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Protective effects of *Curcuma longa* on ischemia-reperfusion induced myocardial injuries and their mechanisms

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Abstract

The present study was undertaken to evaluate the cardioprotective potential of *Curcuma longa* (Turmeric) in the ischemia-reperfusion (I/R) model of myocardial infarction (MI). Wistar rats were divided into three groups and received saline orally (sham, control I/R group) and *Curcuma longa* 100 mg/kg (CL-100 group) respectively for one month. On the 31st day, rats of the control I/R and Cl treated groups were subjected to 45 min of occlusion of the LAD coronary artery and were thereafter reperfused for 1 h. I/R resulted in significant cardiac necrosis, depression in left ventricular function, decline in antioxidant status and elevation in lipid perodixation in the control I/R group as compared to sham control. Myocardial infarction produced after I/R was significantly reduced in the Cl treated group. Cl treatment resulted in restoration of the myocardial antioxidant status and altered hemodynamic parameters as compared to control I/R. Furthermore, I/R-induced lipid peroxidation was significantly inhibited by Cl treatment. The beneficial cardioprotective effects also translated into the functional recovery of the heart. Cardioprotective effect of Cl likely results from the suppression of oxidative stress and correlates with the improved ventricular function. Histopathological examination further confirmed the protective effects of Cl on the heart.

Keywords: Myocardial infarction; Medicinal Herbs; Antioxidants; *Curcuma longa*; Oxidative stress

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Introduction

Myocardial ischemia–reperfusion is clinically relevant to 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 (Ferrari et al., 1990). Extensive studies show that myocardial ischemia and reperfusion is associated with increased generation of reactive oxygen species (ROS) (Bernier et al., 1986). These oxygen free radicals may result in depression in contractile function, arrhythmias, depletion of endogenous antioxidant network, membrane permeability changes resulting in an increase in myocardial malondialdehyde (MDA) content (Curello et al., 1986). Oxidative stress may also depress the sarcolemmal Ca^{2+} transport and result in the development of intracellular Ca^{2+} overload and heart dysfunction (Tappia et al., 2001). There is comprehensive experimental and clinical evidence that antioxidants attenuate the myocardial injury following myocardial infarction (Hearse, 1991; Bernier et al., 1989). Several medicinal plants have been reported to possess strong antioxidant activity and their health benefits have been suggested to be related to their antioxidant activity (Fugh Berman, 2000).

Turmeric (*Curcuma longa*), a common Indian dietary pigment and spice has been shown to possess a wide range of therapeutic utilities in the traditional Indian Medicine. It's role in wound healing, urinary tract infection, liver ailments are well documented (Dixit et al., 1988). The active component of turmeric identified as curcumin exhibits a variety of pharmacological effects including antioxidant, adaptogenic, anti-inflammatory and anti-infectious activities (Srinivas et al., 1992; Shalini and Srinivas, 1987). However, there are few studies presently available that document its cardioprotective potential (Nirmala and Puvarakrishnan, 1996). The preventive effects of *Curcuma longa* (Cl) were studied on ischemia-reperfusion injury in rats as a model of antioxidant-based composite therapy. Biochemical indicators of oxidative damage, lipid peroxidation product; MDA (malondialdehyde),endogenous antioxidant: glutathione (GSH), antioxidant enzymes {superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx)} and myocardial enzyme {creatine phosphokinase (CPK)} have been evaluated. We also examined the effect of Cl on cardiac function {mean arterial pressure (MAP), heart rate (HR), left ventricular end-diastolic pressure (LVEDP), left ventricular (LV) peak positive (+) dP/dt (rate of pressure development) and negative (−) dP/dt (rate of pressure decline)} after coronary artery ligation and reperfusion. Protective action of Cl was also confirmed by assessing the ischemia and reperfusion induced myocardial injury histopathologically.

Materials and methods

All chemicals were obtained from Sigma Chemicals, USA and were of analytical grade. *Curcuma longa* was procured from Sanat Laboratories, India.

Wistar male albino rats, weighing 150–200 g, were used in the study. The study protocol was approved by the institutional Ethics Committee 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. They were kept in standard laboratory conditions under natural light and dark cycle. The animals were fed normal diet (Hindustan Lever Ltd; Chandigarh) and water ad libitum.

Treatment protocol

The animals were randomly divided into three main groups comprising of eight animals each.

Group 1–saline control group-Sham group

Rats were administered 0.9% normal saline for a month and then sacrificed on the 31st day. The animals were subjected to the entire surgical procedure and thread was passed beneath the coronary artery but the LAD coronary artery was not ligated.

Group 2–Ischemia and reperfusion group - I/R control group

The rats were administered 0.9% normal saline for a month and in addition, on the 31st day, subjected to 45 min LAD coronary artery ligation and 60 min reperfusion induced myocardial injury.

Group 3–Curcuma longa treated group–Cl-100 group

Aqueous extract of *Curcuma longa* (100 mg/kg) was administered orally for 1 month. The dose of *Curcuma longa* (100 mg/kg) was selected on the basis of a pilot study in the isoproterenol model of MI. The doses screened were 25,50,100 and 200 mg/kg and 100 mg/kg was found to be the most effective in restoring biochemical and histopathological markers of injury (data not given). On the 31st day, rats 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. Hearts were then removed and immediately processed for histopathological studies. For performing biochemical estimations, hearts were stored in liquid nitrogen till further analysis.

Surgical procedure: infarction protocol

Rats of all the experimental groups were anesthetized intraperitoneally with pentobarbitone sodium (60 mg/kg). Atropine was co-administered with the anesthetic to keep the heart rate elevated especially during the surgery protocol and reduce broncho-tracheal 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 rats were ventilated with room air from a positive pressure ventilator (Inco, India) using compressed air at a rate of 70 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 continuos infusion of 0.9% saline solution. The right carotid artery was cannulated for the measurement of MAP and HR. A left thoractomy was performed at the fifth intercostal space and the pericardium was opened to expose the heart. A wide bore (1.5 mm) sterile metal cannula connected to a pressure transducer (Gould Statham P23ID, USA) was inserted into the cavity of the left ventricle from the posterior apical region of the heart for recording left ventricular pressure dynamics on Polygraph (Grass 7D, USA). The left anterior descending coronary artery (LAD) was ligated 3–4 mm from its origin by a 5–0 silk suture with a atraumatic needle and ends of this ligature were passed through a small vinyl 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 induced by one stage occlusion of the LAD by pressing the polyethylene tubing against the ventricular wall and then fixing it in place by clamping the vinyl tube with a hemostat. A bolus of heparin (30 IU) was administered immediately before coronary artery occlusion for prophylaxis against thrombus formation around the snare. Electrocardiographic leads were attached to subcutaneous electrodes to monitor limb lead II. This was designated time point 0. The animals then underwent 45 min of ischemia, confirmed visually in situ by the appearance of regional 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, appearance of hyperemia over the surface of the previously ischemia cynotic segment 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 blood pressure; MAP and HR. A wide bore (1.5 mm) sterile metal cannula was inserted into the cavity of the left ventricle from the posterior apical region of the heart for recording LVEDP, (+) LVdP/dt and (−) LVdP/dt.

Biochemical studies

A ten-percent homogenate of myocardial tissue was prepared in 50 mM phosphate buffer, pH 7.4 and an aliquot was used for the assay of malondialdehyde according to the method described by Ohkawa et al. (1979). The homogenate was centrifuged at 7000 rpm for 15 minutes and the supernatant was used for the estimation of the biochemical parameters: glutathione (Moron et al., 1979); glutathione peroxidase (Paglia and Valentine, 1967), superoxide dismutase (Mishra and Fridovich, 1976) catalase (Aebi, 1974) and protein (Lowry et al., 1951). Creatine phosphokinase was estimated spectrophotometrically using a kit from Randox Laboratories, USA (Lamprecht et al., 1974).

Histopathological studies

At the end of the experiment, myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. The fixed tissues were embedded in paraffin and serial sections were cut. The sections were examined under light microscope (Nikon. Tokyo, Japan) after hematoxylin and eosin staining and photomicrographs were taken.

Statistical analysis

Descriptive statistics such as mean and standard deviation were calculated for each and every variable for each group. In the I/R group, one-way Analysis of Variance (ANOVA) was applied for statistical

analysis with post-hoc analysis (Bonferroni Multiple Range Test). Student's t test was applied for statistical analysis in the ischemia and reperfusion model and p value <0.05 has been considered as statistical significance level.

Results

Hemodynamic variables

In the control I/R group, a continuous and significant fall in MAP was observed 25 min after coronary artery ligation and throughout the reperfusion period compared to sham group (Fig. 1). Cl treatment significantly restored MAP as compared to control group at the end of reperfusion period. Similarly, the HR was significantly depressed throughout the experimental duration in the control I/R group compared to sham (Fig. 2). The fall in HR in the Cl treatment group was less pronounced as compared to control group. Nonetheless, Cl failed to restore HR significantly as compared to I/R control.

A significant fall in (+) LVdP/dt was observed in the reperfusion period and a slight but non-significant fall during ischemia in the control I/R group as compared to sham (Fig. 3). Cl treatment significantly improved this hemodynamic parameter as compared to control I/R group during the reperfusion duration. Similarly, a marked depression in (−) LVdP/dt was recorded in the control I/R group as compared to sham (Fig. 4). The observed fall in diastolic function was more significant as compared to systolic dysfunction. Cl treatment significantly restored (−) LVdP/dt as compared to control I/R during both ischemia and reperfusion period. A significant elevation in LVEDP marked the

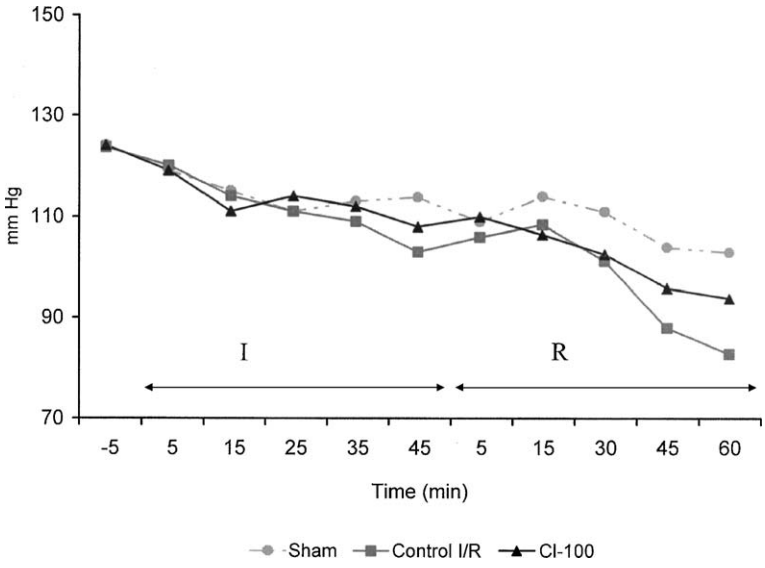

Fig. 1. Time course of changes in MAP during ischemia-reperfusion. Values are mean ± SEM of eight experiments. I = Ischemia, R = Reperfusion.

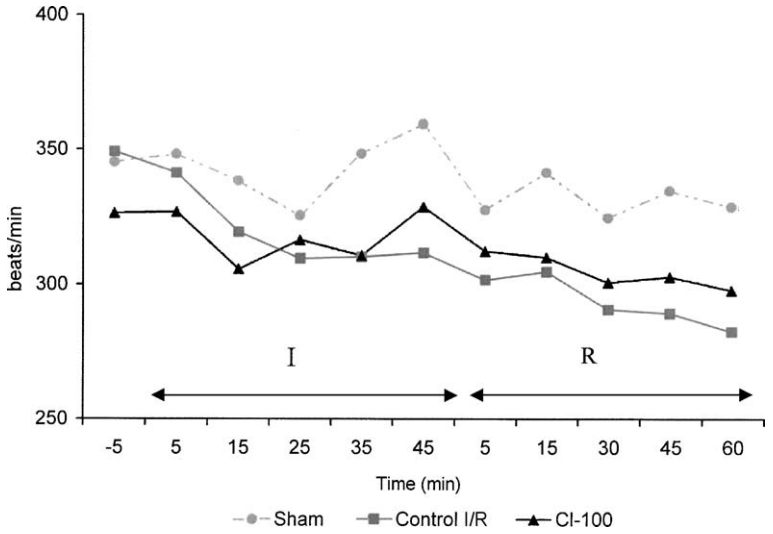

Fig. 2. Time course of changes in HR during ischemia-reperfusion. Values are mean ± SEM of eight experiments. I = Ischemia, R = Reperfusion.

onset of ischemia and remained elevated throughout the ischemic period (Fig. 5). Although on reperfusion, there was a marked decline in the LVEDP, it remained slightly increased compared to the sham control group. Significant correction in LVEDP was seen in the Cl treated group compared to the control I/R group.

Biochemical parameters

Effect of Curcuma longa on antioxidant parameters

A significant decrease in GSH levels ($p < 0.05$) as well as in the activities of GSHPx, CAT and CPK ($p < 0.05$) and an increase in MDA level ($p < 0.05$) were observed in the control I/R group as compared

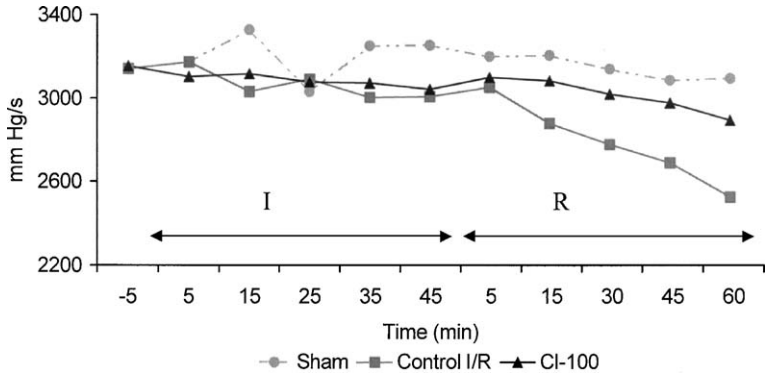

Fig. 3. Time course of changes in (+) LVdP/dt during ischemia-reperfusion. Values are mean ± SEM of eight experiments. I = Ischemia, R = Reperfusion.

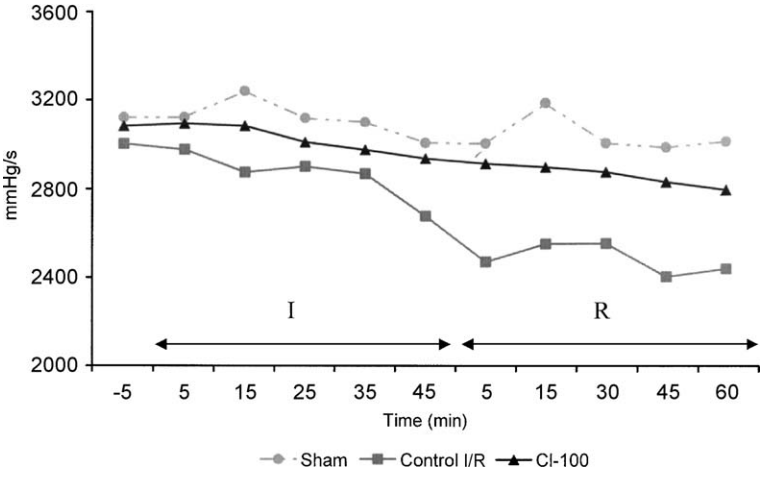

Fig. 4. Time course of changes in (−) LVdP/dt during ischemia-reperfusion. Values are mean ± SEM of eight experiments. I = Ischemia, R = Reperfusion.

to sham group (Table 1 and Fig 6). Cl treatment resulted in a significant repletion of these biochemical markers compared to the control I/R group. A marked restoration in antioxidant enzyme GSHPx ($p < 0.05$), SOD ($p < 0.05$) and myocardial enzyme CPK ($p < 0.05$) was observed in the Cl treated I/R group as compared to control I/R group. Cl also markedly reduced lipid peroxidation ($p < 0.05$) as evidenced by reduction in MDA levels as compared to control I/R group (Table 1). Cl treatment however failed to restore the activities of CAT and GSH level significantly as compared to control I/R group (Table 1).

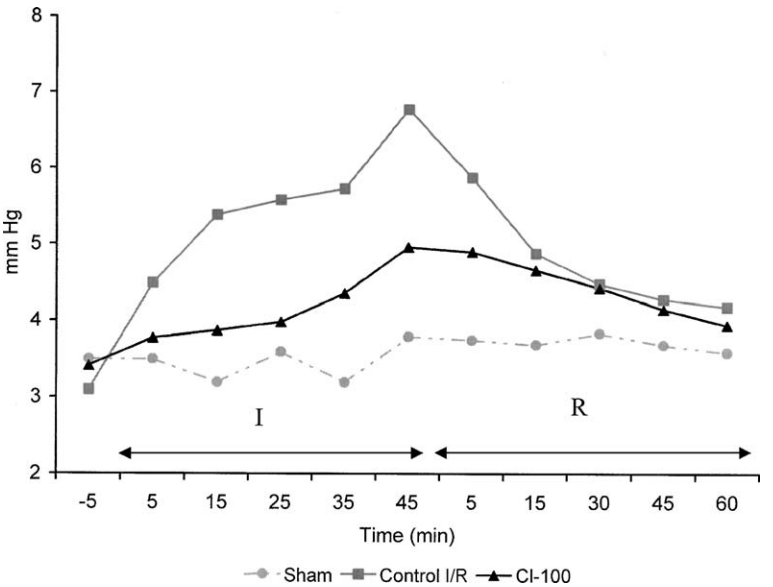

Fig. 5. Time course of changes in LVEDP during ischemia-reperfusion. Values are mean ± SEM of eight experiments. I = Ischemia, R = Reperfusion.

Table 1
Myocardial endogenous antioxidants in the different experimental groups

	Sham	control I/R	I/R+ Cl (100 mg/kg)
GSH	1.86 ± 0.69	0.60 ± 0.07#	1.29 ± 0.15
SOD	7.94 ± 2.90	3.77 ± 1.07#	5.09 ± 1.64*
GSHPx	0.33 ± 0.12	0.18 ± 0.05#	0.36 ± 0.10*
CAT	21.10 ± 4.20	14.80 ± 2.56#	17.05 ± 6.36
MDA	63.4 ± 8.10	79.32 ± 12.62#	68.52 ± 9.12*

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

Histopathological assessment

On histopathological examination, control I/R group 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 chronic inflammatory cells were observed in the control I/R group compared to sham control (Plate 1). *Curcuma longa* treatment showed marked improvement in the degree of myonecrosis, infiltration of inflammatory cells vacuolar changes as well as edema compared to the control I/R group (Plate 1).

Discussion

Myocardial ischemia initiated by occlusion or blockade of a major coronary artery leads to a complex series of cellular events that can result in myocardial cell death. Although reperfusion can produce salvage of ischemic tissue, it may also contribute to myocardial cellular injury (Jennings et al., 1990). Reperfusion can accelerate necrosis in irreversibly injured myocytes because of an increase in cell swelling, disruption of cell ultrastucture, formation of contraction bands, and deposition of intra-mitochondrial calcium

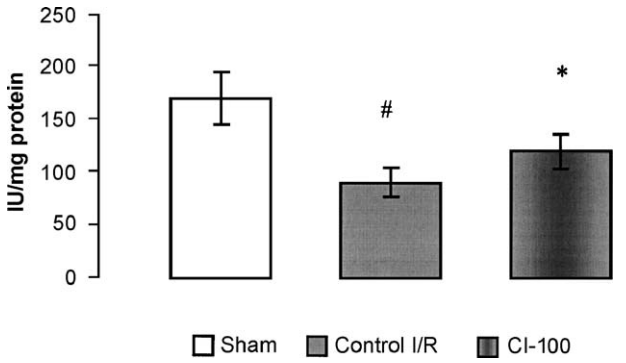

Fig. 6. Myocardial CPK activity in the different experimental groups. Values are mean ± SEM of eight experiments. One unit of CPK will transfer 1 umol of phosphate from phosphocreatine to ADP per min at pH 7.4 at 30°C. #$p < 0.05$ vs. Sham; *$p < 0.05$ vs. control I/R.

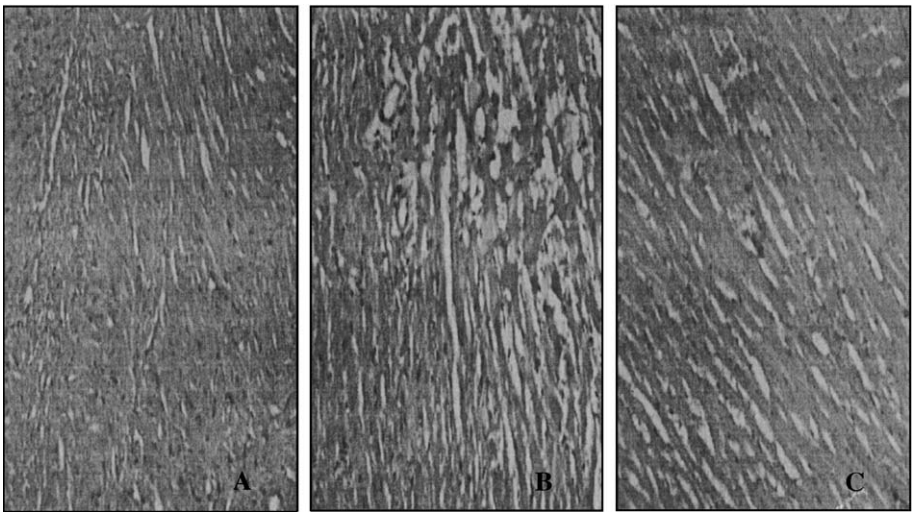

Plate 1. A: Photomicrograph showing normal architecture of rat heart of sham control group. Endocardium and pericardium are seen within normal limits with no inflammatory cells (H&E × 100). B: Photomicrograph of rat heart of the control I/R group subjected to ischemia and reperfusion injury showing extensive areas of focal myonecrosis, edema with fibroblastic proliferation. In sub-endocardium vacuolar changes and prominent edema along with chronic inflammatory cells are clearly visible (H&E × 100). C: Photomicrograph of rat heart of *Curcuma longa* (100 mg/kg) treated group showing transmural patchy area of necrosis but significantly lesser edema and muscle damage compared to control I/R group. (H&E × 100).

phosphate granules. Sarcolemmal damage may also occur, leading to impairment of fluid regulation and ion flux balance (Ferrari et al., 1990; Bernier et al., 1986; Curello et al., 1986).

Several mechanisms have been proposed to explain the myocardial injury observed after ischemia and reperfusion. Recent studies have demonstrated that production of free radicals by neutrophils, monocytes and endothelial cells contribute to myocardial cell injury (Dormandy, 1978). Free radicals have been shown to initiate lipid peroxidation resulting in an alteration of membrane integrity, fluidity and permeability (Sevenian and Hochstein, 1985). We observed a significant elevation in MDA levels in the control I/R group as compared to sham group. Besides MDA, a significant decrease in myocardial GSH and endogenous antioxidant enzymes (SOD, CAT and $GSHP_{X,}$) further confirms myocardial oxidative stress. Due to disruption of endogenous antioxidant network, as observed in the study, the myocardium may be more susceptible to any ischemia-reperfusion injury.

Cl exhibited significant antioxidant activity as it restored GSH levels, GSHPx activity and reduced lipid peroxidation compared to control I/R. In addition, the observation that Cl treatment significantly restores the marker enzyme activity of CPK compared to control I/R suggests the protective effect of Cl on the myocardium.

It is well documented that ischemia-reperfusion induces marked ventricular dysfunction (Dormandy, 1978). 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 minutes and reperfused for 60 minutes. In the present study, a significant fall in MAP, HR, (+) LVdP/dt, (−) LVdP/dt and a marked elevation in LVEDP were observed. The (−) LVdP/dt was significantly depressed indicating a diastolic dysfunction. Deteriorating myocardial contractile status following I/R induced injury might be responsible for the significant fall in MAP. In addition, absence of positive chronotropic effect in

the face of a reduced MAP suggests impairment of conduction (A-V block) of the heart following I/R induced injury. Normally, a fall in MAP due to coronary occlusion is expected to reflexly increase HR and myocardial contractility by activating the baroreceptor reflex, which may subsequently result in reflex coronary vasoconstriction thus worsening the imbalance, between myocardial oxygen demand and supply. However, none of these effects have been observed in the study due to I/R induced injury to the inotropic and chronotropic function of the heart. The heart rate was depressed throughout the ischemia-reperfusion duration in the control I/R group as compared to sham control, clearly depicting the injured state of myocardium following ischemia-reperfusion induced injury. As outlined above, the histopathological findings further confirmed the cardioprotective effects of Cl on the heart similar to biochemical and hemodynamic observations.

Cl induced myocardial protection resulted in preserved left ventricular function as reflected in an increase in indices of contractility (+ LVdP/dt), relaxation (-LVdP/dt), restoration of MAP and decrease in preload (LVEDP). Increase in (+)LVdP/dt and (−)LVdP/dt reflects an overall enhancement of myocardial contractility and relaxation respectively. Another consequence of the reduction in LVEDP is to 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 sub-endocardial regions of the heart, which are subjected to the greatest extra-vascular compression during systole. Cl may indirectly tend to restore blood flow in these regions towards normal by correcting the elevated LVEDP. However, Cl did not have significant affect on the HR during the ischemia and reperfusion period. Cardioprotection afforded by Cl cannot be only explained by these hemodynamic variables as Cl did not have significant effect on HR, that determines myocardial O_2 demand.

The present investigation indicates that Cl has significant cardioprotective activity as shown by its mitigating effects on several myocardial injury induced biochemical, hemodynamic and histopathological perturbations. Improved hemodynamics, restored endogenous antioxidant network along with improved histopathological characteristics assures its cardioprotective potential. The present study supports the hypothesis that myocardial ischemia-reperfusion injury can be attenuated by intervention of antioxidants; suffice to say that the early intervention by antioxidant drugs may attenuate the myocardial ischemia-reperfusion injury. The antioxidant activity of Cl is probably mediated through a mixture of curcuminoids such as curcumin, demothoxycurcumin, bis-demothoxycurcumin, the active ingredients of the Cl rhizome. Curcumin is reported to inhibit nitrite radical induced oxidation of hemoglobin, prostaglandin biosynthesis, scavenge free radicals, inhibit lipid peroxidation, protect SH group of GSH and to activate glutathione-s-transferase (Kunchandy and Rao, 1990). The study also provides scientific rationale of the use of Cl in Ayurveda, the ancient Indian System of Medicine. However, further studies need to be carried out to ascertain whether these results can be reproduced in humans. In view of the safety, efficacy and traditional acceptability of Cl, well-controlled prospective clinical trials of Cl should be contemplated to establish its efficacy in the treatment of ischemic heart diseases.

Conclusion

The present study demonstrates a significant protective effect of *Curcuma longa* in the ischemia and reperfusion model of myocardial infarction. *Curcuma longa* significantly maintained the myocardial antioxidant status and corrected, the altered hemodynamic variables. Histopathological findings further confirmed the cardioprotective effect of *Curcuma longa*.

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