JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1445–1451

Received March 17, 2005
Accepted June 15, 2005
DOI 10.1211/jpp.57.11.0010
ISSN 0022-3573

Dietary palm olein oil augments cardiac antioxidant enzymes and protects against isoproterenol-induced myocardial necrosis in rats

D. Narang, S. Sood, M. Thomas, A. K. Dinda and S. K. Maulik

Abstract

Wistar rats, 150–200 g, of either sex, were fed daily with commercial rat diet supplemented with palm olein oil in two doses (5% v/w (n = 16) and 10% v/w (n = 16) of diet) for 30 days. Control rats (n = 16) were fed with normal diet. On the 29th and 30th days, 8 rats from each group were administred isoproterenol (85 mg/kg, s.c., 24-h interval). On the 31st day, all rats were sacrificed and myocardial tissues were studied for thiobarbituric acid reactive substances (TBARS), antioxidant enzymes and light microscopic changes, along with the ferric-reducing ability of plasma (FRAP). A significant rise in myocardial superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activity and FRAP level were observed in rats fed with palm olein oil. Isoproterenol caused an increase in myocardial oxidative stress in control rats, as evidenced by an increase in myocardial TBARS level, reduction in FRAP and myocardial SOD, catalase and GPx activity, along with focal necrosis of cardiac muscle fibres on light microscopy. The rise in myocardial TBARS and depletion of SOD and catalase activity following isoproterenol administration were prevented in palm-olein-oil-supplemented diet-fed rats at both doses. Isoproterenol-induced myocardial light-microscopic changes were also prevented in the treated groups. The results suggest that dietary palm olein oil caused augmentation of myocardial antioxidant enzymes and protected against isoproterenol-induced myocardial necrosis and associated oxidative stress.

Introduction

Diet and nutrition have a substantial impact on reducing the incidence of ischaemic heart disease (IHD), where oxidative stress is an important aetiopathological factor (Hornstra et al 1998). Oxidative stress involves any condition where reactive oxygen species (superoxide radical, hydrogen peroxide) exert toxic effects either because of their increased production or altered metabolism (Ceconi et al 2003). Several antioxidants have been shown to possess beneficial effects in the prevention and treatment of various oxidative-stress-related disease conditions. Their beneficial role in the aetiopathogenesis of IHD has been extensively studied and the most active antioxidant enzymes involved in this process are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ferrari et al 1985; Ceconi et al 2003). In addition to this, diet constitutes an important source of exogenous antioxidants, including alpha-tocopherol or vitamin E, vitamin C and betacarotene (Diplock 1985; Sies & Murphy 1991).

Palm oil is obtained from the fruit of the tropical plant *Elaeis guineensis*. Palm olein oil, a liquid fraction obtained from the refining of palm oil, is rich in oleic acid (42.7–43.9%), betacarotene and vitamin E (tocopherols and tocotrienols). It is widely used as edible oil in many parts of the world, including India. Palm oil has been reported to have antioxidant effects in experimental hypertension (Osim et al 1996; Ganafa et al 2002) and arterial thrombosis (Pereira et al 1990). However, scientific studies on the antioxidant effects of palm olein oil in ischaemic heart disease are lacking. Recently, we have reported beneficial effects of palm olein oil against oxidative stress associated with ischaemia-reperfusion injury in the rat heart (Narang et al 2004).

Catecholamines are important regulators of myocardial contractility and metabolism. However, it has been known for a long time (Raab 1960) that excessive catechol-

Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029, India

D. Narang, S. Sood, M. Thomas, S. K. Maulik

Department of Pathology, All India Institute of Medical Sciences, New Delhi-110029, India

A. K. Dinda

Correspondence: S. K. Maulik, Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029, India. E-mail: subirmaulik@yahoo.com

Funding: The study was supported by a financial grant from the Department of Science and Technology, Ministry of Science and Technology, Government of India (Grant no. SP/SO/B-15/99).

amines cause cellular damage by depleting the energy reserve of cardiac muscle cells in clinical conditions like acute coronary insufficiency, stress cardiomyopathy (Pavin et al 1997; Ueyama et al 2003), etc. This leads to complex biochemical and structural changes that cause irreversible cellular damage, which is a prelude to necrosis (Rona 1985). Since catecholamines undergo rapid oxidation, it has been suggested that the oxidative products of catecholamines might also be responsible for myocardial changes (Yates & Dhalla 1975). Therefore, this study was designed to evaluate the effects of dietary palm olein oil on myocardial antioxidant enzymes and on oxidative stress associated with isoproterenol-induced myocardial necrosis in the rat heart.

Materials and Methods

Chemicals

All chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Sigma Chemicals (St Louis, MO). Double-distilled water (DDW) was used in all biochemical assays.

Animals

The study was approved by Institute Animal Ethics Committee (137/IAEC/01) and all animal care and experimental protocols were in compliance with the NIH guidelines for the care and use of the laboratory animals (NIH Publication no. 85-23, 1985). Laboratory-bred Wistar rats, 150–200 g, of either sex, were maintained under standard laboratory conditions of $25 \pm 2^{\circ}C$, relative humidity $50 \pm 15\%$ and a 12-h light–dark cycle. Diet and water were freely available.

Preparation of diet

Palm olein oil (registration no. RU/Edible/949; Ruchi Gold, India) was obtained from the local market. Palm olein oil contains oleic acid (42.7–43.2%) linoleic acid (4–7%), palmitic acid (45–46%) and stearic acid (3–5%), along with vitamin E (600–1000 ppm), betacarotene (400–600 ppm) and other compounds, like sterols. Palm olein oil was added to commercial rat diet (Ashirwad, India) containing: protein, 24%; fat, 5%; fibre, 4%; carbohydrates, 55%; calcium, 0.6%; phosphorus, 0.3% w/w. The diet was ground up and palm olein oil was supplemented in a dose of 5% and 10% v/w (i.e., 5 mL and 10 mL, respectively, in 100 g of diet) and was mixed uniformly. The pellets were again made and dried under shade at a temperature of $22 \pm 2^{\circ}C$ to avoid the oxidation of tocotrienols and fatty acids at high temperature. The doses were selected from previous studies (Manorma & Rukmini 1991; Ganafa et al 2002).

Experimental protocol

After one week of acclimatization, rats were randomly divided into three groups, each group containing sixteen rats. In group C (control), rats were fed with normal diet for 30 days. In groups P5 and P10, rats were fed with palm-olein-oil-supplemented commercial rat diet for 30 days in two doses (5% v/w and 10% v/w of diet). Changes in body-weight patterns of rats in all the groups were noted throughout the experimental period. On the 29^{th} and 30^{th} days, eight rats from each group were administered isoproterenol $85\,mg\,kg^{-1}$, subcutaneously (dissolved in DDW), twice, at a 24-h interval (Rona 1985) (groups: C ISO; P5 ISO; P10 ISO). At 24 h after the last injection, all rats from each group were fasted overnight (18 h), anaesthetized with pentobarbital sodium ($60\,mg\,kg^{-1}$, i.p.) and euthanised. Hearts were excised and stored in liquid nitrogen for estimation of biochemical parameters and in 10% buffered formalin (pH 7.2) for light-microscopic studies.

Experimental groups

Experimental groups were as follows: C, rats fed on a normal diet (n = 8); P5, rats fed on a diet supplemented with 5% v/w palm olein oil (n = 8); P10, rats fed on a diet supplemented with 10% v/w palm olein oil (n = 8); ISO, rats fed on a normal diet and administered subcutaneously with two doses of isoproterenol $85\,mg\,kg^{-1}$ daily at a 24-h interval (n = 8); P5 ISO, rats fed on a diet supplemented with 5% v/w palm olein oil and administered subcutaneously with two doses of isoproterenol $85\,mg\,kg^{-1}$ daily at a 24-h interval (n = 8); P10 ISO, rats fed on a diet supplemented with 10% v/w palm olein oil and administered subcutaneously with two doses of isoproterenol $85\,mg\,kg^{-1}$ daily at a 24-h interval (n = 8).

Collection of blood

Blood samples were collected from the retro orbital venous plexus on day 0 and at the time of sacrifice from all the six groups into heparinised glass tubes. Blood samples were centrifuged at 3000 *g* for 30 min and plasma was separated and stored at $-20^{\circ}C$ for the estimation of the total antioxidant capacity of plasma by the ferric-reducing ability of plasma (FRAP) assay.

Biochemical parameters

Myocardial thiobarbituric acid reactive substances (TBARS) were estimated as a marker of lipid peroxidation, along with myocardial antioxidant enzymes (e.g., superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx)). Decrease in all or some of the antioxidant enzymes, with or without rise in TBARS, was considered to indicate increase in oxidative stress. The ferric-reducing ability of plasma (FRAP) was estimated as a marker of the total antioxidant capacity of plasma.

Myocardial TBARS

The method of Ohkawa et al (1979) was followed. The heart was homogenized in 10% trichloroacetic acid (TCA) at $4^{\circ}C$. The homogenate (0.2 mL) was pipetted into a test tube, followed by addition of 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% thiobarbituric acid (TBA), and

the volume was made up to 4.0 mL with DDW. Test tubes were heated at 95°C for 60 min and then cooled. DDW (1.0 mL) and n-butanol–pyridine (15:1 v/v, 5.0 mL) was added to the test tubes and centrifuged at 4000 *g* for 10 min. The absorbance of developed colour in the organic layer was measured at 532 nm.

Data is expressed as nmol (mg protein)$^{-1}$.

Myocardial catalase

The method of Aebi (1974) was followed. The heart was homogenized at 4°C in 50 mM potassium phosphate buffer (pH 7.4) and centrifuged at 3000 *g* for 10 min. Supernatant (50 μL) was added to a 3.0-mL cuvette that contained 1.95 mL of 50 mM phosphate buffer (pH 7.0), 1.0 mL of 30 mM hydrogen peroxide was added and changes in absorbance were measured for 30 s at 240 nm at an interval of 15 s. Catalase activity is expressed as U (mg protein)$^{-1}$.

Myocardial SOD

The method of Kakkar et al (1984) was followed. The heart was homogenized in 0.25 M Tris sucrose buffer and centrifuged at 10 000 *g* for 15 min at 4°C. The supernatant was fractionated by 50% ammonium sulfate and dialysed overnight. Samples of the supernatant (100 μL) were added to sodium pyrophosphate buffer (pH 8.3), followed by addition of 0.1 mL of 186 μM phenazine methosulfate, 0.3 mL of 300 mM nitroblue tetrazolium and 0.2 mL of 780 μM reduced nicotinamide adenine dinucleotide (NADH). The reaction mixture was incubated for 90 s at 30°C and the reaction was stopped by adding 1.0 mL of glacial acetic acid. n-Butanol (4.0 mL) was then added and mixture was centrifuged at 3000 *g* for 10 min. The absorbance of the organic layer was measured at 560 nm. SOD activity is expressed as U (mg protein)$^{-1}$.

Myocardial GPx

The method of Paglia & Valentine (1967) was followed. The heart was homogenized at 4°C in 0.25 M phosphate buffer saline (pH 7.0). The homogenate was centrifuged at 15 000 *g* for 60 min at 4°C and the supernatant was assayed for GPx activity. GPx activity was measured in a 1.0-mL cuvette containing 400 μL of 0.25 M potassium phosphate buffer (pH 7.0), 200 μL of sample, 100 μL of 10 mM reduced glutathione (GSH), 100 μL of 2.5 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 100 μL of glutathione reductase (6 U mL^{-1}). Hydrogen peroxide (100 μl of 12 mM) was then added and the change in absorbance was measured at 366 nm for 5 min at an interval of 1 min. GPx activity is expressed as U (mg protein)$^{-1}$.

Ferric-reducing ability of plasma (FRAP) assay

The method of Benzie & Strain (1996) was followed. The FRAP reagent was prepared by adding 1.0 mL of 10 mM TPTZ (2,4,6 tripyridyl-s-triazine) solution in 40 mM HCl, 1.0 mL of 20 mM $FeCl_3.6H_2O$ and 10.0 mL of 0.3 M acetate buffer (pH 3.6). Freshly prepared and warm (37°C) FRAP reagent (3.0 mL) was mixed with 375 μL of DDW and 25 μL of plasma sample. The absorbance of the developed colour in the organic layer was measured at 593 nm for 5 min. The ferric-reducing activity is expressed as nmol Fe^{2+}/L.

Protein concentration was measured by the method of Bradford (1976).

Histopathological studies

Heart tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (3 μm) were cut and stained with haematoxylin and eosin (H&E) and periodic acid Schiff (PAS) reagent and were examined under a light microscope (Nikon, Japan). Histopathological study was carried out by one of the authors blinded to the groups.

Statistical analysis

All values are expressed as mean ± s.e. One-way analysis of variance followed by Bonferroni's post-hoc test was applied to test for the significance of biochemical data of the different groups. Paired Student's *t*-test was used for significance of FRAP values. Significance was set at $P < 0.05$.

Results

There was no mortality and no change in body weight pattern of rats in any group.

Changes in palm-olein-oil-supplemented diet-fed rats per-se

Myocardial TBARS level

There was no significant change in myocardial TBARS level in group P5 (7.7 ± 1.0 nmol (mg protein)$^{-1}$) and P10 (7.2 ± 0.7 nmol (mg protein)$^{-1}$) as compared with group C (5.7 ± 0.4 nmol (mg protein)$^{-1}$) (Figure 1).

Myocardial catalase activity

There was no significant change in myocardial catalase activity in group P5 (27.8 ± 1.2 U (mg protein)$^{-1}$), while a significant increase in group P10 (31.2 ± 0.7 U (mg protein)$^{-1}$; $P < 0.05$) was observed as compared with group C (26.3 ± 2.1 U (mg protein)$^{-1}$) (Figure 1).

Myocardial SOD activity

There were a significant rise ($P < 0.05$) in myocardial SOD activity in both groups P5 (13.6 ± 0.4 U (mg protein)$^{-1}$) and P10 (13.9 ± 0.4 U (mg protein)$^{-1}$) as compared with group C (10.1 ± 1.4 U (mg protein)$^{-1}$) (Figure 1).

Myocardial GPx activity

There was a significant rise in myocardial GPx activity in group P5 (0.36 ± 0.02 U (mg protein)$^{-1}$; $P < 0.05$), while no significant change in group P10 (0.27 ± 0.02 U (mg protein)$^{-1}$) was observed as compared with group C (0.30 ± 0.01 U (mg protein)$^{-1}$) (Figure 1).

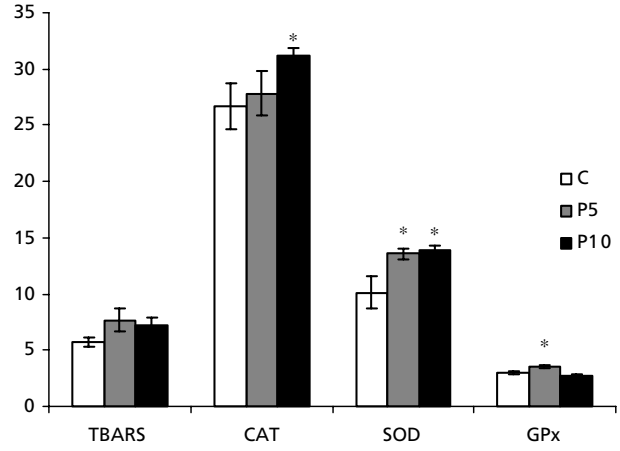

Figure 1 Effect of palm-olein-oil-supplemented diet on myocardial TBARS (nmol (mg protein)$^{-1}$), catalase (U (mg protein)$^{-1}$), SOD (U (mg protein)$^{-1}$) and GPx ($\times$10 U (mg protein)$^{-1}$) in rats. Values are expressed as mean $\pm$ s.e., n = 6. *$P < 0.05$; vs C (one-way analysis of variance followed by Bonferroni's post-hoc test).

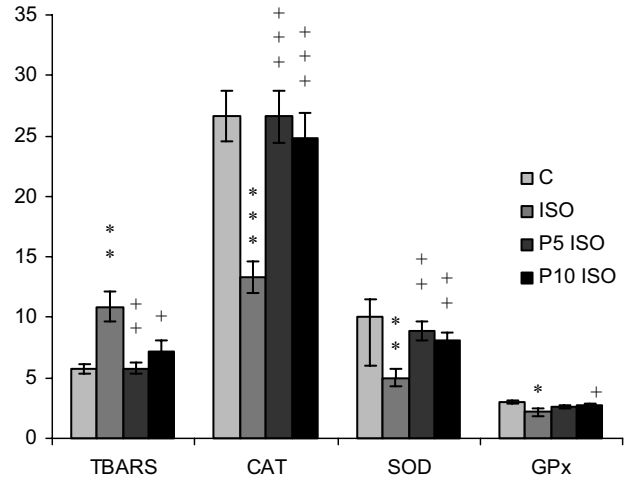

Figure 2 Effect of palm-olein-oil-supplemented diet on myocardial TBARS (nmol (mg protein)$^{-1}$), catalase (U (mg protein)$^{-1}$), SOD (U (mg protein)$^{-1}$) and GPx ($\times$ 10 U (mg protein)$^{-1}$) in rats following isoproterenol administration. Values are expressed as mean $\pm$ s.e., n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs C; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs ISO (one-way analysis of variance followed by Bonferroni's post-hoc test).

Ferric-reducing ability of plasma (FRAP) level

There was a significant ($P < 0.01$) increase in FRAP level in both groups P5 (130.1 ± 5.7 nmol $Fe^{2+}L^{-1}$) and P10 (128.8 ± 3.8 nmol $Fe^{2+}L^{-1}$) on the 31st day as compared with day 0 in groups P5 (100.7 ± 3.0 nmol $Fe^{2+}L^{-1}$) and P10 (97.7 ± 5.4 nmol $Fe^{2+}L^{-1}$) (Table 1). There was no significant change in FRAP level in group C on the 31st day (116.1 ± 4.5 nmol $Fe^{2+}L^{-1}$) as compared with day 0 (110.6 ± 4.3 nmol $Fe^{2+}L^{-1}$).

Changes in palm-olein-oil-supplemented diet-fed rat heart following isoproterenol administration

Myocardial TBARS level

There was a significant ($P < 0.01$) increase in myocardial TBARS level in the group ISO (10.9 ± 1.2 nmol (mg protein)$^{-1}$) as compared with group C (5.7 ± 0.4 nmol (mg protein)$^{-1}$) (Figure 2). The increase in myocardial TBARS level was significantly prevented by supplementation of diet with palm olein oil in both P5 ISO (5.8 ± 0.4 nmol (mg protein)$^{-1}$; $P < 0.01$) and P10 ISO groups (7.2 ± 0.8 nmol (mg protein)$^{-1}$; $P < 0.05$) as compared with group ISO.

Table 1 Changes in ferric reducing ability of plasma (FRAP) level (nmol $Fe^{2+}L^{-1}$) measured by Fe^{2+} equivalent after dietary-palm-olein-oil supplementation of rat diet at days 0 and 31

Group	Day 0	Day 31
C	110.6 ± 4.3	116.1 ± 4.5
ISO	110.6 ± 4.3	83.6 ± 4.4**
P5	100.7 ± 3.0	130.1 ± 5.7**
P5 ISO	100.7 ± 3.0	109.1 ± 2.7
P10	97.7 ± 5.4	128.8 ± 3.8**
P10 ISO	97.7 ± 5.4	113.4 ± 4.5*

All values are expressed as mean $\pm$ s.e., n = 6. *$P < 0.05$, **$P < 0.01$ vs day 0 (paired Student's *t*-test).

Myocardial catalase activity

There was a significant ($P < 0.001$) decrease in myocardial catalase activity in group ISO (13.3 ± 1.3 U (mg protein)$^{-1}$) as compared with group C (26.3 ± 2.1 U (mg protein)$^{-1}$) (Figure 2). The fall in myocardial catalase activity was significantly ($P < 0.001$) prevented by supplementation of the diet with palm olein oil in both groups P5 ISO (26.6 ± 2.2 U (mg protein)$^{-1}$) and P10 ISO (24.7 ± 2.1 U (mg protein)$^{-1}$).

Myocardial SOD activity

There was a significant decrease ($P < 0.01$) in myocardial SOD activity in group ISO (5.0 ± 0.7 U (mg protein)$^{-1}$) as compared with group C (10.1 ± 1.4 U (mg protein)$^{-1}$) (Figure 2). Supplementation of the diet with palm olein oil significantly prevented ($P < 0.01$) the fall in myocardial SOD activity in both groups P5 ISO (8.9 ± 0.8 U (mg protein)$^{-1}$) and P10 ISO (8.2 ± 0.5 U (mg protein)$^{-1}$).

Myocardial GPx activity

There was a significant ($P < 0.05$) decrease in myocardial GPx activity in group ISO (0.22 ± 0.03 U (mg protein)$^{-1}$) as compared with group C (0.30 ± 0.01 U (mg protein)$^{-1}$) (Figure 2). There was no significant change in myocardial GPx activity in group P5 ISO (0.26 ± 0.01 U (mg protein)$^{-1}$) but there was a significant ($P < 0.05$) prevention of the fall in myocardial GPx activity in group P10 ISO (0.28 ± 0.01 U (mg protein)$^{-1}$) as compared with group ISO.

Ferric-reducing ability of plasma (FRAP) level

There was a significant ($P < 0.01$) decrease in FRAP level in group ISO (83.6 ± 4.4 nmol $Fe^{2+}L^{-1}$) on the 31st day as compared with day 0 (110.6 ± 4.3 nmol $Fe^{2+}L^{-1}$) (Table 1). There was no significant change in FRAP level in group P5 ISO (109.1 ± 2.7 nmol $Fe^{2+}L^{-1}$) on the 31st day as compared with day 0 (100.7 ± 3.0 nmol $Fe^{2+}L^{-1}$), while a significant prevention ($P < 0.01$) of the fall in FRAP level in group P10 ISO was observed on the 31st day (113.4 ± 4.5 nmol $Fe^{2+}L^{-1}$) as compared with day 0 (97.7 ± 5.4 nmol $Fe^{2+}L^{-1}$).

Histopathological changes

Figure 3A shows a light micrograph of control rat heart with normal architecture. There was no light microscopic evidence of any cellular injury in hearts from rats fed with the palm-olein-oil-supplemented diet (groups P5 and P10). A significant confluent necrosis of cardiac muscle fibres with infiltration of inflammatory cells was observed in group ISO (Figure 3B). There was mild-to-moderate inflammation with occasional loss of muscle fibres in group P5 ISO (Figure 3C) and only mild inflammation in group P10 ISO without any focal necrosis of cardiac muscle fibres (Figure 3D).

Discussion

Oxidative stress plays a major role in the aetiopathogenesis of isoproterenol-induced myocardial necrosis. The metabolism of isoproterenol produces quinones, which react with oxygen to produce reactive oxygen species (ROS), leading to oxidative stress and depletion of the endogenous antioxidant system along with myocardial injury (Bors et al 1978; Rathore et al 2000).

Isoproterenol is a synthetic catecholamine widely used for experimental models of myocardial necrosis. The myocardial injury produced by isoproterenol resembles that observed in clinical conditions, such as myocardial infarction, coronary-vasospasm-induced myocardial ischaemic damage (Maseri et al 1978), some forms of sudden death described as stress cardiomyopathy (Cibllis & Hirstat 1980), stone heart syndrome (Hutchins & Silverman 1979) and concentric haemorrhagic myocardial necrosis (Rona 1985). In this study, isoproterenol caused an increase in oxidative stress in the rat heart as evidenced by a fall in myocardial endogenous antioxidant (CAT, SOD and GPx) activity, along with a rise in myocardial TBARS level and reduction in FRAP. These changes were accompanied with focal necrosis of cardiac muscle fibres and neutrophil infiltration in light microscopic studies. Similar observations about isoproterenol-induced oxidative

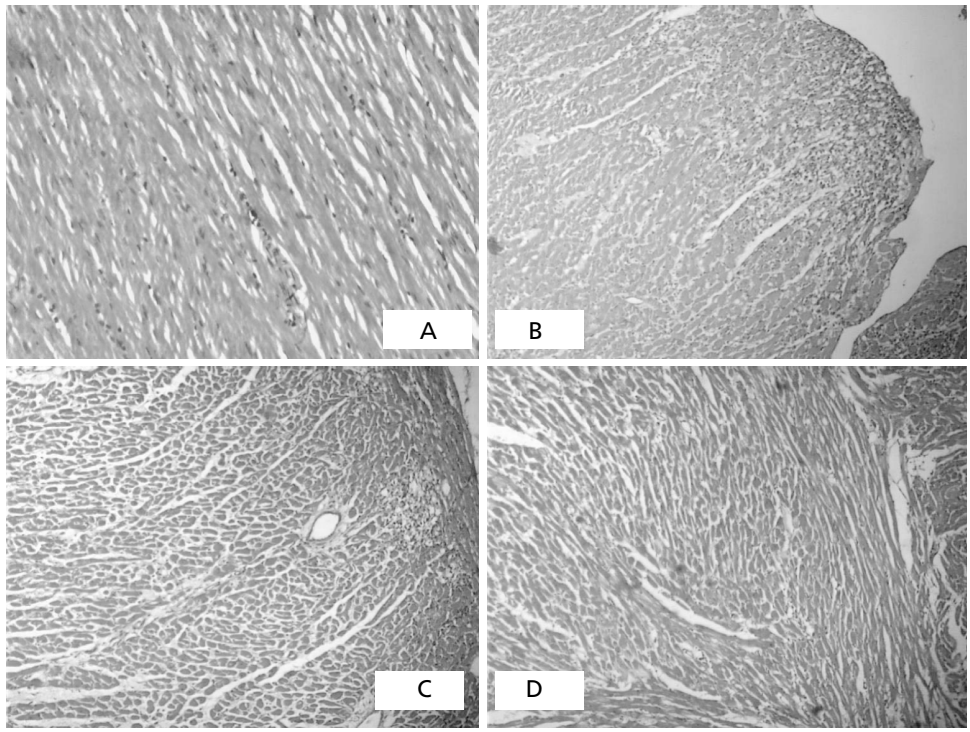

Figure 3 A. Light micrograph of control (C) rat heart showing normal architecture (×10, H&E). B. Light micrograph of rat heart following isoproterenol administration (ISO) with confluent necrosis of cardiac muscle fibres and infiltration of inflammatory cells (×10, H&E). C. Light micrograph of rat heart fed with 5% v/w of palm-olein-oil-supplemented diet following isoproterenol administration (P5 ISO) showing mild to moderate inflammation with occasional loss of muscle fibres (×10, H&E). D. Light micrograph of rat heart fed with 10% v/w of palm-olein-oil-supplemented diet following isoproterenol administration (P10 ISO) showing mild inflammation with no evidence of any focal necrosis (×10, H&E).

stress have also been reported in earlier studies (Kaul & Kapoor 1989; Ithayarasi & Devi 1997; Banerjee et al 2003). Isoproterenol-induced depletion of myocardial SOD, catalase and GPx activity, along with a rise in TBARS, as well as a reduction in FRAP, was significantly prevented by palm olein oil supplementation of the rat diet.

Augmentation of antioxidant enzymes (SOD, CAT, GPx) has been recognized as an important pharmacological property, present in natural, as well as many synthetic, compounds (Tosaki et al 1998; Gauthaman et al 2001; Rajak et al 2004). This is helpful in the therapy of disease conditions where oxidative stress plays an aetiological role. Significant rises in basal levels of FRAP and myocardial SOD, catalase and GPx activity were observed in palm-olein-oil-fed rats in this study. The most abundant reactive oxygen species generated in living systems is the superoxide radical, which is acted upon by SOD to produce hydrogen peroxide, which in turn is inactivated by catalase or GPx into water and oxygen. Thus, a simultaneous increase in catalase or GPx activity is more beneficial than an increase in SOD activity (Harman 1991; Das & Maulik 1995; Engelman et al 1995).

FRAP assesses the total antioxidant capacity of plasma, which is measured by the reduction of ferric ions to ferrous ions in the presence of water-soluble antioxidants. Since antioxidants, like vitamin E and betacarotene, present in palm olein oil, are mostly fat soluble, an increase in FRAP level in the treated rats is an interesting observation and might reflect the augmented status of antioxidant enzymes. Any acute effect of palm olein oil on FRAP was ruled out by the fact that blood for the FRAP assay was collected after overnight fasting (18 h).

A dose-dependent effect on the augmentation of myocardial antioxidant enzymes in the palm olein oil supplemented diet groups was not observed, however. Similar observations were made earlier (Baker et al 1980; Dimitrow et al 1991) and the possible mechanisms suggested are a decrease in intestinal absorption with the higher dose (Schmandke et al 1969) and newly absorbed vitamin E in part replacing the alpha-tocopherol in circulating lipoproteins (Traber et al 1998).

Hearts from rats fed on a diet supplemented with palm olein oil were protected against the rise in TBARS and depletion of antioxidant enzymes following isoproterenol administration. The observed protective effects against isoproterenol might have been mediated through augmentation of the antioxidant enzymes reserve of the heart. These salutary antioxidants effects were reflected by an absence of cellular injury in light microscopy.

The observations made in this study show important antioxidant effects of dietary palm olein oil in relation to oxidative-stress-related disease conditions. However, further studies are required to establish the mechanism underlying the augmentation of tissue antioxidants.

Conclusion

This study, for the first time, demonstrated that supplementation of the rat diet with palm olein oil caused augmentation of the antioxidant enzymes reserve of the heart, which subsequently protected against isoproterenol-induced myocardial necrosis and associated oxidative stress.

References

Aebi, H. (1974) Catalase. In: Bergmeyer, H. U. (ed.), *Methods of enzymatic analysis*. Vol. 2, Chemic Academic Press Inc., Verlag, New York, pp 673–685

Baker, H., Frank, O., DeAngelis, B., Feingold, S. (1980) Plasma tocopherols in man at various times after free and acetylated tocopherol. *Nutr. Rep. Int.* **21**: 531–536

Banerjee, S. K., Dinda, A. K., Manchanda, S. C., Maulik, S. K. (2002) Chronic garlic administration protects rat heart against oxidative stress induced by ischemic-reperfusion injury. *BMC Pharmacol.* **2**: 16

Banerjee, S. K., Sood, S., Dinda, A. K., Das, T. K., Maulik, S. K. (2003) Chronic oral administration of raw garlic protects against isoproterenol-induced myocardial necrosis in rat. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **136**: 377–386

Benzie, I. F. F., Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Ann. Biochem.* **239**: 70–76

Bors, W., Michael, C., Saran, M., Lengfelder, E. (1978) The involvement of oxygen radicals during the autooxidation of adrenaline. *Biochim. Biophys. Acta* **540**: 162–172

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254

Ceconi, C., Boraso, A., Cargnoni, A., Ferrari, R. (2003) Oxidative stress in cardiovascular disease: myth or fact? *Arch. Biochem. Biophys.* **420**: 217–221

Cibllis, M. C., Hirsta, C. S. (1980) Human stress cardiomyopathy. *Hum. Pathol.* **11**: 123–132

Das, D. K., Maulik, N. (1995) Cross talk between heat shock and oxidative stress inducible genes during myocardial adaptation to ischemia. In: Lemasters, J. L., Oliver, C. (eds) *Cell biology of trauma*. CRC Press, Boca Raton, pp 193–211

Dimitrow, N. V., Meyer, C., Gilliland, D., Ruppenthal, M., Chenoweth, W., Malone, W. (1991) Plasma tocopherols concentrations in response to supplemental vitamin E. *Am. J. Clin. Nutr.* **53**: 723–729

Diplock, A. T. (ed.) (1985) *Fat soluble vitamins. Their biochemistry and applications*. Heinemann, London, pp 154–224

Engelman, D. T., Watanbe, M., Engelman, R. M., Rousou, J. A., Kisin, E., Kagan, V., Maulik, N., Das, D. K. (1995) Hypoxic preconditoning preserves antioxidant reserve in the working rat heart. *Cardiovasc. Res.* **29**: 133–140

Ferrari, R., Ceconi, C., Curello, S., Guarnieri, C., Caldarera, C. M., Albertini, A., Visioli, O. (1985) Oxygen-mediated myocardial damage during ischaemia and reperfusion: role of the cellular defenses against oxygen toxicity. *J. Mol. Cell. Cardiol.* **17**: 937–945

Ganafa, A. A., Socci, R. R., Eatman, D., Silvestrov, N., Abukhalaf, I. K., Bayorh, M. A. (2002) Effect of palm oil on oxidative stress induced hypertension in Sprague-Dawley rats. *Am. J. Hypertens.* **15**: 725–731

Gauthaman, K., Maulik, M., Kumari, R., Manchanda, S. C., Dinda, A. K., Maulik, S. K. (2001) Effect of chronic treatment with bark of *Terminalia arjuna*: a study on the isolated ischemic-reperfused rat heart. *J. Ethnopharmacol.* **75**: 197–201

Harman, D. (1991) The aging process: major risk factor for disease and death. *Proc. Natl Acad. Sci. USA* **88**: 5360–5363

Hornstra, G., Barth, C. A., Galli, C., Mensink, R. P., Mutanen, M., Riemersma, R. A., Roberfroid, M., Salminen, K., Vansant, G., Verschuren, P. M. (1998) Functional food science and the cardiovascular system. *Br. J. Nutr.* **80** (Suppl. 1): S113–S146

Hutchins, G. M., Silverman, K. J. (1979) Pathology of the stone heart syndrome. Massive myocardial contraction band necrosis and widely patent coronary arteries. *Am. J. Pathol.* **95**: 745–752

Ithayarasi, A. P., Devi, C. S. S. (1997) Effect of α-tocopherol on lipid peroxidation in isoproterenol-induced myocardial infarction in rats. *Indian J. Physiol. Pharmacol.* **41**: 369–376

Kakkar, P., Das, B., Viswanathan, P. N. (1984) A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem. Biophys.* **21**: 130–132

Kaul, S., Kapoor, N. K. (1989) Reversal of changes of lipid peroxides, xanthine oxidase and superoxide dismutase by cardio-protective drugs in isoproterenol induced myocardial necrosis in rats. *Indian J. Exp. Biol.* **27**: 625–627

Manorma, R., Rukmini, C. (1991) Nutritional evaluation of crude palm oil in rats. *Am. J. Clin. Nutr.* **53**: 1031S–1033S

Maseri, A., Severi, S., Nes, M. D., L'Abbate, A, Chierchia, S., Marzilli, M., Ballestra, A. M., Parodi, O., Biagini, A., Distante, A. (1978) Variant angina: one aspect of a continuous spectrum of vasospastic myocardial ischemia. Pathogenetic mechanisms, estimated incidence and clinical and coronary arteriographic findings in 138 patients. *Am. J. Cardiol.* **42**: 1019–1035

Narang, D., Sood, S., Thomas, M. K., Dinda, A. K., Maulik, S. K. (2004) Effect of dietary palm olein oil on oxidative stress associated with ischemic-reperfusion injury in isolated rat heart. *BMC Pharmacol.* **4**: 29

Ohkawa, H., Ohishi, N., Yagi, K. (1979) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351–358

Osim, E. E., Owu, D. U., Etta, K. M. (1996) Arterial pressure and lipid profile in rats following chronic ingestion of palm oil diets. *Afr. J. Med. Med. Sci.* **25**: 335–340

Paglia, D. E., Valentine, W. N. (1967) Studies on the quantitative and qualified characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**: 1–58

Pavin, D., Le Breton, H., Daubert, C. (1997) Human stress cardiomyopathy mimicking acute myocardial syndrome. *Heart* **78**: 509–511

Pereira, T. A., Shahi, G. S., Das, N. P. (1990) Effects of dietary palm oil on serum lipid peroxidation, antithrombin III, plasma cyclic AMP and platelet aggregation. *Biochem. Med. Metabol. Biol.* **45**: 326–332

Raab, W. (1960) Key position of catecholamines in functional and degenerative cardiovascular pathology. *Am. J. Cardiol.* **5**: 571–578

Rajak, S., Banerjee, S. K., Sood, S., Dinda, A. K., Gupta, Y. K., Gupta, S. K., Maulik, S. K. (2004) *Emblica officinalis* causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury. *Phytother. Res.* **18**: 54–60

Rathore, N., Kale, M., John, S., Bhatnagar, D. (2000) Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat erythrocyte. *Indian J. Physiol. Pharmacol.* **44**: 161–166

Rona, G. (1985) Catecholamine cardiotoxicity. *J. Mol. Cell. Cardiol.* **17**: 291–300

Schmandke, H., Sima, C., Maune, R. (1969) Absorption of alpha-tocopherol in man. *Int. Z. Vitaminforsch.* **39**: 296–298

Sies, H., Murphy, M. E. (1991) Role of tocopherols in the protection of biological systems against oxidative damage. *J. Photochem. Photobiol.* **8**: 211–218

Tosaki, A. K., Maulik, N., Elliott, G. T., Blasig, I. E., Engelman, R. M., Das, D. K. (1998) Preconditioning of rat heart with monophosphoryl lipid A: a role of nitric oxide. *J. Pharmacol. Exp. Ther.* **285**: 1274–1279

Traber, M. G., Rader, D., Acuff, R. V., Ramakrishnan, R., Brewer, H. B., Kayden, H. J. (1998) Vitamin E dose-response studies in humans with use of deuterated RRR-alpha-tocopherol. *Am. J. Clin. Nutr.* **68**: 847–853

Ueyama, T., Senba, E., Kasamatsu, K., Yamamoto, K., Nishio, I., Tsuruo, Y., Yoshida, K. (2003) Molecular mechanism of emotional stress-induced and catecholamine-induced heart attack. *J. Cardiovasc. Pharmacol.* **41** (Suppl 1): S115–S118

Yates, J. C., Dhalla, N. S. (1975) Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol. *J. Mol. Cell. Cardiol.* **7**: 807–816