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Full Length Research Paper

A study of the protective effects of betaine administration against isoprenaline-induced myocardial infarction in rats

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The protective effect of betaine administration on changes in the levels of protein, glycoproteins and amino acids was studied in isoprenaline-induced myocardial infarction in rats as an animal model of myocardial infarction in man. Oral pre-treatment with betaine significantly (P<0.05) attenuated the isoprenaline-induced rise in the levels of troponin-T and creatine phosphokinase (CPK). Oral supplementation of betaine also significantly (P<0.05) counteracted the isoprenaline-induced alterations in the levels of amino acids (taurine, aspartate, glutamate, arginine, hydroxy proline and homocysteine), protein content, glycoprotein components (hexose and hexosamine) and lipid peroxidation in the heart tissue and maintained their levels comparable to that of control animals. The results indicated that the overall cardioprotective effect of betaine was probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action or to a counteraction of free radicals by its antioxidant property.

Key words: Betaine, myocardial infarction, isoprenaline, glycoproteins, lipid peroxidation.

INTRODUCTION

Myocardial infarction (MI) is one of the most widespread manifestations of cardiovascular disease. The morbidity and mortality due to myocardial infarction has reached epidemic proportion in the world, accounting for 16.7 million deaths / year world wide (Mackay and Mensha, 2004). The World Health Organization (WHO) envisages that heart disease and stroke will become the leading cause of both death and disability all over the world by the year 2020, with the number of fatalities projected to increase to more than 20 million a year and to more than 24 million a year by 2030 (Farvin et al., 2006). In India myocardial infarction typically occurs 10 to 15 years earlier than in the west. Low- density lipoprotein (LDL) is the major cholesterol carrying lipoprotein in the plasma and is the causal agent in many forms of heart diseases (Nabel, 2003). Although major advances have been made in the treatment of cardiovascular diseases, myo-cardial infarction remains an important public health con-cern in many developing countries and will soon attain that status in several others (Abdallah et al., 2006). Epi-demiological studies (Cooper et al., 2000) have endowed with compelling evidence that occurrence of myocardial infarction is largely preventable.

Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to biochemical and pharmacological investigations and general biological screening programs for natural products all over the world. Betaine is found in microor-ganisms, plants, and animals and is a significant compo-

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nent of many foods, including wheat, shellfish, spinach, and sugar beets (Zeisel et al., 2003). Betaine, also kno-wn as trimethylglycine or glycine betaine, is a quaternary ami-ne, closely related to the amino acid, glycine. It is hypo-thesized that betaine is non-perturbing to cellular metabo-lism; highly compatible with enzyme functions, and stabili-zes cellular metabolic function under different kinds of stress in various organisms and animal tissues (Lammers et al., 2005) . Reports by Craig (2004) indica-ted that admi-nistration of betaine exerted significant role within tissue as a methyl donor, which in turn may be used for the synthe-sis of methionine, carnitine, phosphatidylcholine, creatine, and these substances playing a key role in protein and energy metabolism in the myocardium.

Betaine has been reported to play a role in reducing blood levels of homocysteine, a toxic breakdown product of amino-acid metabolism that is believed to promote athe-rosclerosis and osteoporosis. Betaine is now attracting increased research attention not only because of potential effects on lipid metabolism, but also re-partitioning agent and modulator of protein metabolism (Guilland et al., 2003). Though the beneficial properties of betaine are pro-mising and well studied in hepatotoxicity, the protective effects of betaine on protein metabolism in experimentally induced myocardial infarction condition have not yet been explored.

Isoprenaline (ISO) is a synthetic β-adrenergic agonist that causes severe stress in the myocardium resulting in infarct like necrosis of the heart muscle (Prabhu et al., 2006). Isoprenaline-induced myocardial infarction serves as a well standardized model to study the beneficial effects of many drugs and cardiac functions (Mohanty et al., 2004). Isoprenaline-induced myocardial necrosis sho-wed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes (Sabeena Farvin et al., 2004). It is also well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irrever-sible damage to the myocardial membrane (Karthick and Prince, 2006).

In the present study, an attempt has been made to assess the protective effects of betaine administration aga-inst isoprenaline-induced myocardial infarction in rats with respect to changes in the levels of creatine phospho-kinase (CPK), protein, glycoproteins, amino acids and lipid peroxidation.

MATERIALS AND METHODS

The present study was performed during March 2007 at Biochemis-try and Nutrition Division, Central Institute of Fisheries Technology (ICAR), Cochin, Kerala, India. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Super-vision of Experiments on Animals (CPCSEA), New Delhi, India.

Procurement of chemicals and experimental animals

Amino acids, isoprenaline (isoproterenol) and betaine used in this study were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

Wistar strain male albino rats, with weight range 150 – 180 g, were selected for the study. The animals were housed individually in poly-propylene cages with stainless steel grill top under hygienic and stan-dard environmental conditions. The prevailing environmental tem-perature was 28 ± 2^{O}C, relative humidity of 60-70% with 12 h light-/-dark cycle. The animals were allowed a standard diet [M/s Sai Feeds, Bangalore, India] and water *ad libitum*.

Induction of myocardial infarction and experimental procedure

The myocardial infarction was induced in experimental rats by intra-peritoneally injecting with isoprenaline at 11mg/100g body weight in physiological saline for 2 days (Anandan et al., 2003).

Five days after acclimatization, the experimental animals were divi-ded into four groups, comprising six rats each. Rats in Group I (nor-mal control) received 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 isoprenaline [11 mg (dissolved in physiological saline)/100 g body wt/day], i.p. for 2 days for the induction of myocardial infarction. In Group IV, the ani-mals were pre-treated with betaine [250 mg/kg body weight/day, pre-oral for 30 days] before the induction of myocardial infarction as des-cribed for Group III. Control animals (Group I and Group II) were injected with physiological saline alone for 2 days.

At the end of the experiment, that is, 24 h after the last dose of iso-prenaline, the experimental animals were killed and blood was coll-ected with ethylenediaminetetraacetate (EDTA) as 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 electro-chemiluminescence immunoassay (EC-LIA) using Modular Analytics E170 (Elecsys module) immunoassay analyzer. Homocysteine (tHcy) concentration in plasma was assayed by using Microtiter Plate Assay package (Diazyme Laboratories). The levels of taurine, asparate, glutamate, arginine and hydroxy proline in the heart tissue were determined according to the method of Ishida et al. (1981) using Shimadzu LC 10 AS Amino Acid Analyzer. The protein content was estimated by the method of Lowry et al., (1951). The glycoprotein components, hexose and hexosamine, were deter-mined by the methods described by Niebes (1972) and Wagner (1979), respectively. Lipid peroxidation (LPO) in the heart tissue was assayed by the method of Ohkawa et al. (1979) in which the malondialdehyde (MDA) released served as the index of LPO.

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 for Windows. Data obtained were expressed as mean ± SD. Multiple comparisons of the means were separated using the Duncan Multiple Range Test at 5% probability.

RESULTS AND DISCUSSION

There was a significant increase (P<0.05) in the troponin T plasma concentration for rats injected with isoprenaline (Group III) when compared to rats in the control Group I. This indicate a damage to the contractile protein which is responsible for the release of structurally bound troponin T used as a marker for the detection of cardiac injury in laboratory animals (O"Brien et al., 1997). Reports by

Robertson et al. (1982) indicated that phosphorylation of troponins reduced myofilament sensitivity to Ca^{2+} ions, thereby contributing to higher rate of relaxation during β-adrenergic stimulation (Table 1). Troponins are regulatory

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

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Troponin T	0.024±0.001[a]	0.021±0.001[a]	0.65±0.04[b]	0.071±0.003[c]
Homocysteine	2.32±0.15[a,b]	2.05±0.11[a]	6.53±0.48[c]	2.61±0.18[b]
CPK	132±7.81[a]	127±7.56[a]	314±24.3[b]	149±8.76[c]

(A): Betaine, 250 mg/kg body wt/day, p.o. for 30 days.(B): Isoprenaline, 11 mg/100g body wt/day, i.p. for 2 days. Results are mean ± SD for 6 animals. Values expressed: Troponin T, nanogram/ml. Homocysteine, µmol/l. Cratine Phosphokinase,µmol creatine liberated h^{-1} l^{-1}. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05; Duncan's multiple range test).

proteins essential for contraction and relaxation processes in myocardium.

In the present study, administration of betaine significantly prevented the isoprenaline-induced release of troponin T from myocardium into the blood stream, thereby demonstrating its protective action on the cell membrane. This perhaps is possible through the maintenance of the delicate balance of tonicity in cells in the myocardium. Betaine plays a major role in cell volume regulation by modulating the elasticity of plasma membrane (Farnum et al., 2002). 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. Studies by Wettstein et al. (2000) have shown that betaine can overt severe osmolar changes associated with possible cell death.

Recent prospective investigations (Senaratne et al., 2000) revealed that even mild hyperhomocysteinemia is associated with an increased risk of cardiovascular diseases independently of classical risk factors. In the present study, a significant increase (P<0.05) was noted in the level of homocysteine in the plasma of Group III rats compared to Group I animals (Table 1). This is in accordance with an earlier report of Hagar et al. (2002). Elevated level of homocysteine has also been reported to be associated with increased interleukin production in monocytes and up regulation of vascular cell adhesion molecules (Silverman et al., 2002).

Oral treatment with betaine significantly reduced the level of homocysteine in plasma of Group IV rats compared to Group III rats. This might be attributed to the inhibition of 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 (Berwanger et al., 1995). The highly lipotropic molecules like 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 betaine is more lipotropic than these statins and easily permeable to vascular smooth muscle cells, it is possible that likewise betaine may also inhibit both homocysteine and interleukin production.

The plasma concentration of CPK, a cardiac diagnostic marker enzyme was significantly higher (P<0.05) in isoprenaline administered rats (Group III) compared to the control group (Table 1) . This is in line with earlier reported studies of Nirmala and Puvanakrishnan (1998) which showed that the level of CPK released from the ischemic myocardium into the blood stream to be directly proportional to the number of necrotic cells present in the heart tissue. The release of CPK reflects non-specific alterations in the plasma membrane integrity and permeability as a response to β-adrenergic stimulation.

Table 1 showed that betaine significantly (P<0.05) prevented the isoprenaline-induced concentration of CPK in plasma of Group IV animals compared to Group III rats, indicating the cytoprotective activity of betaine. The membrane stabilizing action of betaine 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 (Saitoh et al., 2000) . It is possible that likewise betaine may also prolong the viability of myocardial cell membranes from necrotic damage by its membrane stabilizing property (Ji and Kaplowitz, 2003).

Taurine makes up more than 50% of the total amino acid pool in the mammalian heart (Satsu et al., 2002). There was a significant depletion in the level of taurine content in heart tissue of Group III rats compared to Group I controls (Table 2). Studies by Warskulat et al. (2004) demonstrated that pathology develops in the myocardium if the animal is depleted of taurine stores either through a taurine deficient diet or use of taurine transport antagonists. Pion et al. (1992) were the first to explain the role of dietary taurine deficiency associated with a dilated cardiomyopathy observed in experimental animals. Other studies by Lake (1994) have explored the relationship between taurine deficiency and cardiac contractility, loss of cardiac myofibrils and arrhythmogenesis. The Group IV rats pre-treated with betaine showed a significant increase in cardiac taurine content compared to Group III rats. Previous reports (Zapadniuk et al., 1987) indicated

Table 2. Levels of taurine, aspartate, glutamate, arginine and hydroxyproline in heart tissue (µmol/g wet tissue) of control and experimental groups of rats.

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Taurine	43.4±0.42^{a}	50.7±0.28^{b}	26.9±1.45^{c}	42.3±2.65^{a}
Aspartate	12.7±0.94^{a}	13.2±1.08^{a}	6.85±0.43^{b}	10.9±0.86^{c}
Glutamate	16.3±1.18^{a}	15.8±1.12^{a}	5.91±0.47^{b}	15.4±1.24^{a}
Arginine	19.2±1.36^{a,b}	18.4±1.21^{a}	28.4±1.76^{c}	20.7±1.18^{b}
Hydroxyproline	952±85^{a}	995±90^{a}	743±65^{b}	940±82^{a}

(A): Betaine, 250 mg/kg body wt/day, p.o. for 30 days. (B): Isoprenaline, 11mg/100g body wt/day, i.p. for 2 days. Results are mean± SD for 6 animals. Values expressed: Taurine, Aspertate, Glutamate, Arginine and Hydroxyproline µmol/g wet tissue. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05; Duncan's multiple range test).

that administration of betaine increased the fecal excretion of cholesterol without the involvement of taurine- mediated bile acid conjugation reactions. Supplementation of betaine might have preserved the cellular taurine content for other biological processes such as cell membrane stabilization, anti-oxidation, detoxification and osmoregulation in the myocardium.

Significant (*P*< 0.05) decrease was noted in the levels of aspartate and glutamate are important metabolites of the tricarboxylic acid cycle in heart tissue of Group III rats compared to Group I control rats (Table 2). Aspartate and glutamate concentrations have been reported to decrease in human hearts subjected to cardioplegic arrest followed by reoxygenation (Pisarenko et al., 1988). These findings suggested that the myocardial levels of these amino acids were closely associated with its energy state following ischemia and thus may affect the recovery of cardiac contractility. Although such decrease in this amino acid concentration has been primarily attributed to metabolism, they might also partly due to loss into extracellular space (Song et al., 1996).

Prior treatment with betaine significantly prevented the isoprenaline-induced decrease in the levels of aspartate and glutamate levels in Group IV rats compared to Group III rats. Betaine regulated osmolarity without causing additional perturbations of cellular tonicity (Kramer et al., 2002). Betaine attenuated aspartate release by regulation of mitochondrial Ca^{2+} sequestration by activation of a chloride channel and also suppressed the synaptic release of aspartate evoked by the voltage-gated sodium channel opener veratridine (Beck et al. 1995). Neutral and acidic amino acids are taken up into cells by Na^{+} dependant co-transport systems (Gegelashvili et al., 2006). Goldstein and Davis (1994) reported that different chemical classes of organic osmolytes shared a common volume-sensitive transporter.

Arginine is a conditionally essential amino acid involved in various metabolic functions in the body. Significant (*P*<0.05) increase in the level of arginine was observed in heart tissue of Group III rats compared to Group I rats (Table 2). This is in accordance with an earlier study by Gustafsson and Brunton (2000). Isoprena-line-mediated -AR stimulation has been reported to result in a phenotypic up regulation of iNOS in the heart and to enhance the release of pro-inflammatory mediators, which trigger increased NO production through arginine metabolism (Hu et al., 2006). Though NO is short-lived and relatively unreactive radical, it combines with super-oxide to form potent oxidant -ONOO- (peroxynitrite), which plays a significant role in iNOS- mediated postischemic cells damage (Arstall et al., 1999). In the present study, prior administration of betaine maintained the cardiac arginine content at near normal in Group IV rats compared to Group III rats apparently by blockage of the availability of NO radicals and subsequent inhibition of iNOS activity (Arakawa, 2007). Studies by Sjakste et al. (2004) showed that betaine inhibited inducible nitric oxide synthase and tumor necrosis factor- gene expression in activated alveolar macrophages, leading to a decrease in the production of peroxynitrite radicals.

A significant decrease (*P*<0.05) in the hydroxyproline content in the heart tissue of Group III rats compared to Group I rats (Table 2), indicated aberrations in the structural and functional integrity of the collagen fibre network that connects the myocytes in the myocardium that may have ruptured due to pathophysiological expansion of infarction (Whittaker, 1995). These fibre are very important in the maintenance and transmission of the contractile force from the myocytes to the ventricular lumen as reported by Weber (1989). Degradation of collagen occurs in the ischemic myocardium as a result of increase in the activities of lysosomal enzymes, enhanced lysosomal fragility and infiltration of inflammatory cells (Nirma Oral prior administration of betaine significantly (*P*<0.05) prevented the isoprenaline-induced reduction in hydroxyproline content in the heart tissue of Group IV animals compared to Group III rats. This may be due to the enhancement of structural integrity of the cellular and subcellular membranes. Betaine has been reported to possess the most potent membrane stabilizing action as a result of distinct biophysical interactions with membrane lipid bilayer (Kanbak et al., 2001) la and Puvanakishnan, 1996).

Protein synthesis and protein degradation are highly regulated cellular processes essential for cell viability. Alteration in steady state protein metabolism is an important factor in regulating cellular homeostasis in response to oxida-

Table 3. 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 Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Plasma				
Protein	6.52 ± 0.54^{a}	6.71 ± 0.48^{a}	7.85 ± 0.69^{b}	6.82 ± 0.57^{a}
Hexose	98.4 ± 7.32^{a}	87.8± 7.94^{b}	121 ± 9.54^{c}	101 ± 8.72^{a}
Hexosamine	52.4 ± 2.93^{a}	47.5 ± 2.52^{b}	69.3 ± 3.37^{c}	53.9 ± 2.76^{a}
Heart				
Protein	176 ± 14.5^{a,b}	183 ± 15.2^{b}	132 ± 10.7^{c}	165 ±14.7^{a}
Hexose	22.7 ± 1.43^{a}	24.1± 1.24^{b}	15.3 ± 0.84^{c}	22.5 ± 1.08^{a}
Hexosamine	7.38 ± 0.54^{a}	7.25 ± 0.49^{a}	5.11 ± 0.32^{b}	7.88 ± 0.45^{c}

(A): Betaine, 250 mg/kg body wt/day, p.o. for 30 days. (B): Isoprenaline, 11mg/100g body wt/day, i.p. for 2 days. Results are mean± SD for 6 animals. Values expressed: as mg/dl for plasma; mg/g for wet heart tissue. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05; Duncan's multiple range test).

Table 4. Levels 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 (nmol malondialdehyde released/mg protein) of normal and experimental groups of rats.

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
LPO:				
Basal	0.91± 0.05^{a}	0.82 ± 0.04^{b}	1.79 ± 0.09^{c}	0.96 ± 0.06^{a}
Ascorbic acid	2.94 ± 0.18^{a}	2.71 ± 0.15^{b}	5.11 ± 0.24^{c}	3.04 ± 0.16^{a}
$FeSO_4$	4.48 ± 0.28^{a}	4.39 ± 0.25^{a}	7.15 ± 0.55^{b}	4.97 ± 0.33^{c}
t-BH	6.15 ± 0.31^{a}	5.98 ± 0.27^{a}	8.46 ± 0.51^{b}	6.29 ± 0.38^{a}

(A): Betaine, 250 mg/kg body wt/day, p.o. for 30 days. (B): Isoprenaline, 11mg/100g body wt/day, i.p. for 2 days. Results are mean ± SD for 6 animals. Values expressed: LPO, nmol malondialdehyde released/ mg protein. Values that have a different superscript letter (a,b,c) differ significantly with each other (P<0.05; Duncan's multiple range test).

oxidative damage in myocardial infarction condition. In the present investigation, there was a significant (*P*<0.05) increase in the levels of protein and glycoprotein components in plasma with a concomitant decline in their levels in heart tissue of Group III isoprenaline-administered rats compared to Group I rats (Table 3). This is in accordance with the earlier reported study (Dudnakova et al., 2002), which indicated that the increase observed in the protein content in plasma of isoprenaline-administered rats is, at least in part, due to leakage of enzymes and protein-bound components from the damaged myocardium into the systemic circulation. The reduction in the hexose and hexosamine content observed in heart tissue of isoprenaline-induced myocardial infarction might be due to inhibition of glycoprotein synthesis (Table 3). Oxidation of protein is a common phenomenon mediated by highly reactive agents in myocardial infarction condition and oxidized proteins are in turn capable of inducing oxidative stress, a potential mediator of the pathogenesis. This protein oxidation might also be a possible reason for the decline noted in the protein and glycoprotein levels in the heart tissue of Group III rats.

In the present study, oral pretreatment with betaine marginally (*P* <0.05) prevented the isoprenaline-induced alterations in the levels of protein content and glycoprotein components in plasma and heart tissue of Group IV rats compared to Group III rats, possibly by inhibiting the disaggregation of polyribosomes or by attenuating the isoprenaline-induced oxidation of myocardial proteins. Balkan et al., (2004) reported that supplementation of betaine protected the structural and functional integrity of the cell membranes by counteracting reactive oxygen species-mediated lipid peroxidation and protein carbonyl formation. Reports by Erman et al. (2004) indicated that betaine administration protected proteins and lipids from ethanol-induced oxidative damage by its antiperoxidative property.

Isoprenaline-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. Lipid peroxidation 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 level of lipid peroxidation in heart tissue of Group III isoprenaline-administered rats was significantly (*P*<0.05) higher compared with Group I animals (Table 4). Lack of antioxidant defense might

have led to an increase in lipid peroxidation and subsequent deleterious effects on the myocardial membrane in isoprenaline-induced myocardial infarction condition. Synthetic catecholamine isoprenaline, by its property of generating free radicals during the course of its metabolism, alters redox homeostasis and causes damage to cell structure and function (Kukreja and Hess, 1992). In the present study the generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging enzymes to dismute isoprenaline-generated free radical formation resulting in myocyte lesions and reduction of scavengers.

Prior oral administration of betaine resulted in significant ($P<0.05$) reduction in the level of lipid peroxidation compared with the levels in the Group III rats which might be a counteracting effect of isoprenaline-generated free radicals by its antioxidant property. Betaine is highly lipotropic and, when administered exogenously, it can readily pass across the membrane lipid bilayer (Kanbak et al., 2001). The ability of betaine to diffuse into intracellular compartments aids the capabilities of this natural product as an antioxidant. Report by Balkan et al. (2005) indicated that betaine supplementation was effective in prevention of 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 (Barak et al., 1997). 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 (Kim and Kim, 2002).

In conclusion, the results of the present investigation indicated that oral pre-treatment with betaine ameliorates isoprenaline-induced aberrations in the levels of protein content, glycoprotein components and amino acids in isoprenaline-induced myocardial infarction in rats. The overall cardioprotective effect of betaine is probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action or to a counteraction of free radicals by its antioxidant nature.

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