Department of Pharmacology, Acharya & B. M. Reddy College of Pharmacy, Bangalore, Karnataka, India

Cardioprotective effect of tetrahydrocurcumin and rutin on lipid peroxides and antioxidants in experimentally induced myocardial infarction in rats

MD. SULTAN ALI, M. P. MUDAGAL, D. GOLI

Received May 26, 2008, accepted July 18, 2008

P. M. Manjunatha, Dept. of Pharmacology, Acharya & B. M. Reddy College of Pharmacy, Soladevanahalli, Chikkabanavara Post, Bangalore-560090, Karnataka, India
mudagal@yahoo.co.in

Pharmazie 64: 132–136 (2009) *doi: 10.1691/ph.2008.8628*

The present study was undertaken to evaluate the cardioprotective potential of tetrahydrocurcumin (THC) and rutin in an *in vivo* rat ischemia-reperfusion (I/R) model of myocardial infarction (MI). Male wistar rats were divided into six groups receiving saline (control MI/R group), vehicle control MI/R group, THC (5 mg kg^{-1} and 10 mg kg^{-1}) and rutin (5 mg kg^{-1} and 10 mg kg^{-1}) i.p. injection respectively. At the day of the experiment, each group was subjected to acute ischemia for 30 min by occlusion of the left anterior descending coronary artery (LAD). Thereafter reperfusion was allowed for 4 h. MI/R resulted in significant cardiac necrosis, elevation in lipid peroxidation, elevation in cardiac marker enzymes AST, ALT and decline in antioxidant status catalase, reduced glutathione in the normal control MI/R group and vehicle control MI/R group. Myocardial infarction produced after MI/R was significantly reduced in tetrahydrocurcumin and rutin of the myocardial antioxidant status, infarct size reduction compared to control and vehicle control MI/R group. Furthermore, MI/R induced lipid peroxidation was significantly reduced by tetrahydrocurcumin and rutin. Cardioprotection in the treatment group was probably a result from suppression of oxidative stress. Histopathological examination further confirmed the protective effect of tetrahydrocurcumin and rutin on the MI/R heart.

1. Introduction

A commonly used model of experimental heart failure is produced by surgical ligation of the left coronary artery in rat. This procedure is comparable to the human clinical situation of myocardial infarction (MI) where the blood supply to the ventricle is interrupted by blockade of the coronary artery, resulting in ischemia (Kompa and Summers 2000).

In India curcumin is popularly known as 'Haldi'. It belongs to the family Zingibaraceae. *Curcuma longa* (Turmeric) is extensively used as a spice, food preservative and coloring material in India, China and south East Asia. Traditionally it is used as household remedy for various diseases including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders and sinusitis (Ammon et al. 1992).

Curcumin is attracting strong attention due to its antioxidant potential and it's low toxicity (Osavwa et al. 2005; Sugiyama et al. 1996). Curcumin acts as a scavenger of oxygen free radicals (Ruby et al. 1995; Subramanian et al. 1994) and can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, hydrogen peroxide and nitrite radical generation by activated macrophages, which play an important role in inflammation (Joe and Lokesh 1994). Tetrahydrocurcumin, one of the major colorless metabolites of curcumin, in the form of glucuronide conjugate, has shown stronger antioxidant activities than curcumin in several *in vitro* systems (Sugiyama et al. 1996; Osawa et al. 1995).

Rutin is a well-known flavonoid (Duke 1992) which is a potent superoxide radical scavenger (Chang et al. 1993). Rutin can chelate metal ions, such as ferrous cations. Rutin may also modulate the respiratory burst of neutrophils. Rutin may also help to maintain the levels of the biological antioxidants, reduced glutathione. Rutin is known to exhibit multiple pharmacological actions like antibacterial and antiviral, antiprotozoal, antitumor, antiallergic, anti-inflammatory and antiplatelet activities. Moreover, antidiarrhoeal, antiulcer, antispasmodic, antimutagenesis, myocardial protecting, vasodilator and immunomodulator activities have been reported (Janbaz et al. 2002). This study addresses the protective effect of tetrahydrocurcumin and rutin on infarct size, lipid peroxidation and antioxidants in experimentally induced myocardial infarction in rats.

2. Investigations and results

Table 1 illustrates the effect of tetrahydrocurcumin and rutin on percentage left ventricle necrosis (PLVN), tissue and serum malondialdehyde (MDA) lipidperoxidation in control, vehicle control and treatment groups. The control and vehicle control groups showed significant increase in percentage infarct size and lipidperoxidation levels in tissue, serum. Treatment groups with tetrahydrocurcumin (5 mg kg^{-1} and 10 mg kg^{-1}) and rutin (5 mg kg^{-1} and 10 mg kg^{-1}) significantly ($p < 0.001$) decreased both the levels of percentage left ventricle in-

Table 1: Effect of tetrahydrocurcumin and rutin on percentage left ventricle necrosis (PLVN), tissue lipidperoxidation and serum lipidperoxidation in control, vehicle control and treatment group-experimentally induced myocardial infarction in rats

Groups	PLVN	nmol MDAg^{-1} wet tissue	nmol MDA mL^{-1} in serum
Normal control MI/R	41.26 ± 1.93	129.95 ± 5.62	19.08 ± 1.37
Vehicle control MI/R	30.46 ± 1.30	101.91 ± 5.78	16.74 ± 0.61
Tetrahydrocurcumin MI/R-5 mg/kg	8.46 ± 0.83[a]	82.5 ± 6.88[a]	13.11 ± 0.84[a]
Tetrahydrocurcumin MI/R-10 mg/kg	5.28 ± 0.51[a]	32.05 ± 2.04[a]	6.52 ± 0.75[a]
Rutin MI/R-5 mg/kg	4.36 ± 0.22[a]	65.94 ± 4.06[a]	10.71 ± 1.01[a]
Rutin MI/R-10 mg/kg	2.15 ± 0.37[a]	27.36 ± 3.17[a]	5.31 ± 0.69[a]

Values are mean ± S.D. for n = 6, superscript ([a]) significant at $p < 0.001$

Table 2: Effect of tetrahydrocurcumin and rutin on serum aspartate transaminase (AST) and alanine transaminase (ALT) in control, vehicle control and treatment group-experimentally induced myocardial infarction in rats

Groups	AST (IU L^{-1})	ALT (IU L^{-1})
Normal control MI/R	85.66 ± 18.7	82.33 ± 27.5
Vehicle control MI/R	66.33 ± 13.5	69.33 ± 17.17
Tetrahydrocurcumin MI/R-5 mg/kg	45.66 ± 18.3[a]	44.16 ± 18.07[c]
Tetrahydrocurcumin MI/R-10 mg/kg	29.33 ± 10.9[a]	28.33 ± 18.46[b]
Rutin MI/R-5 mg/kg	50 ± 14.3[b]	34.33 ± 23.41[b]
Rutin MI/R-10 mg/kg	38.33 ± 9.58[a]	25 ± 19.26[a]

Values are mean ± S.D. for n = 6, superscript ([a]) significant at $p < 0.001$, ([b]) significant at $p < 0.01$, (c) significant at $p < 0.05$

farct size and malondialdehyde (MDA) lipid peroxidation in tissue and serum.

Table 2 shows the effect of tetrahydrocurcumin a (5 mg kg^{-1} and 10 mg kg^{-1}) and (5 mg kg^{-1} and 10 mg kg^{-1}) on the activity of AST and ALT in the serum of control, vehicle control and treatment groups MI/R rats. Control group and vehicle control group showed a significant increase in the activity of AST and ALT in serum. The treatment group tetrahydrocurcumin (5 mg kg^{-1} and 10 mg kg^{-1}) and rutin (5 mg kg^{-1} and 10 mg kg^{-1}) significantly ($p < 0.001$) decreased the activity of these enzymes in the serum of MI/R rats.

Table 3 represents the effect on myocardial antioxidants status, such as catalase and GSH, in control, vehicle control and treatment groups. Control group and vehicle control group showed a significant decrease in the activity of these enzymic antioxidants in heart. The treatment group tetrahydrocurcumin (5 mg kg^{-1} and 10 mg kg^{-1}) and rutin (5 mg kg^{-1} and 10 mg kg^{-1}) significantly ($p < 0.001$) increased the enzymic activity of antioxidant status in the heart of MI/R rats.

Table 3: Effect of tetrahydrocurcumin and rutin on catalase and reduced glutathione in heart in control, vehicle control and treatment group-experimentally induced myocardial infarction in rats

Groups	Catalase (units g^{-1} tissue)	GSH (μg g^{-1} wet tissue)
Normal control MI/R	6.265 ± 0.41	37.92 ± 3.95
Vehicle control MI/R	7.88 ± 0.533	49.58 ± 7.71
Tetrahydrocurcumin MI/R-5 mg/kg	8.88 ± 0.934[c]	87.18 ± 7.29[a]
Tetrahydrocurcumin MI/R-10 mg/kg	10.85 ± 0.965[a]	101.44 ± 6.87[a]
Rutin MI/R-5 mg/kg	9.31 ± 0.457[b]	82.53 ± 11.62[a]
Rutin MI/R-10 mg/kg	13.41 ± 2.947[a]	94.94 ± 5.17[a]

Values are mean ± S.D. for n = 6, superscript ([a]) significant at $p < 0.001$, ([b]) significant at $p < 0.01$, ([c]) significant at $p < 0.05$

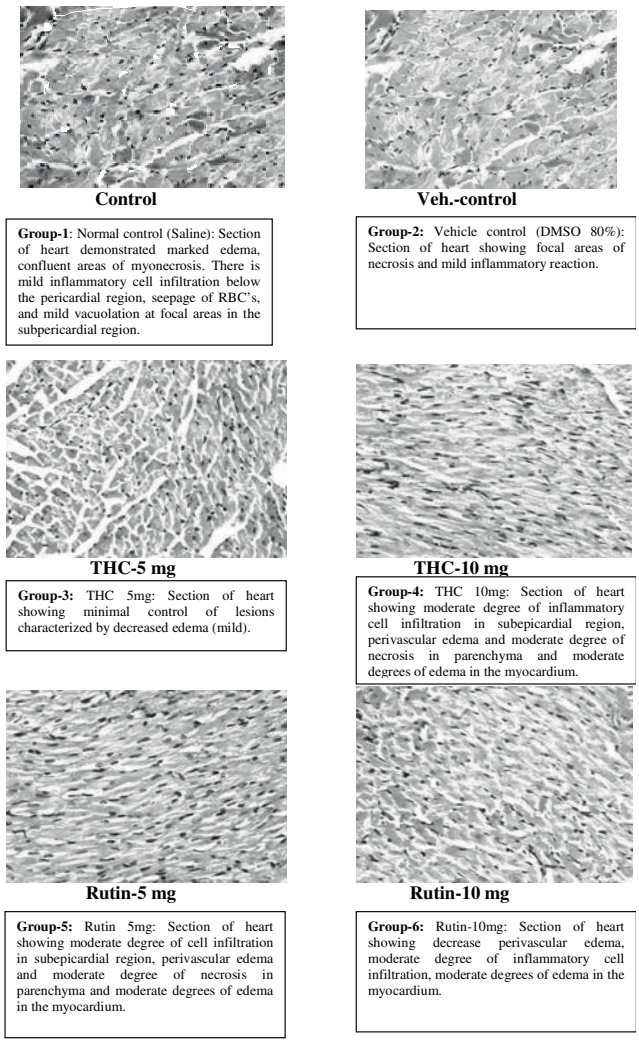

Fig.: Histopathology slides

3. Discussion

Oxygen-derived free radicals are known to play a vital role in the genesis of various cardiovascular disorders (Marx 1987; Mayers et al. 1985). Myocardial ischemia occurs when myocardial oxygen demand exceeds the oxygen supply. If this condition is not reversed, myocardial infarction precipitates. Reperfusion of the ischemic myocardium can restore oxygen supply and causes a burst of oxygen consumption with the consequent generation of free radicals, resulting in imbalance of antioxidative pro-

cesses. This process may result in a loss of contractile function of the heart and lead to severe myocardial cell damage, known as reperfusion injury (Jacobson 1974).

Recently, there has been an increasing interest in the protective function of dietary antioxidants for extending life span. Several antioxidants such as vitamin A, vitamin C, β-carotene, uric acid, ubiquinols and flavanoids, have been found to play an important role in the non-enzymatic protection against oxidative stress (Okada et al. 2001). Oxidative stress can damage many biological molecules, proteins and DNA; they are more significant targets of injury than the lipids, with lipid peroxidation often occurring late in the injury process (Halliwell and Chirico 1993). Oxygen derived free radicals also play a significant role in a large variety of cardiovascular diseases, including atherosclerosis and ischemic heart disease (Marx 1987). Our studies show that tetrahydrocurcumin (5 mg kg^{-1}, 10 mg kg^{-1}) and rutin (5 mg kg^{-1}, 10 mg kg^{-1}) treatment in myocardial infarcted animals prevented the raise in infarct size, lipidperoxidation, serum marker enzymes and decrease in antioxidants status.

In our study, infarct size was one of the major parameters. Tetrahydrocurcumin and rutin groups showed cardioprotective effects by significantly reducing the infarct size when compared to normal control MI/R and vehicle control MI/R groups.

Lipid peroxides play an important role in myocardial cell damage. Enormous amounts of reactive oxygen species (ROS), like superoxide, hydrogen peroxide and hydroxyl radicals, are produced during MI. Significant elevation in the concentration of MDA was observed in normal control MI/R and vehicle control MI/R groups. Lipid peroxidation is an important pathogenic event in MI and accumulation of lipid peroxides reflects the various stages of this disease and its complications (Neely et al. 1973). On treatment with tetrahydrocurcumin and rutin, the concentrations of MDA were significantly decreased. Tetrahydrocurcumin and rutin inhibit lipid peroxide formation and free radical production (Hamberg et al. 1974). The free radical inhibitory activity of rutin is attributed to its antioxidant properties which effectively scavenges the ROS and decreases lipid peroxidation end products. Curcuminoids also exhibited antioxidant activities in some in-vitro lipid peroxidation systems (Okada et al. 2001).

The cardiac marker enzymes of MI are AST and ALT. Rats of normal control (MI/R) and vehicle control (MI/R) groups showed an increase in the activity of these enzymes in serum. The increase in the activity of marker enzymes in serum could be due to leakage of these enzymes from the heart as a result of MI/R injury. Treatment with tetrahydrocurcumin and rutin had a significant effect on these enzymes in serum of treatment group MI/R rats.

In vivo and *in vitro* studies, as well as epidemiological studies, suggest an inverse correlation between the severity of oxidative-stress-induced diseases and levels of antioxidants. Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of cellular defence against oxidative injury. The equilibrium between these enzymes is an important factor for the effective removal of ROS in intracellular organelles (Ferrari et al. 1992). Rats of normal control (MI/R) and vehicle control (MI/R) groups showed a decreased activity of catalase in heart, decrease in the activity of this antioxidant enzyme can lead to formation of oxygen and hydrogen peroxide, which in turn can form the toxic hydroxyl radical ($OH^{\cdot}$). The decrease in the activity of catalase may be due to myocardial cell damage. The increased activity of myocardial catalase is associated with decreased levels of lipid peroxidation in the tetrahydrocurcumin (5 mg kg^{-1}, 10 mg kg^{-1}) and rutin (5 mg kg^{-1}, 10 mg kg^{-1}) treated MI/R rats. This can result in decreased formation of toxic intermediates.

The second line of defence consists of the non-enzymatic scavengers, viz. reduced glutathione and ascorbic acid containing compounds, which scavenge residual free radicals escaping decomposition by the antioxidant enzymes (Kloner et al. 1974).

Reduced glutathione (GSH) is one of the major constituent of erythrocytes and plays an important role in providing protection against oxidative damage. It has been proposed that antioxidants, which maintain the concentration of GSH, may restore the cellular defence mechanism, block lipid peroxidation and protect the tissue against oxidative damage (Chugh et al. 1999). A decrease in the concentration of GSH in normal control (MI/R) and vehicle control (MI/R) rats might be due to its utilization by the glutathione-dependent antioxidant process.

During normal control (MI/R) and vehicle control (MI/R), the level of enzymatic and non-enzymatic antioxidants decreases significantly leading to increased free radical formation. These radicals cascade a number of reactions that could be harmful to the myocardium (Samuelson 1997). The significant increase in the activity of GSH in tetrahydrocurcumin (5 mg kg^{-1}, 10 mg kg^{-1}) and rutin (5 mg kg^{-1}, 10 mg kg^{-1}) treatment groups MI/R rats could prevent free radical formation during myocardial necrosis. These results show the antioxidant activity of tetrahydrocurcumin (5 mg kg^{-1}, 10 mg kg^{-1}) and rutin (5 mg kg^{-1}, 10 mg kg^{-1}) treatment groups of MI/R rats, further confirmed by the histopathological report.

Our results show that tetrahydrocurcumin (5 mg kg^{-1}, 10 mg kg^{-1}) and rutin (5 mg kg^{-1}, 10 mg kg^{-1}) prevented the increase in infarct size and lipid peroxides, prevented the decrease in both enzymatic and non-enzymatic antioxidants in experimentally induced myocardial ischemia/reperfusion injury induced rats, histopathological examination further confirmed the protective effect of tetrahydrocurcumin and rutin on the MI/R heart. This effect might be due to the antioxidant activity of tetrahydrocurcumin and rutin.

4. Experimental

4.1. Animals

All the experiments were carried out with adult male albino Wistar rats, 200–250 g (Bioneeds, Tumkur, Bangalore). Rats were housed in polyacrylic cages (38 × 23 × 10 cm) with not more than four animals per cage. They were housed in an air conditioned room and were kept under standard laboratory conditions under natural light and dark cycle (approximately 14 h light/10 h dark) and maintained humidity 60 ± 5% and an ambient temperature of 25 ± 2 °C. All experiments were performed between 9 : 00 am and 4 : 00 pm, the animals had free access to standard diet (Amrut rat feed, Bangalore) and tap water and were allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22% protein, 4% fat, 4% fiber, 36% carbohydrates and 10% ash w/w. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee of Acharya and B. M. Reddy College of Pharmacy, Bangalore (Approval No. IAEC /PP //03/2006–2007).

4.2. Chemicals

Rutin was procured from the Sigma- Aldrich chemicals Ltd, Bangalore. Tetrahydrocurcumin was supplied as gift sample from Sami labs, Bangalore. All the other chemicals procured from Merck laboratories, Nice chemicals, Loba chemie, Sd. fine chemicals used were of highly analytical grade.

4.3. Experimental design

The rats were divided into 6 groups of 18 rats as follows: *Group-1*, normal control group animals treated 0.2 ml saline intraperitoneally; *group-2*, vehicle control group animals treated 0.2 ml of 80% dimethyl sulfoxide (DMSO) intraperitoneally; *group-3*, test group animals treated with tetrahydrocurcumin 5 mg kg^{-1}; *group-4*, test group animals treated with tetrahydrocurcumin 10 mg kg^{-1}; *group-5*, test group animals treated with rutin 5 mg kg^{-1}; *group-6*, test group animals treated with rutin 10 mg kg^{-1}.
Note: Tetrahydrocurcumin and rutin were dissolved in 80% DMSO and given as an i.p. injection 10 min before removing coronary occlusion.

4.4. Surgical preparation: infarction procedure

Male albino rats were anaesthetized with thiopentone sodium (30 mg kg^{-1} intraperitoneally), before performing trachectomy and then they were ventilated with room air using an Inco positive pressure respirator. Ventilator parameters were adjusted to maintain normal pH and satisfactory oxygenation. The body temperature was monitored and maintained at 31 °C throughout the experiment. The left thoractomy and pericardiotomy were performed, and located the left anterior descending coronary artery (LAD).

4.5. Coronary artery occlusion and reperfusion

Left anterior descending coronary artery (LAD) was ligated 4–5 mm from the origin by a silk thread with help of suture needle; LAD was occluded with that silk thread for 30 min. After 30 min of occlusion, the silk thread was removed to allow reperfusion of the ventricle for 4 h. In normal control group animals, saline was administered intraperitoneally 10 min before reperfusion. In vehicle control group animals, DMSO was administered intraperitoneally 10 min before reperfusion. Tetrahydrocurcumin and rutin were administered intraperitoneally 10 min before reperfusion in drug treated groups.
After 4 h reperfusion, blood was collected by cardiac puncture; serum was separated by centrifugation and used for the biochemical estimations. The heart was removed, washed immediately in ice-chilled physiological saline, blotted dry and weighed. First phase: in six rats of each group the left ventricle was separated from the heart and weighed. A 10% heart tissue homogenate was prepared using 1.15% potassium chloride buffer solution; homogenate was centrifuged at 2000 rpm for 5 min. The supernatant was used for the estimation of various biochemical parameters. Second phase: in the other six rats in each group the left ventricle was separated from the heart and was weighed, heart tissue was homogenized in ice-cold 0.1 M tris-buffer (pH-7.4) to produce 10% w/v homogenate. The homogenate was centrifuged at 2000 rpm for 5 min; the supernatant was used for the estimation of biochemical parameters. Third phase: in rats of each group hearts were removed and immediately processed for histopathological studies.

4.6. Quantification of infarct size

In all the groups, after sacrificing the animal by injecting 2.56 M potassium chloride directly into the left ventricle, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and was weighed. It was sliced parallel to the atrioventricular groove to 0.1 cm thick sections and the slices were incubated in 1% TTC solution prepared in phosphate buffer pH 7.4 for 30 min at 37 °C (Johnson et al. 1990). In viable myocardium TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue dark red (Fishbein and Meerbaum 1981). The infarcted myocardium that does not take TTC stain where the dehydrogenase enzymes are drained off remains pale in colour. The pale necrotic tissue was separated from the stained portions and weighed on an electronic balance. Myocardial infarct size was expressed quantitatively in terms of percent left ventricle necrosis (PLVN).

4.7. Lipid peroxidation

4.7.1. Estimation of MDA in tissue

MDA levels in the myocardium were measured by the method developed by Ohkawa et al. (1975). Briefly, the infarcted left ventricular tissues were homogenized with 1.15% KCl (10% w/v). The assay mixture consisting of 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 ml of 0.8% aqueous solution of TBA (thiobarbituric acid) was added, mixed thoroughly and 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. v/v). After centrifugation at 4000 rpm for 10 min, the organic phase was assayed spectrophotometrically at 532 nm. Tetraethoxypropane standard solution (0.1 nmol–1 nmol of 1,1,3,3-tetraethoxy-propane) in a 5 ml volume and a blank containing 0.2 ml of distilled water were processed along with the test samples.

4.7.2. Estimation of MDA in serum

Before sacrificing the animal at the end of fourth hour of the reperfusion, blood sample was collected from the left ventricle. A blood sample (2 ml) was taken into a 2 ml centrifuge tube and centrifuged at 4000 rpm for 10 min. From this, 0.1 ml serum was taken into the centrifuge tube containing 4 ml of 0.083 N sulphuric acid. Then 0.5 ml of 10% phosphotungstic acid was added to the tube and mixed, kept at room temperature for 5 min. The mixture was centrifuged at 4000 rpm for 10 min. The sediment was suspended in 2 ml of 0.083 N sulphuric acid and 0.3 ml of 10% phosphotungstic acid and the mixture was centrifuged at 4000 rpm for 10 min. The sediment was suspended in 4 ml of distilled water and 1 ml of TBA reagent was added. The reaction mixture was heated for 60 min at 95 °C. After the mixture was cooled to room temperature, 5 ml of n-butanol were added and the mixture was centrifuged at 4000 rpm for 10 min. The n-butanol layer was separated, and used for colorimetric measurement at 532 nm. Standard solution (0.1 nmol–2 nmol of 1,1,3,3-tetraethoxy-propane) in a 5 ml volume and blank containing 0.2 ml of distilled water were processed along with the test samples (Yagi 1976).

4.8. Assay of aspartate transaminase (AST)

Aspartate transaminase in serum was assayed by the method of Reitman and Frankel (1957) using a reagent kit (span diagnostics, product no.0590 Mumbai, India). Buffered substrate (0.5 mL) was incubated at 37 °C for 3 min and 0.1 mL of serum of homogenate was added, mixed well and incubated at 37 °C for 30 min. Then 0.5 mL of 2,4-dinitrophenyl hydrazine (DPNH) reagent was added, mixed well and allowed to stand at room temperature for 20 min and 0.5 mL of 4N working sodium hydroxide was added, and kept at room temperature for 10 min. Blank and standards were also processed in the same way and absorbance was measured spectrophotometrically at 505 nm (Toro and Ackermann 1975).

4.9. Assay of alanine transaminase (ALT)

Alanine transaminase in serum was assayed by the method of Reitman and Frankel (1957) using a reagent kit (span diagnostics, product no. 1167 Mumbai, India). Buffered substrate (0.5 mL) was incubated at 37 °C for 3 min and 0.1 mL of serum of homogenate was added, mixed well and incubated at 37 °C for 30 min. Then 0.5 mL of 2,4-dinitrophenyl hydrazine (DPNH) reagent was added, mixed well and allow to stand at room temperature for 20 min and 0.5 mL of 4N working sodium hydroxide was added, and kept at room temperature for 10 min. Blank and standards were processed in the same way and absorbance was measured spectrophotometrically at 505 nm (Tonhazy et al. 1950).

4.10. Catalase

Homogenate (0.6 ml) was added to 2.9 ml of H_2O_2-phosphate buffer and measured at 240 nm using blank as 0.6 ml of homogenate and H_2O_2 free phosphate buffer. Note the time Δt required for a decrease in absorbance from 0.45–0.40 (Luck 1974).

4.11. Reduced glutathione (GSH)

Reduced glutathione was estimated by the method of Ellman et al. (1959). Heart tissue was homogenized in ice-cold tris-buffer to produce 10% w/v homogenate. The homogenate was centrifuged at 10 000 rpm at 4 °C for 15 min. Protein free supernatant was obtained by addition of equal volume of 10% TCA and the tissue homogenate, centrifuged at 5000 rpm for 10 min. To 0.1 ml of supernatant, 2.0 ml of 0.6 mM DTNB reagent and 1.9 ml of 0.2 M phosphate buffer (pH-9.0) were added and vortexed. The absorbance was measured at 412 nm against a blank containing TCA instead of supernatant. A series of standards treated the same way also were run to determine the glutathione content. The standard graph was plotted with reduced glutathione at concentrations of (100–600 μg) and compared to test O.D. with standard graph, the amount of glutathione was expressed as μg/g wet tissue.

4.12. Histopathological studies

At the end of the experiment, myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. The tissue was carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plate to allow paraffin to solidify. Cross section (5 μm thick) of the fixed myocardial tissues was cut. These sections were stained with hematoxylin and eosin (H&E) and visualized under a light microscope to study the microscopical architecture of the myocardium. The investigators performing the histological evaluation were blind to quantification of infarct size, MDA estimation in tissue and serum and biochemical estimations (Luna 1960).

References

Ammon HPT, Anazodo, Safayhi H, Dhawan BN., Srimal RC (1992) Curcumin: a potent inhibitor of leukotriene B_4 formation in rat peritoneal polymorphonuclear neutrophils. Planta Med. 58: 26.
Kompa AR, Summers RJ (2000) Lidocaine and surgical modification reduces mortality in a rat model of cardiac failure induced by coronary artery ligation. J Pharmacol Toxicol Meth 43 : 199–203.

Chan WS, Lee YJ, Lu FJ, Chaiang H (1993) Inhibitory effects of flavonoids on xnathine oxidase. Anticancer Res 13: 2165–2170.
Chugh SN, Kakkar R, Kalra S, Sharma A (1999) An evaluation of oxidative stress in diabetes mellitus during uncontrolled state after Vitamin E supplementation. J Assoc Physicians India 47: 380–383.
Duke JA (1992) Handbook of phytochemical constituents of GRAS herbs and other economics plants, London CRC press, p. 75.
Ellman GL (1959) Tissue sulfahydryl groups. Arch Biochem Biophys 82: 70–77.
Ferrari R, Ceconi C, Curello S, Curgnonin A, De Giuli F (1992) Occurrence of oxidative stress during myocardial reperfusion. Mol Cell Biol 22: 61–69.
Fishbein MC, Meerbaum S, Rit J (1981) Early phase acute myocardial infarct size Quantification. Am Heart J 101: 593–600.
Halliwell B (1993) Lipid peroxidation: It′s mechanism, measurement and Significance. Am J Clin Nutr 57: 715–724.
Hamberg M, Svensson J, Wakabayashi T, Samuelsson B (1974) Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc Natl Acad Sci 71: 345–349.
Jacobson MD (1974) Reactive oxygen species and programmed cell death. Trends Biochem J. 243: 43–48.
Janbaz KH, Saeed SA, Gilani AH (2002) Protective effect of rutin on paracetamol and ccl_4-induced hepatotoxicity in rodants. Fitotherapia 73: 557–563.
Joe B, Lokesh BR (1994) Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. Biochim Biophys Acta 1224: 255–263.
Johnson G, Tsao PBS, Lefer AM (1990) Synergism between superoxide dismutase and sodium nitrate in cardioprotection following ischemia and reperfusion. Am Heart J 119: 530–538.
Kloner RA, Ganote CE, Whalen DA, Jennings RB (1974) Effects of transient period of ischemia on myocardial cells II. Fine structure during the first few minutes of reflow. Am J Pathol 74: 399–422.
Luck H (1974) Methods in enzymatic Analysis. Aca Press, p. 885.
Luna LC (1960) Manual of histological screening methods of armed forces institute of pathology, Mc. Graw Hill book Co., New York, p 125.
Marx JL (1987) Oxygen free radicals linked to many diseases. Science 235: 529–531.
Mayers ML, Bolli R, Lekich RF, Hartley CJ, Roberts R (1985) Enhancement of recovery of myocardial function by oxygen free radical scavengers after reversible regional ischemia. Circulation. 72: 915–921.
Neely JR, Rovetto MJ, Whitmer JT, Morgan HE (1973) Effects of ischemia on function and metabolism of the isolated working heart. Am J Physiol 225: 651–658.
Ohkawa H, Ohoshi N, Tagi K (1975) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358.
Okada K, Wangpoengtrakul C, Tanaka T, Toyokuni S, Uchida K, Osawa T. (2001) Curcumin and especially tetrahydrocurcumin amiliorate oxidative stress induced renal injury in mice. J Nutrition 131: 2090–2095.
Osavwa, T, Sugiyama, Y, Inayoshi, M and kawakishi, S. 2005. Anti oxidative activity of tetrahydro curcuminoids, Biosci. Biotech. 59: 1609–1612.
Osawa T, Sugiyama Y, Inayoshi M, Kawakishi S (1995) Antioxidative activity of tetrahydrocurcuminoids. Biosci Biotech Biochem 59: 1609– 1612.
Reitman S, Frankel SA (1957) Colorimetric method for the determination of serum glutamic acetacetic and glutamic pyruvic transaminases. Am J Clin Path. 28: 56.
Ruby AJ, Kuttan G, Dinesh Babu K, Rajasekharan KN, Kuttan R (1995) Antitumor and antioxidant activity of natural curcuminoids. Cancer Lett 94: 79–83.
Samuelsson B (1997) Biochemical aspects of prostaglandins and thromboxanes, Academic Press N York, pp. 133–139.
Subramanian M, Sreejayan, Rao MN, Devasagayam TP, Singh BB (1994) Diminuition of singlet oxygen induced DNA damage by curcumin and related antioxidants. Mutat Res 311: 249–255.
Sugiyama Y, Kawakishi, S, Osawa T (1996) Involvement of the β-diketone moiety in the antioxidant mechanism of tetra hydro curcuminoids. Biochem Pharmocol 52: 519–525.
Tonhazy NE, White NG, Umbreit WW (1950) A rapid method for the estimation of the glutamic aspartic transaminase in tissues and its application to radiation sickness. Arch Biochem 28: 36–42.
Toro G, Ackermann PG (1975) Practical clinical Chemistry, Brown & Company, Boston, p. 484.
Yagi K (1976) A simple fluorometric assay for lipid peroxide in blood plasma. Biochem Med 15: 212–216.