Molecular and Cellular Biochemistry **289:** 1–9, 2006.
DOI: 10.1007/s11010-006-9141-7

Cardioprotective effect of lycopene in the experimental model of myocardial ischemia-reperfusion injury

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Received 15 December 2005; accepted 18 January 2006

Abstract

The efficacy of lycopene to limit myocardial injury after ischemia and reperfusion was explored in the present study. Adult male albino Wistar rats were divided into three experimental groups and orally received olive oil as vehicle (sham and control I-R) or lycopene 1 mg/kg dissolved in olive oil (lycopene treated group) respectively for 31 days. On the 31st day, animals of the control I-R and lycopene treated groups were subjected to 45 min of occlusion of the LAD coronary artery and were thereafter reperfused for 1 h. The ischemia-reperfusion injury resulted in significant cardiac necrosis, depression in hemodynamics, decline in antioxidant status and rise in lipid peroxidation product levels in the control I-R group as compared to sham control. In histopathological examinations myocardial damage produced after I-R was significantly prevented in the lycopene treated group. Lycopene treatment resulted in preservation of the myocardial antioxidant status and altered hemodynamic parameters as compared to control I-R group. Furthermore, I-R-induced lipid peroxidation was significantly inhibited in the lycopene treated group. These beneficial cardioprotective effects also translated into the functional recovery of the heart. The beneficial effect of lycopene likely results from the suppression of oxidative stress, which results in the reduction of myocardial injury. (Mol Cell Biochem **289:** 1–9, 2006)

Key words: lycopene, myocardial infarction, ischemia reperfusion injury, nutritional antioxidants

Introduction

Cardiovascular disease remains the leading cause of morbidity and mortality in most industrialized nations and is rapidly becoming a major disease entity in many developing countries [1]. Extensive research through epidemiological techniques has provided information on risk factors and preventive approaches for the treatment of myocardial infarction (MI). Epidemiological studies have shown that nutritional antioxidants slow down the progression and consequences of MI [2, 3]. Diets rich in carotenoids have been of interest because of their potential health benefit against cardiovascular diseases [4, 5]. However, the failure of two large-scale prospective intervention trials involving β-carotene has refocused interest on a number of other carotenoids [6]. Among these is the hydrocarbon carotenoid lycopene, and

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its rich sources are tomatoe, pink grapefruit and watermelon [7, 8]. Interest particularly in lycopene is growing rapidly following the recent publication of epidemiological studies that have associated high serum lycopene levels with reductions in cardiovascular disease incidence [9]. Lycopene is associated with reduced risk of neurodegenerative disorders, serum lipid oxidation and cancers of the lung, bladder, cervix and skin. Health benefits of lycopene have been suggested to be considerably related to its free radical scavenging and antioxidant activity [7, 8].

Scientific evidence supports the role of reactive oxygen species (ROS) in the pathophysiology of MI based on the ability of antioxidants to reduce injury in various experimental models of MI [10]. These ROS may result in depression in contractile function, arrhythmias, depletion of endogenous antioxidant network, membrane permeability changes resulting in an increase in myocardial malondialdehyde content [11]. Oxidative stress may also depress the sarcolemmal Ca^{2+} transport and result in the development of intracellular Ca^{2+} overload and heart dysfunction [12]. These potentially deleterious reactions are controlled in part by antioxidants that eliminate pro-oxidants and scavenge free radicals [13]. The ability of lycopene to quench ROS may explain its potential as a cardioprotective agent. An open-chest coronary artery occlusion and reperfusion procedure to produce ischemia/reperfusion-induced left ventricle infarction was used in the present study to evaluate the cardioprotective potential of lycopene. Myocardial ischemia–reperfusion is clinically relevant to situations such as myocardial infarction, coronary angioplasty, thrombolytic therapy, coronary revascularization and heart transplantation [14]. The reperfusion period, although clearly beneficial for the heart, is associated with myocardial injury. This reperfusion injury is primarily produced by toxic oxygen-derived free radicals [15].

In the present study, *in vivo* cardioprotective activity of lycopene has been evaluated using various biochemical, hemodynamic parameters and further confirmed by histopathological studies. The effect of lycopene on modulation of biochemical parameters: lipid peroxidation product, malondialdehyde (MDA), endogenous antioxidant: reduced glutathione (GSH), antioxidant enzymes {superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx)} and myocardial enzyme creatine phosphokinase-MB isoenzyme (CK-MB) has been evaluated. In order to correlate biochemical and functional changes in the myocardium subjected to ischemia and reperfusion induced damage, hemodynamic parameters; mean arterial pressure (MAP) and heart rate (HR) were also recorded throughout the ischemia and reperfusion duration. Beneficial effects of lycopene have also reconfirmed by assessing the ischemia and reperfusion induced myocardial injury histopathologically.

Materials and methods

Chemicals

All chemicals were obtained from Sigma Chemicals, St. Louis, MO, USA and were of analytical grade. Double distilled water was used for biochemical estimations. Olive oil was purchased from local chemist shop. Lycopene was the kind gift by Jagsonpal Pharmaceuticals, India.

Animals

Adult male albino Wistar rats, weighing 150–200 g, were used in the present study. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of All India Institute of Medical Sciences, New Delhi, India and conducted according to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals. Rats were obtained from the Central Animal House Facility of All India Institute of Medical Sciences, New Delhi, India. The animals were housed in polyacrylic cages ($38 \times 23 \times 10$ cm) with not more than four rats per cage. They were housed in an air-conditioned room and were kept in standard laboratory conditions under natural light and dark cycles (approximately 12 h light/12 h dark) maintained at humidity of $50 \pm 10\%$ and an ambient temperature of $25 \pm 2\,^{\circ}C$. All experiments were performed between 9.0 and 16.0 h. The animals were fed standard pellet diet (Ashirvad Industries; Chandigarh) and given tap water ad libitum, unless otherwise mentioned. The commercial pellet diet contains 24% protein, 5% fat, 55% carbohydrate, 0.6% calcium, 0.3% phosphorous, 4% fiber, 10% moisture and 9% w/w ash.

Experimental design and protocol

The animals were allowed to acclimatize for one week before the experiments. The animals were randomly divided into three main experimental groups sham; control I-R and lycopene treated comprising of eleven animals each. Animals of group 1 served as Sham ($n = 11$) were administered vehicle olive oil (1 mg/kg) once orally for 31 days and then sacrificed on the 31st day. Rats were subjected to the entire surgical procedure and thread was passed beneath the coronary artery but the left anterior descending (LAD) coronary artery was not ligated. Animals of group 2 served as control I-R ($n = 11$) were administered vehicle olive oil (1 mg/kg) once orally for 31 days and in addition, subjected to 45 min LAD coronary artery occlusion and 60 min reperfusion. Animals of group 3 served as lycopene treated group ($n = 11$) administered lycopene (1 mg/kg) dissolved in vehicle olive oil (1 ml/kg) freshly prepared from stock of lycopene 6%

oleoresin was administered once orally using a intragastric feeding tube and administered for 31 days. The dose of lycopene (1 mg/kg) in the present study was selected on the basis of a pilot study in the isoproterenol model of myocardial necrosis. The doses screened were 0.5, 1.0 and 1.5 mg/kg/day and on the basis of biochemical and histopathological study, 1 mg/kg of lycopene exhibited maximum cardioprotective effects. Therefore, this dose was selected for further evaluation in the ischemia-reperfusion model of myocardial infarction. On the 31st day, animals were subjected to 45 min LAD coronary artery ligation and 60 min reperfusion.

In all the groups, hemodynamic parameters were recorded throughout the ischemia and reperfusion period on the 31st day. After the completion of the reperfusion, the animals were sacrificed with an overdose of anesthesia (pentobarbitone 100 mg/kg/i.v.). Hearts were then excised and immediately processed for histopathological and biochemical studies. Hemodynamic and biochemical studies were undertaken for six rats, while the rest (5 each in sham, control I-R group and lycopene treated group) were used for histopathological studies.

Induction of myocardial ischemic reperfusion injury

Animals of all the experimental groups were anesthetized intraperitoneally with pentobarbitone sodium (60 mg/kg). Atropine (0.1 mg/kg) was administered along with the anesthetic to keep the heart rate elevated especially during the surgery protocol and reduce tracheo-bronchial secretions. The body temperature was monitored and maintained at 37 °C throughout the experimental protocol. The neck was opened with a ventral midline incision, and a tracheostomy was performed and the animals were ventilated with room air from a positive pressure ventilator (Inco, India) using compressed air at a rate of 90 strokes/min and a tidal volume of 10 ml/kg. Ventilator setting and PO_2 were adjusted as needed to maintain the arterial blood gas parameters within the physiological range. The left jugular vein was cannulated with polyethylene tube for continuous infusion of 0.9%normal saline solution. The right carotid artery was cannulated for the measurement of MAP and HR. A left thoractomy was performed through the fifth intercostal space and the heart was exposed. The LAD was ligated 3–4 mm from its origin and ends of this ligature were passed through a polyethylene tube to form a snare. After the completion of the surgical procedure, the heart was returned to its normal position in the thorax. The thoracic cavity was covered with saline-soaked gauze to prevent the heart from drying. The animals were then allowed to stabilize for 15 min before LAD ligation. Myocardial ischemia was produced by one stage occlusion of the LAD by pressing the polyethylene tubing against the ventricular wall. This was designated time point 0. The animals then underwent 45 min of ischemia, confirmed by the appearance of epicardial cynosis and ST-segment elevation. Baseline hemodynamic parameters were measured before LAD occlusion and continued according to the experimental protocol throughout ischemia and reperfusion period. The myocardium was reperfused by releasing the snare gently for a period of 60 min. Successful reperfusion was confirmed by visualization of arterial blood flow through the artery, disappearance of the cyanosis and rapid resolution of the ST-segment changes. Brief episodes of ventricular arrhythmia frequently occurred within the first 10 min period of occlusion and within the first 5 min of reperfusion. At the end of reperfusion period, animals were sacrificed for biochemical and histological studies by an overdose of anesthesia.

Experimental parameters studied

Hemodynamic studies

The right carotid artery was cannulated and the cannula filled with heparinized saline and connected with CARDIOSYS CO-101 (Experimentria, Hungary) using a pressure transducer for the measurement of systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and heart rate (HR).

Biochemical studies

Preparation of tissue homogenate. The rats were sacrificed by an overdose of anesthesia (pentobarbitone 100 mg/kg/i.v.). Hearts were excised immediately, washed with normal chilled saline, blotted dry and weighed. A ten-percent homogenate was prepared in phosphate buffer (50 mM, pH 7.4) and an aliquot of 0.2 ml was used for the assay of MDA. The homogenate was centrifuged at 7000 rpm for 15 min. The supernatant thus obtained contained the cytosolic fraction and used for the estimation of the following biochemical parameters: GSH; GSHPx, SOD, CAT, CK-MB isoenzyme and protein. The whole procedure was carried out at 0–4 °C

Determination of reduced glutathione (GSH). Reduced glutathione was estimated by the method of Maron *et al.* [16]. To 0.5 ml of the supernatant, 4.0 ml of 0.3 M Na_2HPO_4 (pH 8.0) and 0.5 ml of dithiobis-2-nitrobenzoic acid (DTNB, 0.6 mM) prepared in 1% tri-sodium citrate were added and vortexed. The absorbance of the resultant yellow colour was recorded spectrophotometrically at 412 nm. Parallel blank and standards were run for the estimation of GSH in the samples.

Determination of glutathione peroxidase (GSHPx) activity. Glutathione peroxidase activity was measured by the method described by Paglia and Valentine [17]. To a 1.0 ml cuvette containing 400 μl of potassium phosphate buffer (pH 7.0, 0.25 M), 100 μl of 10 mM GSH, 100 μl of 2.5 mM NADPH

and 100 μl of glutathione reductase (6 U/ml), 200 μl of supernatant and hydrogen peroxide (100 μl of 12 mM) was added in succession and change in absorbance was recorded spectrophotometrically at 340 nm for 120 s at an interval of 15 s. GSHPx activity is expressed as units/mg protein as compared to the standard. One unit of GSHPx enzyme activity is defined as 1 nmol of NADPH utilized per min at 37 °C.

Determination of superoxide dismutase (SOD) activity. Superoxide dismutase activity was measured by the method of Misra and Fridovich [18]. To a cuvette containing 0.75 ml of carbonate buffer (100 mM, pH 10.2) and 10 μl of epinephrine (3 mM), 50 μl of supernatant was added. The change in absorbance of each sample was then recorded spectrophotometrically at 480 nm for 120 s at an interval of 15 s. Parallel blank and standards were run for the determination of SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation.

Determination of Catalase (CAT) activity. Catalase activity was determined by the method of Aebi [19]. Briefly, supernatant (50 μl) was added to a 3.0 ml cuvette containing 1.95 ml phosphate buffer (pH 7.0, 50 mM) and 1.0 ml of 30 mM hydrogen peroxide. Changes in absorbance were recorded spectrophotometrically at an interval of 5 s for 30 s at 240 nm. Catalase activity is expressed as units/mg protein as compared to the standard. One unit of CAT activity represents one μmol of H_2O_2 decomposed per min at 25 °C.

Determination of lipid peroxides (MDA). Malondialdehyde, a measure of lipid peroxidation, was estimated by method of Ohkawa *et al*. [20]. Briefly, to 0.2 ml of heart tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA) were added in succession. Volume was made up to 4 ml with double distilled water. The mixture was incubated at 95 °C. in a temperature controlled water bath for 60 min and then allowed to cool. A mixture of 5 ml of *n*-butanol and pyridine (15:1) was added to it and vortexed and centrifuged. Organic layer was separated and absorbance was read spectrophotometrically at 515 nm. Blank and standard were measured simultaneously.

Determination of creatine phosphokinase-MB isoenzyme (CK-MB) activity. Creatine phosphokinase-MB was estimated spectrophotometrically using a kit from Randox Laboratories, Crumlin, USA, according to the method of Lamprecht *et al*. [21]. The sample (50 μl) was added to cuvette containing 1 ml of prepared imidazole buffer consisting of 5.2 mM adenosine-mono-phosphate, 2.1 mM adenosine-di-phosphate, 2.1 mM nicotinamide adenine dinucleotide phosphate, 1.6 U/L glucose-6-phosphate dehydrogenase, 31.2 mM creatine phosphate and 21 mM N-acetyl cysteine. It was incubated for 2 min at room temperature and the absorbance was recorded at 340 nm for 180 s at 60 s intervals. One unit of CK-MB isoenzyme is defined as the amount of enzyme that will transfer 1 μmol of phosphate from phosphocreatine to ADP per min at pH 7.4 and a temp of 30 °C.

Determination of protein. Protein was estimated by the method of Lowry *et al*. [22]. To appropriately diluted samples, 5 ml of copper sulphate reagent, which consisted of 1% Na_2CO_3, 2% sodium potassium tartrate and 1% $CuSO_4$, was added. The solution was vortexed and kept for 10 min. To this solution, 0.5 ml of Folin-Ciocalteau phenol reagent was added. The solution was then vortexed and kept for 30 min. Absorbance was read at 620 nm on Beckman's spectrophotometer. The protein content was calculated by using Bovine Serum Albumin as standard.

Histopathological studies. For histopathological studies, myocardial tissue obtained from the excised heart was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. Each section was stained with hematoxylin and eosin (H & E stain). The sections were examined under light microscope (Nikon. Tokyo, Japan) and photomicrographs were taken.

Statistical analysis. Descriptive statistics such as mean and standard deviation were calculated for each and every variable for six experiments in each group. The significance of difference in the I-R model between the data pairs were evaluated by one-way Analysis of Variance (ANOVA) followed by Bonferroni Multiple Range Test post-hoc analysis for statistical comparison between sham control, control I-R and lycopene treated group. A p value <0.05 has been considered as statistical significance level.

Results

Effect of lycopene on hemodynamic variables in experimental model of myocardial ischemia-reperfusion injury

In the control I-R group, a continuous and significant fall in MAP was observed after coronary artery ligation and throughout the reperfusion period compared to sham group (Fig. 1). Similarly, the heart rate was significantly depressed throughout the experimental duration in the control I-R group compared to sham group (Fig. 2). Lycopene treatment corrected MAP and HR as compared to control I-R group at different time course during the ischemia and reperfusion period.

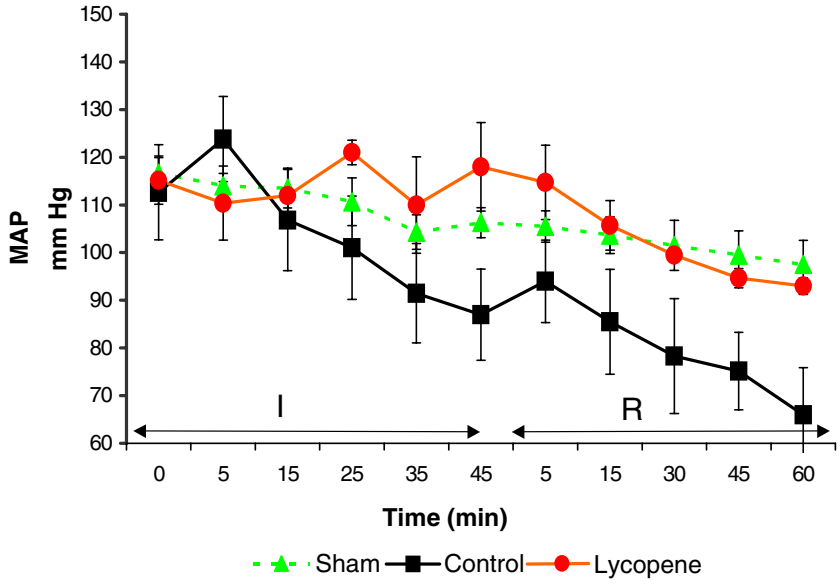

Fig. 1. Time course of changes in mean arterial pressure (MAP) during ischemia-reperfusion. Values are mean ± SEM of six experiments. *I* = Ischemia, *R* = Reperfusion.

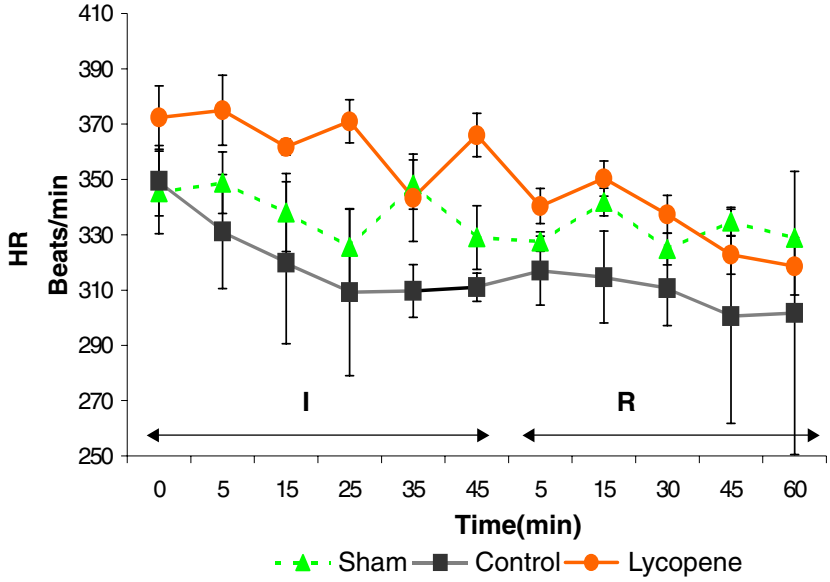

Fig. 2. Time course of changes in heart rate (HR) during ischemia-reperfusion. Values are mean ± SEM of six experiments. *I* = Ischemia, *R* = Reperfusion.

Effect of lycopene on antioxidant parameters in experimental model of myocardial ischemia-reperfusion injury

A significant decrease in GSH levels ($p < 0.05$) as well as in the activities of GSHPx, SOD, CAT and an increase in MDA level ($p < 0.05$) were observed in the control I-R group as compared to sham group (Table 1). A marked rise in level of GSH content ($p < 0.05$) and antioxidant enzyme GSHPx ($p < 0.001$) was observed in the lycopene treated group as compared to control I-R group. Lycopene treatment also markedly reduced lipid peroxidation as evidenced by reduction in MDA levels ($p < 0.001$) as compared to control I-R group. Lycopene treatment however slightly restored the activities of SOD and CAT enzymes (Table 1).

Table 1. Effect of lycopene on biochemical parameters in ischemia-reperfusion model of myocardial infarction in different experimental groups

Biochemical parameters	Experimental groups		
	Sham	Control I-R	Lycopene (1 mg/kg/day)
GSH (μmol/g tissue)	1.86 ± 0.56	$0.60 \pm 0.19^{\#}$	$3.49 \pm 1.95^{*}$
SOD (Units/mg protein)	7.77 ± 0.95	$3.50 \pm 1.07^{\#}$	3.82 ± 0.64
CAT (Units/mg protein)	21.60 ± 1.40	$14.76 \pm 2.60^{\#}$	18.50 ± 6.12
GSHPx (Units/mg protein)	0.33 ± 0.04	$0.18 \pm 0.05^{\#}$	$0.41 \pm 0.10^{***}$
MDA (nmol/g tissue)	63.09 ± 5.31	$79.09 \pm 7.36^{\#}$	$53.72 \pm 6.70^{***}$

The values are expressed as mean $\pm$ SD. Each value represents a mean of six experiments. $\#p < 0.05$ vs Sham; $^{*}p < 0.05$, $^{***}p < 0.001$ vs control I-R. GSH: reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; GSHPx: Glutathione peroxidase; MDA: Malondialdehyde. One unit of SOD inhibits the rate of auto-oxidation of epinephrine by 50% at pH 7 at 25 °C. One unit of CAT activity represents amount of enzyme required to decompose 1 umol of H_2O_2 /min. One unit of GSHPx activity is defined as amount of enzyme required to utilize 1 nmol of NADPH/min at 25 °C.

Effect of lycopene on myocardial specific enzyme marker in experimental model of myocardial ischemia-reperfusion injury

A significant decrease in myocardial specific CK-MB isoenzyme ($p < 0.05$) was observed in the control I-R group as compared to sham group (Table 2). Lycopene treatment resulted in significant prevention of myocardial enzyme CK-MB isoenzyme ($p < 0.001$) concomitant with reduced lipid peroxidation compared to the control I-R group (Table 2).

Effect of lycopene on histopathological changes in experimental model of myocardial ischemia-reperfusion injury

On histopathological examination, control I-R group hearts showed myocardial membrane damage and infiltration of inflammatory cells as compared to those in sham control group. Significant myonecrosis with fibroblastic proliferation and presence of inflammatory cells were observed in the control group (Fig. 3B) compared to that of sham control (Fig. 3A). Lycopene treatment showed marked improvement evidenced by reduced degree of myonecrosis, edema, infiltration of inflammatory cells and lesser vacuolar changes compared to the control I-R group (Fig. 3C).

Discussion

Reperfusion of the ischemic myocardium results in irreversible tissue injury and cell necrosis, leading to decreased cardiac performance. While early reperfusion of the heart is essential in preventing further tissue damage due to ischemia, reintroduction of blood flow can expedite the death of vulnerable, but still viable, myocardial tissue, by initiating a series of events involving both intracellular and extracellular mechanisms [11]. In the last decade, extensive efforts have focused on the role of cytotoxic reactive oxygen species generated during myocardial reperfusion injury [11, 13]. Thus targeting free radicals can be an important therapeutic target, which could scavenge the excess free radicals produced at the time of reperfusion [10].

Epidemiological and clinical studies suggest that dietary antioxidants may reduce the risk of cardiovascular disorders [3]. Consumption of carotenoid rich food is often associated with several health benefits [4, 5]. These benefits are correlated with their antioxidant properties. Among carotenoids, lycopene because of its high number of conjugated dienes

Table 2. Myocardial creatine kinase-MB (CK-MB) isoenzyme activity in different experimental groups

Experimental groups	Total myocardial CK-MB isoenzyme activity (IU/mg of protein)	CK-MB isoenzyme activity depleted from the myocardium (%)
Sham	151.20 ± 2.50	–
Control I-R	$91.20 \pm 4.89^{*}$	39.69%[a]
Lycopene (1 mg/kg/day)	$128.01 \pm 3.38^{\#}$	15.34%[a]

Values are expressed as mean $\pm$ SD. Each value represents mean of six experiments. $^{*}p < 0.05$ vs sham; $^{\#}p < 0.001$ vs control I-R group.
[a]Percentage of CK-MB isoenzyme activity depleted from myocardium as compared to sham group. One unit of CK-MB isoenzyme will transfer 1 umol of phosphate from phosphocreatine to ADP per min at pH 7.4 at 30 °C.

is the most potent singlet oxygen quencher [7, 8]. Due to its potential antioxidant activity, it may have potential cardioprotection activity against ischemia-reperfusion induced myocardial injury. Keeping this in view, the study was designed to evaluate the cardioprotective potential of lycopene in experimental *in vivo* ischemia-reperfusion model of myocardial infarction.

It is now well established that a burst of oxygen free radicals occurs immediately after restitution of blood flow to a previously ischemic myocardium which may result in enhanced lipid peroxidation as indicated by increase in MDA levels documented both in clinical and experimental studies in conditions of myocardial ischemia-reperfusion injury [23]. In the present study, an elevated level of MDA in control I-R

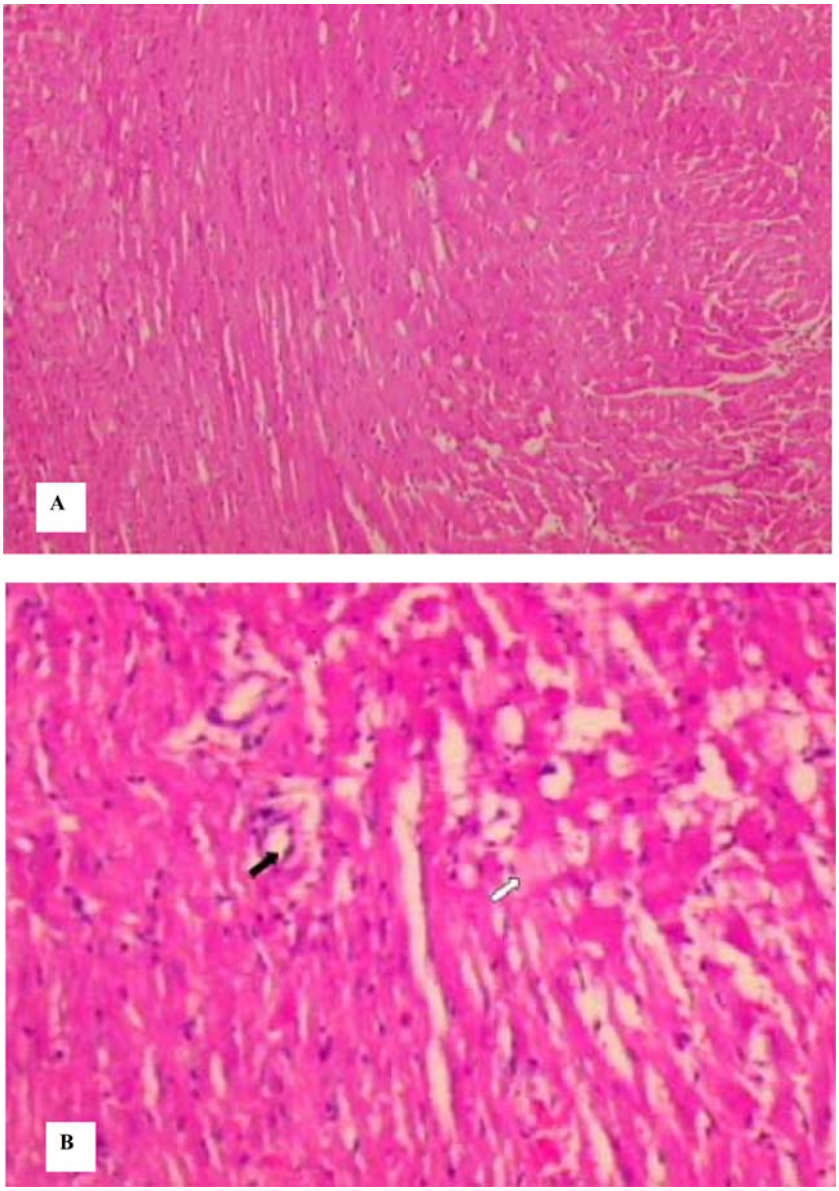

Fig. 3. Photomicrograph showing [A] normal architecture of rat heart of sham control group. Endocardium and pericardium are seen within normal limits with no inflammatory cells [B] extensive areas of focal myonecrosis (white arrow), edema with fibroblastic proliferation (black arrow) in the control I-R group subjected to ischemia-reperfusion injury. In subendocardium vacuolar changes and prominent edema along with inflammatory cells are clearly visible [C] transmural patchy area of necrosis but significantly lesser edema and muscle damage compared to control I-R group in lycopene (1 mg/kg/day p. o.) treated group (H & E X 100).

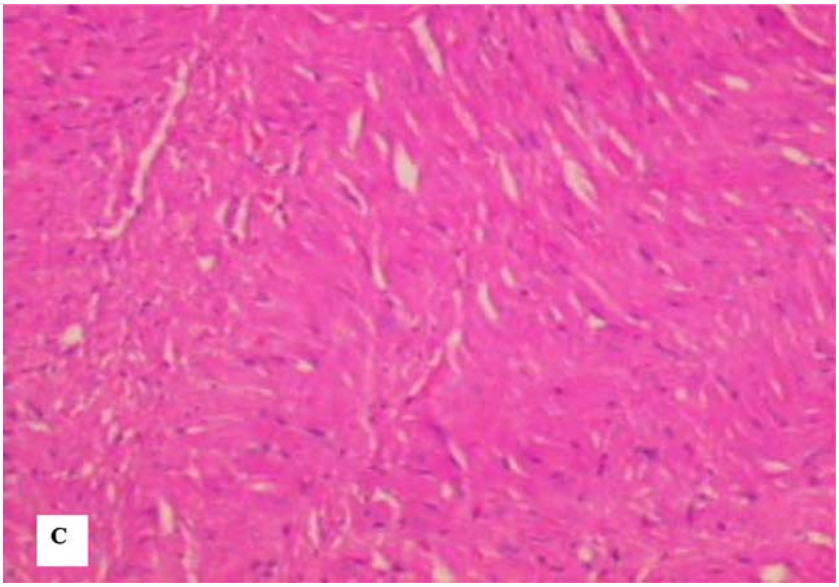

Fig. 3. (*Continued*)

group indicates increased oxidative stress due to ischemia-reperfusion induced injury, as compared to sham animals. On the contrary, lycopene treatment group demonstrated decreased level of lipid peroxides and this could be imparted to reduced formation of lipid peroxides from fatty acids.

A significant decrease in myocardial GSH and endogenous antioxidant enzymes (SOD, CAT and $GSHP_X$) in the present study further confirms the presence of oxidative stress. Due to disruption of endogenous antioxidant network, as observed in the study, the myocardium may be more susceptible to the ischemia-reperfusion injury. In the present study, lycopene exhibited significant antioxidant activity as it increased GSH levels, GSHPx activity and reduced lipid peroxidation. A significant inhibition of lipid peroxidation evidenced by reduced MDA level and enhanced level of reduced GSH along with slight improvement in the activities of SOD and CAT indicate the attenuation of oxidative stress associated with ischemia-reperfusion injury.

Besides antioxidant enzymes and physiological antioxidants, alterations in CK-MB isoenzyme have been considered as an important marker of myocardial infarction [24]. In the present study, CK-MB isoenzyme was estimated in heart tissue and a significant fall in its levels was observed in control I-R group as compared to that of sham group. This observation is in conformity with the previous reports and can be attributed to the fact that CK-MB isoenzyme, being a cardiospecific isoenzyme, leaked out from the tissue to plasma on development of degenerative changes in myocardial cell membranes, due to lipid peroxidation [24]. The observation that lycopene treatment significantly restored the activity of CK-MB isoenzyme compared to control I-R suggests the protective effect of lycopene on the myocardium.

It is well documented that ischemia-reperfusion induces a marked ventricular dysfunction [25]. Similar observations were also recorded in this study when ischemia-reperfusion injury was produced in rat heart by occlusion of LAD coronary artery for 45 min and reperfused for 60 min. Significant increase in systolic arterial pressure at the onset of ischemia indicates the activation of sympathetic nervous system. This increase in SAP might be a compensatory mechanism of the myocardium to increase perfusion in order to meet the increased myocardial energy demand. However, it decreased significantly with progression of ischemia and throughout the reperfusion period indicating the deteriorated metabolic and functional state of the ischemic reperfused myocardium. The heart rate was depressed throughout the ischemia-reperfusion duration in the control I-R group as compared to sham, clearly depicting the injured state of myocardium following ischemia-reperfusion induced myocardial injury. Histopathological findings further confirmed the biochemical and hemodynamic observations. Cardioprotection afforded by lycopene may also be explained by the significant correction of MAP and HR that may increase blood flow through the sub-endocardial region of the ventricular muscle that bears the maximum brunt of the ischemic insult. Under ischemic conditions, there is a disproportionate reduction in blood flow to the subendocardial regions of the heart, which is subjected to the greatest extra-vascular compression during systole.

On the basis of present study findings, it is suffice to say that early intervention by antioxidant drugs may attenuate the myocardial ischemia-reperfusion injury. A number of epidemiological studies have shown an inverse association between β-carotene and the risk of cardiovascular diseases, whereas only a few are available concerning the association

of lycopene with the risk of coronary events. Hemodynamic, biochemical and histopathological results in the ischemia and reperfusion model of MI in the present study emphasize the beneficial action of lycopene (1 mg/kg/day p. o.) as a cardioprotective agent.

Conclusions

In the present study, improved hemodynamics, endogenous antioxidant network along with improved histopathological characteristics demonstrates cardioprotective potential of lycopene. However, further well-controlled studies are required to assess its potential therapeutic utility in human beings.

Acknowledgments

The authors gratefully acknowledge the gift of lycopene from Jagsonpal Pharmaceuticals, India. The authors also thank Mr. B.M. Sharma for his valuable technical assistance.

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