JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 921–926

Received January 15, 2004
Accepted April 08, 2004
DOI 10.1211/0022357023745
ISSN 0022-3573

Cardioprotective effect of tincture of *Crataegus* on isoproterenol-induced myocardial infarction in rats

R. Jayalakshmi and S. Niranjali Devaraj

Abstract

Tincture of *Crataegus* (TCR), an alcoholic extract of the berries of hawthorn (*Crataegus oxycantha*), is used in herbal and homeopathic medicine. The present study was done to investigate the protective effect of TCR on experimentally induced myocardial infarction in rats. Pretreatment of TCR, at a dose of 0.5 mL/100 g bodyweight per day, orally for 30 days, prevented the increase in lipid peroxidation and activity of marker enzymes observed in isoproterenol-induced rats (85 mg kg^{-1} s.c. for 2 days at an interval of 24 h). TCR prevented the isoproterenol-induced decrease in antioxidant enzymes in the heart and increased the rate of ADP-stimulated oxygen uptake and respiratory coupling ratio. TCR protected against pathological changes induced by isoproterenol in rat heart. The results show that pretreatment with TCR may be useful in preventing the damage induced by isoproterenol in rat heart.

Introduction

Myocardial infarction is the number one cause of death worldwide. It occurs when a coronary artery is severely blocked such that there is a significant reduction in the blood supply, causing damage or death to a portion of the myocardium. Depending on the extent of the heart muscle damage, the patient may experience significant disability or die as a result of myocardial infarction.

Isoproterenol (isopropyl norepinephrine) is a potent non-selective β-adrenergic agonist with very low affinity for α-adrenergic receptors. Isoproterenol-induced myocardial infarction in rats has been shown to be accompanied by hyperlipidaemia, increased activity of serum creatine kinase, alanine amino transferase, aspartate amino transferase and lactate dehydrogenase (LDH) (Young et al 1993). Damage to the myocardium could be owing to the induction of free-radical-mediated lipid peroxidation by isoproterenol. The increased level of lipid peroxides is a causative factor in the irreversible damage in isoproterenol-induced myocardial infarction (Golikov et al 1989).

Oxidative stress is implicated in the aetiopathogenesis of a variety of human diseases, including cardiovascular diseases (Frei 1994). The antioxidant status has a major influence on the development of coronary artery diseases. Higher plasma concentrations of the antioxidant vitamins, ascorbic acid and α-tocopherol, are correlated to a lower incidence of coronary artery disease (Riemersma et al 1991).

Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and non-toxic nature. Many of these medicines are a complex mixture of similar or different chemical substances. Tincture of *Crataegus* (TCR), an alcoholic extract of the berries of hawthorn, *Crataegus oxycantha* L. Rosaceae, is used in herbal medicine as a heart tonic (Shanthi et al 1994). TCR has a number of pharmacological properties, possibly owing to its major constituents: flavonoids, triterpene saponins and some cardioprotective amines (Willard 1991). Recent epidemiological studies have found an association between dietary flavonoid intake and reduced risk of heart disease, myocardial infarction and stroke (Hertlog et al 1995). The primary cardioprotective activity of this plant is generally attributed to its flavonoid and oligomeric proanthocyanidines (Miller 1998). In the present study, the activity of marker enzymes, levels of antioxidants (enzymic and non-enzymic),

Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, India

R. Jayalakshmi,
S. Niranjali Devaraj

Correspondence: S. Niranjali Devaraj, Department of Biochemistry, University of Madras, Guindy Campus, Chennai 600 025, India.
E-Mail: niranjali@yahoo.com

lipid peroxides, mitochondrial respiratory coupling ratio and histological changes were examined in serum and heart of rats pretreated with TCR to determine its protective role against experimentally induced myocardial infarction.

Materials and Methods

Experimental animals

Male Albino Wistar rats, 150–200 g, were obtained from Tanuvas, Madhavaram, Chennai, India. Ethical clearance for the study was obtained from the Institutional Animal Ethics Committee (IAEC no: 01/008/02). The rats were housed in solid-bottomed polypropylene cages and received commercial rat chow (Hindustan Lever Ltd, Bangalore, India) and water ad libitum. The rats were divided into five groups with six rats in each group. Group 1: control rats received normal rat diet and water ad libitum. Group 2: drug control, received normal rat diet and were given TCR (0.5 mL/100 g) (Shanthi et al 1994), orally for 30 days. Group 3: isoproterenol (85 mg kg^{-1}; Sigma Chemical Co, USA) was given by subcutaneous injection for 2 days at an interval of 24 h (Day 31 and 32). Group 4: TCR (same dosage as above) plus isoproterenol (same dosage as above). Group 5: captopril (10 mg kg^{-1}) (Sanbe et al 1995) plus isoproterenol (same dosage as above). Captopril was used as a positive control and was given orally for 30 days before isoproterenol injection.

After the experimental period, the animals were killed by ether anaesthesia and decapitation. Blood was collected and serum was separated. The heart was dissected out immediately: one portion of heart was used for histological examination and the other portion was washed in ice-cold saline and homogenized in Tris-HCl buffer (0.1 M), pH 7.4. The homogenate was used for biochemical estimations.

Biochemical analysis

Serum and heart proteins were estimated (Lowry et al 1951). Serum marker enzymes, such as alanine transaminase, aspartate transaminase, LDH (King 1965), creatine kinase (Okinaka et al 1961) and lipid peroxidation products (Santos et al 1980), were assayed. Antioxidants, such as reduced glutathione (Moron et al 1979), glutathione peroxidase (Rotruck et al 1973), glutathione-*S*-transferase (Habig et al 1974), glutathione reductase (Staal et al 1969), glucose-6-phosphate dehydrogenase (Baquer & McLean 1972), superoxide dismutase (Misra & Fridovich 1972), catalase (Beers & Seizer 1952), α-tocopherol (Desai 1984) and ascorbic acid (Omaye et al 1979), were assayed in the tissue homogenate. Mitochondria were isolated from heart. Briefly, the heart was excised, rinsed in ice-cold isotonic saline, blotted with filter paper, weighed, homogenized in 0.25 M sucrose at 4°C and mitochondria were isolated by the method of Johnson & Lardy (1967). The rate of oxidation of succinate was followed using Clark's oxygen electrode. Oxygen uptake in mitochondria was measured by the method of Katyare et al (1971).

Separation of LDH isoenzymes by native polyacrylamide gel electrophoresis

LDH isoenzymes in serum were separated by native polyacrylamide gel electrophoresis (Laemmli 1970). Equal amounts of protein from heart homogenate were loaded onto a 10% polyacrylamide gel for electrophoresis in the absence of sodium dodecyl sulfate. The separated isoenzymes were stained with 1.0 M lithium lactate, 0.1 M NaCl, 5 mM $MgCl_2$, 0.1% of NBT, NAD^+ and phenazine methosulphate in 0.05 M phosphate buffer (pH 7.4). After 15–30 min, blue bands characteristic of isoenzymes of LDH appeared (Dietz & Lubrano 1967).

Histological examination

The hearts were removed, washed immediately with saline and then fixed in 10% buffered formalin. The hearts were embedded in paraffin, sections cut at 5 μm and stained with haematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical analysis

Values are expressed as mean $\pm$ s.e.m. The results were statistically evaluated using one-way analysis of variance by SPSS software for repeated measurements and, when indicated, a post-hoc Tukey's test for multiple comparisons. The mean difference is significant at the 0.05 level.

Results

The effects of TCR on serum marker enzymes, lipid peroxidation products and the activity of creatine kinase in heart of control and experimental animals are shown in Table 1. The activity of serum marker enzymes such as alanine transaminase, aspartate transaminase and lactate dehydrogenase did not change significantly in the drug control group (Group 2). This observation showed that TCR had no damaging effects on the heart. Isoproterenol-treated rats (Group 3) showed a significant increase in serum LDH, creatine kinase and lipid peroxide levels when compared with control rats. TCR- and captopril-treated rats (Groups 4 and 5) showed significantly decreased levels of serum and heart lipid peroxidation and creatine kinase activity compared with Group 3 rats. Lipid peroxides in serum and heart were decreased in Groups 4 and 5 compared with Group 3 rats.

Antioxidant enzyme activities in heart of control and experimental animals are shown in Table 2. Glutathione reductase was significantly increased in TCR-treated rats (Group 4) when compared with Group 3 rats. Glutathione peroxidase, glutathione-*S*-transferase, glucose-6-phosphate dehydrogenase, superoxide dismutase and catalase activities were significantly lowered owing to the myocardial infarction in Group 3 rats. TCR administration successfully prevented the decrease in activity of these enzymes in Group 4 rats and this effect was probably owing to removal of excess free radicals generated by isoproterenol.

Table 1 Activity of marker enzymes and levels of lipid peroxides in serum and heart of control and experimental rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Alanine transaminase[a]	23.5 ± 0.9	26.9 ± 0.9*	52.8 ± 1.1*	43.8 ± 1.0*†	42.8 ± 0.9*†
Aspartate transaminase[a]	26.7 ± 1.7	27.8 ± 1.1*	46.2 ± 1.3*	30.8 ± 1.6*†	33.7 ± 1.2*†
Lactate dehydrogenase[a]	75.6 ± 0.9	72.5 ± 1.4*	97.8 ± 1.0*	82.5 ± 1.2*†	84.6 ± 0.8*†
Creatine kinase[b] (serum)	287.8 ± 2.1	282.4 ± 0.9*	389.5 ± 1.7*	299.3 ± 2.3*†	289.9 ± 1.9*†
Lipid peroxides[c] (serum)	3.2 ± 0.2	3.4 ± 0.1*	9.2 ± 0.2*	5.8 ± 0.2*†	6.2 ± 0.3*†
Lipid peroxides[d] (heart)	0.3 ± 1.9	0.3 ± 1.8*	1.45 ± 1.6*	0.5 ± 2.4*†	0.9 ± 2.2*†
Creatine kinase[b] (heart)	14.3 ± 0.6	13.9 ± 0.4*	7.9 ± 0.3*	9.9 ± 0.2*†	10.3 ± 0.5*†

Group 1: control rats. Group 2: drug control, rats received tincture of *Crataegus* (TCR) (0.5 mL/100 g) orally for 30 days. Group 3: isoproterenol (85 mg kg^{-1} s.c.) was given for 2 days at an interval of 24 h (Day 31 and 32). Group 4: TCR (same dosage as above) plus isoproterenol (same dosage as above). Group 5: captopril (10 mg kg^{-1}) plus isoproterenol (same dosage as above). Captopril was used as a positive control and was given orally for 30 days before isoproterenol injection. [a]IU L^{-1}; [b]nmol pyruvate liberated min^{-1}; [c]nmol malondialdehyde mL^{-1}; [d]nmol malondialdehyde (mg protein)$^{-1}$. Values are expressed as mean ± s.e.m. *$P < 0.05$ compared with Group 1; †$P < 0.05$ compared with Group 3.

Table 2 Activity of antioxidant enzymes in the heart of control and experimental rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Glutathione reductase[a]	10.3 ± 0.4	11.4 ± 0.6*	8.9 ± 0.4*	10.1 ± 0.5*†	9.6 ± 0.4*†
Glutathione peroxidase[b]	84.6 ± 0.6	85.5 ± 0.7*	68.3 ± 1.21*	76.7 ± 0.7*†	78.8 ± 0.6*†
Glutathione-*S*-transferase[c]	809.0 ± 0.7	814.4 ± 0.9*	169.7 ± 1. 2*	562.0 ± 1.1*†	639.1 ± 1.0*†
Glucose-6-phosphate dehydrogenase[d]	65.7 ± 0.6	65.9 ± 0.8*	51.3 ± 0.8*	59.9 ± 1.3*†	60.1 ± 0.8*†
Superoxide dismutase[e]	26.5 ± 0.8	27.9 ± 0.8*	16.5 ± 0.7*	22.8 ± 0.9*†	27.6 ± 0.9*†
Catalase[f]	5.5 ± 0.3	5.6 ± 0.4*	3.5 ± 0.3*	4.8 ± 0.5*†	4.6 ± 0.2*†
Ascorbic acid[g]	5.3 ± 0.2	5.2 ± 0.1*	3.4 ± 0.2*	4.2 ± 0.1*†	4.1 ± 0.2*†
α-Tocopherol[h]	4.3 ± 0.2	4.7 ± 0.2*	2.6 ± 0.2*	3.6 ± 0.2*†	3.9 ± 0.1*†
Reduced glutathione[i]	4.6 ± 0.3	4.5 ± 0.3*	2.9 ± 0.3*	3.7 ± 0.2*†	3.6 ± 0.2*†

See Table 1 for details of groups. [a]nmol NADH oxidized min^{-1} (mg protein)$^{-1}$; [b]nmol glutathione oxidized min^{-1} (mg protein)$^{-1}$; [c]nmol 1-chloro-2,4-dinitrobenzene conjugated min^{-1} (mg protein)$^{-1}$; [d]units (mg protein)$^{-1}$; [e]units (mg protein)$^{-1}$; [f]nmol H_2O_2 released min^{-1} (mg protein)$^{-1}$; [g]μg (mg protein)$^{-1}$; [h]μg (mg protein)$^{-1}$; [i]nmol glutathione reduced (mg protein)$^{-1}$. Values are expressed as mean ± s.e.m. *$P < 0.05$ compared with Group 1; †$P < 0.05$ compared with Group 3.

Isoproterenol-treated rats (Group 3) showed significantly decreased ascorbic acid, α-tocopherol and reduced glutathione levels when compared with control rats (Group 1). In TCR- and captopril-treated animals (Groups 4 and 5), these levels were significantly increased when compared with Group 3 animals. Group 4 rats had significantly elevated α-tocopherol, reduced glutathione and ascorbic acid when compared with Group 3 rats. Captopril treatment significantly increased α-tocopherol, ascorbic acid and reduced glutathione levels when compared with Group 3 rats.

Figure 1 shows the histology of control (Plate a), drug control (Plate b) and experimental (Plates c, d and e) rat heart. The isoproterenol-induced rat heart (Group 3; Plate c) showed the separation of muscle fibres with inflammatory infiltration. The drug-treated group (Group 4; Plate d) showed the normal appearance of cardiac muscle fibres and the positive control also showed normal architecture.

Figure 2 shows the separation of LDH isoenzymes by native polyacrylamide gel electrophoresis. Lanes 1 and 2 are control (Group 1) and drug control (Group 2) serum. There was a significant increase in the LDH1 and LDH2 isoenzymes in the isoproterenol-treated group, which was not seen in the TCR- or captopril-treated groups.

ADP-stimulated oxygen uptake and respiratory control ratio in rat heart mitochondria of control and experimental animals are shown in Table 3. The ADP-stimulated oxygen utilized using succinate as substrate was significantly diminished in isoproterenol-induced rat heart mitochondria when compared with control rats. The respiratory control ratio decreased in heart mitochondria of isoproterenol-treated rats. TCR-treated rats (Group 4) significantly increased the state 3 and state 4 oxidation, and respiratory control ratio in heart mitochondria when compared with isoproterenol-treated rats (Group 3).

Discussion

Creatine kinase, LDH, alanine transaminase and aspartate transaminase are released during myocardial infarction

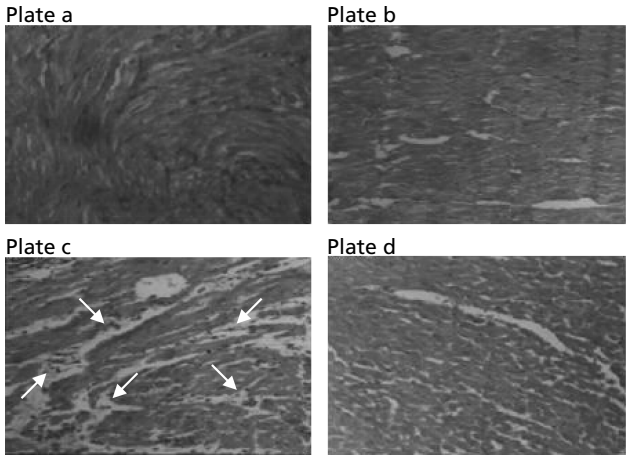

Figure 1 Histological examination of control and experimental heart. Plate a: section of control rat heart showing normal architecture (Group 1). Plate b: section of drug control rat heart showing normal architecture (Group 2). Plate c: section of rat heart treated with isoproterenol showing the separation of muscle fibres with inflammatory infiltration (Group 3). Plate d: section of tincture of *Crataegus* pretreated and isoproterenol administered rat heart showing normal architecture (Group 4).

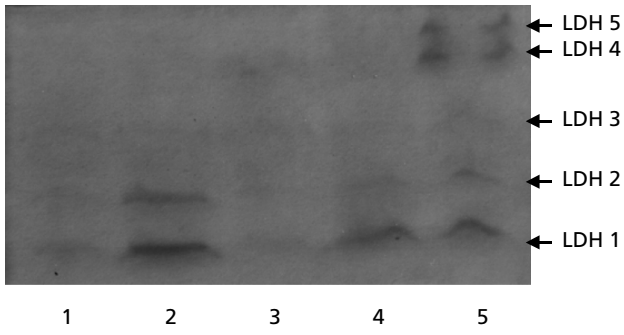

Figure 2 Separation of lactate dehydrogenase (LDH) isoenzymes by native polyacrylamide gel electrophoresis. Lane 1: control (Group 1); Lane 2: drug control (Group 2); Lane 3: isoproterenol-induced myocardial infarction (Group 3); Lane 4: treated with tincture of *Crataegus* (Group 4); Lane 5: positive control (captopril) (Group 5).

into the bloodstream (Narayanan 1999). Animal studies have shown that LDH released from isolated hearts during ischaemia and reperfusion was significantly decreased after pretreatment with *Crataegus* extract, suggesting a protective effect of the extract on the myocardium (Al Makdessai et al 1996). Lipid peroxides may play a very important role in myocardial cell damage during infarction. TCR, by providing antioxidative action, protects the heart from lipid peroxidative damage. Lipid peroxidation is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflect the various stages of the disease and its complications. Damage to the myocardium could be owing to the induction of free-radical-mediated lipid peroxidation by isoproterenol. Increased levels of lipid peroxidation products injure blood vessels, causing increased adherence and aggregation of platelets to the injured sites. Flavonoids have been shown to inhibit lipid peroxide formation in rat tissue and also inhibit the free radical production in cells at various stages. They can interact with superoxide anions, chelate metal ions and react with peroxy radicals (Afanasev et al 1988). Thus, the flavonoids in TCR probably protected the heart from myocardial damage by scavenging free radicals and thereby suppressing the peroxidation of lipids. The triterpene saponins, ursolic and oleanolic acid, present in TCR may also contribute to the antioxidant effect of TCR, since these also have remarkable antioxidant activities (Balanehru & Nagarajan 1991). α-Tocopherol sequesters free radicals and acts as a chain-breaking antioxidant. A diminution of tissue α-tocopherol in the myocardial infarcted rats should have resulted in enhanced lipid peroxidation, leading to cardiac damage. TCR maintained the concentration of α-tocopherol in rat heart and thereby protected the heart from isoproterenol-induced damage. Ascorbic acid is said to augment the antioxidant action of α-tocopherol by reducing the α-tocopherol radical to α-tocopherol (Leung et al 1981). TCR probably assisted the above process indirectly, since its flavonoids have an ascorbic acid sparing property (Middleton 1984).

Histological examination revealed that the cardiac muscle fibres appeared normal in TCR-treated rats. Isoproterenol-induced rat heart showed separation of muscle fibres with inflammatory infiltration and necrosis. The blood supply to the heart muscle was severely reduced, as during isoproterenol induction muscle cells die and liberate LDH 1 into the bloodstream.

Table 3 ADP-stimulated oxygen uptake and respiratory control ratio in rat heart mitochondria of control and experimental rats

Rate of oxidation (nmol O_2 utilized min^{-1} (mg protein)$^{-1}$)	Group 1	Group 2	Group 3	Group 4	Group 5
State 3 (+ ADP)	36.9 ± 0.3	36.0 ± 1.5*	22.4 ± 0.7*	42.0 ± 0.7*†	32.2 ± 0.8*†
State 4 (− ADP)	9.6 ± 0.2	9.4 ± 0.2*	6.3 ± 0.2*	7.9 ± 0.2*†	7.8 ± 0.2*†
Respiratory control ratio (state 3/state 4)	3.8 ± 0.2	3.7 ± 0.2*	3.5 ± 0.2*	5.3 ± 0.3*†	4.1 ± 0.3*†

See Table 1 for details of groups. Values are expressed as mean ± s.e.m. *$P < 0.05$ compared with Group 1; †$P < 0.05$ compared with Group 3.

The electron transferring molecules of the respiratory chain and the enzymes that synthesize ATP from ADP and phosphate in mitochondria are embedded in the inner membrane. The ADP formed from ATP during energy-requiring activities in the cytosol must pass into the mitochondrial matrix to be phosphorylated to ATP. The new ATP passes back to the cytosol. The inner membrane has an intricate mosaic structure the integrity of which is essential in ATP generation (Whittaker & Danks 1978). The membrane must be intact and form a completely enclosed vesicle for effective oxidative phosphorylation to take place (Boyer et al 1977). If the membrane is damaged, loss of mitochondrial oxidative phosphorylating capacity, which probably equates with cell death, occurs (Nishimura et al 1986). The subcutaneous administration of isoproterenol reduces the respiratory coupling ratio, an index of membrane integrity (Capozza et al 1992).

The drastic decrease in respiratory activity during state 3 respiration after isoproterenol administration indicates severe impairment of electron transport capability. This may have led to a decrease in ADP/oxygen ratio, showing that utilization of oxygen is unlinked from oxidative phosphorylation and thus there will be decreased synthesis of ATP (Ceconi et al 1988). A decrease in respiratory rate (in state 3 and state 4) and respiratory control index has been reported in ischaemic myocardium (Capozza et al 1992). TCR pretreatment resulted in an increase in the oxidation of succinate, ADP/oxygen ratio and respiratory control index, suggesting an enhancement of blood flow to myocardium that could lead to increased mitochondrial respiration.

Conclusion

The present study indicates that TCR prevents the increase in lipid peroxidation and corrects the imbalance in the antioxidant status produced as a result of myocardial infarction in rats. It also prevents the defective oxidative phosphorylation and diminished energy production. To conclude, pretreatment with TCR protects against experimentally induced myocardial infarction in rats.

References

Afanasev, I. B., Dorozhko, A. I., Brodskii, A. V., Kostyuk, V. A., Potaporitch, A. I. (1988) Chelating and free radical scavenging mechanisms of inhibitory actions of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **38**: 1763–1769

Al Makdessai, S., Sweidan, H., Mullner, S., Jacob, R. (1996) Myocardial protection by pretreatment with *Crataegus oxycantha*: an assessment by means of the release of lactate dehydrogenase by the ischemic and reperfused Langendorff heart. *Arzneimittelforschung* **46**: 25–27

Balanehru, S., Nagarajan, B. (1991) Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. *Biochem. Int.* **24**: 981–990

Baquer, N. J., McLean, P. (1972) Evidence for the existence and functional activity of pentose phosphate pathway enzymes in the large particle fraction isolate from rat tissues. *Biochem. Biophys. Res. Commun.* **46**: 167–174

Beers, R. F., Seizer, I. W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **115**: 133–140

Boyer, P. D., Chance, B., Ernster, L., Mitchell, P., Racker, E., Slater, E. C. (1977) Oxidative phosphorylation and photophosphorylation. *Annu. Rev. Biochem.* **46**: 955–966

Capozza, G., Muolo, L., Jirillo, E., Guarnieri, F. (1992) Isoproterenol causes changes in the mitochondrial energy metabolism in the rat heart. *Cardiologia* **31**: 663–665

Ceconi, C., Curello, S., Albertini, A., Ferrari, R. (1988) Effect of lipid peroxidation on heart mitochondria oxygen consuming and calcium transporting capacities. *Mol. Cell. Biochem.* **81**: 131–135

Desai, I. D. (1984) Vitamin E analysis methods for animal tissues. *Methods Enzymol.* **105**: 138–147

Dietz, A., Lubrano, T. (1967) Separation and quantitation of LDH isoenzymes by disc electrophoresis. *Anal. Biochem.* **20**: 246–257

Frei, B. (1994) Reactive oxygen species and antioxidant vitamins: mechanism of action. *Am. J. Med.* **97** (Suppl. 3A): 5S–13S

Golikov, P. A., Polumsikov, V. I. V., Davydov, B. V., Karev, V. A., Bashkatov, V. G., Belezerov, G. E., Golikov, P. P., Berestova, A. A. (1989) Lipid peroxidation and the major factor for its activation in patients with myocardial infarction. *Kardiologia* **29**: 53

Habig, W. H., Pabst, U. J., Jakoby, W. B. (1974) Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**: 7130–7139

Hertlog, M. G. L., Kromhout, D., Aravanis, C. (1995) Flavonoid intake and long term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **155**: 381–386

Johnson, D., Lardy, H. (1967) Isolation of liver or kidney mitochondria. In: Estabrook, R. W. (ed.) *Methods in enzymology*, vol. 10, Academic Press, London, pp 94–96

Katyare, S. S., Fatterparker, P., Sreenivasan, A. (1971) Effect of 2,4-dinitrophenol (DNP) on oxidative phosphorylation in rat liver mitochondria. *Arch. Biochem. Biophys.* **144**: 209–215

King, J. (1965) Phosphatases. In: *Practical clinical enzymology*. Van Nostrant Company, New Jersey, p. 191

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685

Leung, H. W., Vang, M. J., Mavis, R. D (1981) The cooperative interaction between vitamin E and vitamin C in suppression of peroxidation of membrane phospholipids. *Biochim. Biophys. Acta* **664**: 266–272

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Middleton, E., Jr (1984) The flavonoids. *Trends Pharmacol. Sci.* **5**: 334–338

Miller, N. D. (1998) Botanical influences on cardiovascular disease. *Altern. Med. Rev.* **3**: 423–430

Misra, H. P., Fridovich, I. (1972) The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**: 3170–3175

Moron, M. S., Diefierre, J. W., Mannervik, K. B. (1979) Levels of glutathione, glutathione reductase and glutathione-S-tranferase activities in rat lung and liver. *Biochem. Biophys. Acta* **582**: 67–78

Narayanan, S. (1999) Current perspectives on laboratory markers for the assessment of cardiovascular disease and myocardial damage. *Indian J. Clin. Biochem.* **14**: 117–128

Nishimura, T., Yoshida, Y., Watanabe, F., Koseki, M., Nishida, T., Tagawa, K., Kawashima, Y. (1986) Blood level of mitochondrial aspartate aminotransferase as an indicator of the extent of ischemic necrosis of the rat liver. *Hepatology* **6**: 701–707

Okinaka, S., Kumogai, H., Ebashi, S., Sugita, H., Momoi, H., Toyokura, Y. (1961) Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease. *Arch. Neurol.* **4**: 520–525

Omaye, S. T., Turnball, J. D., Sauberlich, H. E. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods Enzymol.* **62**: 3–11

Riemersma, R. A., Wood, D. A., Macintyre, C. C., Elton, R. A., Gey, K. F., Oliver, M. F. (1991) Risk of angina pectoris and plasma concentration of vitamins A, C, E and carotene. *Lancet* **337**: 1–5

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., Hoekstra, W. G. (1973) Selenium: biochemical role as a component of glutathione peroxidase purification and assay. *Science* **179**: 588–590

Sanbe, A., Tanonaka, K., Kobayasi, R., Takeo, S. (1995) Effects of long-term therapy with ACE inhibitors, captopril, enalapril and trandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. *J. Mol. Cell. Cardiol.* **27**: 2209–2222

Santos, M. T., Valles, J., Aznar, J., Vilches, J. (1980) Determination of plasma malondialdehyde-like material and its clinical implication in stroke patients. *J. Clin. Pathol.* **33**: 973–976

Shanthi, S., Parasakthy, K., Deepalakshmi, P. D., Niranjali Devaraj, S. (1994) Hypolipdemic activity of tincture of *crataegus* in rats. *Indian J. Biochem. Biophys.* **31**: 143–146

Staal, G. E. J., Visser, J., Veeger, C. (1969) Purification and properties of glutathione reductase of human erythrocytes. *Biochim. Biophys. Acta* **185**: 39–48

Whittaker, P. A., Danks, S. M. (1978) *Mitochondria structure, function and assembly*. Longman, London

Willard, T. W. (1991) The Wild Rose Scientific Herbal Wild Rose College of Natural Healing Ltd, Calgary, Alberta

Young, I. S., Purvis, J. A., Lightbody, J. H., Adyry, A. A., Trimble, E. R. (1993) Lipid peroxidation and antioxidant status following thrombolytic therapy for acute myocardial infarction. *Eur. Heart J.* **14**: 1027–1033