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Protective effect of propyl gallate against myocardial oxidative stress-induced injury in rat

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Abstract

This study was designed to investigate the effect of chronic administration of propyl gallate on myocardial oxidative stress-induced injury. Propyl gallate was administered orally to Wistar albino rats (150–200 g) in three different doses, by gastric gavage (250 mg kg^{-1} (P1), 500 mg kg^{-1} (P2) and 750 mg kg^{-1} (P3)), 6 days a week for 5 weeks. At the end of this period, all the rats, except the normal untreated rats that served as the control group, were administered isoproterenol (ISO), 85 mg kg^{-1} subcutaneously, for 2 consecutive days to induce myocardial injury. After 48 h, rats (n = 6 per group) were anaesthetized with anaesthetic ether, sacrificed and the hearts were harvested for the estimation of thiobarbituric acid reactive substances (TBARS), endogenous antioxidants (reduced glutathione (GSH), superoxide dismutase (SOD) and catalase) and for the assessment of histological changes. In the P2 BL group (BL = baseline), there was a significant ($P < 0.001$) rise in baseline TBARS and SOD when compared with the saline-treated group, while no such changes were observed in the other baseline-treated groups. However, there was a significant ($P < 0.001$) increase in TBARS and endogenous antioxidants (GSH, SOD and catalase) in the P2 ISO and P3 ISO groups, when the hearts were subjected to in-vivo myocardial oxidative stress-induced injury. We observed no such changes in the P1 ISO group. This study showed that propyl gallate modulates the levels of endogenous antioxidants present at the myocardial site. Whether these modifications are a result of direct interference at this site or a remote effect is not immediately clear. In conclusion, from the results it could be stated that chronic administration of 500 mg kg^{-1} of propyl gallate offers significant protection against myocardial oxidative stress-induced injury.

Introduction

Ischaemic heart disease (IHD) has emerged as a major world health problem and it is predicted that by the year 2020 this disease will persist as the major and the most common threat to human life (Anonymous 1997). The pathogenic mechanism of myocardial ischaemic damage is still not completely understood, but the role of oxygen-derived free radicals (OFRs) in myocardial ischaemia has been established, although not completely characterized. Pathological free-radical production in ischaemia or hypoxia may mainly result from reflow or reoxygenation (McCord 1985). Vigorous global research is underway in an effort to develop pharmacological means to control morbidity and mortality arising from IHD. As far as the aetiological factors are concerned, presently, oxidative stress enjoys the widest scientific popularity (Neelam et al 1995; Karthikeyan et al 2003), which plays a major role in causing this cardiac failure, alone or in conjunction with several other equally important factors. While much research has been done on this topic, there is no clear understanding of the underlying causal factors associated with injury following an ischaemic episode (Sabari et al 2002).

Recently, there has been renewed interest in medicinal plants and food products derived from medicinal plants that have been found to have certain preventive measures in the treatment of cardiovascular disease. Propyl gallate, a major component of many medicinal plants, has been reported to have antioxidant properties (Lepran & Lefer 1985; Bhatnagar 1995; Lin et al 2000). A high degree of in-vivo and in-vitro OFR scavenging activity of the different forms of gallates, such as epigallocatechin gallate, has been

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documented (Ajay et al 2003; Buttemeyer et al 2003; Furukawa et al 2003). This might be instrumental in their cardioprotective effects.

Based on the antioxidant properties of gallates, this study in rats was designed to investigate the effect of chronic administration of propyl gallate on endogenous antioxidant levels and on oxidative stress arising out of isoproterenol administration.

Materials and Methods

Experimental procedure

Male Wistar albino rats, 120–150 g, were obtained from the Tamil Nadu Veterinary and Animal Science University, Chennai, India, and were housed at 25 ± 5°C in a well-ventilated animal house under a 12-h light–dark cycle. All protocols were approved by the local standing committee and authority on animal research and the experiments were carried out according to the guidelines of the animal ethics committee of the institute (IAEC No: 01/00/02). Rats were divided into three groups and were administered propyl gallate (Sigma Chemical Co., St Louis, MO) in three doses (250 mg kg^{-1} (P1), 500 mg kg^{-1} (P2) and 750 mg kg^{-1} (P3)) in saline or were administered vehicle (saline) alone, by oral gavage, 6 days a week for 5 weeks. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Bangalore, India; containing protein 22.06%, oil 4.28%, fibre 3.02%, ash 7.8%, sand (silica) 1.37% w/w) and water. There were no significant differences in the body weights of the treated rats when compared with control, either at the beginning or at the end of the study period. The treated rats did not offer any abnormal resistance to drug administration. The treatment schedule did not cause any change in food and water intake pattern. Forty-eight hours after the last dose, rats were heparinized (375 U/200 g, i.p.) (Neely et al 1972), anaesthetized with ether, sacrificed and subjected to any one of the protocols.

Protocol I

Hearts from six rats in each group were harvested and stored in liquid nitrogen for the estimation of basal endogenous antioxidants and in 10% buffered formalin for histological studies.

The groups studied were: Group C, vehicle-treated rats (saline); Group P1 BL, rats treated with 250 mg kg^{-1} of propyl gallate; Group P2 BL, rats treated with 500 mg kg^{-1} of propyl gallate; Group P3 BL, rats treated with 750 mg kg^{-1} of propyl gallate.

Protocol II

At the end of the treatment period six rats from each group were administered isoproterenol (ISO), receiving one subcutaneous injection of 85 mg kg^{-1} daily for 2 consecutive days to induce myocardial injury (Rona et al 1959; Seth et al 1998). Forty-eight hours after the first dose of isoproterenol, the rats were sacrificed; hearts were harvested and immediately frozen in liquid nitrogen for biochemical estimations and in 10% buffered formalin for histological studies.

Groups were as follows: Group C, rats treated with vehicle + saline; Group ISO, rats treated with vehicle + ISO; Group P1 ISO, rats treated with propyl gallate 250 mg kg^{-1} + ISO; Group P2 ISO, rats treated with propyl gallate 500 mg kg^{-1} + ISO; Group P3 ISO, rats treated with propyl gallate 750 mg kg^{-1} + ISO.

Biochemical parameters

Myocardial thiobarbituric acid reactive substances

Myocardial thiobarbituric acid reactive substances (TBARS) were determined by a modified version of the method described by Ohkawa et al (1979). Rat hearts were homogenized in 10% trichloroacetic acid (TCA) at 4°C. Homogenate (0.2 mL) was pipetted into a test tube, followed by the addition of 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). Tubes were boiled for 60 min at 95°C and then cooled on ice. Double-distilled water (1.0 mL) and 5.0 mL of *n*-butanol–pyridine (15:1 v/v) mixture were added to the tubes and centrifuged at 4000 *g* for 10 min. The absorbance of the developed colour in the organic layer was measured at 532 nm. TBARS levels were determined from the standard curve of TBA adduct formation when various concentrations of commercially available 1,1,3,3-tetraethoxypropane were subjected to the above procedure. Data are expressed as nmol (g wet weight)$^{-1}$.

Myocardial reduced glutathione

Myocardial reduced glutathione (GSH) was estimated by the method of Ellman (1959). The rat hearts were homogenized with 10% TCA and centrifuged at 3000 *g* for 10 min. The reaction mixture contained 0.1 mL of supernatant, 2.0 mL of 0.3 M phosphate buffer (pH 8.4), 0.4 mL of double-distilled water and 0.5 mL of 5,5 dithiobis 2-nitrobenzoic acid (DTNB). The reaction mixture was incubated for 10 min and the absorbance was measured at 412 nm. The level of reduced GSH was determined from the standard curve of DTNB's reaction with commercially available GSH (Sigma Chemical Co.). Data are expressed as μg (g wet weight)$^{-1}$.

Myocardial superoxide dismutase

Superoxide dismutase (SOD) levels in the hearts were determined by the method of McCord & Firdovich and modified by Kakkar et al (1984). The hearts were 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, mixed vigorously and the reaction mixture was incubated at 4°C for 4 h. After incubation, the samples were centrifuged at 10 000 *g* for 30 min at 4°C; the supernatant was then kept overnight for dialysis in 0.0025 M Tris–HCl buffer. The supernatant (100 μL) was 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 NADH. The reaction mixture was incubated for 90 s at 30°C, the reaction was stopped by adding 1.0 mL of acetic acid, 4.0 mL of *n*-butanol was then added

and centrifuged at 3000 *g* for 10 min. The absorbance of the organic layer was measured at 560 nm. SOD levels were determined by the standard curve obtained using known concentrations of commercially available SOD (Sigma Chemical Co.), subjected to the above treatment. Data are expressed as IU (mg protein)$^{-1}$.

Myocardial catalase

Catalase was estimated by the method described by Aebi (1974). Hearts were homogenized at 4°C (1:10) 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). Then 1.0 mL of 30 mM hydrogen peroxide was added and changes in absorbance were followed for 30 s at 240 nm at an interval of 15 s. Catalase levels were determined by the standard curve obtained using known concentrations of commercially available catalase (Sigma Chemical Co.), subjected to the above treatment. Catalase levels are expressed as IU (mg protein)$^{-1}$.

Estimation of protein

Tissue protein was estimated by the method of Bradford (1976). Rat hearts were homogenized at 4°C (1:10) in 50 mM potassium phosphate buffer (pH 7.4) and centrifuged at 3000 *g* for 10 min. The supernatant (2 μL) was made up to 20 μL with double-distilled water, 50 μL of 0.1 M NaOH and 1 mL of Bradford reagent were added, vortexed and kept for 10 min, and the absorbance was measured at 595 nm. Protein levels were determined by the standard curve obtained using known concentrations of commercially available bovine serum albumin (Sigma Chemical Co.), subjected to the above treatment.

Histological examination

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

Statistical analysis

All values are expressed as mean ± s.e. One-way analysis of variance was applied to test for significance of biochemical data of the different groups. Significance is set at $P < 0.001$.

Results

Baseline changes

Baseline changes brought about by treatment of rats for 30 days with either propyl gallate (250, 500 or 750 mg kg^{-1}) or vehicle alone are presented in Table 1.

The myocardial baseline TBARS level was not significantly changed in the P1 BL and P3 BL groups compared with group C. However, in the P2 BL group there was a significant rise ($P < 0.001$) in the level of myocardial baseline TBARS compared with group C.

Compared with group C: myocardial baseline GSH levels were not significantly altered in the P1 BL, P2 BL or P3 BL groups; myocardial baseline SOD levels were significantly ($P < 0.001$) increased in the P2 BL group but not in the P1 BL and P3 BL groups; and myocardial baseline catalase levels were not significantly changed in the P1 BL, P2 BL and P3 BL groups.

Oxidative stress-induced changes

The results obtained in the different groups subjected to in-vivo myocardial oxidative stress-induced injury are presented in Table 1.

Myocardial TBARS levels in the ISO group were significantly ($P < 0.001$) higher than in the group C. In the P1 ISO group the levels were not significantly different to

Table 1 Effect of propyl gallate on TBARS, GSH, SOD and catalase in rat heart

Parameters	C	ISO	P1 BL	P1 ISO	P2 BL	P2 ISO	P3 BL	P3 ISO
TBARS (nmol (g wet weight)$^{-1}$)	45.9 ± 3.9	70.3 ± 4.9*	48.3 ± 5.8	73.6 ± 2.5	57.9 ± 3.1*	52.6 ± 5.6$^{\#}$	46.9 ± 3.4	59.2 ± 6.4$^{\#}$
GSH (μg (g wet weight)$^{-1}$)	327.0 ± 18.1	164.9 ± 20.8*	316.0 ± 16.7	135.2 ± 16.9	336.2 ± 18.4	332.8 ± 16.9$^{\#}$	335.1 ± 17.2	298.6 ± 12.6$^{\#}$
SOD (IU (mg protein)$^{-1}$)	2.5 ± 0.3	1.6 ± 0.4*	2.3 ± 0.3	1.9 ± 0.1	3.7 ± 0.2*	5.9 ± 0.5$^{\#}$	2.4 ± 0.2	3.0 ± 0.4$^{\#}$
CAT (IU (mg protein)$^{-1}$)	45.4 ± 4.2	25.8 ± 5.2*	46.5 ± 2.4	24.8 ± 3.6	46.1 ± 3.7	46.4 ± 4.3$^{\#}$	48.2 ± 1.4	47.5 ± 3.4$^{\#}$

All values are expressed as mean ± s.e., n = 6. *$P < 0.001$ vs C; $^{\#}P < 0.001$ vs ISO (one-way analysis of variance). Rats received the following treatments: Group C, vehicle (saline); Group ISO, vehicle and 85 mg kg^{-1} isoproterenol; Group P1 BL, 250 mg kg^{-1} of propyl gallate; Group P1 ISO, 250 mg kg^{-1} propyl gallate and 85 mg kg^{-1} isoproterenol; Group P2 BL, 500 mg kg^{-1} of propyl gallate; Group P2 ISO 500 mg kg^{-1} propyl gallate and 85 mg kg^{-1} isoproterenol; Group P3 BL, 750 mg kg^{-1} of propyl gallate; Group P3 ISO, 750 mg kg^{-1} propyl gallate and 85 mg kg^{-1} isoproterenol. Vehicle or saline was administered by oral gavage, at the indicated dosage, 6 days a week for 5 weeks; isoproterenol was then given, at the indicated dosage, by subcutaneous injection once daily for two days.

those in the ISO group, although there was a significant ($P < 0.001$) fall in the TBARS levels in the P2 ISO and P3 ISO groups.

The myocardial GSH level was significantly ($P < 0.001$) lower in the ISO group than in group C. In the P1 ISO group, the myocardial GSH levels were not significantly different to those in the ISO group. However, there was a significant rise ($P < 0.001$) in the levels of myocardial GSH in the P2 ISO and P3 ISO groups compared with the ISO group.

Myocardial SOD levels were significantly lower ($P < 0.001$) in the ISO group than in group C. In the P1 ISO group, myocardial SOD levels were not significantly different to those in the ISO group. However, there was a significant rise ($P < 0.001$) in the levels of myocardial SOD in the P2 ISO and P3 ISO groups compared with the ISO group.

The myocardial catalase level was significantly lower ($P < 0.001$) in the ISO group than in group C. There was no significant difference in myocardial catalase level between the P1 ISO and the ISO group. However, the level of myocardial catalase was significantly higher ($P < 0.001$) in the P2 ISO and P3 ISO groups than in the ISO group.

Histological changes

Light microscopy of the tissue sections taken from group C rat hearts showed a normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils (Figure 1A). Group ISO tissues showed oedema, focal haemorrhage and leucocyte infiltration with fragmentation of muscle fibres suggestive of necrosis (Figure 1B). The tissue sections from all baseline groups, P1 BL, P2 BL and P3 BL, showed normal myofibrillar structure with some leucocyte infiltration and continuity with adjacent myofibrils (Figures 2A, 3A, 4A). The tissue sections of all treated groups, P1 ISO, P2 ISO and P3 ISO, showed some leucocyte infiltration, mild oedema and some discontinuity with adjacent myofibrils but the morphology of cardiac muscle fibres was relatively well preserved with no evidence of focal necrosis (Figures 2B, 3B, 4B).

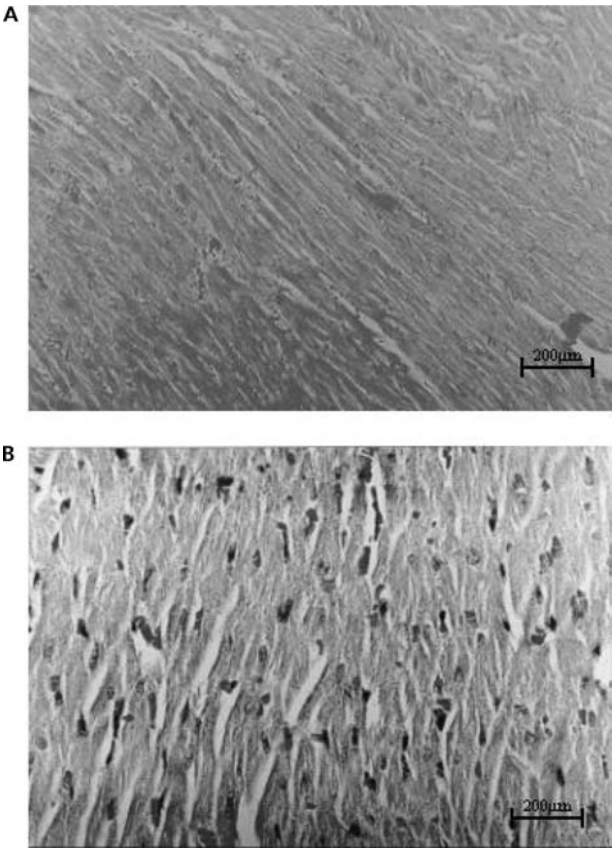

Figure 1 A. Haematoxylin and eosin (× 400) stained microscopic section of group C rat heart showed normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils. Group C rats received saline by oral gavage 6 days a week for 5 weeks. B. Haematoxylin and eosin (× 400) stained microscopic section of group ISO rat heart showed oedema, focal haemorrhage and leucocyte infiltration with fragmentation of muscle fibres, suggestive of necrosis. Group ISO rats received saline by oral gavage 6 days a week for 5 weeks and then isoproterenol 85 mg kg^{-1} by subcutaneous injection once daily for two days.

Discussion

Propyl gallate is used extensively as an antioxidant in fats and oils to prevent rancidity and spoilage. Experimentally, propyl gallate has been shown to protect against free radical injury in-vitro in lung reperfusion models and when given as a dietary supplementation in in-vivo studies (Bhatnagar 1993; Bains et al 1996). Propyl gallate, a synthetic polyhydric phenol, is a member of the gallic acid ester family and has the capacity to act as a free radical scavenger in biological systems. It is a good scavenger of electrophilic radicals and $O_2^{-\cdot}$ (Ponti et al 1978). Propyl gallate protects against CCl_4-induced liver injury by inhibiting the microsomal enzyme system and lipid peroxidation processes (Torrielli & Ugazio 1975). It inhibits lipoxygenase products such as leukotrienes generated via arachidonic acid oxidation (Lepran & Lefer 1985). It is amphipathic and can readily accumulate near the surface of cell membranes and is thought to prevent the oxidation of α-tocopherols (Terao et al 1994). Propyl gallate compounds have been shown to scavenge oxyradicals such as $O_2^{-\cdot}$ and $^{\cdot}OH$ formed enzymatically from xanthine oxidase and non-enzymatically (Wu et al 1994). These biochemical functions may explain, in part, its cardioprotective action seen in this study.

Studies on the antioxidant changes and their significance during heart injury have provided a new insight into the pathogenesis of heart disease. Interestingly, in this study, there was a significant increase in the basal levels of myocardial TBARS on administration of propyl gallate, at a dosage of 500 mg kg^{-1} (P2 BL). It is generally accepted that OFRs are key mediators associated with myocardial oxidative stress-induced injury (Maxwell et al 1987; Jennings et al 1995). The increase in TBARS is indicative of an enhanced oxidative stress, which, in the

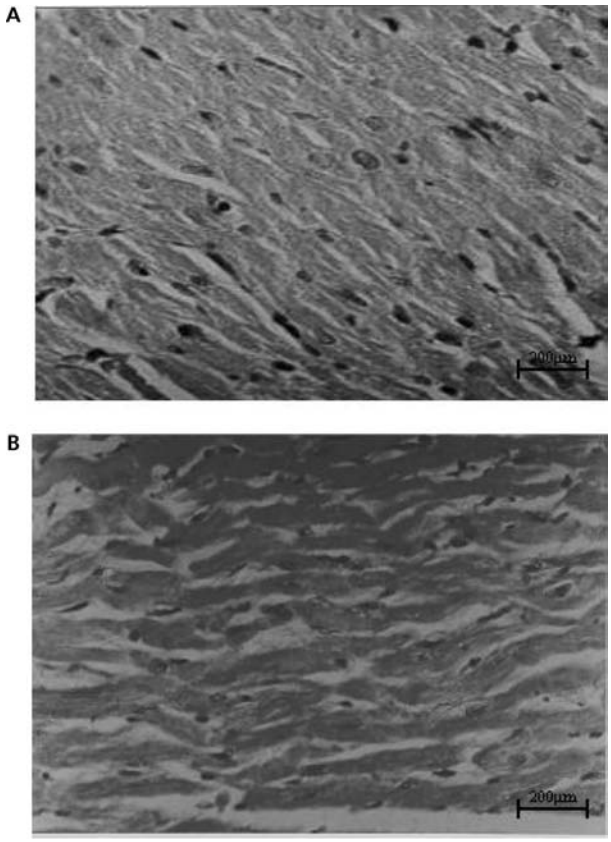

Figure 2 A. Haematoxylin and eosin (× 400) stained microscopic section of group P1 BL rat heart. Group P1 BL rats received propyl gallate 250 mg kg^{-1} by oral gavage 6 days a week for 5 weeks. B. Haematoxylin and eosin (× 400) stained microscopic section of group P1 ISO rat heart. Group P1 ISO rats received propyl gallate 250 mg kg^{-1} by oral gavage 6 days a week for 5 weeks and then isoproterenol 85 mg kg^{-1} by subcutaneous injection once daily for two days.

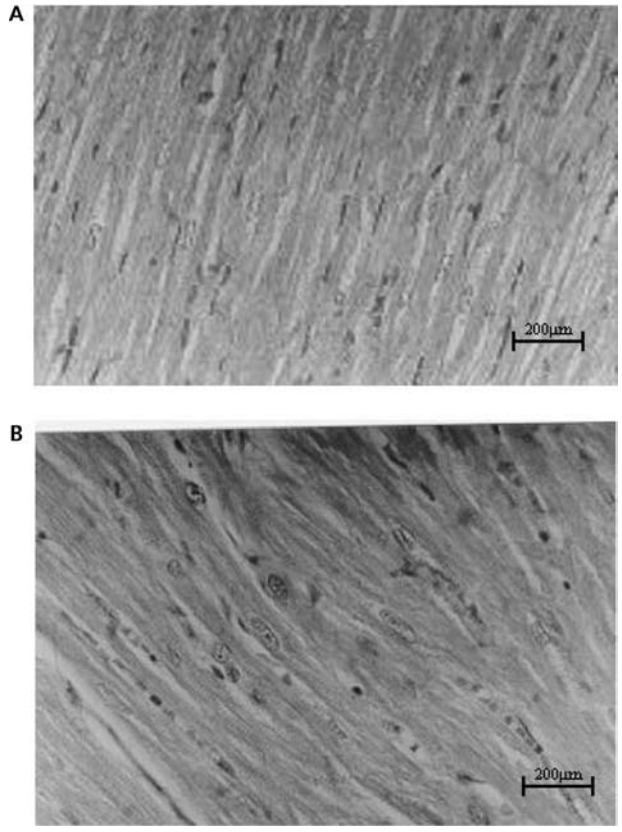

Figure 3 A. Haematoxylin and eosin (× 400) stained microscopic section of group P2 BL rat heart. Group P2 BL rats received propyl gallate 500 mg kg^{-1} by oral gavage 6 days a week for 5 weeks. B. Haematoxylin and eosin (× 400) stained microscopic section of group P2 ISO rat heart. Group P2 ISO rats received propyl gallate 500 mg kg^{-1} by oral gavage 6 days a week for 5 weeks and then isoproterenol 85 mg kg^{-1} by subcutaneous injection once daily for two days.

absence of any cellular injury (as evidenced by histological studies), is considered to be non-lethal. Previously it has been reported that myocardial adaptation occurs in response to various kinds of obnoxious stimuli, such as ischaemia (Lawson et al 1993), certain endotoxins (Maulik et al 1995) and reactive oxygen species (Sun et al 1996; Karthikeyan et al 2003), and protects the heart from subsequent exposure to injuries of similar or more severe nature (Li et al 1990; Asimakis et al 1992; Singal et al 1993; Nilanjana et al 1998; Banerjee et al 2002). However, no significant change in baseline GSH and catalase was noticed in propyl- gallate-treated groups, although a significant rise in SOD activity was seen in group P2 BL. The mechanism of increase in SOD could possibly be oxidative stress induced (de Zwart et al 1999). It is, therefore, possible that the increase in oxidative stress was non-lethal and might be responsible for cellular adaptive mechanisms, leading to increased synthesis of endogenous antioxidants.

In this study, when the rats pre-treated with propyl gallate were subjected to myocardial oxidative stress-induced injury, there was a significant decrease in TBARS in groups P2 ISO and P3 ISO and the levels of the endogenous antioxidants, namely GSH, SOD and catalase, were preserved at normal levels in the P2 ISO and P3 ISO groups. One reason why the exogenous antioxidant systems have limited success in the prevention of myocardial oxidative stress-induced injury may be due to the inaccessibility of large molecules to key intracellular sites of oxidative damage (Halliwell 2000), although the results of this study showed that propyl gallate modulates the levels of endogenous antioxidants present at this site. Whether these modifications are a result of direct interference at this site or a remote effect is not immediately clear from this study. On the other hand, with the P1 ISO treatment group (250 mg kg^{-1}) there was no such protection by propyl gallate and the exact mechanism(s) of such a loss of protective effect is not clearly understood from this study.

Conclusion

This study showed that pre-treatment of rats with 500 mg kg^{-1} of propyl gallate offers significant protection against myocardial oxidative stress-induced injury. Thus, propyl gallate has been proved to be beneficial in preventing

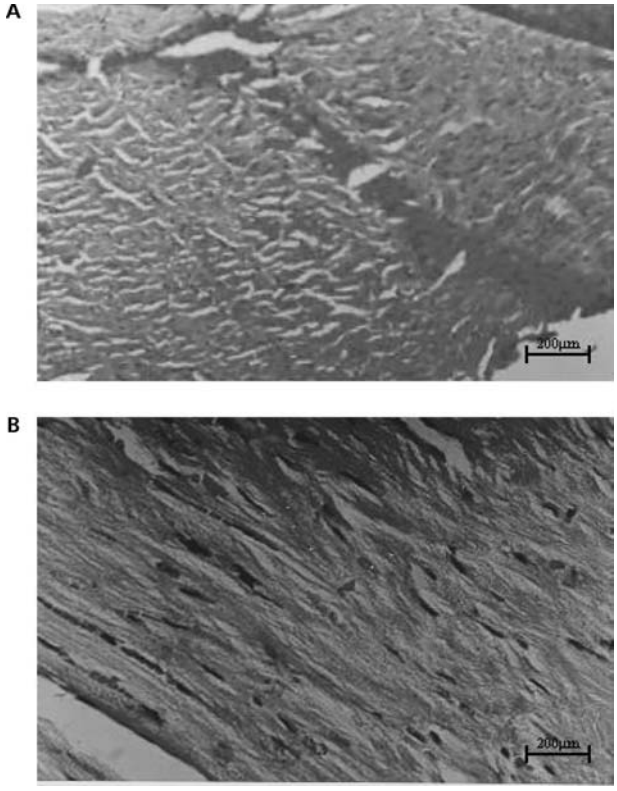

Figure 4 A. Haematoxylin and eosin (× 400) stained microscopic section of group P3 BL rat heart. Group P3 BL rats received propyl gallate 750 mg kg^{-1} by oral gavage 6 days a week for 5 weeks. B. Haematoxylin and eosin (× 400) stained microscopic section of P3 ISO rat heart. Group P3 ISO rats received propyl gallate 750 mg kg^{-1} by oral gavage 6 days a week for 5 weeks and then isoproterenol 85 mg kg^{-1} by subcutaneous injection daily for two days.

cardiovascular disorders. Further studies are needed to elucidate the exact mechanism(s) of action of this compound in the prevention and treatment of cardiovascular disease.

References

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

Ajay, M., Gilani, A. U., Mustafa, M. (2003) Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. *Life. Sci.* **74**: 603–612

Anonymous (1997) From what will we die in 2020? *Lancet* **349**: 1263

Asimakis, G. K., Inners-McBride, K., Medellin, G., Conti, V. R. (1992) Ischemic preconditioning attenuates acidosis and post-ischemic dysfunction in isolated rat heart. *Am. J. Physiol.* **263**: 887–894

Bains, J. S., Kakkar, R., Sharma, S. P. (1996) Gender specific alterations in antioxidant status of aging Zaprionus paravittiger fed on propyl gallate. *Biochem. Mol. Biol. Int.* **40**: 731–740

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

Bhatnagar, A. (1995) Attenuation of reperfusion injury by the antioxidant n-propyl gallate. *J. Cardiovasc. Pharmacol.* **26**: 343–347

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.* **7**: 248–254

Buttemeyer, R., Philipp, A. W., Schlenzka, L., Beissenhirtz, M., Lisdat, F. (2003) Epigallocatechin gallate can significantly decrease free oxygen radicals in the reperfusion injury *in vivo*. *Transplant. Proc.* **35**: 3116–3120

de Zwart, L. L., Meerman J. H., Commandeur, J. N., Vermeulen, N. P. (1999) Biomarkers of free radical damage: applications in experimental animals and in humans. *Free Radic. Biol. Med.* **26**: 202–226

Ellman, G. L. (1959) Tissue sulphydryl groups. *Arch. Biochem. Biophys.* **82**: 70–77

Furukawa, A., Oikawa, S., Murata, M., Hiraku, Y., Kawanishi, S. (2003) Epigallocatechin gallate causes oxidative damage to isolated and cellular DNA. *Biochem. Pharmacol.* **66**: 1769–1778

Halliwell, B. (2000) The antioxidant paradox. *Lancet* **355**: 1179–1180

Jennings, R. B., Steenbergen, C., Reimer, K. A. (1995) Myocardial ischemia and reperfusion. *Monogr. Pathol.* **37**: 47–80

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

Karthikeyan, K., Sarala Bai, B. R., Gauthaman, K., Sathish, K. S., Niranjali Devaraj, S. (2003) Cardioprotective effect of the alcoholic extract of *Terminalia arjuna* bark in an *in vivo* model of myocardial ischemic reperfusion injury. *Life. Sci.* **73**: 2727–2739

Lawson, C. S., Coltart, D. J., Hearse, D. J. (1993) The antiarrhythmic action of ischemic preconditioning in rat heart does not involve functional GI proteins. *Cardiovasc. Res.* **27**: 681–687

Lepran, I., Lefer, A. M. (1985) Protective actions of propyl gallate: a lipoxygenase inhibitor, on the ischemic myocardium. *Circulation* **15**: 79–88

Li, G. C., Vasquez, B. S., Gallagher, K. P., Lucchesi, B. R. (1990) Myocardial protection with preconditioning. *Circulation* **82**: 609–619

Lin, J. K., Chen, P. C., Ho, C. T., Lin-Shiau, S. Y. (2000) Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by the flavin-3, 3′-digallate, -epigallocatechin-3-gallate and propylgallate. *J. Agric. Food Chem.* **48**: 2736–2743

Maulik, N., Watanabe, M., Engelman, D. T., Engelman, R. M., Kagan, V. E., Kisin, E., Tyrin, V., Cordis, G. A., Das, D. K. (1995) Myocardial adaptation to ischemia by oxidative stress-induced by endotoxin. *Am. J. Physiol.* **38**: C907–C916

Maxwell, M. P., Hearse, D. J., Yellon, D. M. (1987) Species variation in the coronary collateral circulation during regional myocardial ischemia: a critical determination of the rate of evolution and extent of myocardial infarction. *Cardiovasc. Res.* **21**: 737–746

McCord, J. M. (1985) Oxygen-derived free radicals in post ischemic tissue injury. *N. Engl. J. Med.* **312**: 159–163

Neelam, S., Arvindar, K. D., Charita, S., Pawan, K. S. (1995) Oxidative stress and heart failure. *Mol. Cell. Biochem.* **47**: 77–81

Neely, J. R., Denton, R. M., England, P. J., Randle, P. J. (1972) The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in the perfused rat heart. *Biochem. J.* **1**: 147–159

Nilanjana, M., Motoaki, S., Brenda, D. P., Dipak, K. D. (1998) An essential role of NFκB in tyrosine kinase signaling of P38

MAP kinase regulation of myocardial adaptation to ischemia. *FEBS Lett*. **429**: 365–369

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

Ponti, V., Dianzani, M. U., Cheeseman, K., Slater, T F. (1978) Studies on the reduction of nitroblue tetrazolium chloride mediated through the action of NADH and phenazine methosulphate. *Chem.-Biol. Interact*. **23**: 281–291

Rona, G., Chappel, C. T., Balazs, T., Gaundry, R. (1959) An infarct like myocardial lesion and other true manifestation produced by isoproterenol in the rat. *Arch. Pathol*. **67**: 443–455

Sabari, D., Dinesch, Y., Rajiv, N., Nibhriti, D. (2002) Interrelation between lipid peroxidation, ascorbic acid and superoxide dismutase in coronary artery disease. *Curr. Sci*. **83**: 488–491

Seth, S. D., Maulik, M., Katiyar, C. K., Maulik, S. K. (1998) Role of lipistat in protection against isoproterenol induced myocardial necrosis in rats: A biochemical and histopathological study. *Indian. J. Pharmacol*. **42**: 101–106

Singal, P. K., Dhalla, A. K., Hill, M., Thomas, T. P. (1993) Endogenous antioxidant changes in the myocardium in response to acute and chronic stress conditions. *Mol. Cell Biochem*. **129**: 179–186

Sun, J. Z., Tang, X. L., Park, S. W., Qin, Y., Turrent, J. F., Bolli, R. (1996) Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pig. *J. Clin. Invest*. **97**: 562–576

Terao, J., Piskula, M., Yao, Q. (1994) Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys*. **308**: 278–284

Torrielli, M. V., Ugazio, G. (1975) Biochemical aspects of the protective action of propyl gallate on liver injury in rats poisoned with carbon tetrachloride. *Toxicol. Appl. Pharmacol*. **34**: 151–169

Wu. T.-W., Fung, K.-P., Zeng, L.-H., Wu, J., Nakamura, H. (1994) Propyl gallate as a hepatoprotector in vitro and in vivo. *Biochem. Pharmacol*. **48**: 419–422