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(–)-Epigallocatechin gallate (EGCG) prevents isoprenaline-induced cardiac toxicity by stabilizing cardiac marker enzymes and membrane-bound ATPases

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Abstract

Intake of tea flavonoids has been reported to reduce the incidence of cardiovascular disease. The present study was undertaken to investigate the preventive effect of (–)epigallocatechin gallate (EGCG) on heart weight, cardiac marker enzymes, membrane-bound ATPases and electrolytes in isoprenaline (ISO)-induced myocardial infarcted (MI) Wistar rats. Rats subcutaneously administered ISO 100 mg kg^{-1} at intervals of 24 h for 2 days resulted in significant increases in heart weight and the activities of cardiac marker enzymes such as creatine kinase, creatine kinase-MB, lactate dehydrogenase (LDH), aspartate transaminase and alanine transaminase in serum, and significant decreases in the activities of these enzymes in the myocardium. ISO injection also increased levels of LDH isoenzymes (LDH 1 and LDH 2). The activity of Na^+/K^+ ATPase was decreased significantly and the activities of Ca^{2+} and Mg^{2+} ATPases were increased significantly in ISO-induced MI rats. Furthermore, the levels of potassium were lowered and the levels of sodium and calcium were increased in ISO-induced MI rats. Prior treatment with EGCG (10, 20 and 30 mg kg^{-1}) daily for a period of 21 days reduced the effects of ISO on heart weight, activities of cardiac marker enzymes and membrane bound-ATPases and levels of LDH 1 and LDH 2 and electrolytes. Thus, EGCG exhibits beneficial effects on these enzymes and electrolytes. The observed effects may be due to the antioxidant and membrane-stabilizing effects of EGCG in ISO-induced MI rats.

Introduction

Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (De Bono & Boon 1992). The catecholamine isoprenaline (ISO) was administered to rats in this study, as it serves as a standard model to study the protective effect of various drugs on cardiac function. It causes severe stress in the myocardial tissue and at high doses produces acute myocardial necrosis (Rona 1985). The induction of MI in experimental animals by ISO is probably due to action on the sarcolemmal membrane, stimulation of adenylate cyclase, activation of Na^+ and Ca^{2+} channels and exaggerated Ca^{2+} inflow and energy consumption, leading to cellular death (Milei et al 1978). It has been reported that the free radicals produced by ISO could initiate peroxidation of membrane-bound polyunsaturated fatty acids, leading to both functional and structural myocardial injury (Thompson & Hess 1986). ISO-induced MI has been reported to show many metabolic and morphological aberrations in the heart tissue of experimental animals that are similar to those observed in human MI (Nirmala & Puvanakrishnan 1996).

Dietary factors play a key role in the development of various human diseases, including cardiovascular disease (CVD). Tea is one of the most popular beverages consumed worldwide, and the bioactive ingredients, mainly the catechin flavonols, have been shown to be protective against various diseases, including CVD. Catechins are well-known examples of natural polyphenolic antioxidants (Rice-Evans et al 1996). They are widely present in fruits and plants in high concentrations and may function as scavengers of active oxygen species in biological systems. In particular, green and black teas contain considerable amounts of catechins such as (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin and (–)epigallocatechin-gallate (EGCG). EGCG (Figure 1) is the major polyphenolic constituent found

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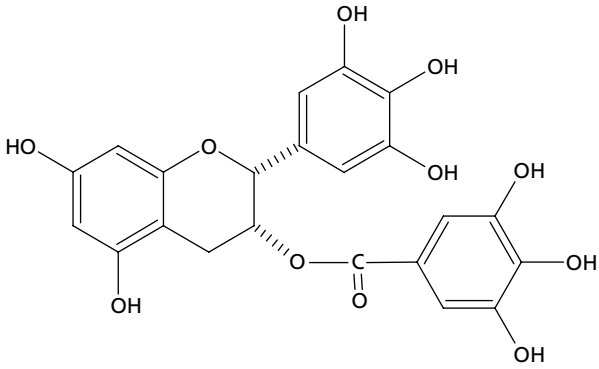

Figure 1 Structure of (–)-epigallocatechin gallate.

in freshly dried leaves of the plant *Camellia sinensis* L. – green tea (Bettuzzi et al 2006). More than 50% of the mass of this catechin combination is composed of EGCG, and a mass of scientific research suggests that EGCG is responsible for the majority of the potential health benefits attributed to consumption of green tea (Dale-Nagle et al 2006).

Numerous epidemiological studies have reported an inverse association between tea consumption and cardiovascular events (Vita 2003). Other studies indicate that polyphenolic flavonoids in tea may mediate the observed cardiovascular benefits (Hertog et al 1993). It has been demonstrated that ingestion of green and black teas significantly increased human plasma antioxidant capacity in-vivo (Serafini et al 1996). EGCG possesses antithrombotic activity, primarily due to its antiplatelet activities (Kang et al 1999). Other studies have shown that EGCG exhibits anti-apoptotic, anti-cancer, anti-mutagenic, anti-neurodegenerative, free radical scavenging, antioxidant and anti-colitis effects (Koh et al 2003; Mochizuki & Hasegawa 2005).

Arts et al (2001) showed that catechin intake was inversely associated with mortality from ischaemic heart disease in elderly men in the Netherlands. It has also been shown that black and green tea polyphenols attenuate blood pressure increase in stroke-prone spontaneously hypertensive rats through their antioxidant properties (Negishi et al 2004). Inhibition of nuclear factor-κB and activator protein-1 pathways by EGCG has also been reported in association with the treatment of reperfusion-induced myocardial damage (Aneja et al 2004). Another potential mechanism of protection against myocardial ischaemia reperfusion injury was suggested by Townsend et al (2004), who reported a reduction in STAT-1 activation and Fas receptor expression by EGCG and green tea extracts. Green tea has been found to be superior to black tea in terms of antioxidant activity, owing to its higher content of EGCG. Green-tea polyphenols show beneficial effects on atherosclerosis, coronary heart disease, hypertension, diabetes mellitus and obesity. The cardioprotective effects of flavonoids from green tea can be attributed not only to antioxidant activity but also to anti-endothelial function, antithrombogenic and anti-inflammatory properties and improvement of coronary flow velocity reserve. Green tea exhibits the strongest cardioprotective effects of all the teas studied (Cheng 2006). EGCG inhibits mitochondrial Ca^{2+} elevation and decreases caspase-3 induced apoptosis and also quenches the activity of active oxygen radicals in guinea-pig hearts subjected to ischaemia and reperfusion (Hirai et al 2007). Stangl et al (2007) have hypothesized that tea polyphenols act as either activators or inhibitors of signal transduction kinases, depending on the status (i.e. activated or resting) of the cells. Evidence is accumulating that a galloyl group can interfere with multiple pathways of signal transduction in relevant cardiovascular cells, primarily by modifying kinase activities. The induction of multiple effects may play a crucial role in the prevention and treatment of CVD. Another report has shown that EGCG mitigates cellular damage by reducing the inflammatory reaction and reducing the lipid peroxidation and nitric-oxide (NO)-generated radicals that lead to oxidative stress in CVD (Tipoe et al 2007).

Tea consumption increases activities of cytochromes P450A1 and A2 and glutathione-S-transferase in chemically induced cancer (Maliakal et al 2001). Yokozawa et al (1999) have reported that green-tea tannin eliminated oxidative stress and was beneficial to renal function. Green-tea polyphenols and partially hydrolysed guar gum also protect rats from streptozotocin-induced kidney damage. Another report showed that tea flavanols inhibit angiotensin-converting enzyme activity and increase NO production in human endothelial cells and prevent CVD (Persson et al 2006).

Studies have also shown that at high concentrations EGCG generates reactive oxygen species (ROS) by its pro-oxidant effect. In particular, metal-mediated auto-oxidation of EGCG was shown to promote generation of H_2O_2, leading to oxidative DNA damage (Furukawa et al 2003; Oikawa et al 2003; Nakagawa et al 2004).

Cardiac marker enzymes are proteins from cardiac tissue found in the blood. These proteins are released into the blood stream when damage to the heart occurs, as in the case of MI. The activities of membrane-bound enzymes such as Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase maintain the integrity of the mitochondrial membrane. ATPases of cardiac cells play a significant role in the contraction and relaxation cycles of cardiac muscle by maintaining normal ion levels (Ca^{2+}, Na^+, K^+, Mg^{2+}) within the myocytes. Changes in the properties of these ion pumps affect cardiac function. Cellular injury is associated with alterations in ion homeostasis. Thus, these enzymes and ions play a vital role in the pathology of MI. Hence, we undertook the present study to evaluate the preventive effect of EGCG on these enzymes and ions in ISO-induced MI rats.

Materials and Methods

Experimental animals

All experiments were carried out on male albino Wistar rats weighing 140–160 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. Rats were housed in polypropylene cages (47 cm × 34 cm × 20 cm) lined with husk

(replaced every 24 h), in a 12 h light–dark cycle at around 22°C. Rats had free access to tap water and standard pellet diet (Pranav Agro Industries Ltd, Pune, Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen-free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. Experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Animal Ethical Committee of Annamalai University (Approval No. 359; 18.10.2006).

Drug and chemicals

EGCG was purchased from Sigma Aldrich (Deisenhofen, Germany). Isoprenaline (isoproterenol) hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO, USA). Lithium lactate, sodium chloride, magnesium chloride, adenosine triphosphate and trichloroacetic acid (TCA) were purchased from Himedia Laboratories Private Ltd (Mumbai, India). Nitroblue tetrazolium, phenazine methosulphate and nicotinamide adenine dinucleotide (NAD) were purchased from S. D. Fine Chemical Company (Mumbai, India). All the other chemicals used in the study were of analytical grade.

Induction of experimental MI

Isoprenaline (100 mg kg^{-1}) was dissolved in normal saline and injected subcutaneously to rats at intervals of 24 h for 2 days.

Dose determination

Different doses of EGCG (5, 10, 20 and 30 mg kg^{-1} body weight) were dissolved in DMSO and administered orally using an intragastric tube at 7, 14, 21 and 28 days before ISO to determine the dose dependence and duration of treatment against ISO-induced MI in rats, based on the activities of creatine kinase (CK), CK-MB and lactate dehydrogenase (LDH). Prior treatment with EGCG at doses of 10, 20 and 30 mg kg^{-1} showed significant effects against ISO-induced MI. Therefore, these doses were chosen for our investigation.

Experimental design

Rats were divided into groups of eight. Group I was normal control rats; groups 2, 3 and 4 were treated with EGCG, 10, 20 and 30 mg kg^{-1}, respectively; group 5 was the ISO control rats (100 mg kg^{-1}); groups 6, 7 and 8 were pretreated with EGCG, 10, 20 and 30 mg kg^{-1}, respectively, and were induced with MI. Normal control rats and ISO-control rats received DMSO only. EGCG was dissolved in DMSO and administered to rats orally using an intragastric tube, daily for a period of 21 days.

On the 22nd day at 10.00 am, the first dose of ISO was administered to rats. Twelve hours after the second injection of ISO, rats were anaesthetized and decapitated. Blood was collected and the serum separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal saline and the whole heart was weighed.

Processing of heart tissue

A known weight of heart tissue was homogenized in 5.0 mL 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant used for estimation of various biochemical parameters.

Assay of marker enzymes

Activities of CK and CK-MB were measured in serum and heart tissue using commercial kits purchased from Agappe Diagnostics (Kerala, India). The activities of LDH, aspartate transaminase (AST) and alanine transaminase (ALT) were assayed in serum and heart tissue using commercial kits purchased from Qualigens Diagnostics (Mumbai, India).

Assay of Na^+/K^+ATPase

The activity of Na^+/K^+ ATPase in heart tissue was measured using the method of Bonting (1970). The incubation mixture contained 1.0 mL buffer, 0.2 mL magnesium sulphate, 0.2 mL potassium chloride, 0.2 mL sodium chloride, 0.2 mL EDTA and 0.2 mL ATP. After incubation at 37°C for 10 min, the reaction was initiated by the addition of 0.2 mL tissue homogenate and the contents were incubated at 37°C for 15 min, after which 1.0 mL 10% TCA was added to stop the reaction. The tubes were centrifuged and supernatant was used for the estimation of phosphorus by the method of Fiske & Subbarow (1925): 1.0 mL supernatant was made up to 4.3 mL with distilled water to which was added 1.0 mL ammonium molybdate reagent. The tubes were incubated at room temperature for 10 min, after which 0.4 mL amino napthol sulphonic acid was added. The colour developed was read at 640 nm after 20 min.

Assay of Ca^{2+} ATPase

The activity of Ca^{2+} ATPase in heart tissue was determined by the method of Hjerten & Pan (1983). The incubation mixture contained 0.1 mL buffer, 0.1 mL calcium chloride, 0.1 mL ATP, 0.1 mL distilled water and 0.1 mL tissue homogenate. The contents were incubated at 37°C for 15 min, after which the reaction was stopped by the addition of 0.5 mL ice-cold 10% TCA. The amount of phosphorous generated was estimated as described above.

Assay of Mg^{2+} ATPase

The activity of Mg^{2+} ATPase in heart tissue was determined by the method of Ohnishi et al (1982). The incubation mixture consisted of 0.1 mL Tris-HCl buffer, 0.1 mL magnesium chloride, 0.1 mL ATP, 0.1 mL distilled water and 0.1 mL tissue homogenate. The contents were incubated at 37°C for 15 min, after which the reaction was stopped by adding 0.5 mL 10% TCA. The phosphorus generated was estimated as described above.

Estimation of Na^+, K^+, Ca^{2+} and protein concentration

The concentrations of Na^+ and K^+ were estimated using commercial kits (Monozyme India Ltd, Secunderabad, India). Ca^{2+} in the heart was measured by the O-cresolphthalein complexone method using a reagent kit (Span Diagnostic Ltd, Gujarat, India). The protein content in the heart tissue homogenate was estimated by the method of Lowry et al (1951).

Separation and quantification of LDH isoenzymes

LDH isoenzymes were separated and quantified by agarose gel electrophoresis (Mckenzie & Henderson 1983). Agarose gel (1%) was prepared and applied immediately to a glass slide. Once the gel had set, 10 μL aliquots of serum samples were applied into the wells. After the run, the gels were removed and stained. The stain contained 1.0 mL 1 M lithium lactate, 1.0 mL 1 M sodium chloride, 1.0 mL 5 mM magnesium chloride, 2.5 mL 0.1% (w/v) nitroblue tetrazolium, 0.25 mL 0.1% phenazine methosulphate, 2.5 mL 0.5 M phosphate buffer (pH 7.5) and 10 mg NAD^+ in a total volume of 10 mL. The gels were incubated with the stain at 37°C in the dark for a suitable period. The separated LDH isoenzymes appeared as purple bands. The gels were washed with 7.5% acetic acid, preserved in 5% acetic acid and scanned using a densitometer.

Statistical analysis

Statistical analysis was performed by one-way analysis of variance followed by Duncan's multiple range test using Statistical Package for the Social Sciences (SPSS) software (version 12.00; SPSS Inc. Chicago, IL, USA). Results were expressed as mean ± s.d. for eight rats in each group. P values below 0.05 were considered significant.

Results

The effect of EGCG on the heart weight in normal and ISO-induced MI rats is shown in Figure 2. The heart weight of ISO-induced MI rats was significantly higher than of normal control rats. Pretreatment with EGCG (10, 20 and 30 mg kg^{-1}) to ISO-induced MI rats daily for a period of 21 days significantly reduced the increase in heart weight compared with untreated ISO-induced MI rats.

Table 1 shows the effect of EGCG on the activities of serum CK, CK-MB, LDH, AST and ALT in normal and ISO-induced MI rats. ISO-induced MI rats showed significant increase in the activities of these enzymes in serum compared with normal control rats. Pretreatment with EGCG (10, 20 and 30 mg kg^{-1}) significantly decreased the activities of these enzymes in serum of ISO-induced MI rats compared with untreated ISO-induced MI rats.

Table 2 shows the effect of EGCG on the activities of CK, LDH, AST and ALT in the hearts of normal and ISO-induced MI rats. ISO-induced MI rats showed significant decrease in the activities of these enzymes in the heart compared with normal control rats. Pretreatment with EGCG (10, 20 and 30 mg kg^{-1}) significantly increased the activities of these enzymes in hearts of ISO-induced MI rats compared with untreated ISO-induced MI rats.

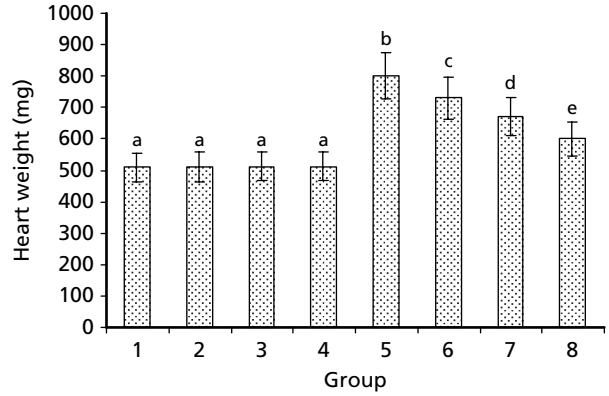

Figure 2 Effect of (–)-epigallocatechin gallate (EGCG) on heart weight in normal and isoprenaline (ISO)-induced myocardial infarcted (MI) rats. Group 1, normal control rats; groups 2, 3 and 4 were treated with EGCG, 10, 20 and 30 mg kg^{-1}, respectively; group 5, ISO control rats; groups 6, 7 and 8 were ISO-induced MI rats pretreated with EGCG, 10, 20 and 30 mg kg^{-1}, respectively, daily for 21 days. Each column is mean ± s.d. (n = 8 rats). Columns that do not share a common letter (a, b, c, d, e) differ significantly from each other ($P < 0.05$, Duncan's multiple range test).

The pattern of LDH isoenzymes in serum of normal and experimental groups of rats (as separated by agarose gel electrophoresis) are shown in Figure 3. ISO-induced MI caused an increased expression of LDH isoenzyme bands, predominantly LDH 1 and LDH 2 compared with normal control rats. EGCG pretreatment (10, 20 and 30 mg kg^{-1}) decreased the intensity of LDH 1 and LDH 2 in ISO-induced MI rats compared with untreated ISO-induced MI rats.

The activity of Na^+/K^+ ATPase was decreased significantly and the activities of Ca^{2+} and Mg^{2+} ATPases increased significantly in the hearts of ISO-induced MI rats compared with normal control rats (Table 3). Pretreatment with EGCG (10, 20 and 30 mg kg^{-1}) significantly increased the activity of Na^+/K^+ ATPase and significantly decreased the activities of Ca^{2+} and Mg^{2+}-ATPases in the hearts of ISO-induced MI rats compared with untreated ISO-induced MI rats.

Figure 4 shows that there was significant increase in the levels of sodium and calcium and a significant decrease in the level of potassium in the hearts of ISO-induced MI rats compared with normal control rats. Pretreatment with EGCG (10, 20 and 30 mg kg^{-1}) of ISO-induced MI rats significantly decreased the levels of sodium and calcium and increased the levels of potassium in the heart compared with untreated ISO-induced MI rats.

For all the biochemical parameters studied, EGCG at a dose of 30 mg kg^{-1} showed higher significant effects than the two lower doses (10 and 20 mg kg^{-1}). Oral administration of EGCG at all three doses to normal rats did not show any significant effect on any of the parameters studied.

Table 1 Effect of (–)-epigallocatechin gallate (EGCG) on the activities of creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in the serum of normal and isoprenaline (ISO)-induced myocardial infarcted (MI) rats. EGCG was administered to rats daily for a period of 21 days before MI induction with ISO

	Normal rats				ISO-induced MI rats			
	Control	EGCG dose ($mg kg^{-1}$)			ISO control	EGCG dose ($mg kg^{-1}$)		
		10	20	30		10	20	30
CK ($IU L^{-1}$)	181.0 ± 16.1^a	180.4 ± 16.1^a	181.2 ± 15.9^a	180.1 ± 16.1^a	320.4 ± 18.6^b	280.3 ± 25.0^c	240.5 ± 21.8^d	200.1 ± 18.8^e
CK-MB ($IU L^{-1}$)	71.0 ± 6.2^a	70.5 ± 6.2^a	70.4 ± 6.2^a	70.3 ± 6.2^a	180.7 ± 16.0^b	150.7 ± 13.4^c	120.1 ± 10.8^d	90.1 ± 8.0^e
LDH ($IU L^{-1}$)	72.9 ± 6.2^a	72.6 ± 6.5^a	72.5 ± 6.4^a	72.2 ± 6.4^a	145.4 ± 12.9^b	128.3 ± 9.8^c	105.2 ± 9.4^d	84.9 ± 7.4^e
AST ($IU L^{-1}$)	26.1 ± 2.2^a	25.7 ± 2.2^a	25.6 ± 2.2^a	25.4 ± 2.2^a	47.5 ± 4.1^b	42.8 ± 3.8^c	36.5 ± 3.2^d	29.9 ± 2.1^e
ALT ($IU L^{-1}$)	16.9 ± 1.4^a	16.7 ± 1.3^a	16.3 ± 1.2^a	16.1 ± 1.2^a	32.4 ± 3.1^b	28.1 ± 2.5^c	24.1 ± 2.2^d	20.1 ± 2.0^e

Values are mean ± s.d. (n = 8 rats). Values that do not share a common superscript (a, b, c, d, e) differ significantly from each other ($P < 0.05$, Duncan's multiple range test).

Table 2 Effect of (–)-epigallocatechin gallate (EGCG) on the activities of creatine kinase (CK; (μmol phosphorus generated min^{-1} (mg protein)$^{-1}$), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) (all in nmol pyruvate generated min^{-1} (mg protein)$^{-1}$) in the hearts of normal and isoprenaline (ISO)-induced myocardial infarcted (MI) rats. EGCG was administered to rats daily for a period of 21 days before MI induction with ISO

	Normal rats				ISO-induced MI rats			
	Control	EGCG dose ($mg kg^{-1}$)			ISO controls	EGCG dose ($mg kg^{-1}$)		
		10	20	30		10	20	30
CK	17.4 ± 1.5^a	17.5 ± 1.5^a	17.7 ± 1.5^a	17.8 ± 1.5^a	9.5 ± 0.8^b	11.8 ± 0.9^c	13.4 ± 1.1^d	15.2 ± 1.1^e
LDH	102.1 ± 8.9^a	102.3 ± 9.2^a	102.3 ± 9.1^a	102.6 ± 9.1^a	63.2 ± 5.6^b	73.1 ± 6.5^c	84.2 ± 7.8^d	92.1 ± 8.2^e
AST	36.4 ± 3.3^a	36.7 ± 3.3^a	36.8 ± 3.0^a	36.9 ± 3.2^a	20.4 ± 1.8^b	24.1 ± 2.1^c	26.9 ± 2.1^d	32.2 ± 2.7^e
ALT	25.2 ± 2.1^a	25.4 ± 2.2^a	25.6 ± 2.2^a	26.0 ± 2.2^a	15.5 ± 1.3^b	17.5 ± 1.5^c	19.1 ± 1.6^d	21.1 ± 1.8^e

Values are mean ± s.d. (n = 8 rats). Values that do not share a common superscript (a, b, c, d, e) differ significantly from each other ($P < 0.05$, Duncan's multiple range test).

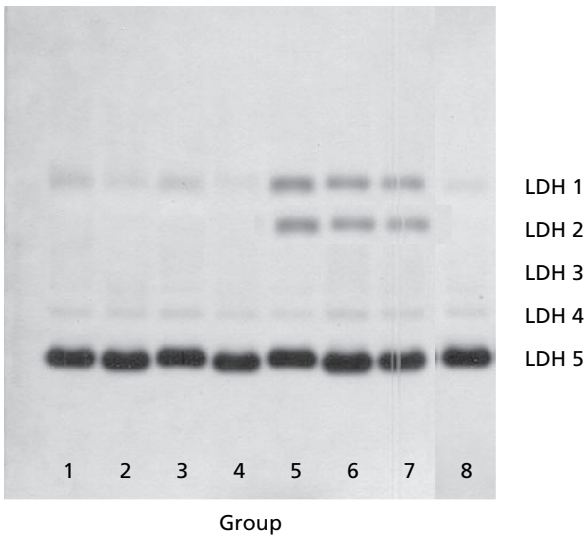

Figure 3 Effect of (–)-epigallocatechin gallate (EGCG) on lactate dehydrogenase (LDH) isoenzymes in the serum of normal and isoprenaline-induced myocardial infarcted rats. Descriptions of the groups are given in the legend to Figure 2.

Discussion

It has been reported that EGCG has beneficial effects in reperfusion-induced ischaemic guinea-pig hearts, which is closely related to effects on NO, active oxygen radicals and biological systems in mitochondria (Hirai et al 2007). Aneja et al (2004) observed attenuation of myocardial ischaemia reperfusion injury after treatment with EGCG. Intravenous application of 10 mg kg^{-1} EGCG during reperfusion significantly decreased IkB kinase activity, resulting in reduction of IkBα degradation and NFκB activity. Moreover, EGCG treatment diminished phosphorylation of C-Jun and consequently AP-1 activity. In-vivo treatment with EGCG also reduced myocardial damage and myeloperoxidase activity. Plasma interleukin-6 and creatine phosphokinase levels were decreased after EGCG administration. A reduction in STAT-1 activation and Fas receptor expression by treatment with EGCG and green-tea extracts has also been reported (Townsend et al 2004).

Even though a number of studies have been carried out on the beneficial effects of EGCG on myocardial damage, there is no scientific report detailing the preventive effect of EGCG on cardiac marker enzymes, membrane-bound ATPases and

Table 3 Effect of (–)-epigallocatechin gallate (EGCG) on the activities of Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases (measured as (μmol inorganic phosphate generated min^{-1} (mg protein)$^{-1}$) in the hearts of normal and isoprenaline (ISO) induced myocardial infarcted (MI) rats. EGCG was administered to rats daily for a period of 21 days before with MI induction with ISO

	Normal rats				ISO-induced MI rats			
	Control	EGCG dose (mg kg^{-1})			ISO controls	EGCG dose (mg kg^{-1})		
		10	20	30		10	20	30
Na^+/K^+ ATPase	0.47 ± 0.04^a	0.47 ± 0.04^a	0.48 ± 0.04^a	0.49 ± 0.03^a	0.17 ± 0.01^b	0.22 ± 0.02^c	0.27 ± 0.02^d	0.31 ± 0.03^e
Mg^{2+}ATPase	4.8 ± 0.4^a	4.5 ± 0.3^a	4.4 ± 0.3^a	4.2 ± 0.4^a	7.9 ± 0.7^b	7.1 ± 0.6^c	6.2 ± 0.5^d	5.7 ± 0.5^e
Ca^{2+}ATPase	0.98 ± 0.08^a	0.97 ± 0.09^a	0.96 ± 0.08^a	0.95 ± 0.08^a	3.10 ± 0.30^b	2.51 ± 0.15^c	2.01 ± 0.12^d	1.54 ± 0.10^e

Values are mean ± s.d. (n = 8 rats). Values that do not share a common superscript (a, b, c, d, e) differ significantly from each other ($P < 0.05$, Duncan's multiple range test).

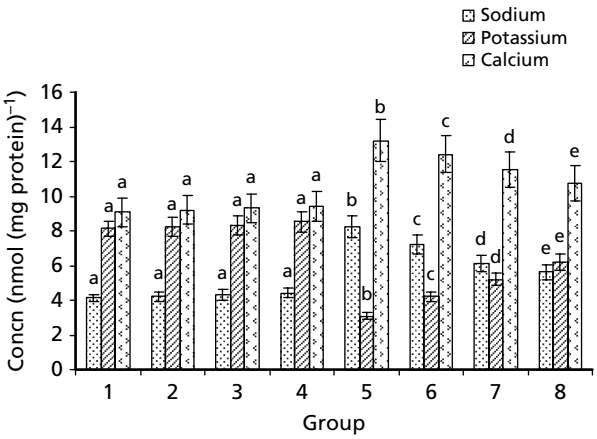

Figure 4 Effect of (–)-epigallocatechin gallate (EGCG) on the levels of sodium, potassium and calcium in the hearts of normal and isoprenaline-induced myocardial infarcted rats. Each column is mean ± s.d. (n = 8 rats). Columns that do not share a common letter (a, b, c, d, e) differ significantly from each other ($P < 0.05$, Duncan's multiple range test). Descriptions of groups are given in the legend to Figure 2.

ions in MI. Hence, the present study investigated the preventive effect of EGCG on these enzymes and ions in experimentally induced MI in rats.

We observed a significant increase in the heart weight in ISO-induced MI rats. Nirmala & Puvanakrishnan (1996) have reported that such an increase may be due to increased water content, oedematous intramuscular spaces and extensive necrosis of cardiac muscle fibres, followed by the invasion of damaged tissues by inflammatory cells. Oral pretreatment with EGCG daily for a period of 21 days significantly decreased the gain in heart weight in ISO-induced MI rats. EGCG may thus protect the myocardium against infiltration by decreasing the oedema. In this context, Henry & Stephens-Larson (1984) have reported that the polyphenols do not favour oedema formation in heart tissues of mice. This effect shows the anti-inflammatory effect of EGCG (Tipoe et al 2007). In the present study, increased levels of diagnostic marker enzymes such as CK, LDH, AST and ALT were found in the serum of ISO-induced MI rats. The increase in the levels of these enzymes is an indication of the severity of ISO-induced necrotic damage to the myocardial membrane. The levels of these enzymes present in plasma is reported to be directly proportional to the number of necrotic cells present in the cardiac tissue (Geetha et al 1990). In ISO-induced MI rats, the activities of CK, LDH, AST and ALT were decreased in the myocardium. The myocardial cells containing CK, LDH, AST and ALT are damaged or destroyed because of deficient oxygen and glucose supply and the cell membrane becomes permeable or may rupture, which results in the leakage of these enzymes. This accounts for the lowered activities of these enzymes in the hearts of ISO-induced MI rats. This may be due to the damage caused to the sarcolemma by ISO, rendering it leaky (Mathew et al 1985).

Assessment of the magnitude and persistence of elevation of CK-MB activity in plasma is a useful way to estimate the extent of infarction (Sobel 1992). ISO-induced cardiac damage was indicated by elevated levels of marker enzymes such as CK-MB in serum. Jaffe et al (1996) have suggested that MI can be differentiated from other types of tissue damage because the LDH isoenzyme begins to rise 12–24 h after MI, peaks after 2–3 days, and gradually dissipates over 5–14 days. In the present study, an increase in LDH 1 and LDH 2 isoenzyme bands in ISO-induced MI rats was observed. The heart-specific isoenzymes LDH 1 and LDH 2 could have been released into the circulation because of necrosis caused by ISO. EGCG ameliorated ISO-induced myocardial damage by inhibiting these cardiac marker enzymes in serum.

A significant protection of heart CK, LDH, AST and ALT levels indicates that EGCG has cardioprotective action and maintains the integrity of myocytes. EGCG is a polyphenol which possesses antioxidant activity, which may explain its ability to protect the myocardium from damage by preventing the leakage of these enzymes into the blood in EGCG-pretreated Wistar rats.

In the present study, a significant decrease in the activity of Na^+/K^+ ATPase and significant increases in the activities of Ca^{2+} and Mg^{2+} ATPases were observed in the hearts of ISO-induced MI rats. Furthermore, significant increases in the levels of sodium and calcium and significant decreases in

the levels of potassium were noted in the hearts of ISO-treated MI rats. Similar results in ISO-induced rats have been reported by other workers (Yogeeta et al 2006b).

Calcium is essential for normal cardiac function, for the maintenance of cell membrane integrity and for coagulation of blood. In the heart, cytosolic calcium is carefully controlled and Ca^{2+} is the key ion for normal activity of many enzymes (Hamet 1995). ISO-induced MI has been reported to enhance adenylate cyclase activity, resulting in increased formation of cAMP (Subash et al 1978). During β-adrenergic stimulation, cAMP phosphorylates several sites on the C-terminal chains of the calcium channel and increases the probability of the calcium channel opening (Varadi et al 1995). This may be the reason for enhanced activity of Ca^{2+}ATPase and increased concentration of Ca^{2+} observed of myocardial tissue in ISO-induced MI rats in this study. Intracellular Ca^{2+} overload can set off a cascade of events that can lead to the formation of ROS, which suggests that ROS formation and Ca^{2+} surge may be involved in the contractile dysfunction of the ischaemic myocardium (Jan & Shu 2005). Furthermore, Sathish et al (2003) have reported decreased content of ATP in ISO-treated rats. The affinity of ATP for magnesium is higher than that of ADP, so that cytosolic magnesium increases during ATP hydrolysis (Leyssens et al 1996). As ATP breaks down, opening of the potassium channel is promoted, leading to decreased concentration of potassium in the myocardial tissue (Ferraro et al 1996).

The Na^+/K^+ ATPase pump is responsible for the active transport of Na^+ and K^+ across the cell membrane. Ahmed & Thomas (1971) reported that increased concentrations of free fatty acids (FFAs) in the myocardium resulted in the non-competitive inhibition of many enzyme systems such as Na^+/K^+ATPase. Inhibition of the sodium pump may precipitate increased levels of intracellular sodium (Jennings et al 1986). The increased levels of FFAs may have resulted in non-competitive inhibition of Na^+/K^+ ATPase, thereby leading to increased accumulation of Na^+ ions in ISO-induced MI rats. In this context, studies have shown that concentrations of FFAs were increased in the myocardium of ISO-treated rats (Yogeeta et al 2006a).

EGCG maintained the electrolyte levels (sodium, potassium and calcium) by the inhibition of lipid peroxidation, preventing the accumulation of lipid peroxidation products and thereby inhibiting the calcium ion transport system. Oral pretreatment with EGCG increased the activity of Na^+/K^+ ATPase and decreased the activities of Ca^{2+} and Mg^{2+} ATPases in ISO-treated MI rats. This effect may be due to membrane stabilizing properties of EGCG. Saffari & Sadrzadeh (2004) have reported that EGCG protects membrane-bound ATPases (Ca^{2+} and K^+ ATPases) against t-butylhydroperoxide-induced damage.

The pharmacokinetic parameters of the tea catechins have been thoroughly determined in mice and rats following oral, intravenous and intragastric administration. Tea flavanols are mainly absorbed into the circulation from the intestine. Mean EGCG plasma concentrations in CD-1 mice 1 h after oral administration of 1600 ng kg^{-1} EGCG to male and female mice were 699.8 and 603.5 ng mL^{-1} (approximately 1.53 and 1.32 μM), respectively. Intravenous injection of 10, 25 and 50 mg EGCG kg^{-1} day^{-1} to rats resulted in much higher plasma concentrations (Isbrucker et al 2006). The absolute bioavailability of EGCG following intragastric administration of decaffeinated green tea was 0.1% (Chen et al 1997). Similarly, another study showed that EGCG levels in the tissues and blood corresponded to 0.0003–0.45% of the injected dose in rats (Nakagawa & Miyazawa 1997).

Studies of [^{3}H]-EGCG have been performed in both the rat and the mouse. Following a single intragastric dose of [^{3}H]-EGCG, radioactivity can be detected throughout the body, with 10% of the initial dose present in the blood after 24 h, and approximately 1% in the brain, lungs, heart, liver, kidneys and other tissues (Suganuma et al 1998). Excretion in the faeces is the major route of elimination, with 25–30% of the radioactivity excreted after 24 h. In the rat, intravenous administration of [^{3}H]-EGCG resulted in 77% of the dose being excreted in the bile and only 2% excreted in the urine (Kohri et al 2001). Interestingly, plasma levels of EGCG increased significantly when the dose was increased from 400 to 600 mg. The protective effect of EGCG in this study may be due to scavenging of peroxides and/or blockade of membrane lipid peroxidation. The latter effect will protect membrane integrity and protects ATPases against lipid radicals generated in the membrane by ISO. In this context, studies have shown that EGCG exhibits strong free-radical-scavenging and antioxidant effects (Koh et al 2003). We have also observed that EGCG exhibits anti-lipoperoxidation action and improves antioxidant system in ISO-induced MI rats.

Conclusion

EGCG protects the myocardium against the leakage of cardiac marker enzymes in ISO-induced MI rats. It also maintains the levels of ATPases and ions. The effects observed in this study are due to the antioxidant and membrane-stabilizing effects of EGCG in experimentally induced MI.

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