Protective Effect of Betaine on Changes in the Levels of Membrane-bound ATPase activity and Mineral Status in Experimentally Induced Myocardial Infarction in Wistar Rats

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Abstract Cardiovascular diseases are emerging as a major public health problem in most parts of the world even in developing countries still afflicted by infectious diseases, undernutrition, and other illnesses related to poverty. In the present study, we investigated the protective effect of betaine, a potent lipotropic molecule, on changes in the levels of membrane-bound ATPase activities, lipid peroxidation, sulfhydryl activities, and mineral status in isoprenaline-induced myocardial infarction in Wistar 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 ($p < 0.05$) reduced the isoprenaline-induced abnormalities noted in the levels of sodium, potassium, and calcium in plasma and heart tissue. Pretreatment with betaine significantly attenuated isoprenaline-induced membrane-bound ATPase depletion in the heart tissue and preserved the myocardial membrane-bound ATPase activities at levels comparable to that of control rats. Oral administration of betaine significantly attenuated the isoprenaline-altered sulfhydryl groups in the heart tissue and preserved the myocardial sulfhydryl activities at levels comparable to that of control rats. It also significantly counteracted the isoprenaline-mediated lipid peroxidation and maintained the level at near normal. In the results of the present study, betaine administration significantly prevented the isoprenaline-induced alterations in the activities of membrane-bound ATPases, lipid peroxides, myocardial sulfhydryl levels, and maintained the mineral status at near normal.

Keywords Isoprenaline · Myocardial infarction · Membrane-bound ATPase ·
Mineral status · Trimethylglycine

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Introduction

Cardiovascular diseases are emerging as a major public health problem in most parts of the world even in developing countries still afflicted by infectious diseases, undernutrition, and other illnesses related to poverty [1]. Every year, about 12 million people throughout the world die of a heart attack and stroke [2]. These diseases have been implicated as leading causes of death for both men and women of all racial and ethnic groups [3]. Women are much more at risk after menopause [4]. The World Health Organization estimates that, over the next two decades, developing countries will bear the brunt of the increasing burden of cardiovascular diseases [5]. Myocardial infarction predominates in Latin America, the Middle East, and Urban India. Over the past 40 years, the prevalence of myocardial infarction in urban India has increased by a factor of six to eight, to about 10% among persons 35–64 years of age [6]. Moreover, it is very much painful and of serious concern to realize that myocardial infarction in India occurs 10–15 years earlier as compared to that of West [7].

There has been increasing recognition that certain natural substances have the potential to reduce the detrimental effect of a number of cardiovascular risk factors. Betaine is found in microorganisms, plants, and animals and is a significant component of many foods including wheat, shellfish, spinach, and sugar beets [8]. Betaine (also known as trimethyl glycine or glycine betaine) (Fig. 1) is an osmolyte and methyl donor shown to protect internal organs and improve vascular risk factors [9].

It has been shown that choline and betaine are structurally very similar, and choline is largely oxidized to betaine in the body. Choline is also metabolized to acetylcholine and phosphatidylcholine, and is essential for the normal function of cells [10]. It has been reported that betaine is non-perturbing to cellular metabolism, highly compatible with enzyme function, and stabilizes cellular metabolic function under different kinds of stress in various organisms and animal tissues [11]. It has also been shown that betaine protected the liver from the damaging effects of CCl_4-induced nephrotoxicity in Sprague–Dawley rats [12]. The principal physiologic role of betaine is either as an organic osmolyte to protect cells under stress or as a catabolic source of methyl groups via transmethylation for use in many biochemical pathways. As a methyl donor, betaine participates in methionine metabolism by its ability to convert homocysteine (Hcy) to methionine [9]. Earlier, we have reported the protective effect of betaine on lysosomal function [13], protein and glycoprotein metabolism [14], and lipid metabolism [15] in isoprenaline-induced myocardial infarction in Wistar rats.
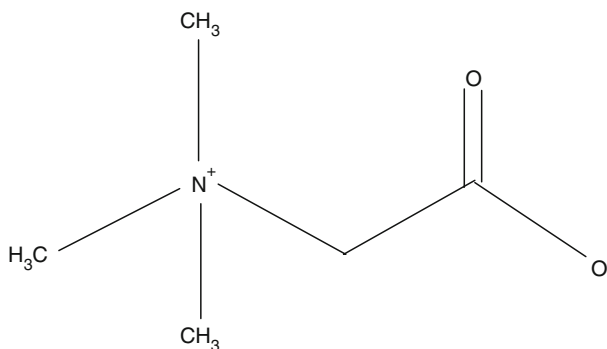

Fig. 1 Chemical structure of betaine

Cardiac marker enzymes are proteins from cardiac tissue found in the blood. These proteins are released into the bloodstream when damage to the heart occurs, as in the case of myocardial infarction. The activities of membrane-bound enzymes such as Na^+/K^+ ATPase, Ca^{2+} ATPase, and Mg^{2+} ATPase maintain the integrity of the myocardial membrane. ATPases of cardiac cells play a significant role in the contraction and relaxation cycles of cardiac muscle by maintaining normal ion (mineral) levels (sodium, potassium, and calcium) within the myocytes. Changes in the properties of these ion pumps affect cardiac function. Cellular injury is associated with alterations in mineral homeostasis. Thus, these enzymes and ions play a vital role in the pathology of myocardial infarction [16]. Hence, we undertook the present study with new cohorts of animals to evaluate the protective effect of betaine on these membrane-bound ATPases, lipid peroxides, myocardial sulfhydryl levels, and mineral status in isoprenaline-induced myocardial infarction in rats.

Isoprenaline (Fig. 2), a synthetic catecholamine and β-adrenergic agonist, causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle. Isoprenaline administration causes intracellular calcium overload which leads to a deleterious high-energy phosphate deficiency by the excessive activation of Ca^{2+}-dependent intracellular ATPase and by impairing the phosphorylating capacity of mitochondria. Isoprenaline-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes [17]. Isoprenaline-induced myocardial infarction there by serves as a well-standardized model to study the beneficial effects of many drugs and cardiac function [18]. The rat model of isoprenaline-induced myocardial necrosis, out of many well-known models, has often been used to evaluate several cardiac dysfunctions [19].

Materials and Methods

Chemicals

Betaine and isoprenaline were obtained from M/s. 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)
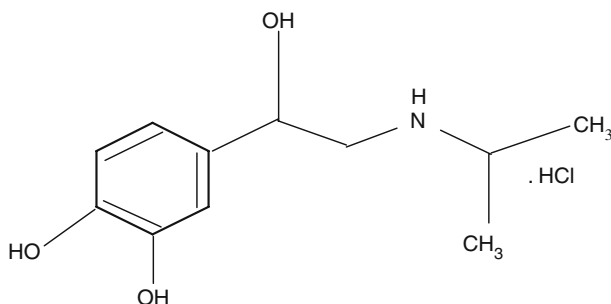

Fig. 2 Structure of isoprenaline hydrochloride

under hygienic and standard environmental conditions ($28\pm2°C$, humidity 60–70%, 12-h light/dark cycle). The animals were allowed a standard diet [M/s Sai Feeds, Bangalore, India] and water *ad libitum*. The diet provided metabolizable energy of 3,600 kcal (Table 1). 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 Ethical Committee of the Central Institute of Fisheries Technology.

Induction of Myocardial Infarction

Myocardial infarction was induced in experimental rats by intraperitoneal (i.p.) injection of isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight/day] for 2 days [20].

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 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 weight/day], i.p. for 2 days for the induction of myocardial infarction. In group IV, the animals were pretreated with betaine [250 mg/kg body weight/day, p.o. for 30 days] before the induction of myocardial infarction as described for group III. Control animals (group I and group II) were injected with physiological saline alone for 2 days.

At the end of the experimental period, i.e., 24 h after last injection of isoprenaline, the experimental animals were killed by using chloroform, blood was collected using sodium citrate as anticoagulant, and the plasma separated was used for the determination of minerals (sodium, potassium, and calcium) by using an atomic absorption spectrophotometer. The heart tissue was dissected out immediately, washed with chilled physiological saline and the heart tissue homogenates prepared in ice-cold 0.1 M Tris–HCl buffer, pH 7.2, were used for the determination of the levels of sodium, potassium and calcium, membrane-bound Na^+/K^+-dependent ATPase activity, Mg^{2+} ATPase activity, Ca^{2+}-dependent ATPase activity, lipid

Table 1 Composition of the Diet

Sample no.	Ingredients	Composition (g/100 g diet)
1	Carbohydrate (nitrogen free)	56.2
2	Crude protein	22.0
3	Ash	7.5
4	Crude oil	4.2
5	Crude fiber	3.0
6	Glucose	2.5
7	Vitamins	1.8
8	Sand silica	1.4
9	Calcium	0.8
10	Phosphorus	0.6

peroxides (LPO), and myocardial sulfhydryl contents (total sulfhydryl content (TSH), non-protein-bound sulfhydryl content (NPSH), and protein-bound sulfhydryl content (PSH)).

Biochemical Assays

Determination of Minerals

Minerals (sodium, potassium, and calcium) were estimated according to the method of the AOAC [21]. Samples' size of 1 g heart tissue and 1 ml plasma were used for the experiment. To the sample-containing flask, 7 ml of nitric acid and perchloric acid (9:4) mixture was added, covered with a watch glass, and left at room temperature overnight. The sample was then digested using a microwave digester (Milestone ETHOS PLUS lab station Closed Vessel Microwave Digestion System). The completely digested samples were allowed to cool at room temperature, filtered (glass wool) carefully transferred into a clean 50-ml volumetric standard flask, and then diluted to the mark with ultra pure water (Milli Q, Millipore). The digested samples were analyzed using Varian Spectra-220AA atomic absorption spectrophotometer equipped with a deuterium background corrector for the determination of minerals viz. sodium, potassium, and calcium.

Assay of Na^+/K^+ ATPase

The activity of Na^+/K^+ ATPase in heart tissue was measured by using the method of Bonting [22]. The incubation mixture contained 1.0 ml buffer, 0.2 ml magnesium sulfate, 0.2 ml potassium chloride, 0.2 ml sodium chloride, 0.2 ml EDTA, and 0.2 ml ATP. After incubation at 37°C for 10 min, the reaction was initiated by the addition of 0.2 ml tissue homogenate and the contents were incubated at 37°C for 15 min, after which 1.0 ml 10% TCA was added to stop the reaction. The tubes were centrifuged and supernatant was used for the estimation of phosphorus (Pi) by the method of Fiske and Subbarow [23]. Supernatant (1.0 ml) was made up to 4.3 ml with distilled water to which was added 1.0 ml ammonium molybdate reagent. The tubes were incubated at room temperature for 10 min, after which 0.4 ml amino napthol sulfonic acid was added. The color developed was read at 640 nm after 20 min. The enzyme activity was expressed as µmol Pi liberated/min/mg protein.

Assay of Mg^{2+} ATPase

The activity of Mg^{2+} ATPase in heart tissue was determined by the method of Ohnishi et al. [24]. The incubation mixture consisted of 0.1 ml Tris–HCl buffer, 0.1 ml magnesium chloride, 0.1 ml ATP, 0.1 ml distilled water, and 0.1 ml tissue homogenate. The contents were incubated at 37°C for 15 min, after which the reaction was stopped by adding 0.5 ml 10% TCA. The phosphorus (Pi) generated was estimated as described above. The enzyme activity was expressed as µmol Pi liberated/min/mg protein.

Assay of Ca^{2+} ATPase

The activity of Ca^{2+} ATPase in heart tissue was determined by the method of Hjerten and Pan [25]. The incubation mixture contained 0.1 ml buffer, 0.1 ml calcium chloride, 0.1 ml ATP, 0.1 ml distilled water, and 0.1 ml tissue homogenate. The contents were incubated at 37°C for 15 min, after which the reaction was stopped by the addition of 0.5 ml ice-cold

10% TCA. The amount of phosphorous (Pi) generated was estimated as described above. The enzyme activity was expressed as μmol of Pi liberated/min/mg protein.

Assay of Lipid Peroxidation (LPO)

Lipid peroxidation (LPO) was assayed by the method of Ohkawa et al. [26] in which the malondialdehyde (MDA) released served as the index of LPO. 1,1,3,3-Tetra ethoxypropane malondialdehyde bis (diethyl acetal) was used as standard. To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4.0 ml with water and then heated in a water bath at 95.8°C for 60 min using a glass ball as condenser. After cooling, 1.0 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of lipid peroxides was expressed as nmol MDA formed/mg of protein.

Estimation of Total Sulfhydryl, Protein-bound, and Non-protein-bound Sulfhydryl Contents

Total sulfhydryl, protein-bound, and non-protein-bound sulfhydryl contents were estimated according to the method of Sedlak and Lindsay [27]. This method is based on the development of a yellow color when DTNB is added to compounds contained sulfhydryl groups to form 2-nitro 5-mercaptobenzoic acid. The total, protein, and non-protein sulfhydryl contents are expressed as nmol/g wet tissue.

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

Figure 3 shows the levels of lipid peroxidation in heart tissue of control and experimental groups of rats. There was a significant ($p<0.05$) increase noted in the level of lipid peroxidation in heart tissue of group III rats as compared with group I control rats. In the present study, prior oral administration of betaine significantly ($p<0.05$) prevented all these isoprenaline-induced alterations and maintained the lipid peroxidation in the myocardium at near normal.

Body Weight and Daily Feed Consumption

Rats were fed commercial diet as shown in Table 1. There is no significant difference observed in the level of body weight, total feed consumption, and feed efficiency ratio in control and experimental groups of rats (Table 2). The group III rats were apparently dull in food intake and fluid consumption after the injection of isoprenaline. However, the behavior of the animals orally administered with betaine was comparable to that of normal controls.

Table 3 depicts the levels of sodium, potassium, and calcium content in plasma of normal and experimental groups of rats. Intraperitoneal administration of isoprenaline caused a significant ($p<0.05$) elevation in the levels of sodium and calcium in the plasma of
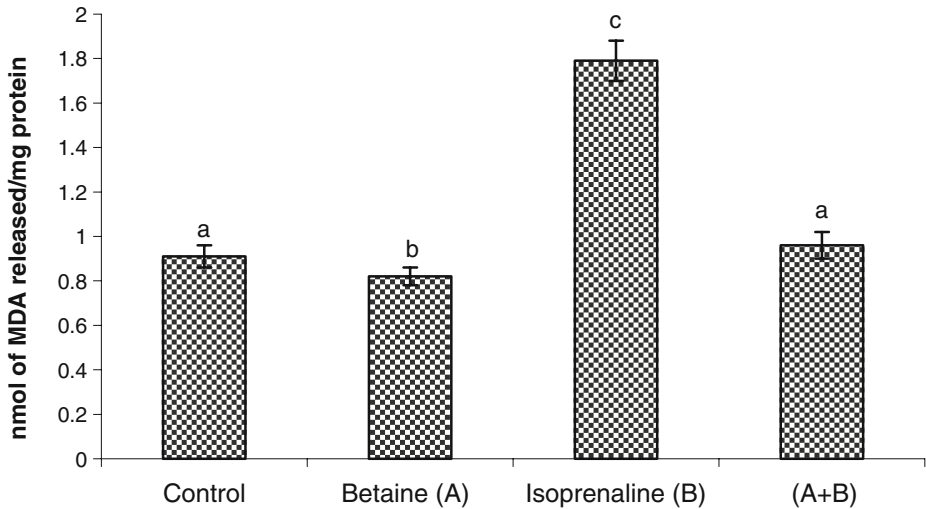
Fig. 3 Level of lipid peroxidation in heart tissue of normal and experimental groups of rats. *(A)* Betaine, 250 mg/kg body weight/day, oral administration for 30 days. *(B)* Isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days. Results are mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (*a, b, c*) differ significantly ($p<0.05$) with each other. Values expressed: LPO, nmol malondialdehyde released/mg protein

group III rats as compared with that of group I control rats. A parallel decline in the level of potassium was also observed. In the present study, oral pretreatment with betaine [250 mg/kg body weight/day for a period of 30 days] significantly ($p<0.05$) prevented the isoprenaline-induced alteration in the levels of sodium, potassium, and calcium content in plasma and also maintained the rats at near normal status.

Table 4 shows the levels of sodium, potassium, and calcium content in heart tissue of normal and experimental groups of rats. There was a significant ($p<0.05$) elevation noted in the levels of potassium and calcium in heart tissue of group III rats as compared with group I animals. A parallel decline in the level of sodium was also observed. Prior oral treatment with betaine significantly ($p<0.05$) prevented the isoprenaline-induced alteration in the levels of sodium, potassium, and calcium content in heart tissue and also maintained the rats at near normal status.

Table 2 Levels of Body Weight, Accumulated Weight Gain, Total Feed Consumption, and Feed Efficiency Ratio in Control and Experimental Groups of Rats

Growth parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Initial body weight (g)	157.0±11.3[a]	160.0±11.5[a]	163.0±11.7[a]	159.0±11.4[a]
Accumulated weight gain (g)	260.0±18.7[a]	262.0±18.8[a]	267.0±19.2[a]	264.0±19.0[a]
Total feed consumption	450.0±32.4[a]	455.0±32.7[a]	460.0±33.1[a]	456.0±32.8[a]
Feed efficiency ratio (g gain/g feed)	0.57±0.04[a]	0.57±0.04[a]	0.58±0.04[a]	0.57±0.04[a]

(A): Betaine, 250 mg/kg body weight/day, oral administration for 30 days. (B): Isoprenaline, 11 mg 100 g/ body weight/day, i.p. for 2 days

Results are mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have superscript letter (*a*) did not significantly ($p<0.05$) differ with each other

Table 3 Levels of Sodium, Potassium, and Calcium Content in Plasma of Control and Experimental Groups of Rats

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Sodium	$99.0 \pm 7.12^{a,c}$	106 ± 7.63^{a}	160 ± 11.52^{b}	91.5 ± 6.58^{c}
Potassium	6.92 ± 0.50^{a}	7.15 ± 0.51^{a}	3.90 ± 0.28^{b}	7.01 ± 0.51^{a}
Calcium	57.5 ± 4.14^{a}	54.0 ± 3.88^{a}	107 ± 7.70^{b}	64.5 ± 4.64^{c}

(A): Betaine, 250 mg/kg body weight/day, oral administration for 30 days. (B): Isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days

Results are mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c) differ significantly ($p < 0.05$) with each other

Table 5 depicts the activities of membrane-bound ATPases (Na^{2+}, K^+ ATPase, Mg^{2+} ATPase, and Ca^{2+} ATPase) in the heart tissue of control and experimental groups of rats. Intraperitoneal administration of isoprenaline induced a significant ($p < 0.05$) reduction in the levels of Na^{2+}, K^+ ATPase, Mg^{2+} ATPase, and Ca^{2+} ATPase in the heart tissue of group III rats as compared with that of group I normal control rats. In the present study, prior oral administration of betaine significantly ($p < 0.05$) prevented all these isoprenaline-induced adverse effects and maintained the membrane-bound ATPases in heart tissue at near normal.

Table 6 shows the levels of sulfhydryl content (total sulfhydryl content (TSH), non-protein-bound sulfhydryl content (NPSH), and protein-bound sulfhydryl content (PSH)) in heart tissue of control and experimental groups of rats. There was a significant ($p < 0.05$) decline noted in the levels of total sulfhydryl content, non-protein-bound sulfhydryl content, and protein-bound sulfhydryl content in heart tissue of group III rats as compared with group I normal control rats. In the present study, prior oral administration of betaine significantly ($p < 0.05$) prevented all these isoprenaline-induced alterations and maintained the sulfhydryl contents in the myocardium at near normal.

Discussion

A significant ($p < 0.05$) rise was observed in the level of calcium in the plasma and heart tissue of isoprenaline-administered group III rats compared with group I normal control rats (Tables 3 and 4). This is in accordance with an earlier reported study [28]. Calcium is

Table 4 Levels of Sodium, Potassium, and Calcium Content in Heart Tissue of Control and Experimental Groups of Rats

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Sodium	350 ± 25.20^{a}	335 ± 24.12^{a}	193 ± 13.89^{b}	300 ± 21.6^{c}
Potassium	133 ± 9.57^{a}	140 ± 10.08^{a}	216 ± 15.55^{b}	153 ± 11.01^{c}
Calcium	77.4 ± 5.57^{a}	82.5 ± 5.90^{a}	146 ± 10.51^{b}	95.5 ± 6.87^{c}

(A): Betaine, 250 mg/kg body weight/day, oral administration for 30 days. (B): Isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days

Results are mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c) differ significantly ($p < 0.05$) with each other

Table 5 The Activities of Membrane-bound ATPases (Na^{2+}, K^+ ATPase, Mg^{2+} ATPase, and Ca^{2+} ATPase) in Heart Tissue of Control and Experimental Groups of Rats

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
Na^{2+}, K^+ ATPase	1.42 ± 0.10^a	1.34 ± 0.09^a	0.85 ± 0.06^b	1.16 ± 0.08^c
Mg^{2+} ATPase	1.06 ± 0.07^a	$1.00\pm0.07^{a\ c}$	0.76 ± 0.05^b	0.98 ± 0.07^c
Ca^{2+} ATPase	0.65 ± 0.05^a	0.73 ± 0.05^b	0.41 ± 0.03^c	0.60 ± 0.04^d

(A): Betaine, 250 mg/kg body weight/day, oral administration for 30 days. (B): Isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days

Results are mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c, d) differ significantly ($p<0.05$) with each other. Values expressed: the membrane-bound ATPase activities are expressed as μmol Pi liberated/min/mg protein

essential for normal cardiac function, for the maintenance of cell membrane integrity, and for coagulation of blood. In the heart, cytosolic calcium is carefully controlled and Ca^{2+} is the key ion for normal activity of many enzymes [29]. Isoprenaline-induced myocardial infarction has been reported to enhance adenylate cyclase activity, resulting in increased formation of cAMP [30]. During β-adrenergic stimulation, cAMP phosphorylates several sites on the C-terminal chains of the calcium channel and increases the probability of the calcium channel opening [31]. This may be the reason for enhanced activity of Ca^{2+} ATPase and increased concentration of Ca^{2+} observed of myocardial tissue in isoprenaline-induced myocardial infarcted rats. Intracellular Ca^{2+} overload can set off a cascade of events that can lead to the formation of reactive oxygen species, which suggests that reactive oxygen species formation and Ca^{2+} surge may be involved in the contractile dysfunction of the ischemic myocardium [32]. Since calcium and sodium ions are competitive at a number of membrane sites, a high concentration of calcium ions in the cells of ischemic heart would compete with sodium-specific sites at the inner surface of the membrane, leading to a decrease in myocardial sodium content [33].

Pretreatment with betaine significantly ($p<0.05$) prevented isoprenaline-induced alterations in the levels of sodium, potassium, and calcium ions both in plasma and heart tissue of group IV rats. The transport of Na^+ and K^+ between intra- and extracellular pools and the maintenance of the transmembrane gradients are important to cell function and integrity.

Table 6 Levels of Sulfhydryl Groups (Total Sulfhydryl Content (TSH), Non-protein-bound Sulfhydryl Content (NPSH), and protein-bound Sulfhydryl Content (PSH)) in Heart Tissue of Control and Experimental Groups of Rats

Parameters	Group I Control	Group II Betaine (A)	Group III Isoprenaline (B)	Group IV (A+B)
TSH	2.43 ± 0.18^a	2.45 ± 0.18^a	1.73 ± 0.12^b	2.32 ± 0.16^a
NPSH	0.53 ± 0.04^a	0.54 ± 0.04^a	0.43 ± 0.03^b	0.51 ± 0.03^a
PSH	1.90 ± 0.14^a	1.91 ± 0.14^a	1.30 ± 0.09^b	1.81 ± 0.13^a

(A): Betaine, 250 mg/kg body weight/day, oral administration for 30 days. (B): Isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days

Results are mean±SD for six animals. Values expressed: TSH, NPSH, PSH contents are expressed as nmol/g wet tissue. One-way ANOVA; Duncan's multiple comparison tests. Values that have a different superscript letter (a, b) differ significantly ($p<0.05$) with each other. Values expressed: The total sulfhydryl, non-protein-bound sulfhydryl, and protein-bound sulfhydryl contents are expressed as nmol/g wet tissue

The Na$^+$/betaine co-transport mechanism might have played an important role in the protection against Na$^+$/K$^+$ imbalance [34] and intracellular calcium overload. Betaine treatment has probably improved Ca^{2+} homeostasis by facilitating the efflux of Ca^{2+} via the Na$^+$/Ca^{2+} exchanger [35]. In addition to this, the ability of betaine to maintain the integrity of membrane-bound ATPases might have contributed significantly to its role in maintaining the ionic equilibrium in group IV rats. This is in agreement with studies by Coelho-Sampaio et al. [36] which indicated that betaine prevented the suppression of membrane-bound ATPases in human erythrocyte plasma membrane. Betaine is also reported to normalize the content of potassium and calcium ions in mammalian cells [37].

There was a significant ($p<0.05$) reduction noticed in the activities of the membrane-bound ATPases (Na$^+$, K$^+$-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase) in the heart tissue of group III isoprenaline-induced myocardial infarcted rats compared with group I normal control rats (Table 5). This is in line with previous findings [38]. The Na$^+$/K$^+$ ATPase pump is responsible for the active transport of Na$^+$ and K$^+$ across the cell membrane. Ahmed and Thomas [39] reported that increased concentrations of free fatty acids (FFAs) in the myocardium resulted in the non-competitive inhibition of many enzyme systems such as Na$^+$/K$^+$ ATPase. Inhibition of the sodium pump may precipitate increased levels of intracellular sodium [40]. The increased levels of FFAs may have resulted in non-competitive inhibition of Na$^+$/K$^+$ ATPase, thereby leading to increased accumulation of Na$^+$ ions in isoprenaline-induced myocardial infarcted rats. ATPases are integral membrane proteins which require thiol groups and phospholipids to maintain their structure and function. According to Hazarika and Sarkar [41], peroxidation of membrane phospholipids not only altered the lipid milieu and structural as well as functional integrity of cell membrane but also affected the activities of various membrane-bound enzymes including Mg^{2+} ATPase, Ca^{2+} ATPase, and Na$^+$, K$^+$ ATPase.

In the present study, the group IV rats pretreated with betaine showed a significant ($p<0.05$) increase in the level of membrane-bound ATPases compared to group III isoprenaline-injected rats. It probably did so by its membrane-stabilizing action. Supplementation of thiol-group-generating substances and free-radical scavengers has been reported to restore the cellular thiol content and membrane functions. Since ATPases require sulfhydryl groups for their structural and functional integrity, the maintenance of thiol content by betaine might have contributed to its protective effect [42]. Reports by Kanbak et al. [43] showed that supplementation of betaine restored the ethanol-induced reduction in the activity of membrane-bound Na$^+$, K$^+$ ATPase by its antioxidant property. Betaine can be considered both as an antioxidant and as a membrane stabilizer. It exerts cellular and subcellular membrane stabilization in the liver by restoring both non-enzymic and enzymic antioxidants [42]. Betaine treatment significantly prevented the inhibition in the activities of Na$^+$, K$^+$ ATPase and Ca^{2+} ATPase in human RBC membranes against hypoosmotic stress [44].

A significant ($p<0.05$) increase noticed in the level of lipid peroxides in the cardiac tissue of group III isoprenaline-administered rats as compared to that of group I normal control rats reflected the oxidative deterioration of myocardial cell membrane (Fig. 3). This is in corroboration with an earlier investigation [45], 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 information of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA, and proteins [46].

In the present investigation, the prior oral administration of betaine resulted in a significant ($p<0.05$) reduction in the level of lipid peroxidation in the heart tissue of group

IV rats. It probably did so by counteracting the isoprenaline-generated free radicals by its antioxidant property [47]. Betaine is highly lipotropic and, when administered exogenously, it can readily pass across the membrane lipid bilayer [43]. The ability of betaine to diffuse into intracellular compartments aids the capabilities of this natural product as an antioxidant. Reports by Balkan et al. [48] 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. Betaine treatment is also found to decrease lipid peroxidation in ethanol-treated rats. As it is known, betaine participates in the synthesis of methionine from homocysteine and restores SAM levels, which has essential roles in phospholipid metabolism and membrane structure.

Thiols are crucial targets for oxidation in cells. These reducing agents are an indirect measure of oxidative damage. The oxidation of sulfhydryl groups in proteins may affect their functional properties. Formation of protein disulfides, mixed disulfides with glutathione, or sulfenic acids can result in changes in enzymatic activity, conformation, or affinity towards other molecules. Such changes contribute to the cell damage caused by oxidative stress. Modulation of thiol redox state also provides a sensitive mechanism for regulation of metabolic processes. Lower amounts of thiols are indicative of an increase in oxidative stress and cellular damage [49]. Oxidative stress is known to play an important role in the pathogenesis of myocardial injury. 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 [50].

Intraperitoneal administration of isoprenaline significantly ($p < 0.05$) reduced the total sulfhydryl content, protein-bound sulfhydryl, and non-protein-bound sulfhydryl content in group III rats (Table 6). This is in line with earlier reported studies [51]. The reactive oxygen species produced by isoprenaline might have induced oxidation of sulfhydryl groups, leading to a decline in thiol concentration in the myocardial tissue. Isoprenaline can be converted into o-quinones and undergoes cyclization into aminochromes enzymatically or through autoxidation. Aminochromes are highly reactive molecules that can cause oxidation of protein sulfhydryl groups [52].

In the present investigation, pretreatment with betaine preserved total sulfhydryl as well as protein-bound and non-protein-bound thiol contents in heart tissue of group IV rats compared to group III rats. It probably did so by shielding the thiol groups of the myocardial membrane from reactive oxygen species by its antioxidant property. This observation concurs with an earlier reported study [42] which showed that betaine protects erythrocyte membrane alterations against chronic ethanol toxicity in rats. It has been shown that betaine has the ability to enhance thiol levels as an antioxidative defense system. Go et al. [53] reported that betaine exerts its efficacy (both in vivo and in vitro) by maintaining thiol status in the regulation of COX-2 and TNFalpha via NF-kappaB activation during aging in rats. There is also evidence that administration of betaine along with ethanol restored glutathione and tissue thiols in rats [54].

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 a period of 30 days maintains the mineral levels (sodium, potassium, and calcium) and the activities of membrane-bound ATPases by the inhibition of lipid peroxidation, preventing the accumulation of lipid peroxidation products and thereby inhibiting the calcium ion transport system at near normal. The effects observed in this study are due to the antioxidant and membrane-stabilizing effects of betaine in experimentally induced myocardial infarction.

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