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Curcumin modulates free radical quenching in myocardial ischaemia in rats

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Abstract

This study was designed to investigate the protective effect of curcumin (CUR) against isoprenaline induced myocardial ischaemia in rat myocardium. The effect of single oral dose of curcumin (15 mg kg^{-1}), administered 30 min before and/or after the onset of ischaemia, was investigated by assessing oxidative stress related biochemical parameters in rat myocardium. Curcumin pre and post-treatment (PPT) was shown to decrease the levels of xanthine oxidase, superoxide anion, lipid peroxides (LPs) and myeloperoxidase while the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-transferase (GST) activities were significantly increased after curcumin PPT. Histopathological and transmission electron microscopical studies also confirmed the severe myocardial damage occurring as a consequence of isoprenaline induced ischaemia and they also showed the significant improvement effected by curcumin PPT. These findings provided evidence that curcumin was found to protect rat myocardium against ischaemic insult and the protective effect could be attributed to its antioxidant properties as well as its inhibitory effects on xanthine dehydrogenase/xanthine oxidase (XD/XO) conversion and resultant superoxide anion production.

Keywords: Anticoagulants; Oxidative stress; Free radicals; Curcumin

1. Introduction

Curcumin (CUR), a major active component of turmeric (Fig. 1), is extracted from the powdered dry rhizome of *Curcuma longa* Linn (*Zingiberaceae*) and it has been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases (Ammon & Wahl, 1991). Recent studies have shown that CUR is a potent inhibitor of tumor initiation in vivo (Huang, Newmark, & Frenkel, 1997) and it possesses antiproliferative activities against tumor cells in vitro (Hanif, Qiao, Shiff, & Rigas, 1997). Besides its anticarcinogenic properties, CUR, at a relatively low concentration, exhibits remarkable antiinflammatory and antioxidant effects (Abe, Hashimoto, & Horie, 1999). Although

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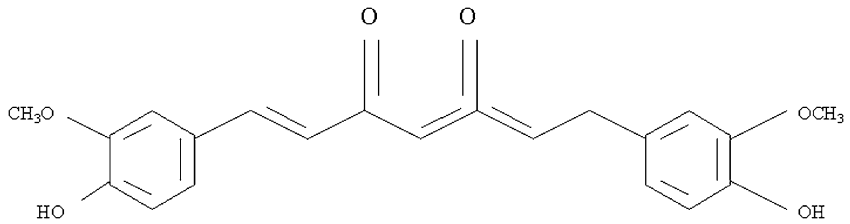

Fig. 1. Chemical structure of curcumin (1,7,-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione).

the exact mechanism by which CUR promotes these effects remains to be elucidated, the antioxidant properties of this yellow pigment appear to underlie its pleiotropic biological activities.

Oxidative stress and formation of reactive oxygen species (ROS) could set off a cascade of biochemical and molecular sequelae such as the xanthine dehydrogenase/xanthine oxidase (XD/XO) conversion, leading to production of ROS (McCord, 1985). Oxidative ischaemic injury is suggested to be a central mechanism of the cellular damage affecting all organs and tissues after ischaemia; however, the mechanisms which trigger and modulate this damage have been partially characterized (Xia & Zweier, 1995).

Hence, in this study, myocardial ischaemia was induced in rats using isoprenaline hydrochloride and curcumin was administered orally before and/or after the onset of ischaemia. Biochemical, histological and electron microscopical studies were carried out to find out the efficacy of CUR on the protection of myocardium from oxidative cell injury by isoprenaline.

2. Materials

This study conforms to the guiding principles of Institutional Animal Ethics Committee (IAEC), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Chemicals

Isoprenaline·HCl (ISO), nitro blue tetrazolium, bovine serum albumin, epinephrine, thiobarbituric acid, *O*-dianisidine, L-ascorbic acid, 1-chloro-2,4-dinitrobenzene, sodium azide, epinephrine, α-α[1]dipyridyl, dinitrophenyl hydrazine, adenosine triphosphate, *N*-acetyl cysteine, creatine phosphate, adenosine-5-diphosphate, thrombin, D-glucose and curcumin were purchased from Sigma (St. Louis, MO, USA). Sodium cacodylate, osmium tetroxide and glutaraldehyde were purchased from Electron Microscopy Sciences (Washington, US). Thiopentone sodium was obtained from May and Baker (UK). All other chemicals used were of analytical grade.

2.2. Animals

Female rats (Wistar), weighing 180–200 g were inbred in a pathogen free facility and they were maintained in environmentally controlled rooms with 12 h light/dark cycle. The animals received commercial rat diet and water ad libitum.

3. Methods

3.1. Physicochemical characterization

3.1.1. Antiplatelet and anticoagulant assays

The antiplatelet and anticoagulant assays were carried out according to Jamaluddin and Krishnan (1987). Briefly, for the antiplatelet assay, human blood from healthy volunteers was collected in 3.8% citrate (9:1, v/v) and rat blood was collected by cardiac puncture in 3.8% citrate (9:1, v/v). The citrated blood was immediately centrifuged at $70 \times g$ for 30 min and the platelet rich plasma (PRP) was collected. PRP was again centrifuged at $24 \times g$ for 20 min to remove the residual erythrocytes and leukocytes. Platelets were counted in a haemocytometer adjusting to a concentration of

6×10^7 ml^{-1}, which corresponded to an absorbance of 0.5 at 540 nM. Spectrophotometric assay of platelet aggregation was carried out using increasing concentrations of CUR.

3.2. Development of the model

Myocardial ischaemia was induced by subcutaneous injection of isoprenaline hydrochloride (ISO, 50 mg kg^{-1} body weight, 0 h), dissolved in physiological saline (Qi, Shi, Bu, Pang, & Tang, 2003). Control rats received 1 ml of physiological saline. Rats administered drug alone received CUR (15 mg kg^{-1} body weight) orally, 30 min before and after saline administration (Asai & Miyazawa, 2000; Dikshit, Rastogi, Shukla, & Srimal, 1995). CUR (15 mg kg^{-1} body weight) was administered to rats orally 30 min before ISO administration (pre-treatment (PRT)). When the drugs were administered 30 min before and after ISO, it was referred to as pre- and post-treatment (PPT) while post-treatment (POT) referred to administration of drug 30 min after ISO induction.

The animals were divided into six groups (six rats per group) as follows:

Group 1	Saline control (normal)
Group 2	CUR (15 mg kg^{-1}) + saline +CUR (15 mg kg^{-1}) (CUR control)
Group 3	ISO induced myocardial ischaemia (50 mg kg^{-1}) (diseased)
Group 4	CUR (15 mg kg^{-1}) + ISO (50 mg kg^{-1}) − PRT
Group 5	CUR (15mg/kg) + ISO (50 mg kg^{-1}) + CUR (15 mg kg^{-1}) − PPT
Group 6	ISO (50 mg kg^{-1}) + CUR (15 mg kg^{-1}) − POT

3.3. Physical parameters

3.3.1. Electrocardiography

Standard ECG was recorded by Student's Physiograph (INCO, India) under anesthesia as described under (Manikandan et al., 2002) for all the groups. ECG was recorded using standard lead at a chart II speed of 10 cm s^{-1} with sensitivity set at 1.

3.4. Gravimetric calculation

Heart weight to body weight ratio (HW/BW) was calculated by dividing heart weight (g) by body weight (g) and multiplying by 100 (Manikandan et al., 2002).

3.5. Tissue sampling

After 48 h of induction of myocardial ischaemia, the animals were sacrificed by cervical decapitation. Blood was collected and the heart tissues were minced into small pieces. Twenty percent (w/v) heart homogenate obtained at 2000 rpm (Ultra-Turrax®, IKA, Germany) in ice-cold saline or phosphate buffer (50 mM, pH 7.8) was used. The homogenate in ice-cold saline was used for the determination of lipid peroxides (LPs) and reduced glutathione (GSH). The homogenate in phosphate buffer containing 1 mM EDTA, 10mM DTT, 1mM PMSF was centrifuged (Himac SCP 70G, Hitachi, Japan) at 25,000 × *g* for 30 min at 4 °C and the supernatant was used for determination of XO, total (XO/XD) activity as well as superoxide anion ($O_2^{\bullet -}$). For the determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-*S*-transferase (GST) the heart homogenized in ice-cold saline was centrifuged at 8500 × *g* for 10 min at 2 °C and the supernatant was used. Protein concentration in homogenate or supernatant was determined according to Lowry, Rosenbrough, Farr, and Randall (1951).

3.6. Biochemical analysis

3.6.1. Reactive oxygen species and myeloperoxidase (MPO)

The heart homogenate and serum content of lipid peroxides (LPs), measured in terms of malondialdehyde (MDA) (Yagi, 1984), GSH (Moran, Drapierre, & Mannervik, 1979) and ascorbic acid (Omaye & Turnbull, 1979) were estimated spectrophotometrically (Shimadzu UV-VIS 2401 PC®, Japan). The neutrophils specific MPO activity was determined in heart tissue according to Henson, Zanolari, Schwartzmann, and Honf (1978). The 25,000 × *g* supernatant from XO homogenizing buffer was used for the determination of XO, total (XO + XD) and %XO (Terada,

Leff, & Repine, 1990; Parks, Williams, & Beckman, 1988). Another aliquot of the 25,000 × *g* supernatant was used for $O_2^{\bullet -}$ estimation (Johnston, 1984), expressed as nmol min^{-1} mg $protein^{-1}$, based on an extinction co-efficient $\Delta\varepsilon_{550} = 21.0 \times 10^3\ M^{-1}\ cm^{-1}$ (Massey, 1959).

3.6.2. Enzymatic and non-enzymatic antioxidants

An aliquot of 8500 × *g* supernatant from the saline homogenate was used for the determination of SOD (Nebot et al., 1993), GPx and GST (Rotruck, Pope, Ganther, Hafeman, & Hoekstra, 1973) and CAT (Aebi, 1984). The levels of non-enzymatic antioxidants such as α-tocopherol (Quaife & Dju, 1948) and ceruloplasmin (Ravin, 1961) were estimated in serum.

3.7. Histopathological examination

The animals in all the groups were sacrificed and the heart tissue was removed. These samples were then separately fixed in 10% formal-saline, dehydrated through graded alcohol series, cleared in xylene and embedded in paraffin wax (melting point −56 °C). Serial sections of 4 μm thick were cut and stained with hematoxylin and eosin (HE). The sections were examined using light microscope and photomicrographs were taken (Sumitra et al., 2001).

3.8. Transmission electron microscopy (TEM)

Thin slices of heart sections were processed for electron microscopic examination as previously described (Jennings et al., 1978). Briefly, multiple cubes measuring 1 mm^3 or less were cut under glutaraldehyde with a sharp blade and they were transferred into 20–30 ml of 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The tissue was fixed with 3% glutaraldehyde in 0.1M phosphate buffer, pH 7.2 and post-fixed with 1% osmium tetroxide. After 1–5 h of fixation, the blocks were dehydrated in a graded ethanol series and embedded in spurr resin medium. Semi-thin sections obtained from about six or more embedded blocks were examined by light microscopy. Ultrathin sections with golden interference were cut and picked up on copper grids, stained using 1% aqueous uranyl acetate and alkaline lead citrate, pH 12.0 and examined using TEM (JEOL 1200 EXII) at 62 kV. At least, three stained grids from each embedded block were examined and the changes were recorded.

3.9. Statistical analysis

Results were reported as mean ± standard deviation (S.D.). Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *F*-value was found to be statistically significant ($P < 0.05$), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using SPSS statistical version 11 software package (SPSS® Inc., USA).

4. Results

4.1. Antiplatelet and anticoagulant studies

CUR inhibited thrombin induced platelet aggregation (human and rat) at an inhibitory concentration of 0.051 ± 0.013 mM (Fig. 2). The anticoagulant activity of CUR with rat plasma was almost similar to that of human plasma. Fig. 3 showed that CUR prolonged the clotting time of both human as well as rat plasma to approximately 1.6-, 2.1- and 1.1-fold as shown by thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (APTT) assays respectively when compared to control.

4.2. In vivo studies

All the ISO administered animals exhibited signs of shock, difficulty in breathing, dyspnea, lethargy and prostration during the first few hours of ISO injection.

Various doses of CUR viz. 3, 5, 10, 20 mg (per kg body weight) were administered and since ED_{50} calculation revealed the effective dose as 8.43 ± 1.54 mg/kg body weight, 15mg/kg body weight was chosen as the optimal dose in vivo studies. Statistical comparison of drug alone (CUR control–Group 2) with control (Group I) revealed its insignificance.

A critical analysis (Table 1) indicated that curcumin PPT treatment effected an increase in *R* amplitude and a decrease in ST segment elevation. Furthermore, curcumin PPT was found to significantly reduce the gravimetric parameters when compared to diseased group.

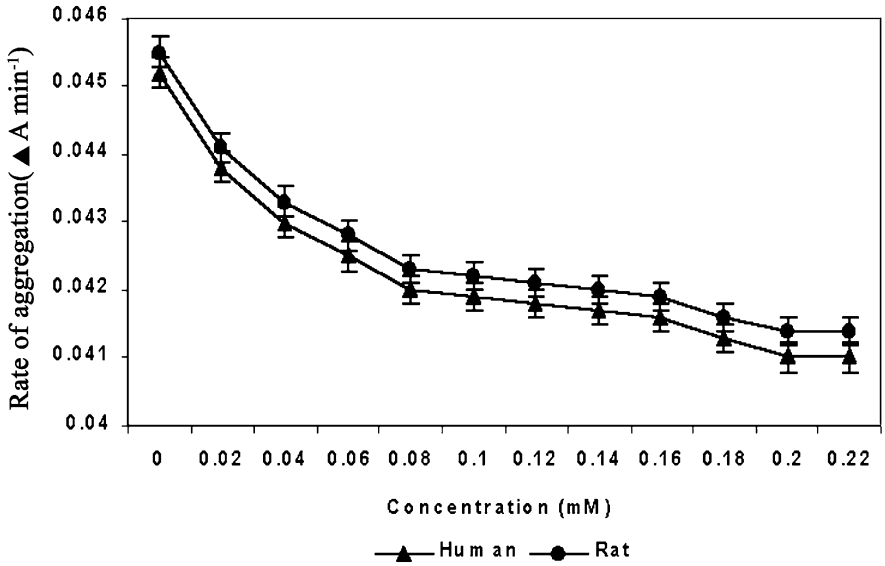

Fig. 2. Effect of curcumin on thrombin induced aggregation. Mean ± S.D. for six separate experiments.

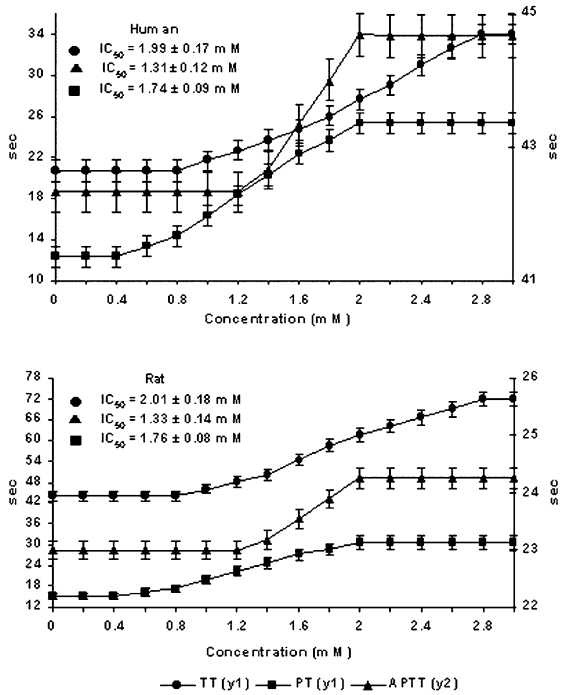

Fig. 3. Coagulation cascade showing the effect of curcumin on thrombin time (TT), prothrombin time (PT) and activated partial thromboplastin time (APTT) assays. Control values for TT, PT and APTT were 20.66, 12.33 and 42.33 s, respectively, for human plasma and 44, 15 and 23 s, respectively, for rat plasma. Mean ± S.D. for six separate experiments.

In the ischaemic rats, XO activity in the myocardium of ischaemic rats was found to increase from 28.4 ± 2.5 mU g^{-1} (control) to 91.3 ± 3.1 mU g^{-1} (Fig. 4i) whereas the XO + XD activity increased from 107 ± 1.6 mU g^{-1} (control) to 181 ± 3.2 mU g^{-1} (Fig. 4ii). Fig. 4iii depicts that ischaemic injury (diseased group) resulted in a two-fold increase in %XO to reach 56.52% of total (XO + XD) activity while CUR administration (15 mg kg^{-1}, PPT) was shown to bring down the levels of total (XO/XD) activity very near to that of control. On the other hand, PRT as well POT of CUR administration did not produce any significant effect on the enzyme activity. Production of $O_2^{\bullet -}$ paralleled the increase in %XO activity in rat myocardium of the diseased group wherein a three-fold increase in $O_2^{\bullet -}$ production was noticed when compared to control (Fig. 5). CUR at a dose of 15 mg kg^{-1} (PPT) significantly blocked this FR burst and the $O_2^{\bullet -}$ level was reduced by 50% of the elevated level of the diseased group. PRT as well as POT did not produce any significant effect on $O_2^{\bullet -}$ production.

Fig. 6 showed heart LPs, serum LPs and heart MPO content which were significantly elevated in rat ischaemic myocardium to approximately 1.8-, 3.3- and 1.6-fold, respectively, due to ischaemic injury. CUR (PPT) significantly decreased the elevated heart LPs, serum LPs and heart MPO content by 37, 42 and 38%,

Table 1
Regulatory effect of curcumin on haemodynamic measurements in isoprenaline induced myocardial ischaemia in rats

Parameters	Control	CUR control	Diseased	CUR (PRT)	CUR (PPT)	CUR (POT)
Heart weight (mg)	921 ± 10	927 ± 11	1350 ± 13[a]	1134 ± 14	943 ± 9[b]	1062 ± 11
Body weight (g)	256 ± 3.0	254 ± 4.0	250 ± 4.0	252 ± 3.0	255 ± 4.0	253 ± 4.0
(Heart weight/body weight) × 100	0.36 ± 0.005	0.36 ± 0.005	0.54 ± 0.005[a]	0.45 ± 0.005	0.37 ± 0.005[b]	0.42 ± 0.005
Heart rate (beats/min)	416 ± 12.36	412 ± 11.57	359 ± 13.03[a]	378 ± 11.56	412 ± 10.15[b]	386 ± 12.41
R amplitude (mV)	1.00 ± 0.020	1.00 ± 0.020	0.81 ± 0.023[a]	0.87 ± 0.022	0.99 ± 0.020[b]	0.94 ± 0.022
ST segment (mV)	0.10 ± 0.009	0.10 ± 0.011	0.26 ± 0.016[a]	0.17 ± 0.013	0.09 ± 0.005[b]	0.15 ± 0.016

Values are presented as mean ± S.D. of six animals.

[a] Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test.

[b] Significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test.

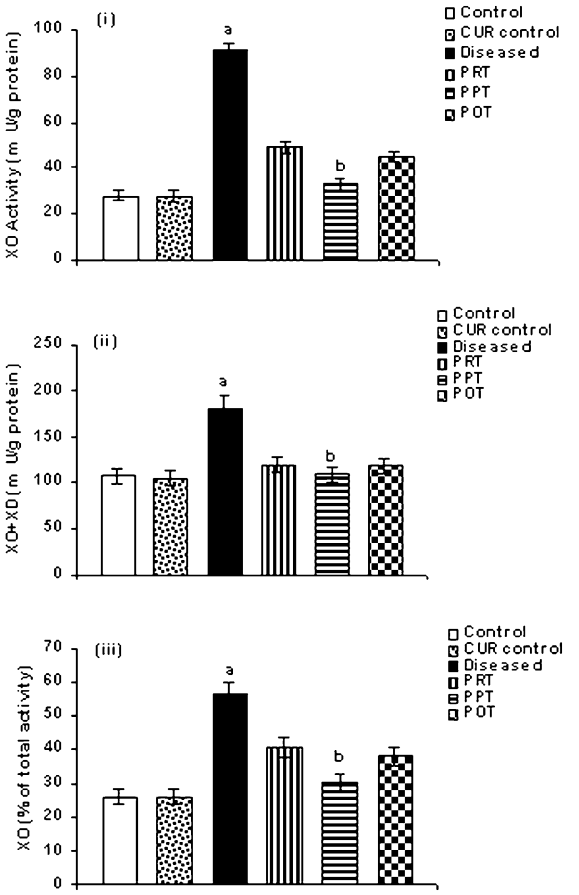

Fig. 4. (i) Effect of curcumin on XO activity in rat ischaemic myocardium. Values are presented as mean ± S.D. of six animals. (a) Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test; (b) significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test. (ii) Effect of curcumin on percentage XO + XD activity in rat ischaemic myocardium. Values are presented as mean ± S.D. of six animals. (a) Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test; (b) significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test. (iii) Effect of curcumin on %XO activity in rat ischaemic myocardium. Values are presented as mean ± S.D. of six animals. (a) Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test; (b) significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test. $\%XO = (XO/(XO + XD)) \times 100$.

respectively, when compared to untreated ischaemic group while, CUR (PRT as well as POT) did not produce any significant change in elevated LPs and MPO content.

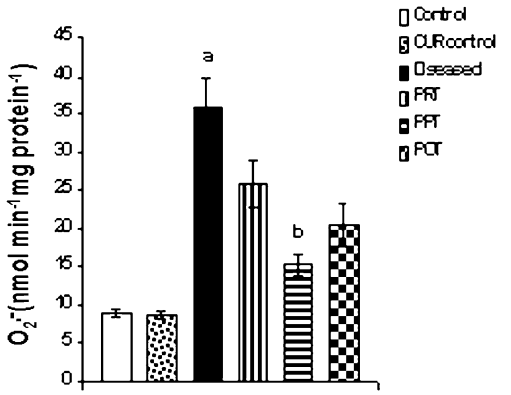

Fig. 5. Effect of curcumin on $O_2^{\bullet-}$ activity in rat ischaemic myocardium. Values are presented as mean ± S.D. of six animals. (a) Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test; (b) significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test.

The heart activities of SOD, CAT, GPx and GST were significantly decreased in untreated diseased group as compared to control by 52, 30, 28 and 41%, respectively. CUR (PPT) increased the activities of these enzymes by 90, 35, 37 and 66%, respectively, when compared to diseased group. On the other hand, increase in the enzymatic antioxidant activities was not significant after treatment with CUR (PRT) as well as CUR (POT) (Table 2). When animals were exposed to ischaemic insult, their heart GSH content

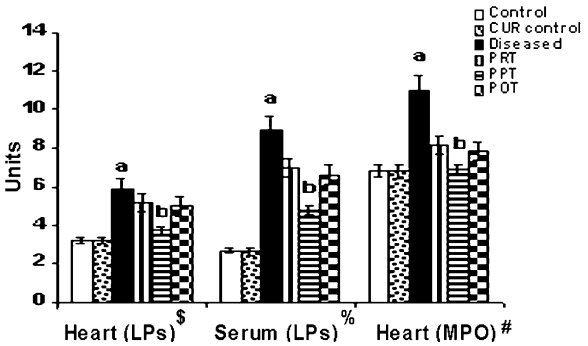

Fig. 6. Effect of curcumin on LPs and MPO levels in rat ischaemic myocardium. Values are presented as mean ± S.D. of six animals. (a) Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test; (b) significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test; ($) nmol/g; (%) nmol/ml; (#) μmol of hydrogen peroxide oxidized min^{-1} protein^{-1}.

Table 2
Regulatory effect of curcumin on enzymatic and non-enzymatic antioxidants in isoprenaline induced myocardial ischaemia in rats

Parameters	Control	CUR control	Diseased	CUR (PRT)	CUR (PPT)	CUR (POT)
Superoxide dismutase (50% of epinephrine autooxidation)[a]	10.53 ± 0.52	10.67 ± 0.57	5.03 ± 0.31[b]	9.19 ± 0.47[c]	9.56 ± 0.45[c]	9.47 ± 0.51[c]
Catalase (μmol of H_2O_2 decomposed min^{-1} mg $protein^{-1}$)[a]	16.33 ± 0.906	16.48 ± 0.932	11.45 ± 0.406[b]	12.14 ± 0.532	15.57 ± 0.440[c]	13.62 ± 0.514
Glutathione peroxidase (μmol of glutathione min^{-1} mg $protein^{-1}$)[a]	1.81 ± 0.029	1.83 ± 0.027	1.31 ± 0.031[b]	1.46 ± 0.031	1.79 ± 0.029[c]	1.54 ± 0.029
Glutathione-*S*-transferase (U min^{-1} mg $protein^{-1}$)[a]	0.36 ± 0.002	0.36 ± 0.002	0.21 ± 0.002[b]	0.28 ± 0.004	0.35 ± 0.002[c]	0.30 ± 0.002
Reduced glutathione (nmol g^{-1})[a]	2.62 ± 0.11	2.60 ± 0.09	0.78 ± 0.05[b]	1.42 ± 0.06	2.30 ± 0.08[c]	1.52 ± 0.07
Ascorbic acid (mg g^{-1})[a]	214.00 ± 4.38	215.42 ± 4.36	129.90 ± 4.18[b]	168.00 ± 4.06	210.00 ± 3.25[c]	185.00 ± 4.21
Reduced glutathione(mg dl $serum^{-1}$)	58.32 ± 2.38	57.42 ± 2.47	31.80 ± 1.93[b]	35.51 ± 3.23	40.18 ± 1.99	36.31 ± 2.25
α-Tocopherol (μg/ml $serum^{-1}$)	4.60 ± 0.12	4.63 ± 0.14	2.18 ± 0.075[b]	2.45 ± 0.166	4.36 ± 0.110[c]	3.54 ± 0.124
Ceruloplasmin (mg/dl $serum^{-1}$)	34.87 ± 0.84	34.61 ± 0.87	17.46 ± 0.921[b]	19.03 ± 0.935	32.14 ± 0.725[c]	26.34 ± 0.857
Ascorbic acid (mg/dl $serum^{-1}$)	1.42 ± 0.23	1.43 ± 0.24	0.59 ± 0.012[b]	0.88 ± 0.048	1.21 ± 0.039[c]	0.92 ± 0.051

Data are presented as mean ± S.D. of six animals.

[a] Heart.

[b] Significantly different from the corresponding control value at $P < 0.05$ using Tukey's post hoc test.

[c] Significantly different from the corresponding diseased value at $P < 0.05$ using Tukey's post hoc test.

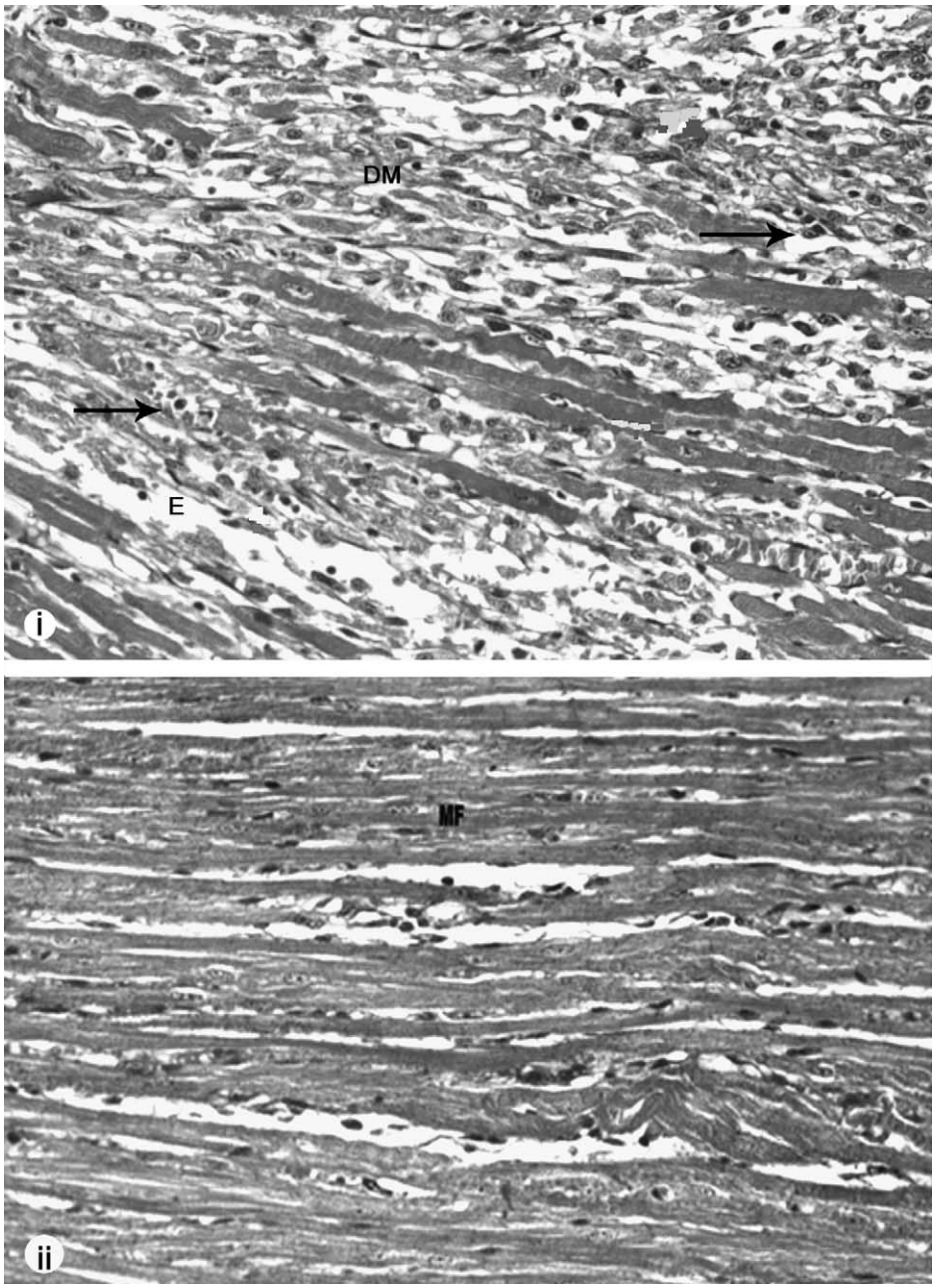

Fig. 7. (i) Histological studies showing degeneration and disruption of myocardial fibres (DM), edema (E) and infiltration of leucocytes (→) in the interstitium of heart tissue in ISO administered rats (HE; 320×). (ii) Heart tissue showing normal architecture of myofibrils (MF) in ISO administered group, pre- and post-treated with 15 mg of curcumin (HE; 320×).

showed a significant reduction, as compared to control group. PRT and POT treated groups did not cause significant change in the levels of GSH whereas a significant increase was observed in CUR (PPT) treated group (Table 2). Table 2 also showed that ascorbic acid, reduced glutathione, α-tocopherol and ceruloplasmin levels were observed to significantly decrease in ISO administered group while PPT was noticed to restore the levels towards normalcy.

4.3. Histopathological examination

Some typical photomicrographs are shown in Fig. 7i and ii. Degenerative changes of myocardial muscle fibres with infiltration of a few mononuclear cells and hyalinization of muscle fibres were observed in heart tissue of ISO administered rats (Fig. 7i). The muscle fibres appeared normal or very close to normal in PPT group (data not shown). In PRT and POT group, certain amount of hyalinization of muscle fibres at focal areas and infiltration of leukocytes were still seen (data not shown). Normal architecture of heart was observed after CUR (PPT) administration (Fig. 7ii).

4.4. TEM studies

Ultrastructural studies of heart tissue (Fig. 8i) showed distortion of myofibrils, distinct disruption of the cristae, double membrane with deposition of amorphous dense particle and vacuole formation in mitochondria in ISO treated group. Normal architecture of the mitochondria as well as the myofibrils was shown to be well preserved (Fig. 8ii) after CUR treatment (PPT).

5. Discussion

Platelet aggregation occurs when fibrinogen molecules bind to the activated GPIIb/IIIa receptor and connect platelet to one another (Lefkovits, Plow, & Topol, 1995). Antiplatelet therapy prevents potential thrombolytic- induced platelet aggregation, coronary artery reocclusion and reinfarction (Frishman et al., 1995). This observation concurred with an earlier finding by Srivastava, Bordia, and Verma (1995) who showed that CUR, an active constituent of *C. longa*, inhibited aggregation and altered eicosanoids metabolism in human blood platelets. Curcumin might inhibit the cyclooxygenase pathway by blocking GPIIb/IIIa receptor, inhibiting platelet aggregation leading to the formation of blood clots (Huang et al., 1991). The IC_{50} shown by the antiplatelet aggregation of CUR in this study might be of great help to develop potent antiplatelet drugs.

The cascade of reactions involved in the coagulation of blood is an autocatalytic and self-limiting process converting zymogen to active form in the presence of proteolytic enzyme thrombin (Frishman et al., 1995). The unique specificity of thrombin led us to search for highly potent and selective inhibitors for this enzyme. The anticoagulant action of CUR was shown by its ability to prolong the clotting time as observed in TT, PT and APTT assays and this could be due to the presence of hydrophobic groups in curcumin moiety.

The ISO administered rats displayed signs of shock, difficulty in breathing, lethargy and flushed skin leading to myocardial ischaemia and these changes resembled the subendocardial laminar necrosis produced by myocardial ischaemia in humans (Qi et al., 2003). ISO induced myocardial damage was due to local deprivation of blood supply, cell membrane dysfunction and tissue hypoxia, caused by an increased heart rate and decreased peripheral vascular resistance (Qi et al., 2003) while in CUR administered rats, these symptoms were found to be less severe as shown by hemodynamic and gravimetric measurements (Table 1).

The consecutive loss of cell membrane function in the regional ischaemic myocardium might be characterized by rapid ST segment elevation followed by significant diminution in the size of the R wave amplitude and our findings were in agreement with an earlier report (Qi et al., 2003). ST segment elevation observed in this study indicated that ISO administration induced acute infarction and curcumin was shown to ameliorate ISO induced myocardial damage. The increase in heart weight after ISO administration as shown in this study might be due to myocardial edema, ventricular hypertrophy, edematous intermuscular space and mild necrosis of cardiac muscle fibres followed by prominent inflammatory infiltrate and this observation concurred with our ultrastructural studies showing distinct distortion of myofibrils and mitochondria due to edema formation. CUR was found to protect the myocardium against inflammatory infiltrate and also to

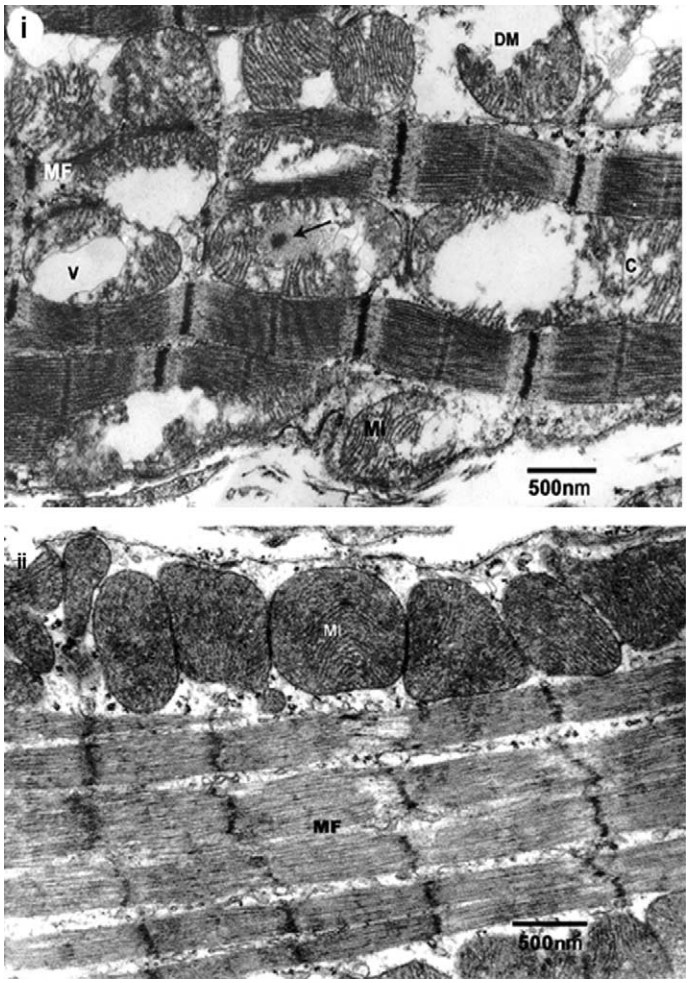

Fig. 8. (i) Transmission electron micrograph of infarcted myocardium showing distortion of myofibrils (MF) and mitochondria (MI) with presence of condensed amorphous dense body (→). A few mitochondria showing vacuole formation (V), ruptured cristae (C) and double membrane (DM) in isoprenaline administered rats. (ii) Transmission electron micrograph of heart tissue showing normal architecture of intact myofibrils (MF) and mitochondria (MI) in isoprenaline administered group, after pre- and post-treatment with 15 mg of curcumin.

decrease the edematous condition of the myocardium as shown by the histological studies and this could be the reason for the observed reduction in the HW/BW ratio after CUR (PPT).

Oxidative injury is associated with the generation of reactive oxygen species. It is well documented that XO is an important prerequisite factor in the process of $O_2^{\bullet -}$ generation in acute ischaemic injury (Saavedra et al., 2002) and this observation concurred with our finding wherein a significant rise in percentage of XO was noticed in rat heart after ischaemic insult followed by a significant increase in $O_2^{\bullet -}$ generation. This finding was also in agreement with that of Terada et al. (1992) who demonstrated increased $O_2^{\bullet -}$ production from endothelial cells exposed to hypoxia. In this study, the elevated level of %XO activity in the diseased group was shown to be effectively counteracted by administration of CUR (PPT). In addition, CUR was noticed to significantly decrease $O_2^{\bullet -}$ burst and the one proposed mechanism underlying this protective effect of CUR could be through its antioxidant properties restoring endogenous GSH levels and thereby detoxifying free radicals (Sreejayan Rao, 1994). An alternative mechanism for such protec-

tion could be through CUR's free radical scavenging activity, particularly against $O_2^{\bullet -}$ radical, which would inhibit sulfhydryl (SH) oxidation, leading to inhibition of reversible XD/XO conversion (Kunchandy & Rao, 1990). CUR, as a free radical scavenger, might also inhibit proteases, which are known to be activated by free radicals and this would eventually lead to inhibition of the irreversible proteolytic XD/XO conversion (Matsuyama, 1997). Thus, the protective role of CUR could be related to inhibition of free radical propagation with subsequent inhibition of XD/XO conversion and resultant decreased production of $O_2^{\bullet -}$ through its antioxidant and/or free radical scavenging activity. Neither PRT nor POT of CUR offered such a significant protection. This could be explained on the basis of the low bioavailability of, as well as heart disposition to CUR (Pan, Huang, & Lin, 1999), and dual treatment (PPT) could result in a concentration sufficient to elicit antioxidant activity (Kunchandy & Rao, 1990).

It is already known that lipids are the most susceptible macromolecules to oxidative stress (Sreejayan Rao, 1994) and our results showed that the level of lipid peroxides, measured in terms of MDA, significantly increased due to ischaemic insult. In this study, CUR (PPT) was shown to significantly reduce the LPs level by scavenging free radicals and inhibiting the propagating chain reaction of LPs and our finding was in consonance with an earlier report (Sreejayan Rao, 1994). It was noticed earlier that the neutrophils, a major source of FRs, characteristically invaded the myocardial tissue during ischaemia (Abe et al., 1999). The observations of this study showing that CUR pre and post-treatment reduced the MPO levels indicated that CUR suppressed neutrophil infiltration into the injured myocardium.

GSH, considered as the most prevalent and important intracellular non-protein thiol, has a crucial role as a free radical scavenger and a decline in GSH could reflect oxidative stress (Thomas, 1994). In this study, GSH content was significantly reduced due to ischaemic insult and this could be explained by the assimilation of GSH by the rapidly generating free radicals. Nevertheless, a number of studies reported that low myocardial GSH levels might not provide evidence for deleterious free radical reactions during and following ischaemia (Seif-El-Nasr & Abd-El-Fattah, 1995). In this study, PRT and POT treated groups did not offer significant protection against ischaemia induced GSH depletion. This observation could be explained on the basis of Michael-type addition reaction of GSH (as a nucleophile) in the chromophore region of CUR (as an electrophile), which would result in the depletion of cellular GSH defense (Mathews & Rao, 1991). However, CUR antioxidant activity still imparted some degree of GSH salvation, as GSH levels were shown to increase significantly in PPT group. ISO administration resulted in an increase in the level of free radicals, which in turn, induced cellular damage, and this observation could be substantiated by the low levels of free radical scavenging enzymes such as CAT, SOD, GPX, GST that formed the first line of cellular defense against the oxidation injury. The second line of defense consisting of ascorbic acid, α-tocopherol and ceruloplasmin, which scavenged residual free radicals escaping decomposition, was also found to be affected and this observation could be substantiated by the low levels of a non-enzymatic antioxidants. Indeed, CUR (PPT) proved beneficial in restoring the levels of both enzymatic and non-enzymatic antioxidants and this finding was in accordance with that of Jovanovic, Boone, Steenken, Trinoga, and Kaskey (2001), who showed regeneration of CUR radicals by α-tocopherol and ascorbic acid.

The antioxidant activity of CUR could be attributed to the phenolic and the methoxy groups in conjunction with the 1,3-diketone conjugated diene system, for scavenging of the oxygen radicals (Sreejayan Rao, 1994; Wright, 2002). In addition, CUR primary metabolite, tetrahydrocurcumin, a major antioxidant with β-diketone moiety, exhibited antioxidant activity by cleavage of the C–C bond at the active methylene carbon between the two carbonyls (Pan et al., 1999). These antioxidant properties seem to have a role in inhibiting $O_2^{\bullet -}$ generation directly or indirectly (via inhibiting XD/XO conversion). However, the impact of this effect on the generation of other damaging species such as hydroxyl radical ($OH^{\bullet}$) or peroxynitrite ($ONOO^{-}$) is yet to be resolved. This study showed that CUR is an effective protector against ischaemic insult in the rat myocardium and this effect could be related to its antioxidant properties and/or its inhibitory effects on XD/XO conversion and resultant $O_2^{\bullet -}$ production. This finding is in accordance with that of Ruby, Kuttan, Babu, Rajasekharan, and Kuttan (1995), who showed that CUR inhibited lipid

peroxidation and effectively scavenged superoxide anion and hydroxyl radical. In addition to its inherent ability to attenuate the reactivity of oxygen free radical species, CUR was shown in vivo to enhance the activities of detoxifying enzymes such as glutathione-*S*-transferase (Piper et al., 1998).

Thus, this study conclusively showed that oxygen free radicals exacerbated cardiac damage and CUR (PPT) induced cardioprotective effect and also inhibited free radical generation.

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