

Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats

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BACKGROUND: There is comprehensive experimental and clinical evidence that either exogenous supplementation of natural antioxidants or augmentation of endogenous antioxidants attenuates myocardial infarction.

OBJECTIVES: To investigate the effects of chronic administration of silymarin against ischemia-reperfusion-induced myocardial infarction in rats.

METHODS: Silymarin was administered orally to Wistar albino rats (200 g to 250 g) in three different doses (100 mg/kg, 250 mg/kg and 500 mg/kg), by gastric gavage for one week. At the end of this period, control (ischemia-reperfusion) groups and silymarin-treated groups were subjected to 30 min occlusion of the left anterior descending coronary artery and thereafter reperused for 4 h.

RESULTS: Ischemia-reperfusion resulted in significant cardiac necrosis, indicated by elevated levels of serum marker enzymes such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, lactate dehydrogenase, creatine kinase-isoenzyme and creatine kinase. A significant rise in the end products

of myocardial lipid peroxides (malondialdehydes [MDAs]), loss of antioxidative enzymes (superoxide dismutase, catalase, glutathione S-transferase and reduced glutathione) and increased levels of myeloperoxidase in heart tissue were observed in the animals subjected to in vivo myocardial ischemia-reperfusion injury. Infarct size was measured by using the staining agent 2,3,5-triphenyltetrazolium chloride. A lead II electrocardiogram was monitored at various intervals throughout the experiment.

DISCUSSION: The present study showed that silymarin protected the endogenous antioxidant enzymes, suppressed the neutrophil infiltration during ischemia-reperfusion and limited the infarct size, with concomitant reduction in serum MDA, tissue MDA and serum marker enzymes in rats subjected to 30 min coronary artery occlusion followed by 4 h of reperfusion. Pretreatment with silymarin also protected rat hearts from a further drop in mean arterial blood pressure during reperfusion, and restored heart rate at the end of the reperfusion period.

CONCLUSIONS: The present study suggests that the phytochemical silymarin has cardioprotective activity against ischemia-reperfusion-induced myocardial infarction in rats.

Key Words: *Infarct size; Myocardial infarction; Reperfusion injury; Silymarin*

Cardiovascular disease, particularly ischemic heart disease, has become a worldwide health problem affecting all economic groups of the society. Ischemia-reperfusion injury is a major pathological factor in the natural course of ischemic heart disease (1). Myocardial ischemia-reperfusion is clinically relevant in situations such as myocardial infarction, coronary angioplasty, thrombolytic therapy, coronary revascularization and heart transplantation. The reperfusion period, although clearly beneficial for the heart, is associated with myocardial injury (2). A major goal in the management of myocardial infarction is to reduce postmyocardial infarction complications and mortality by reversing myocardial ischemia and limiting the infarct size. While much research has been done on this topic, there remains no clear understanding of the underlying causal factors associated with injury following an ischemic episode. Reperfusion can accelerate necrosis in irreversibly injured myocytes because of an increase in cell swelling disruption of cell ultrastructure, formation of contraction bands and deposition of intramitochondrial calcium phosphate granules. Sarcolemmal damage may also occur, leading to impairment of fluid regulation and influx imbalance (2-4). Several mechanisms have been proposed to explain the myocardial injury observed after ischemia and reperfusion

(5). Oxidative stress is one of the mechanisms implicated in the pathogenesis of ischemic-reperfusion injury. There is comprehensive experimental and clinical evidence that antioxidants attenuate myocardial infarction (6,7). Indeed, many antioxidative plants and their isolated components have been reported to possess cardioprotective activity in experimental models of myocardial ischemia-reperfusion injury (5,8-19). Our previous studies (20) also suggested that *Hydrocotyle asiatica* possesses cardioprotective activity against ischemia-reperfusion injury.

Silymarin is a phytochemical isolated from the milk thistle plant *Silybum marianum*. Silymarin consists of a mixture of three bioflavonoids found in the fruit, seeds and leaves of the plant: silybin, silydianin and silychristin (21). Silybin is the main component (60% to 70%) and is believed to have the most biological activity. Silymarin has been shown to have utility in many liver disorders, including toxin-induced liver toxicity, hepatitis and alcoholic liver diseases (22,23). In an animal model of cirrhosis produced by bile duct obliteration, silymarin has an antifibrotic effect (24). Silymarin can prevent lipid peroxidation (25-28), inhibit low-density lipoprotein oxidation (29) and scavenge reactive oxygen species (30-32). It can also inhibit DNA synthesis (33), as well as fibroblast (34) and epidermal cell

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proliferation (28). Silymarin can modulate the activation of nuclear factor-kappa B to stimulate transcription (35), increase antioxidative enzyme levels (36,37) and limit lipid peroxidation (37). It can also enhance superoxide dismutase (SOD) activity and expression in vitro (38). Silymarin has been shown to increase glutathione (GS) levels in the liver by more than 50% in rats. The mechanism of action for these beneficial effects of silymarin is unknown. However, antioxidant activity is a leading theory. Hence, the aim of the present study is to evaluate the cardioprotective activity of silymarin by studying infarct size and levels of lipid peroxides, endogenous antioxidant enzymes (reduced glutathione [GSH]), antioxidant enzymes (SOD), catalase (CAT), glutathione S-transferase (GST) and serum marker enzymes, ie, lactate dehydrogenase (LDH), creatine kinase-isoenzyme (CK-MB), total CK, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) against myocardial ischemia-reperfusion-induced myocardial injury in rats. The dose of silymarin that had an optimal protective effect was chosen to study the myeloperoxidase levels for assessment of neutrophil infiltration.

MATERIALS AND METHODS

Chemicals

2,3,5-triphenyltetrazolium chloride (TTC) was purchased from BDH Chemicals Ltd (England), and 1,1,3,3-tetraethoxypropane from Sigma Chemical Company (USA). Thiopentone sodium was supplied by Abbott Laboratories Ltd (India). Nitroblue tetrazolium, NADH, GSH, oxidized GS, 1-chloro-2,4-dinitrobenzene (CDNB), Folin phenol reagent, Ellman's reagent and TritonX-100 were purchased from Sisco Research Laboratories Pvt Ltd (India). Phenazine methosulphate was purchased from National Chemicals (India). All other chemicals and reagents used were of analytical grade.

Animals

Albino Wistar rats (National Institute of Nutrition, India) of either sex weighing between 200 g and 250 g were selected. Animals were maintained under standard laboratory conditions at $25\pm 2^\circ\text{C}$, relative humidity of $50\pm 15\%$ and normal photoperiod (12 h dark and 12 h light). Commercial pellet diet (Ratan Brothers, India) and water were provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and by the animal regulatory body of the government (Regd No 516/01/A/CPCSEA).

Experimental design

The rats were divided into six groups, each consisting of five animals. Groups 1 and 2 were orally treated with 1% sodium carboxymethylcellulose, which served as a sham control and an ischemia-reperfusion control, respectively. Groups 3, 4 and 5 were treated with silymarin at doses of 100 mg/kg, 250 mg/kg and 500 mg/kg, respectively. Group 6 was treated with ramipril at a dose of 2 mg/kg, which served as a reference standard. On the eighth day, 1 h after administering the above treatments, the rats were subjected to 30 min of left anterior descending coronary artery (LAD) occlusion followed by 4 h reperfusion. The sham control group was not subjected to 30 min LAD occlusion.

Surgical procedure

Coronary artery occlusion and reperfusion was performed according to the method described by Pragada et al (39). In

brief, rats were anesthetized with thiopentone sodium (30 mg/kg intraperitoneally). The neck was opened with a ventral midline incision and intubated through a tracheotomy. The animals were ventilated with room air by a Techno positive pressure respirator (Crompton Parkinson Ltd, England). Body temperature was monitored and maintained at 37°C throughout the experimental protocol. The right carotid artery was cannulated for measuring mean arterial blood pressure (MAP), and a lead II electrocardiogram (ECG) was monitored throughout the study by using Cardiart 408 (BPL) with sensitivity 20 mm/mV at a paper speed of 50 mm/s. A left thoracotomy and a pericardiotomy were performed, followed by identification of the marginal branch of the LAD. A silk thread (4-0) was passed around the artery, which was occluded by a knot for 30 min. The silk thread was removed after 30 min with the help of two-knot releasers to allow reperfusion of the heart for the succeeding 4 h.

Quantification of infarct size

In all the groups, after sacrificing the animal by injecting 2.56 M KCl directly into the left ventricle, the heart was rapidly excised from the thorax and the greater vessels were removed. The left ventricle was separated from the heart and weighed. It was sliced parallel to the atrioventricular groove into 2 mm to 3 mm thick sections and the slices were incubated in 1% TTC solution prepared in phosphate buffer (pH 7.4) for 30 min at 37°C (40). In viable myocardium, TTC is converted by dehydrogenases to a red formazan pigment that stains tissue dark red (41). Areas of the infarcted myocardium that do not take TTC stain, where the dehydrogenases are drained off, remain pale in colour. The pale necrotic tissue was separated from the stained portions and weighed on an electronic balance (Dhona 200D, Dhona Instrument Pvt Ltd, India). Infarct size was calculated as a percentage fraction of nonviable myocardium of the left ventricle.

Functional measurements

ECG changes were derived from standard II-lead ECG. After surgical preparation, rats were allowed 10 min for stabilization, and then control measurements of ECG and MAP were taken. ECG and MAP measurements were taken again before opening the thoracic cavity; before occlusion; after 15 min of occlusion; immediately after release of occlusion; and after 1 h, 2 h, 3 h and 4 h of reperfusion. Mean arterial pressure-rate product (PRP) was calculated as the product of MAP and heart rate (HR), and PRP was reported as $\text{mmHg/beat}/\text{min}\times 10^3$ (42).

Biochemical studies

In all the groups, before sacrificing the animal, blood samples were collected and marker enzymes such as SGOT, SGPT, LDH, CK-MB and CK in serum were estimated spectrophotometrically, using kits from Reckon Diagnostics Pvt Ltd, India, and Randox Laboratories Ltd, United Kingdom, respectively. All these marker enzymes were expressed as U/L. Serum lipid peroxide levels were estimated using the method developed by Yagi (43). Tetraethoxypropane (in amounts of 0.5 nmol, 1 nmol, 2 nmol, 4 nmol, 6 nmol, 8 nmol and 10 nmol) served as the external standard. Lipid peroxide levels in serum were expressed as $\mu\text{mol/L}$.

Estimation of protein

Protein was estimated by the Lowry method (44). The level of protein was expressed as mg protein/g tissue.

Total GSH

GSH was estimated by the method described by Ellman (45). The amount of GS was expressed as $\mu\text{g/g}$ wet tissue.

GST

GST was assayed by the method described by Habig et al (46). To 0.1 mL of homogenate, 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 were added. After incubation at 37.8°C for 15 min, 0.1 mL of GSH was added and change in optical density was read at 340 nm for 3 min at intervals of 3 s. Reaction mixture without the enzyme was used as a blank. The GST activity was expressed as nmol of CDNB conjugated/mg protein/min.

SOD

SOD level was estimated by the method described by Kakkar et al (47). The assay mixture contained 0.1 mL of sample, 1.2 mL of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL phenazine methosulphate (186 μM), 0.3 mL of 300 μM nitroblue tetrazolium and 0.2 mL of NADH (750 μM). The reaction was started by the addition of NADH. After incubation at 30°C for 90 s, the reaction was stopped by the addition of 0.1 mL glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 mL of n-butanol, and the mixture was allowed to stand for 10 min. After centrifuging the mixture, the butanol layer was separated. Colour intensity of the chromogen in the butanol was measured at 560 nm spectrophotometrically, and the concentration of SOD was expressed as U/mg protein.

CAT

CAT level was measured by the method described by Aebi (48). A volume of 0.1 mL of supernatant was added to a cuvette containing 1.9 mL of 50 mM phosphate buffer (pH 7.0). A reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H_2O_2 . The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of CAT was expressed as $\mu\text{mol H}_2\text{O}_2$ metabolized/mg protein/min.

Lipid peroxide levels

Lipid peroxide levels in the myocardium were measured by the method developed by Ohkawa et al (49). Briefly, the infarcted left ventricular tissues were homogenized with 1.15% KCl (10% weight/volume). The assay mixture consisting of 0.1 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mol of 20% acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid was heated for 60 min at 95°C. Thereafter, the mixture was cooled and extracted with 5 mL of mixture of n-butanol and pyridine (15:1 volume/volume). After centrifugation at 4000 rpm for 10 min, the organic phase was assayed spectrophotometrically at 532 nm. Tetraethoxypropane (in amounts of 2 nmol, 4 nmol, 6 nmol and 8 nmol) was served as an external standard. Lipid peroxide levels in myocardium were expressed as nmol/g tissue.

Myeloperoxidase assay

Myeloperoxidase, a green hemoprotein enzyme stored in the granules of neutrophils, can use H_2O_2 generated by NADPH oxidase to oxidize halides (Cl^- , Br^- and I^-) to their corresponding hypohalous acids (an additional class of active oxygen

metabolite). To quantify myocardial neutrophil infiltration, the cardiac activity of myeloperoxidase, an abundant enzyme of neutrophils, was assessed using a modified method (50). Myeloperoxidase activity was used as a marker for neutrophil content in the heart, because it correlates closely with the number of neutrophils (51). The myocardial tissue was homogenized in 50 mM K_2HPO_4 buffer (pH 6) containing 0.5% hexadecyl-trimethylammonium bromide using a Polytron (Ultra-turrax T25, England) homogenizer. After freeze-thawing three times, the samples were centrifuged at 15,000 rpm for 30 min at 40°C, and the resulting supernatant was assayed spectrophotometrically for myeloperoxidase determination. In brief, 40 μL of sample was mixed with 960 μL of 50 mM phosphate buffer (pH 6), containing 0.167 mg/mL O-dianisidine dihydrochloride and 0.0005% H_2O_2 . The change in absorbance at 460 nm was measured with the spectrophotometer. One unit of enzyme activity was defined as the amount of myeloperoxidase present that caused a change in absorbance measured at 460 nm for 3 min. Myeloperoxidase activity data are presented as U/g wet tissue.

Statistical analysis

The results are expressed as mean \pm SEM, and the data were processed by factorial one-way ANOVA. Individual groups were compared using Dunnett's *t* test. Differences with $P < 0.05$ were considered statistically significant.

RESULTS**Effect of silymarin on myocardial infarct size and lipid peroxides**

In control animals (group 2), the infarct size was found to be $50.9 \pm 1.90\%$ and statistically significant compared with the sham control animals (group 1). In silymarin-treated animals (groups 3, 4 and 5), the infarct size was decreased to $25.26 \pm 0.78\%$, $13.93 \pm 0.21\%$ and $8.28 \pm 0.43\%$, respectively, and in the ramipril-treated animals (group 6), the infarct size was decreased to $17.6 \pm 0.8\%$ and the difference was statistically significant ($P < 0.05$) compared with control (Table 1). Lipid peroxide levels in serum and heart tissue in control groups were $27.6 \pm 1.42 \mu\text{mol/L}$ (Figure 1) and $109.2 \pm 2.77 \text{ nmol/g tissue}$ (Figure 2), respectively, and statistically significant compared with the sham control group. In groups 3, 4 and 5, the lipid peroxide levels in serum were found to be $20.18 \pm 0.33 \mu\text{mol/L}$, $11.66 \pm 0.27 \mu\text{mol/L}$ and $4.89 \pm 0.39 \mu\text{mol/L}$, and in heart tissue, the lipid peroxide levels were found to be $55.11 \pm 0.51 \text{ nmol/g tissue}$, $30.97 \pm 0.31 \text{ nmol/g tissue}$ and $18.78 \pm 0.32 \text{ nmol/g tissue}$, respectively. In group 6, ramipril significantly decreased the lipid peroxide levels in serum and heart tissue, and were found to be $4.3 \pm 0.4 \mu\text{mol/L}$ and $30.8 \pm 1.9 \text{ nmol/g tissue}$, respectively. The results are shown in Figures 1 and 2.

Effect of silymarin on hemodynamic variables

In the control group, sudden decreases in MAP and HR were observed after coronary artery ligation and decreased steadily throughout the occlusion and reperfusion period. Similar results were observed in calculated PRP, and the results are given in Figures 3A, 3B and 3C.

Effect of silymarin on myocardial antioxidants

In the control group, the endogenous antioxidant enzymes such as SOD, CAT, GST and GSH levels in heart tissue were

TABLE 1
Effect of silymarin on infarct size in the heart of control and experimental group animals

Group	Dose (mg/kg)	Infarct size (% left ventricle necrosis)
Sham control	–	4.50±0.79*
Control	–	50.91±1.90
Silymarin	100	25.26±0.78*
Silymarin	250	13.93±0.21*†
Silymarin	500	8.28±0.43*††
Ramipril	2	17.60±0.80*

All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ††P<0.001 compared with silymarin 250 mg/kg group

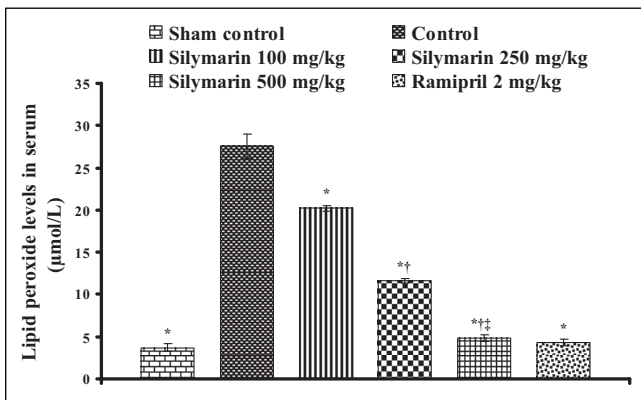


Figure 1) Effect of silymarin on lipid peroxide levels in serum of experimental groups. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ††P<0.01 compared with silymarin 250 mg/kg group

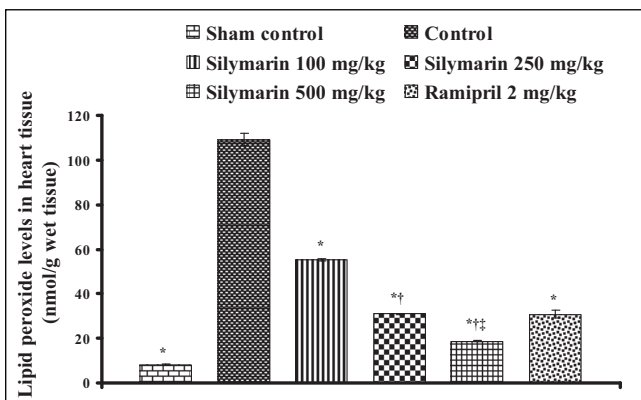


Figure 2) Effect of silymarin on lipid peroxide levels in ischemic heart tissue of experimental groups. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ††P<0.01 compared with silymarin 250 mg/kg group

found to be 13.76±0.57 U/mg protein, 12.42±0.57 µmol H₂O₂ metabolized/mg protein/min, 0.494±0.026 nmol of CDNB conjugated/mg protein/min and 80.42±9.16 µg/g wet tissue, respectively, and the levels were statistically significant compared with the sham control group. In rats pretreated with silymarin at a dose of 100 mg/kg, the antioxidant enzymes levels in heart tissue were found to be 16.41±0.25 U/mg protein,

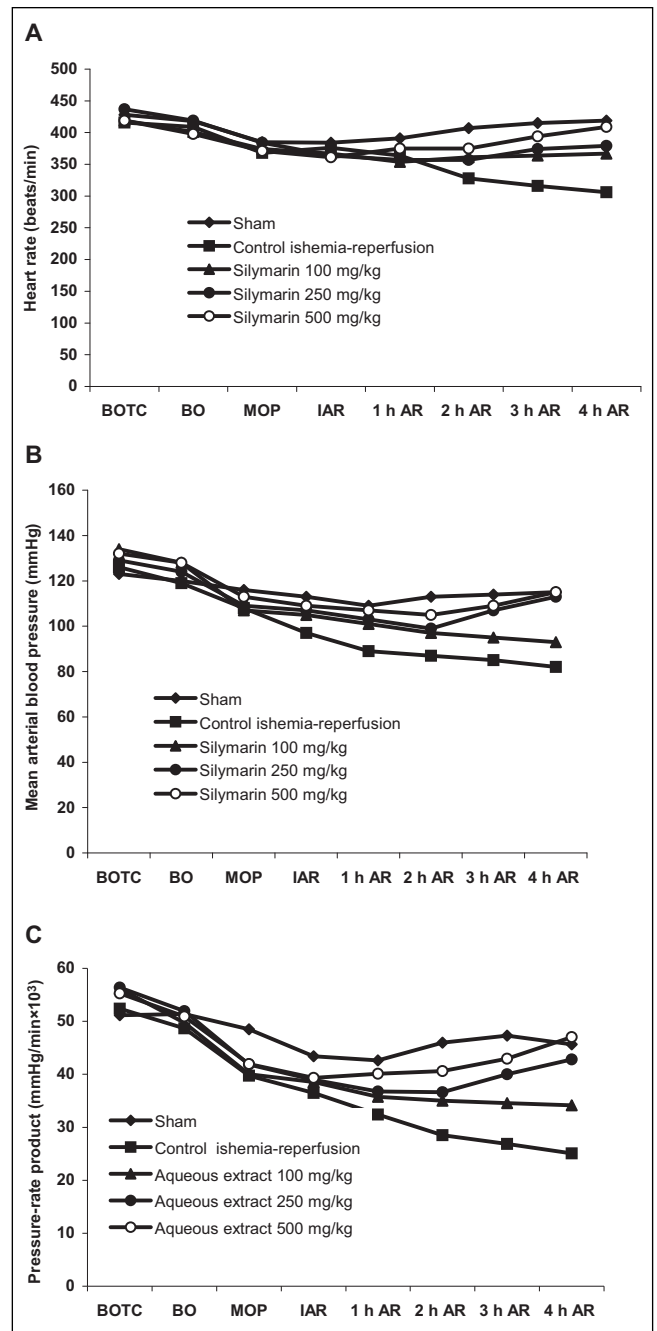


Figure 3) Effect of silymarin at doses of 100 mg/kg, 250 mg/kg and 500 mg/kg on (A) heart rate, (B) Mean arterial blood pressure and (C) pressure-rate product in rats during occlusion of the left anterior descending coronary artery for 30 min and subsequent reperfusion for 4 h. Values are mean ± SD of five animals. AR After reperfusion; BO Before occlusion; BOTC Before opening thoracic cavity; MOP Middle of occlusion period

12.16±0.06 µmol H₂O₂ metabolized/mg protein/min, 0.534±0.01 nmol of CDNB conjugated/mg protein/min and 144.98±10.84 µg/g wet tissue, respectively. At a dose of 250 mg/kg, the antioxidant enzyme levels in heart tissue were found to be 19.99±0.07 U/mg protein, 18.57±0.08 µmol H₂O₂ metabolized/mg protein/min, 0.744±0.001 nmol of CDNB conjugated/mg protein/min and 342.02±9.46 µg/g wet tissue,

respectively. At a dose of 500 mg/kg, the antioxidant enzymes levels were found to be 21.91 ± 0.17 U/mg protein, 20.86 ± 0.20 $\mu\text{mol H}_2\text{O}_2$ metabolized/mg protein/min, 0.834 ± 0.006 nmol of CDNB conjugated/mg protein/min and 408.9 ± 8.14 $\mu\text{g/g}$ wet tissue, respectively, and the difference was also significant ($P < 0.05$) compared with the control group. In group 6, ramipril significantly increased the antioxidant enzymes levels in heart tissue and were found to be 21.61 ± 0.50 U/mg protein, 12.39 ± 0.28 $\mu\text{mol H}_2\text{O}_2$ metabolized/mg protein/min, 0.74 ± 0.01 nmol of CDNB conjugated/mg protein/min and 401.46 ± 11.16 $\mu\text{g/g}$ wet tissue, respectively (Figures 4, 5, 6 and 7).

Effect of silymarin on serum marker enzymes

In the control group, the levels of marker enzymes such as SGOT, SGPT, LDH, CK-MB and CK in serum were found to be 1324 ± 6.64 U/L, 435.5 ± 3.28 U/L, 6037 ± 6.3 U/L, 4378.66 ± 61.23 U/L and 5551 ± 95.19 U/L, respectively, and the levels were statistically significant compared with sham control animals. After pretreatment with silymarin at a dose of 100 mg/kg, the marker enzyme levels were found to be 428.33 ± 4.91 U/L, 135.33 ± 5.46 U/L, 2057 ± 5.21 U/L, 902.66 ± 24.36 U/L and 3045 ± 49.38 U/L, respectively. At a dose of 250 mg/kg, the marker enzyme levels in serum were found to be 271 ± 8.39 U/L, 94.3 ± 3.84 U/L, 1335 ± 23.4 U/L, 464.66 ± 6.74 U/L and 1935 ± 22.94 U/L, respectively. At a dose of 500 mg/kg, the marker enzyme levels were found to be 193.3 ± 4.8 U/L, 63.33 ± 3.84 U/L, 675.3 ± 12.8 U/L, 204.33 ± 5.04 U/L and 883.67 ± 23.59 U/L, respectively, and the difference was significant ($P < 0.05$) compared with control. In group 6, ramipril significantly decreased the marker enzyme levels in serum and were found to be 207.66 ± 8.29 U/L, 116 ± 3.78 U/L, 638 ± 16.86 U/L, 437 ± 8.50 U/L and 1605 ± 27.3 U/L, respectively (Figures 8, 9, 10, 11 and 12).

Effect of silymarin on myeloperoxidase levels

In the control group, the myeloperoxidase level in heart tissue was found to be 11.68 ± 0.18 U/g wet tissue and statistically significant compared with sham control animals (2.4 ± 0.14 U/g wet tissue). After pretreatment with silymarin at a dose of 500 mg/kg, the myeloperoxidase level was found to be 2.48 ± 0.26 U/g wet tissue. In the ramipril-treated (2 mg/kg) group, the myeloperoxidase level was found to be 5.96 ± 0.33 U/g wet tissue. The results are given in Table 2.

DISCUSSION

As a therapeutic agent, silymarin is well tolerated and largely free of adverse effects (52). The main activity of silymarin is an antioxidant effect of its flavolignans and other polyphenolic constituents, which is attributable to radical scavenging of both free radicals and reactive oxygen species (53,54).

Recently, silymarin received attention because of its alternative beneficial activities gained indirectly from its hepatoprotective effects. These benefits include mostly anticancer activities, and hypocholesterolemic, anti-inflammatory and renal protective activities (55-58). These protective actions of silymarin are associated with its antioxidant properties and have been reported to act as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer (59,60).

In the present investigation, we observed a significant elevation of lipid peroxide levels in serum and heart tissue, and a

significant increase of infarct size in the control group, compared with the sham control group. The results clearly show the presence of cytotoxic free radical activity because there is a loss of membrane integrity, with disintegration of polyunsaturated fatty acids in the membrane bilayer, which exerts unfavourable effects on the heart structure and function. In groups treated with silymarin, the elevated lipid peroxide levels were ameliorated in both serum and heart tissue, and significantly reduced the infarct size in a dose-dependent manner, with a 500 mg/kg silymarin dose producing the greatest effect. In serum and heart tissue with the reference drug, ramipril, lipid peroxide levels decreased and significantly reduced the infarct size compared with the control group.

In the control ischemia-reperfusion group, a continuous and significant fall in MAP was observed 15 min after coronary artery occlusion and throughout the reperfusion period compared with the sham control group (Figure 3B). Pretreatment with silymarin significantly restored MAP at the end of the reperfusion period in a dose-dependent manner compared with the control group. Similarly, the HR was significantly decreased throughout the experiment duration in the control ischemia-reperfusion group compared with the 500 mg/kg group and sham control group (Figure 3A). Pretreatment with silymarin significantly restored the HR compared with the control group at the end of the reperfusion period.

The PRP was comparable for all groups before coronary artery occlusion and decreased to a similar extent immediately following occlusion (Figure 3C). Control group animals exhibited a progressively decreasing PRP during the course of the experiment, which was much lower at the end of reperfusion, compared with the value before occlusion. In contrast, treatment with silymarin significantly and dose-dependently suppressed the decrease of PRP at end of the reperfusion (Figure 3C).

Endogenous antioxidant enzymes such as SOD, CAT, GS peroxidase (GPX) and GST are the first-line cellular defense against oxidative stress, decomposing oxygen and H_2O_2 before they interact to form the more reactive hydroxyl radical (OH). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. SOD and CAT are important antioxidant enzymes in mitigating free radical-induced cell injury. A decrease in the activity of SOD and CAT can result in the decreased removal of superoxide ion and H_2O_2 radicals that brings about a number of reactions, which are harmful to myocardium. Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate. An increase in SOD activity is beneficial in the event of increased free radical generation. However, it has been reported that a rise in SOD activity, without a concomitant rise in the activity of CAT and/or GPX, may be detrimental because SOD generates H_2O_2 as a metabolite, which is more cytotoxic than oxygen radicals, and must be scavenged by CAT or GPX (61). Thus, a simultaneous increase in CAT and/or GPX activity is essential for an overall beneficial effect of an increase in SOD activity (62). In the present study, during ischemia-reperfusion injury, the levels of endogenous antioxidant enzymes (SOD, CAT, GSH and GST) in heart tissue were decreased significantly compared with sham control animals. In the treatment groups, silymarin antagonized the decrease in endogenous antioxidant enzyme levels in a dose-dependent manner and produced beneficial effects. In the ramipril-treated group, SOD, GSH and GST were protected.

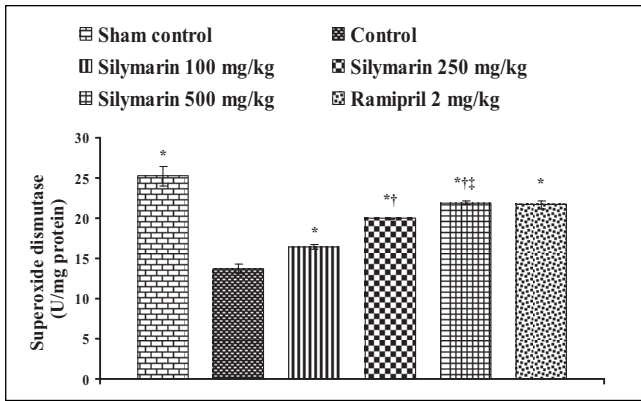


Figure 4) Effect of silymarin on superoxide dismutase levels in heart tissue of experimental group animals. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group

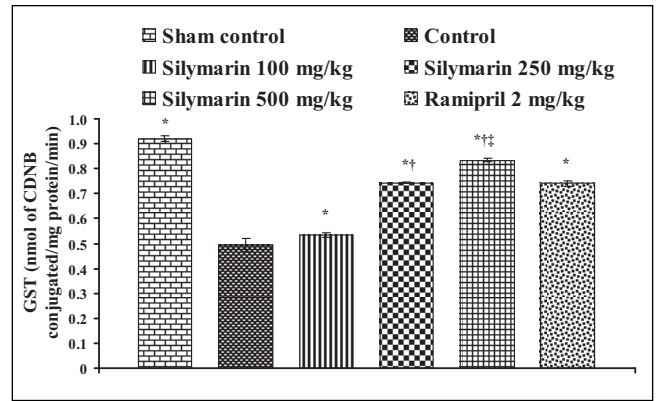


Figure 7) Effect of silymarin on glutathione S-transferase (GST) levels in heart tissue of experimental group animals. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group. CDNB 1-chloro-2,4-dinitrobenzene

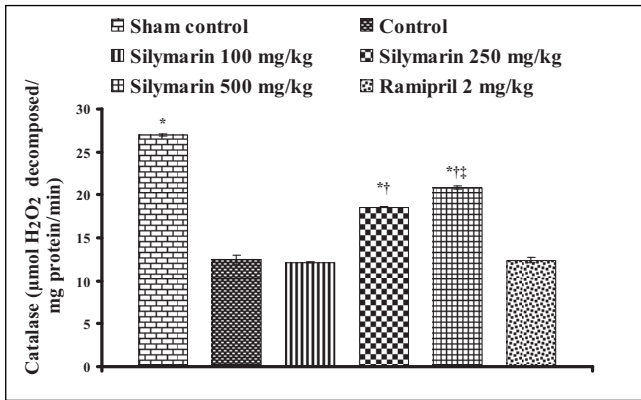


Figure 5) Effect of silymarin on catalase levels in heart tissue of experimental group animals. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group

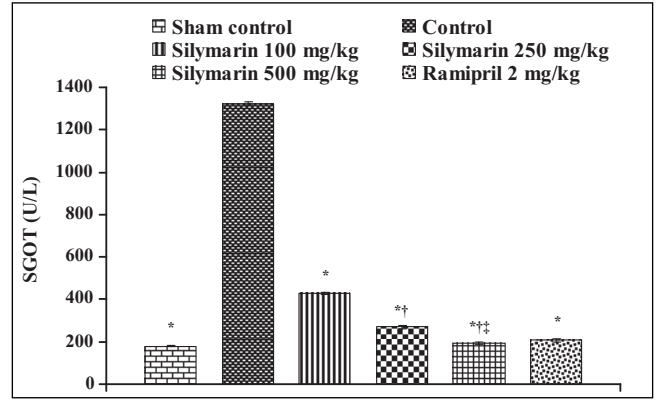


Figure 8) Effect of silymarin on serum glutamate oxaloacetate transaminase (SGOT) levels in serum of experimental groups. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group

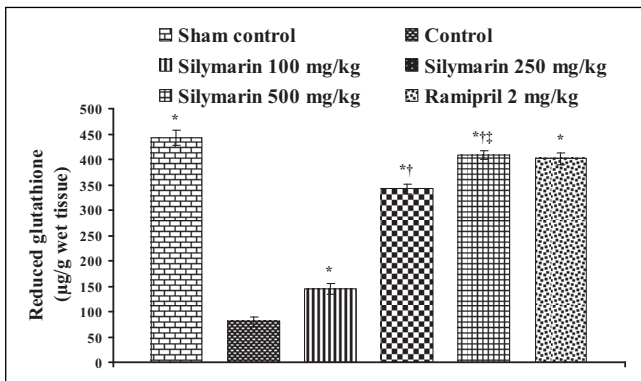


Figure 6) Effect of silymarin on reduced glutathione levels in heart tissue of experimental group animals. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group

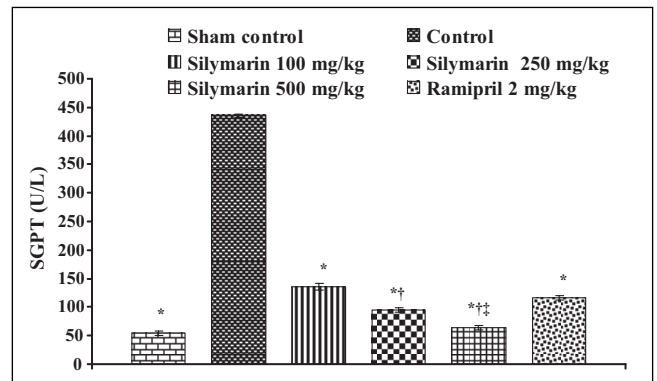


Figure 9) Effect of silymarin on serum glutamate pyruvate transaminase (SGPT) levels in serum of experimental group animals. All values are expressed as mean ± SEM. Each group consists of five albino rats. *P<0.001 compared with control group; †P<0.001 compared with silymarin 100 mg/kg group; ‡P<0.01 compared with silymarin 250 mg/kg group

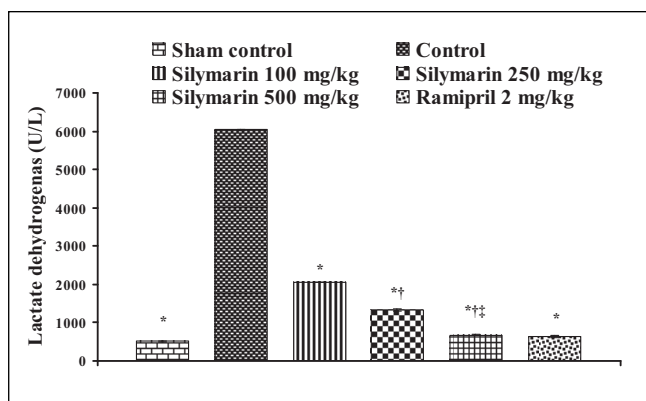


Figure 10 Effect of silymarin on lactate dehydrogenase levels in serum of experimental groups. All values are expressed as mean \pm SEM. Each group consists of five albino rats. * $P < 0.001$ compared with control group; $^{\dagger}P < 0.001$ compared with silymarin 100 mg/kg group; $^{\ddagger}P < 0.01$ compared with silymarin 250 mg/kg group

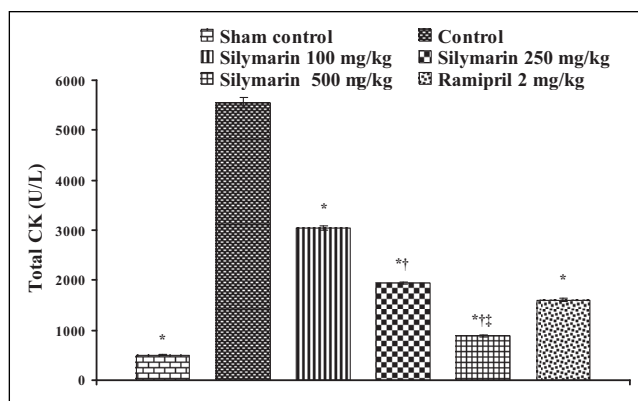


Figure 12 Effect of silymarin on total creatine kinase (CK) levels in serum of experimental group animals. All values are expressed as mean \pm SEM. Each group consists of five albino rats. * $P < 0.001$ compared with control group; $^{\dagger}P < 0.001$ compared with silymarin 100 mg/kg group; $^{\ddagger}P < 0.01$ compared with silymarin 250 mg/kg group

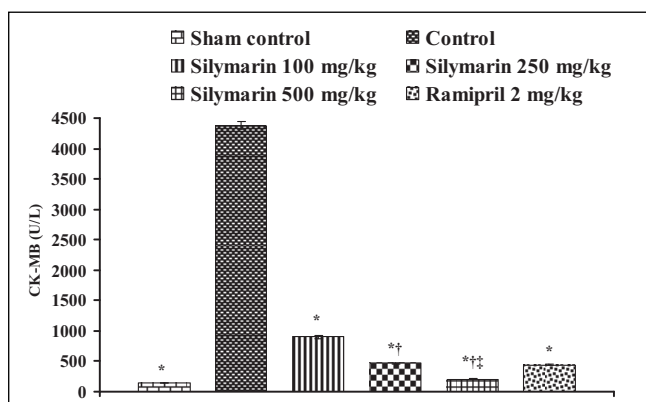


Figure 11 Effect of silymarin on creatine kinase-isoenzyme (CK-MB) levels in serum of experimental group animals. All values are expressed as mean \pm SEM. Each group consists of five albino rats. * $P < 0.001$ compared with control group; $^{\dagger}P < 0.001$ compared with silymarin 100 mg/kg group; $^{\ddagger}P < 0.01$ compared with silymarin 250 mg/kg group

Cytosolic enzymes leak into the intracellular space, where there is cell membrane damage (63,64). Increased levels of SGOT, SGPT, LDH, CK-MB and CK are well-known diagnostic markers of myocardial infarction. In the present study, marked elevations in the level of these enzymes in the serum of the control ischemia-reperfusion group suggest the occurrence of considerable membrane damage compared with the sham control group. Treatment with silymarin resulted in a significant reduction in the levels of these enzymes toward near-normal levels in a dose-dependent manner compared with the control ischemia-reperfusion group.

Because neutrophils represent a potentially important source of oxygen free radicals, myeloperoxidase, an enzyme that is associated with the azurophilic granules of neutrophils, has been measured to form an index of the infiltration of neutrophils into inflamed tissue. In the present study, the myeloperoxidase levels are significantly elevated in the control group compared with the sham control group. In silymarin-treated groups, the reduced levels of myeloperoxidase indicated that pretreatment with silymarin suppressed neutrophil infiltration into the injured myocardium.

TABLE 2
Effect of silymarin on myeloperoxidase induction in ischemia-reperfusion-induced myocardial injury in rat hearts

Group	Dose (mg/kg)	Myeloperoxidase (U/g tissue)	Percentage inhibition
Normal	–	2.04 \pm 0.116	–
Sham control	–	2.4 \pm 0.141	–
Control	–	11.68 \pm 0.185	–
Silymarin	500	2.48 \pm 0.265*	78.76
Ramipril	2	5.96 \pm 0.331*	48.97

All values are expressed as mean \pm SEM. Each group consists of five animals. * $P < 0.001$ compared with the control group

The present findings suggest that silymarin, a well-known liver protectant and phytochemical, possesses dose-dependent cardioprotection against ischemia-reperfusion-induced myocardial injury. Reduction in infarct size, as well as decreased levels of lipid peroxides in serum and heart tissue, clearly indicates that silymarin neutralizes the cytotoxic free radicals generated during ischemia-reperfusion injury, thereby protecting against the loss of membrane integrity and stabilizing the membrane. Hence, silymarin protects myocytes from oxidative stress. This is further confirmed by the investigations on antioxidant enzymes (SOD, CAT, GST and GSH) and serum marker enzymes (SGOT, SGPT, LDH, CK-MB and CK), which leak out from tissues to plasma when degenerative changes develop in myocardial cell membranes. Suppressing the neutrophil infiltration and preventing the fall in mean arterial pressure and HR during ischemia-reperfusion further support the protection offered by silymarin against ischemia-reperfusion injury.

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