

# Biochemical Studies on the Protective Effect of Betaine on Mitochondrial Function in Experimentally Induced Myocardial Infarction in Rats

Balaraman Ganesan,<sup>a,b</sup> Rangasamy Rajesh,<sup>a</sup> Rangasamy Anandan,<sup>\*,b</sup> and Nanjappan Dhandapani<sup>a,c</sup>

<sup>a</sup>Vinayaka Mission's University, Ariyanoor, Sankari Main Road, Salem-636308, Tamil Nadu, India, <sup>b</sup>Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, CIFT Road, Matsyapuri (PO), Cochin-682029, India, and <sup>c</sup>Department of Pharmaceutical Chemistry, R.V.S College of Pharmaceutical Sciences, Trichy Road, Sulur, Coimbatore-641402, India

(Received April 13, 2007; Accepted August 21, 2007)

The present study was designed to examine the cardioprotective effect of betaine on mitochondrial function in isoprenaline-induced myocardial infarction in rats with respect to changes in the mitochondrial energy status and antioxidant defense system. Prior oral treatment with betaine significantly prevented the isoprenaline-induced elevation in the levels of diagnostic marker enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK)] and homocysteine in plasma of the experimental group of rats. Its administration significantly counteracted the isoprenaline-induced aberrations in the myocardial energy status by maintaining the levels of myocardial ATP and betaine contents and the activities of mitochondrial TCA cycle enzymes [isocitrate dehydrogenase (ICDH),  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ -KDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH)] and respiratory marker enzymes (NADH dehydrogenase and cytochrome-*c*-oxidase) at near normalcy. It also exerted an antioxidant effect against isoprenaline-induced myocardial infarction by blocking the induction of mitochondrial lipid peroxidation (LPO). A tendency to minimize the isoprenaline-induced alterations in the level of reduced glutathione (GSH) and in the activities of glutathione-dependent antioxidant enzymes [glutathione peroxidase (GPx) and glutathione-S-transferase (GST)] and antiperoxidative enzymes [superoxide dismutase (SOD) and catalase (CAT)] in the heart mitochondria was also observed. The results of the present study indicate that the overall cardioprotective effect of betaine is probably related to its ability to maintain the myocardial energy status (ATP) at higher level by maintaining the activities of TCA cycle enzymes and respiratory marker enzymes at near normalcy, and/or to its free radical-scavenging ability against isoprenaline-induced lipid peroxidation, which is primarily responsible for the irreversible necrosis of the myocardial membrane.

**Key words** — betaine, isoprenaline, myocardial infarction, diagnostic marker enzyme, tricarboxylic acid cycle enzyme, antioxidant defense system

## INTRODUCTION

Despite understanding more about the etiology and pathophysiology of cardiovascular disease, the burden of cardiovascular disease is likely to worsen rather than improve over the next 20 years. In terms of global burden of disease in 1999, the World Health Organization (WHO) placed myocardial in-

farcion in sixth place and stroke in seventh place, but by 2020 they will have moved to first and fourth place, respectively. According to WHO estimates, cardiovascular disease killed 14.7 million individuals in 1990 and 17 million in 1999.<sup>1)</sup> It is often assumed that myocardial infarction is a disease of affluent, industrialized countries. However, 80% of these deaths occur in low-to-middle income countries of varying size such as China, Russia, Poland, Mauritius, Argentina, and India.<sup>2)</sup> The incidence of myocardial infarction is also high among people with Indian origins who are now living abroad.<sup>3)</sup>

Epidemiological studies and randomized clini-

\*To whom correspondence should be addressed: Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO), Cochin-682029, India. Tel.: +91-484-2666845; Fax: +91-484-2668212; E-mail: kranandan@email.com

cal trials have provided compelling evidence that the occurrence of myocardial infarction is largely preventable.<sup>4)</sup> Hence the high incidence of myocardial infarction requires new ways for prevention. Betaine (*N,N,N*-trimethyl glycine) may be an interesting candidate, all the more because homocysteinemia, which can be treated with betaine, is a risk factor for myocardial infarction. Betaine is distributed widely in animals, plants, and microorganisms and rich dietary sources include seafood, especially marine invertebrates; wheat germ or bran; and spinach.<sup>5)</sup> The principle 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.<sup>6)</sup> Earlier reports indicated that administration of betaine exerted significant hepatoprotection in experimental animals against a wide variety of hepatotoxins including chloroform, lipopolysaccharide, methotrexate, and carbon tetrachloride.<sup>7–9)</sup> Specific effects of betaine on cellular function have been reported to include reduced hepatic lipodosis and necrosis, improved morphology of mitochondria, rough endoplasmic reticulum, Golgi complexes, and nuclear DNA and increased S-adenosyl methionine content.<sup>5, 10)</sup> Betaine has also been reported to protect against bile-induced apoptosis via inhibition of the proapoptotic mitochondrial pathway.<sup>10)</sup> Previous studies have shown that betaine exerts cellular and subcellular membrane stabilization in the liver by restoring both non-enzymic and enzymic antioxidants.<sup>11, 12)</sup> Though the beneficial properties of betaine are promising and well studied, the protective effects of betaine on mitochondrial function in experimentally induced myocardial infarction condition have not yet been explored.

Myocardial infarction induced by isoprenaline [Isoproterenol; *L*- $\beta$ -(3,4-dihydroxyphenyl)- $\alpha$ -isopropylaminoethanol hydrochloride], a  $\beta$ -adrenergic agonist, has been reported to exhibit many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction.<sup>13)</sup> Isoprenaline challenge induces myocardial necrosis by a multiple step mechanism. The primary disturbance of isoprenaline-induced myocardial infarction has been reported to enhance adenylate cyclase activity resulting in increased cAMP formation, which in turn leads to increased lipid accumulation in the myocardium.<sup>14)</sup> It is also well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative

factor for irreversible damage to the myocardial membrane.<sup>15)</sup> Isoprenaline-induced myocardial infarction there by serves as a well-standardized model to study the beneficial effects of many drugs and cardiac function.<sup>16)</sup>

In the present study, an attempt was made to assess the preventive effects of betaine on mitochondrial energy status and antioxidant defense system in isoprenaline-induced myocardial infarction in rats by virtue of its membrane-stabilizing action and antioxidant property.

## MATERIALS AND METHODS

**Chemicals**—Betaine, epinephrine, and isoprenaline were obtained from M/s. Sigma Chemical Company, St. Louis, MO, U.S.A. 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 ( $28 \pm 2^\circ\text{C}$ , humidity 60–70%, 12 hr light/dark cycle). The animals were allowed a standard diet (M/s Sai Feeds, Bangalore, India) and water *ad libitum*. The experiment was carried out as per the guidelines of the 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 isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight, day] for 2 days.<sup>17)</sup>

**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 physi-

ological saline alone for 2 days.

At the end of the experimental period, i.e. 24 hr after last injection of isoprenaline, the experimental animals were killed, blood was collected using sodium citrate as anticoagulant, and the plasma separated was used for the determination of diagnostic marker enzymes [alanine aminotransferase (ALT) (EC 2.6.1.2), aspartate aminotransferase (AST) (EC 2.6.1.1), lactate dehydrogenase (LDH) (EC 1.1.1.27), and creatine phosphokinase (CPK) (EC 2.7.3.2)] and homocysteine. The heart tissue was dissected out immediately, and washed with chilled physiological saline. Mitochondria isolated from the heart tissue by the method of Johnson and Lardy<sup>18)</sup> were used for the determination of TCA cycle enzymes [isocitrate dehydrogenase (ICDH) (EC 1.1.1.42),  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ -KDH) (EC 1.2.4.2), succinate dehydrogenase (SDH) (EC 1.3.99.1), malate dehydrogenase (MDH) (EC 1.1.1.37)], respiratory marker enzymes [NADH dehydrogenase (EC 1.6.99.3), cytochrome-c-oxidase (EC 1.9.3.1), lipid peroxides (LPO), reduced glutathione (GSH)], glutathione-dependent antioxidant enzymes [glutathione peroxidase (GPx) (EC 1.11.1.9), glutathione-S-transferase (GST) (EC 2.5.1.18)] and antiperoxidative enzymes [superoxide dismutase (SOD) (EC 1.15.1.1) and catalase (CAT) (EC 1.11.1.6)].

**Biochemical Assays**—The activity of ALT was assayed by the method of Mohur and Cook.<sup>19)</sup> To 1.0 ml of substrate (0.1 M phosphate buffer, pH 7.4, 0.2 M DL-alanine, 2.0 mM 2-oxoglutarate), 0.2 ml of plasma was added and incubated at 37.8°C for 1 hr. Then 1.0 ml of 0.02% 2,4-dinitrophenylhydrazine (DNPH) was added and kept at room temperature for 20 min. To the control tube, sample was added after arresting the reaction with DNPH. Then 5 ml of 0.4 N NaOH was added and the colour developed was read at 540 nm. The activity was expressed as  $\mu$ moles of pyruvate liberated/l per hour.

AST was assayed by the method of Mohur and Cook.<sup>19)</sup> The assay mixture containing 1.0 ml of buffered substrate (L-aspartic acid and  $\alpha$ -ketoglutaric acid in 0.15 M phosphate buffer, pH 7.4) and 0.2 ml of plasma was incubated at 37.8°C for 1 hr. To the control tubes, sample was added after the reaction was arrested by the addition of 1.0 ml DNPH. The tubes were kept at room temperature for 30 min. Then 5.0 ml of 0.4 N NaOH was added and the colour developed was read at 540 nm. The activity was expressed as  $\mu$ moles of pyruvate

liberated/l per hour.

LDH was assayed according to the method of King.<sup>20)</sup> To 1.0 ml of the buffered substrate (lithium lactate in 0.1 M glycine buffer, pH 10), 0.1 ml of enzyme preparation was added and the tubes were incubated at 37.8°C for 15 min. After adding 0.2 ml of NAD<sup>+</sup> solution, the incubation was continued for another 15 min. The reaction was arrested by adding 0.1 ml of DNPH (2,4-dinitrophenyl hydrazine), and the tubes were incubated at 37.8°C for a further period of 15 min after which 7.0 ml of 0.4 N NaOH was added and the color developed was measured at 420 nm in a Shimadzu (Tokyo, Japan) UV-1601 spectrophotometer. Suitable aliquots of the standards were also analyzed by the same procedure. The activity of the enzyme was expressed as  $\mu$ moles of pyruvate liberated/l per hour.

CPK activity in plasma was determined by the method of Okinaka *et al.*<sup>21)</sup> The reaction mixture comprised of 0.05 ml of plasma, 0.1 ml of substrate, 0.1 ml of ATP solution, and 0.1 ml of cysteine-hydrochloride solution. The final volume was made up to 2.0 ml with distilled water and incubated at 37.8°C for 30 min. The reaction was arrested by the addition of 1.0 ml of 10% trichloroacetic acid (TCA) and the contents were subjected to centrifugation. To 0.1 ml of the supernatant, 4.3 ml distilled water and 1.0 ml ammonium molybdate were added and incubated at room temperature for 10 min. 1-amino-2-naphthol-6-sulphonic acid (ANSA) 0.4 ml was added and the color developed was read at 640 nm after 20 min. The activity of the enzyme was expressed as  $\mu$ moles of phosphorus liberated/l per hour.

ICDH activity was assayed according to the method of Bell and Baron (1960).<sup>22)</sup> Briefly, the reaction mixture consisting of 0.1 M Tris-HCl buffer, 0.1 M trisodium isocitrate, 0.015 M manganous chloride, 0.001 M NADP, and mitochondrial suspension was incubated at 37.8°C for 60 min. Added to this were 1.0 ml of 0.001 M DNPH and 0.5 ml of 5% EDTA. After 20-min incubation, 10 ml of 0.4 N NaOH was added and the color intensity was measured at 390 nm in a Shimadzu UV-1601 spectrometer. The activity of ICDH was expressed as  $\mu$ mol  $\alpha$ -ketoglutarate liberated/min per mg protein.

SDH activity was estimated according to the method of Slater and Bonner (1952).<sup>23)</sup> The rate of reduction of potassium ferricyanide was measured in the presence of sufficient potassium cyanide to inhibit cytochrome oxidase by following the rate of decrease in the optical density at 420 nm. Briefly,

0.2 ml of mitochondrial suspension was added to a reaction mixture containing 0.3 M phosphate buffer, 0.03 M EDTA, 0.4 M sodium succinate, 0.075 M potassium ferricyanide, bovine serum albumin, and 0.03 M potassium cyanide. The enzyme activity was measured at 420 nm in a Shimadzu UV-1601 spectrophotometer using UVPC Software package. The activity of SDH was expressed as  $\mu$ moles of succinate oxidized/min per mg protein.

Malate dehydrogenase activity was assayed by the method of Mehler *et al.* (1948).<sup>24)</sup> The activity determination was based on the measurement of the rate of oxidation of NADH in the presence of the enzyme and excess of oxaloacetate. Briefly, 0.3 ml of 0.25 M Tris-HCl buffer, 0.1 ml of NADH, and 0.1 ml of oxaloacetate were added and the total volume was made to 2.9 ml with water. The reaction was started by adding 0.1 ml of mitochondrial suspension. The enzyme activity was measured at 420 nm in a Shimadzu UV-1601 spectrophotometer using UVPC Software package. The activity of malate dehydrogenase was expressed as  $\mu$ moles of NADH oxidized/min per mg of protein.

NADH dehydrogenase activity was assayed according to the method of Minakami *et al.* (1962).<sup>25)</sup> The reaction mixture contained 1.0 ml of 0.1 M phosphate buffer, 0.1 ml of 0.03 M potassium ferricyanide, 0.1 ml of 0.1% NADH, and 1.6 ml of distilled water in a total volume of 3.0 ml. The temperature was brought to 30°C and 0.1% NADH was added just before the addition of the sample. A suitable aliquot of mitochondrial solution was added and the enzyme activity was measured at 420 nm in a Shimadzu UV-1601 spectrophotometer using UVPC Software package. The activity of NADH dehydrogenase was expressed as  $\mu$ moles of NADH oxidized/min per mg of protein.

$\alpha$ -KDH activity was estimated according to the method of Reed and Mukherjee (1969).<sup>26)</sup> It is based on the calorimetric determination of ferrocyanide produced by the decarboxylation of  $\alpha$ -ketoglutarate with ferricyanide as electron acceptor. To 0.15 ml of 0.1 M phosphate buffer, 0.1 ml each of 0.002 M thiamine pyrophosphate, 0.003 M magnesium sulphate, and 0.01 M potassium ferrocyanide was added. The total volume was made up to 1.2 ml with water. Mitochondrial suspension 0.2 ml was added and incubated at 30°C for 30 min. Aliquots of the supernatant after centrifugation were taken, 0.1 ml of 0.25 M potassium ferricyanide was added, and the volume was made up to 2.4 ml with water. One milliliter of 4% dupanol and 0.5 ml of ferric

ammonium sulphate dupanol reagent were added and incubated at 25°C for 30 min. The colour intensity was measured at 540 nm in a Shimadzu UV-1601 spectrophotometer. The activity of  $\alpha$ -KDH was expressed as nanomoles of potassium ferrocyanide liberated/min per mg protein.

Cytochrome-C-oxidase activity was assayed according to the method of Pearl *et al.*<sup>27)</sup> The enzyme activity was determined utilizing the accumulation of the free radical formed by the enzymatic univalent oxidation of a stable non-toxic substrate, *N*-phenyle-*p*-phenylene diamine. The reaction mixture consisted of 1.0 ml of the buffer, 0.2 ml of 0.2% solution of *N*-phenyle-*p*-phenylene diamine, 0.1 ml of cytochrome solution, and 0.5 ml of distilled water. The reaction mixture was incubated at 25°C for 5 min. Then added 0.2 ml of the enzyme preparation was added and the solution mixed well by inverting the cuvette. A blank containing water instead of enzyme, and control containing all the reagents except cytochrome-C, were also treated similarly. The change in optical density was measured in Shimadzu UV-1601 spectrophotometer at 550 nm at an interval of 15 s for 5 min. The activity of the enzyme was expressed as change in optical density/min per mg protein.

Lipid peroxidation (LPO) was assayed by the method of Ohkawa *et al.*<sup>28)</sup> 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 mitochondrial suspension, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% Thiobarbituric acid (TBA) were added. The mixture was made up to 4.0 ml with water then heated in a water bath at 95.8°C for 60 min using glass ball as a condenser. After cooling, 1.0 ml of water and 5 ml of *n*-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 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 nanomoles of MDA formed/mg protein.

GSH was estimated by the method of Ellman.<sup>29)</sup> Mitochondrial suspension 0.1 ml was precipitated with 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged. To 0.1 ml of supernatant, 2.0 ml of 0.6 mM DTNB [5,5 dithiobis(2-nitrobenzoic acid)] reagent and 0.2 M phosphate buffer (pH 8.0) were added to make up to a final volume of 4.0 ml. The absorbance

was read at 412 nm against a blank containing TCA instead of sample. A series of standards treated in a similar way also were run to determine the glutathione content. The amount of glutathione was expressed as nmol/g heart tissue. 5-Sulphosalicylic acid was used to prevent oxidation of glutathione.

GPx was assayed by the method of Paglia and Valentine *et al.*<sup>30)</sup> The reaction mixture consisted of 0.2 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H<sub>2</sub>O<sub>2</sub>, 0.2 ml of GSH, 0.4 ml of 0.4 M phosphate buffer pH 7.0, and 0.2 ml of mitochondrial suspension and was incubated at 37.8°C for 10 min. The reaction was arrested by the addition of 0.5 ml of 10% Trichloroacetic acid (TCA) and the tubes were centrifuged at 2000 rpm. To the supernatant 3.0 ml of 0.3 mM disodium hydrogen phosphate and 1.0 ml of 0.04% DTNB were added and the colour developed was read at 420 nm immediately. The activity of GPx was expressed as  $\mu$ moles of glutathione oxidized/min per mg protein.

GST was assayed by the method of Habig *et al.*<sup>31)</sup> To 0.1 ml of mitochondrial suspension, 1.0 ml of 0.3 M phosphate buffer pH 6.5, 1.7 ml of water, and 0.1 ml of 30 mM CDNB (1-chloro-2,4-dinitrobenzene) were added. After incubation at 37.8°C for 15 min, 0.1 ml of GSH was added and change in Optical Density (OD) was read at 340 nm for 3 min at an interval of 30 s. Reaction mixture without the enzyme was used as blank. The glutathione-S-transferase activity was expressed as units/min per mg protein.

SOD was assayed by the method of Misra and Fridovich.<sup>32)</sup> Mitochondrial suspension 0.1 ml was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate-bicarbonate (pH 10.2) buffer were added. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured in a Shimadzu UV spectrophotometer. One unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation.

CAT was assayed by the method of Takahara *et al.*<sup>33)</sup> To 1.2 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of mitochondrial suspension was added and reaction was started by the addition of 1.0 ml of 30 mM H<sub>2</sub>O<sub>2</sub> solution. The decrease in absorbance was measured at 240 nm at 30 s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen perox-

ide. The enzyme activity was expressed as  $\mu$ moles of H<sub>2</sub>O<sub>2</sub> decomposed/min per mg protein.

Homocysteine (tHcy) concentration in plasma was assayed by Microtiter Plate Assay package (Diazyme Laboratories, La Jolla, California).<sup>34)</sup> Betaine content in the heart tissue was determined by the method of Mar *et al.*<sup>35)</sup> using Shimadzu LC 10 ATvp HPLC-PDA system with Lichrosorb C8 RP Column (4.6 mm I.D  $\times$  125 mm). The level of ATP in the heart tissue was determined according to the method of Ryder<sup>36)</sup> using Shimadzu LC 10 ATvp HPLC-PDA system with Hypersil C18 RP Column (4.6 mm I.D  $\times$  250 mm).

**Statistical Analysis** — Results are expressed as mean  $\pm$  S.D. Multiple comparisons of the significant analysis of variance (ANOVA) were performed by Tukey's multiple comparison tests. A *p*-value  $< 0.05$  was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

## RESULTS

Table 1 depicts the levels of diagnostic marker enzymes (ALT, AST, LDH, and CPK) in plasma of normal and experimental groups of rats. Intraperitoneal administration of isoprenaline caused a significant (*p*  $< 0.001$ ) elevation in the levels of diagnostic marker enzymes in the plasma of Group III rats as compared with that of Group I control rats. In the present study, oral pretreatment with betaine [250 mg/kg body weight, day, for a period of 30 days] significantly (*p*  $< 0.001$ ) prevented the isoprenaline-induced release of diagnostic marker enzymes from the heart tissue into the systemic circulation and also maintained the rats at near normal status. Figure 1 shows the level of tHcy in normal and experimental groups of rats. There was a significant rise observed in the level of tHcy in plasma of Group III isoprenaline-administered rats as compared with Group I control animals. Prior oral administration of betaine significantly was found to reduce the level of tHcy in plasma of Group IV rats. Figure 2 depicts the level of betaine content in normal and experimental groups of rats. There was a significant decline noted in the level of betaine in heart tissue of Group III isoprenaline-administered rats as compared with Group I control animals. In Group II rats, there was a significant elevation noted in the betaine content in the heart tissue as compared with that of Group I control animals. Oral pre-

**Table 1.** Levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH), and Creatine Phosphokinase (CPK) in Plasma of Normal and Experimental Groups of Rats

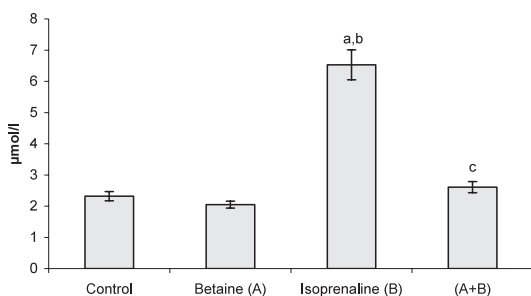
Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
AST	97.7 ± 9.1	91.2 ± 7.6	396 ± 18 <sup>*,**</sup>	129 ± 8 <sup>***</sup>
ALT	86.3 ± 5.9	81.7 ± 6.5	358 ± 16 <sup>*,**</sup>	110 ± 5 <sup>***</sup>
LDH	152 ± 9	149 ± 8	331 ± 16 <sup>*,**</sup>	182 ± 9 <sup>***</sup>
CPK	132 ± 7	127 ± 7	314 ± 24 <sup>*,**</sup>	149 ± 8 <sup>***</sup>

(A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days. Results are mean ± S.D. of 6 animals. Values expressed: ALT, AST, and LDH,  $\mu\text{mol}$  pyruvate liberated  $\text{h}^{-1}\text{l}^{-1}$ ; CPK,  $\mu\text{mol}$  creatine liberated  $\text{h}^{-1}\text{l}^{-1}$ . \* $p < 0.001$  significantly different compared with control animals; \*\* $p < 0.001$  significantly different compared with betaine-administered normal rats; \*\*\* $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.

**Table 2.** Activities of Isocitrate Dehydrogenase (ICDH), Succinate Dehydrogenase (SDH),  $\alpha$ -Ketoglutarate Dehydrogenase ( $\alpha$ -KDH), Malate Dehydrogenase (MDH), NADH-Dehydrogenase, and Cytochrome-*c*-Oxidase in the Heart Mitochondria of Normal and Experimental Groups of Rats

Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
TCA cycle enzymes				
ICDH	718 ± 54	762 ± 51	427 ± 29 <sup>*,**</sup>	673 ± 45 <sup>***</sup>
SDH	292 ± 17	315 ± 23	149 ± 10 <sup>*,**</sup>	284 ± 19 <sup>***</sup>
$\alpha$ -KDH	152 ± 10	169 ± 9	58.7 ± 33.2 <sup>*,**</sup>	133 ± 8 <sup>***</sup>
MDH	347 ± 28	385 ± 32	164 ± 12 <sup>*,**</sup>	327 ± 24 <sup>***</sup>
Respiratory marker enzymes				
NADH-dehydrogenase	41.8 ± 2.9	43.8 ± 2.5 <sup>****</sup>	19.2 ± 1.4 <sup>*,**</sup>	36.5 ± 1.6 <sup>***</sup>
Cytochrome- <i>c</i> -oxidase	4.23 ± 0.27 × 10 <sup>-2</sup>	4.55 ± 0.25 × 10 <sup>-2</sup>	2.11 ± 0.14 × 10 <sup>-2</sup> <sup>*,**</sup>	3.95 ± 0.23 × 10 <sup>-2</sup> <sup>***</sup>

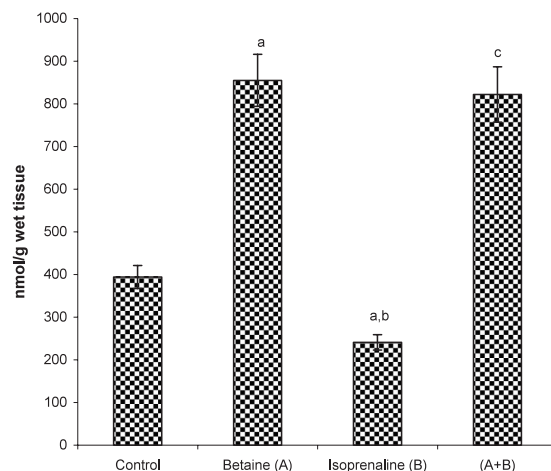
(A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days. Results are mean ± S.D. for 6 animals. Values expressed: ICDH, nmol of  $\alpha$ -ketoglutarate formed/min per mg protein;  $\alpha$ -KDH, nmol of ferrocyanide formed/min per mg protein; SDH,  $\mu\text{mol}$  of succinate oxidized/min per mg protein; MDH,  $\mu\text{mol}$  of NADH oxidized/min per mg protein; NADH-dehydrogenase,  $\mu\text{mol}$  of NADH oxidized/min per mg protein; Cytochrome-*c*-oxidase, change in optical density/min per mg protein. \* $p < 0.001$  significantly different compared with control animals; \*\* $p < 0.001$  significantly different compared with betaine-administered normal rats; \*\*\* $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.

**Fig. 1.** Level of Homocysteine in Plasma of Normal and Experimental Groups of Rats. (A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days

Results are mean ± S.D. for 6 animals. <sup>a</sup> $p < 0.001$  significantly different compared with control animals; <sup>b</sup> $p < 0.001$  significantly different compared with betaine-administered normal rats; <sup>c</sup> $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.

treatment with betaine significantly prevented the isoprenaline-induced betaine depletion in the heart tissue of Group IV rats as compared with that of Group III rats.

Table 2 shows the activities of TCA cycle en-

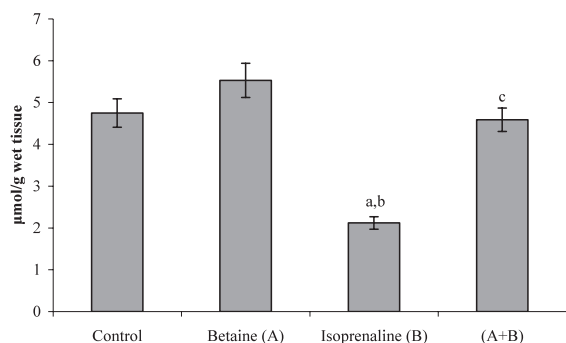
**Fig. 2.** Level of Betaine Content in the Heart Tissue of Normal and Experimental Groups of Rats. (A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days

Results are mean ± S.D. for 6 animals. <sup>a</sup> $p < 0.001$  significantly different compared with control animals; <sup>b</sup> $p < 0.001$  significantly different compared with betaine-administered normal rats; <sup>c</sup> $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.

**Table 3.** Levels of Lipid Peroxides (LPO) and Reduced Glutathione (GSH) and Activities of Glutathione Peroxidase (GPx), Glutathione-S-Transferase (GST), Catalase (CAT), and Superoxide Dismutase (SOD) in the Heart Mitochondria of Normal and Experimental Groups of Rats

Parameters	Control	Betaine (A)	Isoprenaline (B)	(A+B)
LPO	0.79 ± 0.03	0.72 ± 0.04	2.18 ± 0.01 <sup>*,**</sup>	0.94 ± 0.04 <sup>***</sup>
GSH	3.36 ± 0.18	3.74 ± 0.24	1.62 ± 0.12 <sup>*,**</sup>	3.18 ± 0.16 <sup>***</sup>
GPx	2.78 ± 0.23	2.91 ± 0.21	1.21 ± 0.09 <sup>*,**</sup>	2.59 ± 0.18 <sup>***</sup>
GST	1160 ± 75	1225 ± 72	578 ± 46 <sup>*,**</sup>	996 ± 69 <sup>***</sup>
CAT	10.7 ± 0.9	12.4 ± 1.0	4.15 ± 0.29 <sup>*,**</sup>	9.58 ± 0.76 <sup>***</sup>
SOD	5.02 ± 0.28	5.23 ± 0.25	2.02 ± 0.15 <sup>*,**</sup>	4.53 ± 0.21 <sup>***</sup>

(A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days. Results are mean ± S.D. for 6 animals. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH,  $\mu\text{mol g}^{-1}$  wet tissue; GPx, nmol GSH oxidized  $\text{min}^{-1}\text{mg}^{-1}$  protein; GST,  $\mu\text{mol}$  1-chloro-2,4-dinitrobenzene conjugate formed  $\text{min}^{-1}\text{mg}^{-1}$  protein; CAT, nmol  $\text{H}_2\text{O}_2$  decomposed  $\text{min}^{-1}\text{mg}^{-1}$  protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. <sup>\*</sup> $p < 0.001$  significantly different compared with control animals; <sup>\*\*</sup> $p < 0.001$  significantly different compared with betaine-administered normal rats; <sup>\*\*\*</sup> $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.



**Fig. 3.** Level of ATP in the Heart Tissue of Normal and Experimental Groups of Rats. (A): Betaine, 250 mg/kg body wt, day, p.o. for 30 days. (B): Isoprenaline, 11 mg/100 g body wt, day, i.p. for 2 days

Results are mean ± S.D. for 6 animals. <sup>a</sup> $p < 0.001$  significantly different compared with control animals; <sup>b</sup> $p < 0.001$  significantly different compared with betaine-administered normal rats; <sup>c</sup> $p < 0.001$  significantly different compared with isoprenaline-induced myocardial infarcted rats.

zymes (ICDH,  $\alpha$ -KDH, SDH, and MDH) and respiratory marker enzymes (NADH dehydrogenase and cytochrome-*c*-oxidase) in heart mitochondria of normal and experimental groups of rats. There was a significant ( $p < 0.001$ ) reduction noted in the activities of TCA cycle enzymes and respiratory marker enzymes in heart mitochondria of Group III rats as compared with Group I animals. Prior oral treatment with betaine resulted in the maintenance of these mitochondrial enzyme activities at near normalcy as compared with those of Group III rats. It also significantly ( $p < 0.001$ ) prevented the isoprenaline-induced decline in the ATP (Fig. 3) content in the heart tissue of experimental rats.

Table 3 depicts the levels of lipid peroxides and GSH, and the activities of glutathione-dependent

antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) in heart mitochondria of normal and experimental groups of rats. Injection of isoprenaline induced a significant ( $p < 0.001$ ) elevation in the level of lipid peroxides in the heart mitochondria of Group III rats as compared with that of Group I control animals. A parallel decline in the level of GSH and in the activities of the glutathione-dependent antioxidant enzymes and antiperoxidative enzymes was also observed. In the present study, prior oral administration of betaine significantly ( $p < 0.001$ ) prevented all these isoprenaline-induced adverse effects and maintained the heart mitochondrial antioxidant defense system at near normal.

## DISCUSSION

Elevated levels of AST, ALT, LDH, and CPK in plasma are presumptive markers of the occurrence of necrotic lesions in the myocardial membrane. Significant ( $p < 0.001$ ) rises observed in the levels of diagnostic marker enzymes in the plasma of Group III rats as compared with Group I normal controls are indicative of the severity of isoprenaline-induced necrotic damage to the myocardial membrane. This observation concurs with an earlier reported study.<sup>37)</sup> Recent prospective studies have shown that hyperhomocysteinemia is associated with an increased risk of cardiovascular diseases independently of classical risk factors.<sup>38)</sup> Our observations confirm the same pattern and showed a significant ( $p < 0.001$ ) rise in the level of tHcy in plasma of Group III rats as compared with Group I controls.

In the present study, prior administration of betaine maintained the levels of diagnostic marker enzymes and tHcy in plasma of Group IV rats at near normalcy as compared with Group III rats. It probably did so by its membrane-stabilizing property. Betaine plays a significant role in maintaining the structural and functional integrity of cell membranes.<sup>39)</sup> Previous reported studies indicated that oral administration of betaine protected the liver from the necrotic damaging effects of CCl<sub>4</sub> and ethanol through the formation of S-adenosylmethionine (SAM), which is important in the synthesis of phosphotidyl choline, a principal component of cell membranes.<sup>40,41)</sup>

There was a significant reduction noted in the betaine content in heart tissue of Group III rats as compared with Group I normal animals, indicating aberrations in normal functioning of the cellular and subcellular membranes in the myocardium. A previous reported study showed that dietary betaine accumulated in the liver and intestinal tissue and stabilized the intestinal epithelial structure in healthy and coccidia-infected broiler chicks.<sup>42)</sup> In the present study, oral administration of betaine resulted in increased accumulation of betaine in heart tissue of Group II animals as compared with Group I rats. Oral pretreatment with betaine significantly attenuated the isoprenaline-induced betaine depletion in heart tissue of Group IV rats as compared with that of Group III animals, indicating its cytoprotective property. This finding concurs with a previous reported study, which showed that supplementation of betaine ameliorated ischemia-reoxygenation injury, presumably by inhibiting Kupffer cell activation.<sup>43)</sup>

Reduction in the activities of TCA cycle enzymes and respiratory marker enzyme in heart mitochondria of Group III rats as compared with those of Group I animals indicated that the mitochondrial oxidative phosphorylation was operating at a lower level despite the higher energy demand in the ischemic myocardium. Changes in the concentration of respiratory components, phosphorylative activity, cytochrome-*c*-oxidase activity, and adenylate charge level have also been reported in isoprenaline-induced cardiac damage.<sup>44)</sup> Our observations confirm the same pattern and showed a significant ( $p < 0.001$ ) reduction in the level of ATP content in the heart tissue of Group III rats as compared with that of Group I controls.

Prior oral administration of betaine maintained the level of ATP content and the activities of TCA

cycle enzymes and respiratory marker enzymes at near normalcy in Group IV rats as compared with those of Group III rats, reflecting the drug's ability to maintain functional integrity of the heart mitochondria at normal status. The protective action of betaine on mitochondrial energy status is probably related to its ability to modulate the physicochemical properties of the mitochondrial membrane lipid bilayer. Reports by Harita and Axelrod<sup>45)</sup> have demonstrated that betaine through its participation in sequential methylation within the cellular and subcellular membranes maintains the proper balance between phosphotidyl ethanolamine and phosphotidyl choline, hence sustaining proper membrane structure and fluidity for the transport of materials across membranes as well as the transmission of signals across membranes.

Significant elevation observed in the level of lipid peroxidation with a concomitant decline in the level of GSH and the activities of glutathione-dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (CAT and SOD) in heart mitochondria of Group III rats, which is in corroboration with previous investigations,<sup>13,46)</sup> reflected the high vulnerability of mitochondrial membrane to oxidative deterioration in ischemic conditions. Depletion of glutathione is known to result in enhanced LPO and excessive LPO can cause increased glutathione consumption,<sup>17)</sup> as observed in the present study. The generation of free radicals in isoprenaline-induced myocardial infarction might have exceeded the ability of antiperoxidative enzymes (SOD and CAT) to dismutate the radicals, resulting in membrane damage and inactivation of these enzyme activities.<sup>37)</sup> Inhibition in the activities of antioxidant enzymes may lead to the increased generation of O<sup>2-</sup> and H<sub>2</sub>O<sub>2</sub>, which in turn can form hydroxyl radical (OH<sup>·</sup>) and bring about a number of reactions harmful to structural and functional integrity of mitochondrial membranes.<sup>47)</sup>

Oral pretreatment with betaine significantly ( $p < 0.001$ ) prevented the isoprenaline-induced LPO and maintained the level of GSH and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes in heart mitochondria of Group IV rats at near normalcy. It probably did so by its antioxidant action<sup>5)</sup> against isoprenaline-induced lipid peroxidation.<sup>48)</sup> Betaine is highly lipotropic and, when administered exogenously, can readily pass across the membrane lipid bilayer and inhibit LPO in cellular membranes as a result of distinct biophysical interactions with mem-



brane lipid bilayer.<sup>49)</sup>

Reports by Kim and Kim<sup>7)</sup> 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. Earlier reports<sup>6, 50, 51)</sup> indicated that betaine had a potent reducing effect on the production of free radicals in rats exposed to cytotoxicity. Feeding SH-generating substances such as methionine or non-enzymic antioxidants such as glutathione have been reported to protect cellular and subcellular membranes from toxic free radical metabolites.<sup>52)</sup> 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<sup>8)</sup> that protects the cell from reactive metabolites and reactive oxygen species.

In conclusion, the results of the present investigation indicate that oral pretreatment with betaine ameliorates isoprenaline-induced aberrations in the myocardial mitochondrial energy status and antioxidant defense system in experimental 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 its ability to maintain the myocardial energy status (ATP) at higher level by maintaining the activities of TCA cycle enzymes and respiratory marker enzymes at near normalcy, and/or to its free radical-scavenging ability against isoprenaline-induced LPO, which is primarily responsible for the irreversible necrosis of the myocardial membrane. However, further studies have to be carried out in other experimental models such as coronary ligation-induced myocardial infarction to identify the exact mechanism involved in the cardioprotective action of betaine.

## REFERENCES

- 1) WHO (2004) World Health Organization in collaboration with the Centre for Disease Control and Prevention, Geneva, Switzerland. pp. 48–49.
- 2) Bonow, R. O., Smaha, L. A., Smith, S. C., Mensah, G. A. and Lenfant, G. (2002) The international burden of cardiovascular disease: responding to the emerging global epidemic. *Circulation*, **106**, 1602–1605.
- 3) Gupta, R. and Gupta, V. P. (1996) Meta-analysis of coronary heart disease prevalence in India. *Indian Heart J.*, **48**, 241–245.
- 4) Cooper, R., Cutler, J., Desvignes-Nickens, P., Fortmann, S. P., Friedman, L., Havlik, R., Hogelin, G., Marler, J., McGovern, P., Morosco, G., Mosca, L., Pearson, T., Stamler, J., Stryer, D. and Thom, T. (2000) Trends and disparities in coronary heart disease, stroke, and other cardiovascular disease in the United States: Findings of the National Conference on Cardiovascular Disease Prevention. *Circulation*, **102**, 3137–3147.
- 5) Craig, S. A. (2004) Betaine in human nutrition. *Am. J. Clin. Nutr.*, **80**, 539–549.
- 6) Park, E. I. and Garrow, T. A. (1999) Interaction between dietary methionine and methyl donor intake on rat liver betaine-homocysteine methyltransferase gene expression and organization of the human gene. *J. Biol. Chem.*, **274**, 7816–7824.
- 7) Kim, S. K. and Kim, Y. C. (1998) Effects of singly administered betaine on hepatotoxicity of chloroform in mice. *Food Chem. Toxicol.*, **36**, 655–661.
- 8) Kim, S. K. and Kim, Y. C. (2002) Attenuation of bacterial lipopolysaccharide-induced hepatotoxicity by betaine or taurine in rats. *Food Chem. Toxicol.*, **40**, 545–549.
- 9) Junnila, M., Rahko, T., Sukura, A. and Lindberg, L. A. (2000) Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male Han-Wistar rats: a morphometric histological study. *Vet. Pathol.*, **37**, 231–238.
- 10) Graf, D., Kurz, A. K., Reinehr, R., Fischer, R., Kircheis, G. and Haussinger, D. (2002) Prevention of bile acid-induced apoptosis by betaine in rat liver. *Hepatology*, **36**, 829–839.
- 11) Barak, A. J., Beckenhauer, H. C., Junnila, M. and Tuma, D. J. (1993) Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. *Alcohol. Clin. Exp. Res.*, **17**, 552–555.
- 12) Rudolph, A. S., Crowe, J. H. and Crowe, L. M. (1986) Effects of three stabilizing agents-proline, betaine, and trehalose-on membrane phospholipids. *Arch. Biochem. Biophys.*, **245**, 134–143.
- 13) Shiny, K. S., Kumar, S. H. S., Farvin, K. H. S., Anandan, R. and Devadasan, K. (2005) Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. *J. Pharm. Pharmacol.*, **57**, 1313–1317.
- 14) Subhash, D., Narinder, K. K. and Nityanand, S. (1978) Effect of isoproterenol on lipid profile and cardiac enzymes in rats. *Indian J. Exp. Biol.*, **16**, 376–378.

- 15) Ithayarasi, A. P. and Devi, C. S. S. (1997) Effect of alpha tocopherol on lipid peroxidation in isoproterenol-induced myocardial infarction in rats. *Indian J. Physiol. Pharmacol.*, **41**, 369–376.
- 16) Kakreja, R. C. and Hess, M. L. (1992) The oxygen free radical system from equations through membrane–protein interactions to cardiovascular injury and protection. *Cardiovasc. Res.*, **26**, 641–655.
- 17) Anandan, R., Asha, K. K., Ammu, K., Suseela, M. and Nair, P. G. V. (2003) Effects of peroxidised PUFA on tissue defense system in experimentally induced myocardial infarction in rats. In *Seafood Safety* (Surendran, P. K., Mathew, P. T., Thampuran, N., Nambiar, N., Joseph, J., Boopendranath, M. R., Lakshmanan, P. T. and Nair, P. G. V., Eds.) Society of Fisheries Technologists (India), Cochin, pp.330–335.
- 18) Johnson, D. and Lardy, H. (1967) Isolation of liver or kidney mitochondria. In *Methods in Enzymology*, Vol.10, Academic Press, London, pp.94–96.
- 19) Mohur, A. and Cook, I. J. Y. (1957) Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic-pyruvic transaminase in routine laboratories, *J. Clin. Pathol.*, **10**, 394–399.
- 20) King, J. (1965) The dehydrogenase of oxidoreductase -lactate dehydrogenase. In *Practical Clinical Enzymology* (King, J. C. and Van, D., Eds.) Nostrand Co., London, pp.83–93.
- 21) Okinaka, S., Kumogai, H., Ebashi, S., Sugaita, H., Momoi, H., Toyokura, Y. and Fujie, Y. (1961) Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease, *Arch. Neurol.*, **4**, 520–525.
- 22) Bell, J. L. and Baron, D. N. (1960) A colorimetric method for determination of isocitric dehydrogenase, *Clin. Chim. Acta*, **5**, 740–747.
- 23) Slater, E. C. and Borner, W. D. (1952) The effect of fluoride on the succinic oxidase system. *Biochem. J.*, **52**, 185–196.
- 24) Mehler, A. H., Kornberg, A., Grisolia, S. and Ochoa, S. (1948) The enzymatic mechanism of oxidation-reductions between malate or isocitrate and pyruvate. *J. Biol. Chem.*, **174**, 961–977.
- 25) Minakami, S., Ringler, R. L. and Singer, T. P. (1962) Studies on the respiratory chain-linked dihydrodiphosphopyridine nucleotide dehydrogenase. I. Assay of the enzyme in particulate and in soluble preparations, *J. Biol. Chem.*, **237**, 569–576.
- 26) Reed, L. J. and Mukerjee, R. B. (1969)  $\alpha$ -Ketoglutarate dehydrogenase complex from *Escherichia coli*. In *Methods in Enzymology*, Vol.13, Academic Press, London, pp.53–61.
- 27) Pearl, W., Cancercio, J. and Zweifach, B. W. (1963) Microdetermination of cytochrome oxidase in rat tissues by the oxidation of N-phenyl-p-phenylenediamine or ascorbic acid, *J. Histochem. Cytochem.*, **11**, 102–107.
- 28) Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction, *Anal. Biochem.*, **95**, 351–358.
- 29) Ellman, G. L. (1959) Tissue sulfhydryl groups, *Arch. Biochem. Biophys.*, **82**, 70–77.
- 30) Paglia, D. E. and Valentine, W. N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.*, **70**, 158–169.
- 31) Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974) Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation, *J. Biol. Chem.*, **249**, 7130–7139.
- 32) Misra, H. P. and Fridovich, I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase, *J. Biol. Chem.*, **247**, 3170–3175.
- 33) Takahara, S., Hamilton, B. H., Nell, J. V., Ogubra, T. Y. and Nishimura, E. T. (1960) Hypocatalasemia: a new genetic carrier state, *J. Clin. Invest.*, **39**, 610–619.
- 34) Yuan, C. S. (2002) Methods and composition for assaying analytes, *U.S. Patent*, No. US 6,376,210 B. 1.
- 35) Mar, M. H., Ridky, T. W., Garner, S. C. and Zeisel S. H. (1995) A method for the determination of betaine in tissues using high performance liquid chromatography, *J. Nutr. Biochem.*, **6**, 392–398.
- 36) Ryder, J. M. (1985) Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography, *J. Agric. Food Chem.*, **33**, 678–680.
- 37) Farvin, K. H. S., Anandan, R., Kumar, S. H. S., Shiny, K. S., Sankar, T. V. and Thankappan, T. K. (2004) Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats, *Pharmacol. Res.*, **50**, 231–236.
- 38) Senaratne, M. P., Griffiths, J. and Nagendran, J. (2000) Elevation of plasma homocysteine levels associated with acute myocardial infarction, *Clin. Invest. Med.*, **23**, 220–226.
- 39) Harita, F., Viveros, O. H., Diliberto, E. J. and Axelrod, J. (1978) Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 1718–1721.
- 40) Murakami, T., Nagamura, Y. and Hirano, K. (1998) The recovering effect of betaine on carbon tetrachloride-induced liver injury, *J. Nutr. Sci.*

- Vitaminol. (Tokyo)*, **44**, 249–255.
- 41) Barak, A. J., Beckenhauer, H. C., Junnila, M. and Tuma, D. J. (1993) Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration, *Alcohol Clin. Exp. Res.*, **17**, 552–555.
  - 42) Kettunen, H., Tiihonen, K., Peuranen, S., Saarinen, M. T. and Remus, J. C. (2001) Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks, *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **130**, 759–769.
  - 43) Wettstein, M. and Haussinger, D. (1997) Cytoprotection by the osmolytes betaine and taurine in ischemia-reoxygenation injury in the perfused rat liver, *Hepatology*, **26**, 1560–1566.
  - 44) Padma, V. V. and Devi, C. S. (2002) Effect of fish oil on mitochondrial respiration in isoproterenol induced myocardial infarction in rats, *Indian J. Exp. Biol.*, **40**, 268–272.
  - 45) Harita, F. and Axelrod, J. (1980) Phospholipid methylation and biological transmission, *Science*, **209**, 1082–1090.
  - 46) Kumar, S. H. S. and Anandan, R. (2007) Biochemical studies on the cardioprotective effect of glutamine on tissue antioxidant defense system in isoprenaline-induced myocardial infarction in rats, *J. Clin. Biochem. Nutr.*, **40**, 49–55.
  - 47) Anandan, R., Rekha R. D. and Devaki, T. (1999) Protective effect of *Picrorhiza kurroa* on mitochondrial glutathione antioxidant defense system in D-galactosamine-induced hepatitis in rats, *Curr. Sci.*, **76**, 1543–1545.
  - 48) Rathore, N., John, S., Kale, M. and Bhatnagar, D. (1998) Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues, *Pharmacol. Res.*, **38**, 297–303.
  - 49) Junnila, M., Barak, A. J., Beckenhauer, H. C. and Rahko, T. (1998) Betaine reduces hepatic lipidosis induced by carbon tetrachloride in Sprague-Dawley rats, *Vet. Hum. Toxicol.*, **40**, 263–266.
  - 50) Balkan, J., Oztezcan, S., Kucuk, M., Cevikbas, U., Kocak-Toker, N. and Uysal, M. (2004) The effect of betaine treatment on triglyceride levels and oxidative stress in the liver of ethanol-treated guinea pigs, *Exp. Toxicol. Pathol.*, **55**, 505–509.
  - 51) Kanbak, G., Akyuz, F. and Inal, M. (2001) Preventive effect of betaine on ethanol-induced membrane lipid composition and membrane ATPases, *Arch. Toxicol.*, **75**, 59–61.
  - 52) Selvam, R. and Kannabiran, K. (1993) Induction of oxalate binding by dehydroascorbic acid feeding in rat kidney, *Nutr. Res.*, **13**, 667–676.