Indian J Physiol Pharmacol 1997; 41(3) : 369–376

EFFECT OF α-TOCOPHEROL ON LIPID PEROXIDATION IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

A. PARITHA ITHAYARASI AND C. S. SHYAMALA DEVI*

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

(Received on January 27, 1997)

Abstract : The effect of α-tocopherol pretreatment on lipid peroxidation and antioxidant status in isoproterenol induced myocardial infarction was studied in rats. Isoproterenol administered rats showed a significant increase in lipid peroxides in serum, heart and aorta. A significant increase in serum iron level with a significant decrease in iron binding capacity was also observed. The levels of antioxidants such as ceruloplasmin, glutathione and the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase decreased significantly in isoproterenol administered rats when compared to control. The activity of Na^+K^+ATPase decreased significantly and the activity of Ca^{2+}ATPase increased significantly in heart and aorta of isoproterenol administered rats. α-tocopherol pretreated rats maintained the levels of antioxidants, membrane bound enzymes and activities of antioxidant enzymes near normal, on isoproterenol administration, thus establishing its effect as an antioxidant.

Key words : α-tocopherol lipid peroxides isoproterenol myocardial infarction antioxidant enzymes

INTRODUCTION

Cardiovascular diseases including atherosclerosis and cardiac tissue injury after myocardial infarction have been shown to result due to an over-production of free radicals generated at the site of damage (1). Lipid peroxidation is a free radical mediated process and is a result of oxidative deterioration of polyunsaturated lipids (2).

Isoproterenol induced myocardial infarction serves as a well standardised model to study the beneficial effects of many drugs and cardiac function. Isoproterenol is a β-adrenergic agonist and has been reported to increase lipid peroxidation through enhanced free radical formation (3).

α-Tocopherol is a potential free radical scavenger and an effective inhibitor of the autocatalytic process of lipid peroxidation in membrane fatty acids (4). Data from human studies suggest an inverse correlation between plasma levels of vitamin E and mortality from ischaemic heart disease (5, 6).

*Corresponding Author and address : 62, II Main Road, Gandhi Nagar, Adyar, Chennai – 600 020

Since lipid peroxidation appears to be the prime cause in the genesis of myocardial necrosis, α-tocopherol, a proven antioxidant against various free radical mediated complications, has been chosen to study its effect on lipid peroxidation and activities of antioxidant enzymes during experimental myocardial infarction in rats.

METHODS

α-Tocopherol, isoproterenol, epinephrine, 1, 1', 3, 3' tetra methoxy propane, NADPH, glutathione (reduced), para phenylene diamine and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO; USA). All the other chemicals used were of analytical grade.

Adult male albino rats of Wistar strain weighing 100-120 g were used for the study. The rats were fed with commercial pelleted rat chow and given water *ad libitum*, and were maintained in clean, sterile, polypropylene cages. The rats were divided into 4 groups of 6 animals each. Group I served as control. Group II rats were administered isoproterenol (20 mg/100 g, body wt, subcutaneously, twice at an interval of 24 hrs) in sterile saline. Group III rats were given α-tocopherol (6 mg/100 g body wt, daily for a period of 90 days, orally). Group IV rats were orally administered α-tocopherol at the above mentioned dosage for 90 days and were given isoproterenol (20 mg/100 g, body weight, subcutaneously, twice at an interval of 24 hrs) at the end of treatment period.

After the experimental period the rats were killed by cervical decapitation. Blood was collected in two tubes with and without anticoagulant, from which plasma and serum was collected respectively. Heart and aorta were dissected immediately washed in ice-cold saline and homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of glutathione (GSH) (7), superoxide dismutase (SOD) (8), catalase (CAT) (9), glutathione peroxidase (GPX) (10), glutathione-S-transferase (GST) (11), glutathione reductase (GRD) (12), Na^+K^+ATPase (13), and Ca^{2+}ATPase (14). Estimation of iron (15), plasma iron binding capacity (16) and ceruloplasmin activity (17) were done by standard methods. Lipid peroxide levels were determined in serum, heart and aorta in terms of "TBA reactants" using 1, 1', 3, 3' tetramethoxy propane as the standard (18). Protein was determined by the method of Lowry et al (19).

Statistical analysis of the data was performed by applying Students 't' test.

RESULTS

Table I represents the levels of iron and ceruloplasmin in serum, plasma iron binding capacity and glutathione levels in blood of control and experimental animals. Isoproterenol administered rats showed significant increase ($P < 0.001$) in the levels of serum iron with a significant decrease in plasma iron binding capacity, ceruloplasmin and blood glutathione. α-tocopherol pretreatment maintained the levels of ceruloplasmin, serum iron, plasma iron binding capacity and blood glutathione at near normal values in group IV rats.

TABLE I : The levels of serum iron, plasma iron binding capacity, ceruloplasmin activity and blood glutathione in control and experimental animals.

(Values are expressed as mean ± SD for 6 animals in each group).

	Control	*Isoproterenol*	*α-Tocopherol*	*α-Tocopherol + Isoproterenol*
Serum iron (mcg/dl)	42.470 ± 3.6	58.62 ± 5.4***	41.320 ± 3.9^{NS}	48.14 ± 4.66*
Plasma iron binding capacity (mcg/dl)	44.3 ± 2.6	31.3 ± 2.2***	44.100 ± 3.2^{NS}	40.3 ± 2.8*
Glutathione (mg/dl)	70.25 ± 5.86	51.36 ± 4.85***	71.25 ± 6.8^{NS}	63.14 ± 4.27*
Ceruloplasmin (units/ml)	0.960 ± 0.082	0.615 ± 0.04***	0.901 ± 0.06^{NS}	0.883 ± 0.06^{NS}

Statistically significant variations when compared with control are expressed as ***P<0.001, **P<0.01, *P<0.05, ^{NS} – Nonsignificant.

The levels of lipid peroxides (as TBA reactive substances) and the activities of membrane bound Na^+K^+ATPase and Ca^{2+}ATPase in heart and aorta are presented in Table II. Rats administered isoproterenol alone, showed a significant increase ($P < 0.001$) in the levels of TBA reactive substances in serum, heart and aorta.

TABLE II : The levels of lipid peroxides and the activities of membrane bound enzymes such as Na^+K^+ATPase and Ca^{2+}ATPase in heart and aorta of control and experimental animals.

(Values are expressed as mean ± SD for 6 animals in each group).

	Control	*Isoproterenol*	*α-Tocopherol*	*α-Tocopherol + Isoproterenol*
Lipid peroxides				
Serum	2.1 ± 0.14	4.36 ± 0.32***	1.9 ± 0.13*	2.36 ± 0.18*
Heart	3.5 ± 0.28	6.27 ± 0.48***	2.96 ± 0.21*	4.12 ± 0.36*
Aorta	3.7 ± 0.27	4.31 ± 0.36***	3.49 ± 0.23^{NS}	3.91 ± 0.32^{NS}
Na^+K^+ATPase				
Heart	3.8 ± 0.28	2.61 ± 0.18***	4.05 ± 0.35^{NS}	3.38 ± 0.26*
Aorta	2.7 ± 0.23	1.8 ± 0.13***	2.8 ± 0.26^{NS}	2.3 ± 0.21*
Ca^{2+}ATPase				
Heart	2.3 ± 0.17	3.82 ± 0.31***	2.08 ± 0.16*	2.60 ± 0.18*
Aorta	2.24± 0.18	3.48 ± 0.31***	2.08 ± 0.19^{NS}	2.65 ± 0.23**

The levels of lipid peroxides is serum is represented as n moles of TBA reactants/ml. The level of lipid peroxides in heart and aorta are expressed as n moles of TBA reactants/mg protein. The activities of Na^+K^+ATPase and Ca^{2+}ATPase are expressed as μM of phosphorus liberated/hour/mg proten.

Statistically significant variations when compared with control are expressed as ***P<0.001, **P<0.01, *P<0.05, ^{NS} – Nonsignificant.

Isoproterenol administered rats showed a significant decrease in the activity of Na^+K^+ATPase and a significant increase in the activity of Ca^{2+}ATPase in heart and aorta. Group IV rats retained near normal lipid peroxide levels and the activities of membrane bound enzymes in heart and aorta on isoproterenol administration. Rats administered α-tocopherol alone showed a significant decrease ($P < 0.05$) in the lipid peroxide levels in serum and heart when compared to control.

Table III presents the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST), glutathione reductase (GRD) and glutathione in heart and aorta of control and experimental animals. Group

TABLE III : The activities of superoxide dismutase. catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and the levels of glutathione in heart and aorta of control and experimental animals.

(Values are expressed as mean ± SD for 6 animals in each group).

	Control	*Isoproterenol*	*α-Tocopherol*	*α-Tocopherol + Isoproterenol*
Superoxide dismutase				
Heart	3.84 ± 0.23	1.79 ± 0.14***	3.97 ± 0.28^{NS}	3.48 ± 0.23*
Aorta	2.95 ± 0.21	1.39 ± 0.12***	3.16 ± 0.24^{NS}	2.74 ± 0.22^{NS}
Catalase				
Heart	3.81 ± 0.28	2.78 ± 0.21***	3.91 ± 0.31^{NS}	3.62 ± 0.29^{NS}
Aorta	4.12 ± 0.36	3.48 ± 0.29**	4.41 ± 0.34^{NS}	3.87 ± 0.31*
Glutathione peroxidase				
Heart	3.64 ± 0.31	1.94 ± 0.08***	3.89 ± 0.22^{NS}	3.12 ± 0.28*
Aorta	3.13 ± 0.28	2.69 ± 0.21*	3.49 ± 0.34^{NS}	3.01 ± 0.25^{NS}
Glutathione reductase				
Heart	5.40 ± 0.43	3.70 ± 0.28***	5.6 ± 0.48^{NS}	4.77 ± 0.41*
Aorta	4.32 ± 0.38	3.67 ± 0.31**	4.8 ± 0.41^{NS}	3.86 ± 0.37^{NS}
Glutathione-S-transferase				
Heart	872.0 ± 15.2	634.0 ± 21.5***	863.0 ± 18.8^{NS}	834.0 ± 29.4*
Aorta	749.0 ± 19.0	587.0 ± 18.7***	738.0 ± 18.1^{NS}	729.0 ± 22.1^{NS}
Glutathione				
Heart	4.39 ± 0.36	2.97 ± 0.18***	4.56 ± 0.38^{NS}	3.88 ± 0.27*
Aorta	4.21 ± 0.38	3.43 ± 0.27**	4.42 ± 0.39^{NS}	3.96 ± 0.31^{NS}

Activity of superoxide dismutase is expressed as units/mg protein. Activity of catalase is expressed as a n moles of H_2O_2 decomposed/min/mg protein. Activity of glutathione peroxidase is expressed as mcg of GSH utilised/min/mg protein. Activity of glutathione-S-transferase is expressed as n moles of CDNB conjugated/min/mg protein. Activity of glutathione reductase is expressed as mcg of GSSG utilised/min/mg protein. Level of glutathione is expressed as n moles of GSH/g tissues.

Statistically significant variations when compared with control are expressed as ***P<0.001, **P<0.01, *P<0.05, ^{NS} – Nonsignificant

II rats showed a significant decrease in the activities of antioxidant enzymes when compared to control. Group IV rats restored the activities of antioxidant enzymes at near normal values.

DISCUSSION

Mc Cord et al reported an over production of reactive oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals during myocardial infarction (20). Isoproterenol induced myocardial infarction in rats has been shown to be accompanied by alterations in membrane permeability and myocardial integrity with increased Ca^{2+} overload and insufficient oxygen utilisation (21).

The significant increase in the levels of serum iron and a corresponding decrease in iron binding capacity was observed during isoproterenol treatment. During ischaemia, the free iron released from heme dependent proteins and a decreased iron binding capacity has been reported to increase prostaglandin metabolism and *in vivo* lipid peroxidation (22).

Increased mobilisation of iron from ferritin in the heart by xanthine oxidase and over production of hydroxyl radicals and superoxide radicals results in myocardial damage (23). Group IV rats retained the levels of serum iron and iron binding capacity at near normal values. α-Tocopherol being an antioxidant has been reported to prevent hemolysis (24), thereby preventing increased free radical production from the iron mobilised from ferritin.

Serum ceruloplasmin activity decreased significantly in isoproterenol administered rats. Ceruloplasmin, a copper donor and ferroxidase, donates copper ions to superoxide dismutase and act as an inhibitor of lipid peroxidation (25). α-Tocopherol restored the ceruloplasmin activity to near normal in Group IV rats thereby inhibiting the increase in lipid peroxide levels of serum.

Activated lipid peroxidation is an important pathogenic element in myocardial infarction, with lipid peroxide levels reflecting the major stages of disease and its complications (26). The significant increase observed in the levels of lipid peroxides in serum, heart and aorta of isoproterenol administered rats compared to control is in accordance with the observation of Sushma Kumari et al (3). The increased levels of TBA reactive substances indicate the excessive formation of free radicals and activation of lipid peroxidation system resulting in the irreversible damage to the heart and aorta, in animals subjected to isoproterenol stress. Hamberg et al reported an enhanced platelet aggregation following increased lipid peroxides which are usually observed in myocardial infarction (27).

α-Tocopherol pretreated - isoproterenol administered rats maintained the levels of TBA reactive substances to near normal in serum, heart and aorta when compared to control. Rats given α-tocopherol alone showed significantly lower MDA levels when compared to control. This is in accordance with the observation of Kibata M et al and Higuchi Y (28). α-Tocopherol, being a lipid-soluble chain breaking antioxidant reacts

with superoxide and lipid peroxy radicals, thereby inhibiting lipid peroxidation. α-Tocopherol being a potential anti-platelet aggregating factor, reduces the synthesis of thromboxane A_2 which is a potent platelet activating factor (29). It prevents free radical formation by lipoxygenase pathway metabolites through inhibition of phospholipase A_2 and lipoxygenase (30).

Isoproterenol treated rats showed a significant decrease in the glutathione levels and activities of glutathione reductase, glutathione peroxidase and glutathione-S-transferase in heart and aorta. The glutathione levels and activities of glutathione dependent enzymes were restored at near normal levels in Group IV rats, which were pretreated with α-tocopherol. GSH together with GSH dependent systems GPX, GST, GRD and CAT-SOD couple, efficiently scavenge toxic free radicals (31). GRD and GPX are essential for maintaining a constant ratio of reduced glutathione to oxidised glutathione in the cell. Decreased glutathione levels may be due to its increased utilisation in protecting 'SH' containing proteins from lipid peroxides. The unavailability of glutathione reduces the activity of glutathione peroxidase and transferase and is the condition seen in isoproterenol treated rats.

GSSG, the oxidised product of GSH has been reported to accumulate due to the inactivation of glutathione reductase. GSSG inactivates many enzymes containing the 'SH' group and inhibits protein synthesis (33). α-Tocopherol pretreatment has been reported to maintain the cellular thiol status (34). Isoproterenol administered rats showed a significant decrease in the activities of SOD and catalase in heart and aorta. Group IV rats retained the activities of SOD and CAT at near normal values on isoproterenol administration.

The decreased activities of superoxide dismutase and catalase during isoproterenol administration is in accordance with the observation of Manjula et al (35). During myocardial infarction, these enzymes are structurally and functionally impaired by free radicals resulting in myocardial damage (36). In Group IV rats the restoration of the activities of the antioxidant enzymes could be due to the capacity of α-tocopherol to scavenge reactive oxygen species within the lipid region of the membrane as suggested by Mc Cay et al (37). α-Tocopherol has also been reported to interact with selenium containing glutathione peroxidase to prevent the oxidative damage of membranes associated with hydroperoxides of polyunsaturated fatty acids (38).

Evidence suggest that calcium overload in the myocardial cell during ischaemia activate the Ca^{2+} dependent ATPase of the membrane depleting cell of high energy phosphate stores thereby indirectly inhibiting Na^+ and K^+ transport and inactivation of Na^+K^+ATPase (39). Isoproterenol administered rats showed a decreased activity of Na^+K^+ATPase and an enhanced Ca^{2+}ATPase activity in heart and aorta when compared to control. Inactivation of Na^+K^+ATPase could be due to enhanced lipid peroxidation by free radicals on isoproterenol treatment since Na^+K^+ATPase is a 'SH' group containing enzyme and is lipid dependent (40).

Enhanced Ca^{2+}ATPase activity in Group II rats could be due to adenylate cyclase

activation by isoproterenol. α-Tocopherol pretreatment restored the activities of Na^+K^+ATPase and Ca^{2+}ATPase to near normal on isoproterenol administration. This could be due to the ability of α-tocopherol to protect the 'SH' groups from oxidative damage through inhibition of peroxidation of membrane lipids (41). The maintenance of Ca^{2+}ATPase activity of α-tocopherol pretreatment could be mediated by a low membrane permeability of Ca^{2+} in the inward direction and by an active efflux mechanism catalysed by the active membrane-bound Na^+K^+ATPase.

The results obtained from the above study indicate that α-tocopherol pretreatment offers protection to the myocardium by preventing the inactivation of antioxidant enzymes and membrane-bound enzymes thereby inhibiting peroxidation of polyunsaturated fatty acids resulting in the maintenance of the myocardial membrane integrity and cellular thiol status. All these processes will help the myocardium to withstand the stress generated by isoproterenol and thereby prevent myocardial necrosis.

ACKNOWLEDGEMENTS

The authors (API and CSS) thank University Grants Commission for the financial support.

REFERENCES

1. Machlin LJ, Bendich A. Free radical tissue damage – Protective role of antioxidant nutrients. *FASEB J* 1987; 1: 441–445.
2. Niki E. Interaction of ascorbate and α-tocopherol. *Ann NY Acad Sci* 1987; 498; 186–199.
3. Sushmakumari S, Menon PVG. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. *Indian J Exp Biol* 1987; 27: 134–137.
4. Tappel AL. Management of and protection from *in vivo* lipid peroxidation. In "Free Radicals in Biology" by Pryor W.A. 1980, p. 147, Academic Press, New York, USA.
5. Dutta-Roy AK, Gordon MJ, Campbell FM, Duthie GG, James WPT. Vitamin E requirements, transport and metabolism : Role of α-tocopherol-binding proteins. *J Nutr Biochem* 1994; 5: 562–570.
6. Gey KF. Inverse correlation of vitamin E and ischemic heart disease. *Intl J Vitam Nutr Res* 1989; 30 : 224–231.
7. Moron MS, Bepierre JW, Mannerwick B. Levels of glutathione, glutathione reductase and glutathione-S-transferase in rat lung and liver. *Biochem Biophys Acta* 1979; 582 : 67–78.
8. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; 247 : 3170–3185.
9. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of the hydrogen peroxide by catalase. *J Biol Chem* 1952; 195 : 133–140.
10. Rotruck JT, Pope AL, Ganther H, Awanson AB, Hafeman DG, Hoeckstra WG. Selenium - biochemical role as a component of glutathione peroxidase. *Science* 1979; 179 : 588-589.
11. Habig WH, Papst MJ, Jacoby WB. Glutathione-S-transferase the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974; 249: 7130–7139.
12. Pinto PE, Bartley. The effect of age and sex on glutathione reductase and glutathione peroxidase activities on aerobic glutathione oxidation in rat liver homogenate. *Biochem J* 1969; 112 : 109–115.
13. Bonting SL. In membrane and ion transport, (Ed. Bilter, E.E.) 1970; p. 257 Wiley Interscience, London.

14. Hjerton S, Pan H. Purification and characterisation of two forms of a low affinity Ca^{2+}ATPase from erythrocyte membranes. *Biochim Biophys Acta* 1983; 728 : 281-288.

15. Ramsay WNM. Plasma iron. In Advances in Clinical Chemistry (H. Sobotka and C.P. Stewart, eds.) 1969; p 1–3 Academic Press, New York, NY USA.

16. Ramsay WNM. Ramsay's dipyridyl method for iron-binding capacity. In Practical Clinical Biochemistry (H. Varley ed) 1969; p. 475–476, Heinemann, London, UK.

17. Ravin HA. Improved-Colorimetric enzymic assay of ceruloplasmin. *J Lab Clin Med* 1961; 58: 161-168.

18. Okhawa H, Ohishi N, Yagi K. Reaction of linoleic acid hydroperoxides with thio-barbituric acids. *Anal Biochem* 1979; 95 ; 351-354.

19. Lowry OH, Rosebrough NJ, Farr A, Randall R. Protein determination with the folin reagent. *J Biol Chem* 1951; 195 : 133-140.

20. Mc Cord JM. Free radicals and myocardial ischaemia. *Free Radic Biol Med* 1988; 4 : 9-14.

21. Rona G. Catecholamine cardiotoxicity. *J Mol Cell Cardiol* 1985; 17 : 291-306.

22. Halliwell B, Gutteridge JMC. In Free Radicals in Biology and Medicine (Halliwell B and Gutteridge JMC ed.) 1989; pp. 168-176. Claredon, Oxford.

23. Biemond P, Swaak AJG, Beindroff CM, Koster JF. Superoxide dependent and independent mechanisms of iron mobilisation from ferritin by xanthine oxidase. Implications for oxygen radical destruction during ischaemia and inflammation. *Biochem J* 1986; 239 : 169-173.

24. Steven JG, Stephen AL. The effect of vitamin E on red cell hemolysis and bilirubinemia. *Ann NY Acad Sci* 1982; 393 : 315-322.

25. Siner PM, Garber P. Inactivation of the human Cu Zn superoxide dismutase during exposure to O_2 and H_2O_2. *Arch Biochem Biophys* 1981; 212 : 411-416.

26. Golikov PA, Polumiskov VIV, Davydov BV, Karev VA, Bashkatov VG, Belezerov GE, Golikov PP, Berestova AA. Lipid peroxidation and the major factor of its activation in patients with myocardial infarction. *Kardiologiia* 1989; 29 : 53-59.

27. Hamberg M, Svenson J, Wakabayashi T, Samuelssen B. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc Natl Acad Sci* 1974; 71 : 345–355.

28. Kibata M, Higuchi Y. Serum α-tocopherol, coenzyme Q and thiobarbituric acid reactive substances in acute myocardial damage and stroke. *Ann NY Acad Sci* 1982; 393 : 179–182.

29. Steiner M, Anastasi J. Vitamin E an inhibitor of platelet release reaction. *J Clin Inves* 1976; 57 : 732–737.

30. Yoshihara Y, Watanabe Y. Translocation of phospholipase A_2 from cytosol to membranes in rat brain induced by calcium ions. *Biochem Biophys Res Commun* 1990; 170 : 484–491.

31. Poliodoro G, Ilio CDi, Arduini A, Robere GLa, Federici G. Superoxide dismutase, reduced glutathione and TBA reactive products in erythrocytes of patients with multiple sclerosis. *Int J Biochem* 1984; 16 : 505–510.

32. Ferrari R, Ceconi C, Curello S, Guarnieri, Calderera CM, Albertini A, Visioli D. Oxygen mediated myocardial damage during ischaemia and reperfusion. Role of the cellular defenses against oxygen toxicity. *J Mol Cell Cardiol* 1985; 17 : 937–945.

33. Lil JL, Stantman FW, Lardy HA. Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch Biochem Biophys* 1988; 263 : 150–160.

34. Costiogliola C, Iuliano G, Menzione M, Rinaladi E, Vito P, Auricchio G. Effect of vitamin E on glutathione content in red blood cells, aqueous humor and lens of humans and other species. *Exp Eye Res* 1986; 43 : 905–914.

35. Manjula TS, Deepa R, Shyamala Devi CS. Effect of aspirin on lipid peroxidation in experimental myocardial infarction in rats. *J Nutr Biochem* 1994; 5 : 95–98.

36. Guarnieri C, Flamigni F, Calderera CM. Role of oxygen in cellular damage induced by reoxgenation of hypoxic heart. *J Mol Cell Cardiol* 1980; 12 : 797-808.

37. Mc Cay PB, King MM. Vitamin E : its role as a biologic free radical scavenger and its relationship to the microsomal mixed function oxidase system. In Vitamin E, A comprehensive treatise. L.J. Machlin, Ed. 1980; 287–317. Marcel Dekker Inc. New York, N.Y.

38. Putnam MC, Comben N. Vitamin E. *Vet Rec* 1987; 121, 541–545.

39. Fleckenstein A. In calcium antagonism in heart and smooth muscles. Experimental facts and therapeutic prospects. 1983; p. 109. Wiley Interscience Publication, NY, USA.

40. Gubdjarson S, Hallgrimson J, Skuladottir G. In Arterial Pollution (Peters, H. Gresham, G.A. & Paoetti, R.) 1983; p. 101–107. Plenum Publishing Corp, NY.

41. Meister A. Red cell invasion by the malarial parasite. *Trends Biochem Sci* 1981; 7 : 231–232.