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Cardioprotective effect of embelin on isoproterenol-induced myocardial injury in rats: Possible involvement of mitochondrial dysfunction and apoptosis

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ABSTRACT

Aims: Preventive and/or therapeutic interventions using natural products for ischemic heart disease have gained considerable attention worldwide. This study investigated the cardioprotective effect and possible mechanism of embelin, a major constituent of *Embelia ribes* Burm, using isoproterenol (ISO)-induced myocardial infarction model in rats.

Materials and methods: Rats were pretreated for three days with embelin (50 mg/kg, p.o) before inducing myocardial injury by administration of ISO (85 mg/kg) subcutaneously at an interval of 24 h for 2 consecutive days. Serum was analyzed for cardiac specific injury biomarkers, lipids and lipoprotein content. Heart tissues were isolated and were used for histopathology, antioxidant and mitochondrial respiratory enzyme activity assays and western blot analysis.

Key findings: Results showed that pretreatment with embelin significantly decreased the elevated levels of serum specific cardiac injury biomarkers (CK-MB, LDH and AST), serum levels of lipids and lipoproteins and histopathological changes when compared to ISO-induced controls. Exploration of the underlying mechanisms of embelin action revealed that embelin pretreatment restored the myocardial mitochondrial respiratory enzyme activities (NADH dehydrogenase, succinate dehydrogenase, cytochrome c oxidase and mitochondrial redox activity), strengthened antioxidant status and attenuated ISO-induced myocardial lipid peroxidation. Immunoblot analysis revealed that embelin interrupted mitochondria dependent apoptotic damage by increasing the myocardial expression of Bcl-2 and downregulating the expression of Bax, cytochrome c, cleaved-caspase-3 & 9 and PARP. Histopathology findings further strengthened the cardioprotective findings of embelin.

Significance: Result suggested that embelin may have a potential benefit in preventing ischemic heart disease like myocardial infarction.

Introduction

Myocardial infarction refers to a condition in which a portion of the myocardium undergoes damage due to lack of oxygen for a certain period of time followed by reperfusion leading to irreversible damage (Thygesen et al., 2012). Ischemia of the myocardium causes development of arrhythmias and may also lead to cardiac necrosis. The etiology of many cardiovascular disorders depends on a number of interlinked factors such as changing lifestyle, stress, aging, food habits and other socioeconomic determinants of developing nations (Vendrame et al., 2013). Excess generation of free radicals and associated oxidative and apoptotic damage due to ischemia play a major role during ischemic heart diseases by causing qualitative and quantitative alterations in the myocardium (Wang et al., 2009; Prince et al., 2011; Li et al., 2012). Overproduction of catecholamines due to adrenergic overstimulation

Abbreviations: AST, aspartate transaminase; CAT, catalase; CDNB, 1-chloro-2, 4-dinitrobenzene; CK-MB, creatine kinase-MB isoenzyme; DCIP, 2, 6-dichlorophenolindophenol; DTNB, 5, 5-dithio-bis (2-nitrobenzoic acid); GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSH, oxidized glutathione; GST, glutathione S-transferase; HDL, high density lipoprotein; ISO, isoproterenol; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; NADH, β-nicotinamide adenine dinucleotide hydrate; NADPH, β-nicotinamide adenine dinucleotide 3-phosphate reduced form; NBT, nitro blue tetrazolium salt; NQO1, NAD (P) H: quinine oxidoreductase 1; PARP, poly (ADP-ribose) polymerase; SOD, superoxide dismutase; TBA, 2-thiobarbituric acid; TBARS, thiobarbituric acid reactive substance; VLDL, very low density lipoprotein.

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is believed to be a major cause of stress-induced cardiac dysfunction (Shao et al., 2013). It has been reported that supraphysiological plasma concentrations of catecholamines produce myocardial dysfunction through cardiomyocyte apoptosis or necrosis and also have a great influence on myocardial energy metabolism through perturbations of lipid metabolism in the heart (Radhiga et al., 2012; Shao et al., 2013). Isoproterenol (ISO), a β-adrenergic agonist and synthetic catecholamine is employed at sub-maximal dose as a non-invasive method to induce myocardial lesions in rodents (Li et al., 2012). Out of several mechanisms proposed for understanding ISO-induced myocardial injury, production of highly cytotoxic free radicals through auto-oxidation is widely accepted. It induces severe stress and loss of myocardial integrity through hypoxia, calcium overload and increased free radical production (Roy and Prince, 2013).

Phytomedicine is being used for the prevention of many cardiac ailments such as heart failure, coronary insufficiency and atherosclerosis from times immemorial. Many epidemiological studies suggest the protective effect of specific groups of fruits and vegetables against cardiovascular disorders (Yu et al., 2013). Natural compounds rich in antioxidants are of paramount importance in dealing with various pathological conditions. Recently they have been used as adjuvants to treat nephro-, neuro-, hepato- and cardiovascular related disorders (Azofeifa et al., 2013). Embelin (2, 5-dihydroxy-3-undecyl-1, 4-benzoquinone), an alkyl substituted benzoquinone bioactive molecule and represents the major constituents of *Embelia ribes* Burm belonging to the family of Myrsinaceae (Schaible et al., 2013). Embelin was found to possess a wide spectrum of medicinal and pharmacological properties including anti-inflammatory, analgesic, antitumor, antioxidant, antidiabetic, anxiolytic, antibacterial, anticonvulsant and antidepressant properties (Thippeswamy et al., 2011). In a previous study, *Embelia ribes* seed extract has been reported to elicit cardioprotective effect in rats (Bhandari et al., 2008). However, it is not clear, in this study, whether the cardioprotective effect is because of embelin or other active ingredients of *Embelia ribes* seed extract. Inspired by the observed cardioprotective effect of *Embelia ribes* extract on the one hand and the role of oxidative stress in cardiovascular disorders on the other, the current study was designed to study the cardioprotective effect of embelin as a natural antioxidant and to elucidate the mechanism of protection by employing ISO-induced myocardial infarction model in rats.

Materials and methods

Chemicals

Embelin (Purity: 98%), isoproterenol, Bradford reagent, cytochrome c, catalase, reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, 2, 6-dichlorophenolindophenol (DCIP), 2-thiobarbituric acid (TBA), 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB), β-nicotinamide adenine dinucleotide hydrate (NADH), β-nicotinamide adenine dinucleotide 3-phosphate reduced form (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), nitro blue tetrazolium salt (NBT), hydroxylamine hydrochloride, and MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide were purchased from Sigma-Aldrich Co, St Louis, MO, USA. Antibodies against Bax, Bcl-2, cytochrome c, cleaved caspase-3, caspase-9, cleaved PARP, β-actin and HRP-conjugated secondary anti-rabbit and anti-mouse antibody were obtained from Cell Signaling Technology (Boston, MA). All other chemicals and reagents were from Sigma-Aldrich Co, St Louis, MO, USA unless otherwise stated.

Animals

Male Sprague–Dawley rats, weighing between 150 and 180 g, were obtained from the National Institute of Nutrition (NIN), Hyderabad, India. Rats were housed under optimal conditions of temperature, humidity and light-cycle (12 L:12D) and were allowed to acclimatize for 1 week prior to initiation of the experiment (food and water ad libitum). All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of the institute (Permission No: IICT/PHARM/SRK/FEB/2013/11) and the study was conducted according to the ethical norms approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Induction of myocardial infarction and experimental design

As reported in earlier literature, experimental myocardial injury in rats was induced by administering isoproterenol (Isoproterenol was dissolved in normal saline and was administered at the dose of 85 mg/kg body weight) subcutaneously at an interval of 24 h for 2 consecutive days (Wang et al., 2009; Li et al., 2012).

The experimental animals were randomly divided into four groups consisting of 8 rats in each. Group I (Vehicle control, control): Rats received 2% gum acacia suspension (2 ml/kg/day, orally) for 5 consecutive days and normal saline (1 ml/kg/day, s.c) on the 4th and 5th days. Group II (Embelin control, Emb): Rats received 50 mg/kg embelin suspension in 2% gum acacia (2 ml/kg/day, orally) for 5 consecutive days and normal saline (1 ml/kg/day, s.c) on the 4th and 5th days. Group III (ISO control, ISO): Rats received 2% gum acacia suspension (2 ml/kg/day, orally) for 5 consecutive days and 85 mg/kg ISO dissolved in normal saline (1 ml/kg/day, s.c) on the 4th and 5th days. Group IV (ISO + Emb): Rats received 50 mg/kg embelin suspension in 2% gum acacia (2 ml/kg/day, orally) for 5 consecutive days and 85 mg/kg ISO dissolved in normal saline (1 ml/kg/day, s.c) on the 4th and 5th days. The dose of the embelin (50 mg/kg) was selected based on previous studies (Thippeswamy et al., 2011; Kumar et al., 2011) and duration of pretreatment (5 days) of embelin was based on our pilot study (data not shown).

After 48 h of first dose of ISO (i.e. on the 6th day), body weight of all experimental animals was recorded and blood samples were collected through retro-orbital plexus. Serum was separated by centrifugation at 4000 *g* for 15 min and stored at −80 °C for biochemical analyses. Then animals were sacrificed, heart tissue was removed and homogenized in ice cold phosphate buffer saline (50 mM, pH 7.4) to obtain a 10% (w/v) tissue homogenate. Heart tissue homogenate of different experimental animals was centrifuged at 12000 *g* for 45 min at 4 °C and the supernatant obtained was used for estimation of various biochemical parameters. Total protein concentration in tissue samples was measured using Bradford reagent (Sigma-Aldrich) against bovine serum albumin (BSA) as standard.

Estimation of serum myocardial injury markers

Serum levels of CK-MB (creatine kinase-MB isoenzyme), LDH (lactate dehydrogenase) and AST (aspartate transaminase) were estimated using respective commercial kits (Siemens, India) and employing auto blood analyzer (Siemens, Dimension Xpand^{plus}, USA). The relative weight of the heart for each experimental animal was also recorded as indices of cardiac hypertrophy.

Estimation of serum lipids and lipoproteins

Serum levels of total cholesterol, triglycerides and HDL-C were estimated using commercially available enzymatic kits (Siemens, India) and employing auto blood analyzer (Siemens, Dimension Xpand^{plus}, USA). Serum LDL-cholesterol was calculated by the Friedewald formula: LDL-C = Total cholesterol − {HDL-C + (Triglycerides / 5)}. Similarly, VLDL-C was calculated as follows: VLDL-C = Triglycerides / 5.

Measurement of myocardial antioxidant status

Myocardial content of various enzymatic and non-enzymatic antioxidants were estimated in heart tissue of all experimental