner in experimental animals. Earlier reports by Duranti *et al*. (2004) indicated that supplementation of betaine increased the expression of LDL-receptors which plays an important role in the regulation of plasma LDL-cholesterol levels.

Lipoprotein metabolism plays a pivotal role in atherogenesis, leading to myocardial infarction (Goldstein and Brown 1984). Lipoprotein (a) is a complex of apolipoprotein (A) and low density lipoprotein is one of the most powerful and most prevalent independent non-modifiable risk factors for myocardial dysfunction (Simon *et al*. 1993). Apolipoprotein (A), a glycosylated protein attached to apolipoprotein B-100 of low density lipoprotein acts as a competitive inhibitor of tissue type plasminogen activator. In the present study, the levels of lipoprotein (a) and apolipoprotein-B increased significantly ($p < 0.05$) with a concomitant decrease in apolipoprotein AI in plasma of Group III ISO-injected rats compared to Group I control rats (**Table 2**). This is in agreement with a previous study (Yogeeta *et al*. 2006). Apolipoprotein AI is the primary protein constituent of high density lipoprotein and serves the function of preventing the deposition of cholesterol-loaded macrophages on the arterial cell wall as foam cells.

Pretreatment with betaine maintained the levels of these lipoproteins in Group IV animals at near normal compared to Group III ISO-injected animals. Since apolipoprotein AI is associated with HDL and apolipoprotein-B is associated with LDL, the level of apolipoprotein AI might have increased with subsequent reduction in lipoprotein (a) and apolipoprotein-B in betaine treated-groups. Previously, Zeisel (2006) reported that betaine interfered with the first step of lipoprotein (a) assembly, leading to a reduction in plasma concentration of lipoprotein (a).

Significant ($p < 0.05$) depletion was noticed in the levels of phospholipids in heart tissue of Group III animals compared of Group I rats (**Table 3**). This is in line with an earlier report (Rajadurai and Stanely Mainzen Prince 2006), which indicated that ischemic injury-related alterations in lipid composition of myocardial tissue appeared to occur due to the destruction of the myocardial membrane lipid bilayer. The significant elevation noticed in the levels of free fatty acids in plasma and heart tissue of ISO-induced rats might be due to enhanced breakdown of membrane phospholipids both in adipose tissue and myocardium by the lipolytic action of phospholipase A_2 (Anandan *et al*. 2007), which could be very likely the biochemical basis for the irreversible cell injury and ischemia. A previous study (Karthick and Stanely Mainzen Prince 2006) suggested that high lipid accumulation and increased lipid peroxidation in the myocardium might be the key events that determine ISO-induced myocardial infarction. Reports by Cao *et al*. (1996) have shown that the heart has less antioxidant protection than the liver, lung, or kidneys and therefore it may provide conditions conducive to free radical-mediated necrotic damage, as observed in the present study.

The results of the current investigation showed that prior administration of betaine significantly ($p < 0.05$) prevented the ISO-induced degradation of membrane phospholipids in heart tissue of Group IV rats compared to Group III rats, establishing its membrane stabilizing effect. It probably did so by decreasing ISO-induced Ca^{2+} overload in myocardium. An earlier report (Kempson *et al*. 2006) indicated that betaine modulated calcium-mediated cell death by its antioxidant and membrane stabilizing properties. It is possible that lipid peroxides and the spontaneous oxidation products of ISO by their action on the sarcolemma may cause leakiness and contribute to a second phase of calcium accumulation (Chernysheva *et al*. 1980). This presumption is further supported by studies carried out in cultured cardiomyocytes in which inhibition of fatty acid accumulation by phospholipase inhibitors protected the cell membranes from calcium overload and morphological change (Qian *et al*. 2004). Furthermore, the protective effect of phospholipase inhibitors apart from blocking calcium influx may also be due to their antioxidant activity and altered myocardial utilization of fuel from fatty acids to carbohydrates. Hence, it is postulated that like-wise betaine may also pro-

Table 4 Levels of fatty acids in heart tissue of control and experimental group of rats

Fatty acids (%)	Control	Betaine (A)	Isoprenaline (B)	(A+B)
C14:0	3.02 ± 0.20 a	3.53 ± 0.29 b	4.42 ± 0.35 c	3.12 ± 0.25 a
C:16:0	15.5 ± 1.57 ab	15.1 ± 1.3 a	17.2 ± 1.8 b	15.9 ± 1.8 ab
C:18:0	21.8 ± 1.95 a	21.4 ± 1.9 a	25.1 ± 2.15 b	22.1 ± 2.08 a
C:16:1	3.24 ± 0.27 a	3.15 ± 0.26 a	3.67 ± 0.31 b	3.28 ± 0.27 a
C:18:1	12.7 ± 1.15 ab	11.9 ± 0.81 a	13.8 ± 1.3 b	12.5 ± 1.15 a
C:18:2	18.1 ± 1.35 b	18.3 ± 1.31 b	14.9 ± 1.11 a	17.6 ± 1.35 b
C:20:3	0.72 ± 0.052 ab	0.75 ± 0.055 b	0.68 ± 0.051 a	0.72 ± 0.052 ab
C:20:4	11.9 ± 0.9 b	12.3 ± 0.96 b	9.31 ± 0.68 a	12.1 ± 0.9 b
C:20:5	0.26 ± 0.017 b	0.28 ± 0.02 c	0.21 ± 0.013 a	0.26 ± 0.017 b
C:22:6	11.8 ± 0.85 b	12.3 ± 0.85 b	9.85 ± 0.62 a	11.7 ± 0.75 b

(A): Betaine, 250 mg kg^{-1} body wt day^{-1}, oral administration for 30 days
(B): Isoprenaline, 11 mg $100g^{-1}$ body wt day^{-1}, i.p for 2 days.
Results are mean±SD for 6 animals; one way ANOVA; Duncan's multiple comparison test. Values that have a different letter differ significantly ($p < 0.05$) with each other.

tect myocardial cell membrane from necrotic damage by its membrane-stabilizing action and antioxidant property.

Alterations in fatty acid composition of membrane phospholipids and consequent changes in membrane properties play an important role in induction of myocardial infarction. Pepe and McLennan (2002) reported that cardiac mem-brane fatty acid composition modulated myocardial oxygen consumption and post ischemic recovery of contractile function. **Table 4** depicts the myocardial fatty acid composition of normal and experimental groups of rats. In the present study, significant alterations were observed in the com-position of fatty acids in heart tissue of ISO-administered Group III rats compared to Group I control rats. There was a slight increase noticed in the levels of saturated and monounsaturated fatty acids (C14:0, C16:0, C18:0 and C16:1, C18:1) in heart tissue of ISO-administrated Group III rats compared to Group I control rats. This is in line with reports by Al Makdessi *et al*. (1987) which indicated modifications in the distribution of fatty acids evidenced by significant changes of monounsaturated or saturated and of 16:1 *cis*/ 16:1 *trans* ratios in arterial free fatty acids and tissue triglycerides in ISO-administrated experimental animals. Polyunsaturated fatty acids, major components of membrane phospholipids, play a key role in membrane functions. There was a significant ($p < 0.05$) decline observed in the polyunsaturated fatty acid content (C18:2, C20:3, C20:4, C20:5, C22:6) in ISO-administrated Group III rats compared to Group I control rats. A significant ($p < 0.05$) alteration was noticed in the composition of total n6 and n3 polyunsaturated fatty acids in ISO-injected Group III rats compared to Group I control rats. A slight change in the ratio of n6/n3 was also observed in the ISO-administrated rats compared to normal rats. This is in line with earlier reports (Gudbjarnason 1989), which indicated that administration of ISO modified the fatty acid composition and the balance between n-6 and n-3 fatty acids in cellular phospholipid composition. ISO-induced free radicals probably attacked the membrane phospholipids in the ischemic myocardium, resulting in the oxidation of membrane phospholipids rich in polyunsaturated fatty acids.

Pretreatment with betaine significantly ($p < 0.05$) prevented the ISO-induced aberrations in the fatty acid composition of heart tissue in Group IV rats as compared to Group III rats. There was a decrease in the saturated and monounsaturated fatty acid content and an increase in the polyunsaturated fatty acid content in heart tissue of Group IV rats compared to Group III rats. It probably did so by protecting the membrane phospholipids from the ISO-induced free radical attack by virtue of its antioxidant property. This is in agreement with reports by Kanbak *et al*. (2001) which indicated that betaine administration protected membrane phospholipids by enhancing antioxidant defense status on ethanol-induced membrane lipid composition and membrane ATPases in rats. Nageswari *et al*. (1999) reported that polyunsaturated fatty acids exerted an anti-atherogenic effect and offered significant protection against acute myocardial infarction in experimental animals. The increase observed in polyunsaturated fatty acid content in the heart tissue of Group IV rats might have also contributed to the protective effect of betaine. Graf *et al*. (2002) reported that the cytoprotective effect of betaine could be due to its ability to modulate polyunsaturated fatty acid composition in the mitochondrial cell membrane.

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia (Ferrari *et al*. 2004). Lipid peroxidetion of membranes is regulated by the availability of substrate in the form of polyunsaturated fatty acids, 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. The oxidation of polyunsaturated 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. ISO-induced myocardial infarction is generally attributed to the formation of the highly reactive hydroxyl radical ($OH^{\cdot}$), stimulator of lipid peroxidation and source for the destruction and damage to cell membranes (Yogeeta *et al*. 2006). In the present study, the significant ($p < 0.05$) increase noticed in the level of lipid peroxides in the cardiac tissue of Group III ISO-treated rats as compared to that Group I control animals reflected the oxidative deterioration of myocardial cell membrane (**Table 3**). This is in corroboration with an earlier investigation (Shiny *et al*. 2005), which suggested that the high vulnerability myocardium to peroxidative damage is mainly due to a decline in the level of free radicals for scavengers.

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. In the present investigation, the prior oral administration of betaine resulted in a significant ($p < 0.05$) reduction in the level of lipid peroxidation as compared with the levels in the Group III ISO-administered rats. It probably did so by counteracting the ISO-generated free radicals by its antioxidant property. Betaine is highly lipotropic and, when administered exogenously, can readily pass across the membrane lipid bilayer. The ability of betaine to diffuse into intracellular compartments aids the capabilities of this natural product as an antioxidant. A report by Balkan *et al.* (2005) indicated that betaine supplementation was effective in preventing lipopolysaccharide-induced necrotic damage in liver by inhibiting Kupffer cell activation and behaving as an antioxidant. Feeding SH-generating substances like methionine or non-enzymic antioxidants like glutathione have been reported to protect cellular and subcellular membranes from toxic free radical metabolites (Osman *et al*. 1993). Betaine is involved in the synthesis of methionine, which serves as a major supplier of cellular cysteine via transsulfuration pathway for the synthesis of reduced glutathione (Mosharov *et al*. 2000).

In conclusion, the results of the present study indicate that the prior administration of betaine at a concentration of 250 mg/ kg body weight for 30 days prevents the symptoms

of ISO-induced myocardial infarction in rats. The overall cardio protective effect of betaine is probably related to its ability to inhibit the ISO-induced lipid accumulation by its lipotropic property and to its free radical scavenging ability against ISO-induced lipid peroxidation, which is primarily responsible for the irreversible necrosis of the myocardium.

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