Cardioprotective effect of aqueous extract of *Embelia ribes* Burm fruits against isoproterenol-induced myocardial infarction in albino rats

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Received 19 June 2007; revised 5 October 2007

In the present study, cardioprotective effect of aqueous extract of fruits of *Embelia ribes* Burm (ER) was evaluated in a rat model having acute myocardial infarction, induced by isoproterenol (5.25 and 8.5 mg/kg, sc, for two consecutive days). Aqueous ER extract (100 mg/kg) pretreatment orally for 40 days in isoproterenol (ISO)-treated rats significantly decreased the heart rate, systolic blood pressure, increased levels of serum lactate dehydrogenase, serum creatine kinase and myocardial lipid peroxides and significantly increased the myocardial endogenous antioxidants (glutathione, superoxide dismutase and catalase) levels. The results of biochemical observations in serum and heart tissues were supplemented by histopathological examination of rat’s heart sections to confirm the myocardial injury. The results were comparable to that of gliclazide treated group. The present results provide evidence for the first time, that aqueous ER extract pretreatment ameliorated myocardial injury and enhanced the antioxidant defense against ISO-induced myocardial infarction in rats and exhibited cardioprotective property.

**Keywords:** *Embelia ribes*, Isoproterenol, Lipid peroxides, Myocardial infarction

L-isoproterenol (ISO), a synthetic catecholamine causes myocardial cell damage when administered in large doses\(^1\). However, the etiology of ISO-induced myocardial infarction (MI) is not clear. Several mechanisms by which ISO causes myocardial injuries have been proposed\(^3\)-\(^5\). Because of iatrogenic effect of ISO, it is demonstrated that repeated subcutaneous administration of ISO also produces myocardial necrosis in rats and hence, serves as good model for acute MI.

Mechanisms proposed to explain catecholamine-induced necrosis include an increase in cAMP levels\(^6\), intracellular calcium overload and exhaustion of high-energy phosphates\(^7\). Since catecholamines readily undergo oxidation, it has been suggested that oxidation products of catecholamines, rather than catecholamines per se, are responsible for myocardial changes observed following the administration of the parent compounds\(^8\). There is strong evidence that adrenochromes and other oxidation metabolites of catecholamines can cause cell necrosis and contractile failure in rat heart\(^9\),\(^10\).

It is also known that auto-oxidation of catecholamines result in generation of highly cytotoxic free radicals\(^11\). It is, therefore, likely that free radicals may play an important role in catecholamine-induced cardiotoxicity by causing peroxidation of membrane phospholipids, which can result in permeability changes in the membrane as well as intracellular calcium overload.

Myocardial infarction (MI), the most dreaded sequel among ischemic heart diseases is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia etc., leading to qualitative and quantitative alterations of myocardium\(^12\). High blood pressure is considered one of the major risk factors for cardiovascular disease and premature death\(^13\). Oxygen free radicals (OFR) are implicated as mediators of tissue injury in cardiovascular pathology\(^14\). Free radical generation and lipid peroxidation could be involved in ISO-induced cardiac damage\(^15\). ISO-induced myocardial infarction increases lysosomal hydrolase activities, which may be responsible for tissue damage and infarcted heart\(^16\) and also causes alterations in fragility of lysosomal membrane of heart\(^17\).

Despite considerable progress has been made in the management of myocardial infarction by synthetic drugs, the search for indigenous cardioprotective...
agents still continue. Some plant products have also been demonstrated to cause augmentation of myocardial antioxidants.\textsuperscript{18}

*Embelia ribes* Burm (Myrsinaceae), commonly known as vidanga, is a large woody climbing shrub and is widely distributed throughout India. It is esteemed in Ayurveda as a powerful anthelmintic\textsuperscript{19}. Whole plant is used as anti-inflammatory drug to relieve rheumatism and fever\textsuperscript{20}. In a preliminary study, Tripathi\textsuperscript{21} has reported the antihyperglycemic activity of decoction of *E. ribes* fruits in glucose-fed albino rabbits. Further, Bhandari \textit{et al.}\textsuperscript{22} have reported the diabetic dyslipidemic activity of *E. ribes* in streptozotocin-induced diabetes in rats using gliclazide as positive control drug. However, the exact mechanism of its cardioprotective effect with respect to the present knowledge of pathophysiology of cardiotoxicity has not been investigated. Hence, the present study was designed to evaluate the protective effect of aqueous *E. ribes* (ER) extract pretreatment against ISO-induced myocardial infarction in rats.

**Materials and Methods**

Extraction of *Embelia ribes*—The dried fruits of *E. ribes* Burm were purchased locally, identified at Department of Botany, Faculty of Science, Hamdard University, New Delhi, India and voucher specimen was deposited in Department of Pharmacology (voucher specimen no. UB 2). The dried and coarsely powdered fruits (100 g) were packed in a soxhlet apparatus and was subjected to extraction with water (150 ml) for 72 hr. The filtrate was evaporated under vacuum drier (Narang Scientific Works Pvt. Ltd., New Delhi, India) and brown mass residue obtained was stored at 4°C for further use. The average yield of the aqueous ER extract was approximately 7%. For experimental study, the weighed amount of aqueous ER extract (100 mg/Kg) was dissolved in Tween 80 (1%) in normal saline.

Phytochemical analysis of extract — Preliminary phytochemical screening of aqueous extract of dried fruits was carried out for detection of phytoconstituents, using standard chemical tests. Alkaloids, carbohydrates, phenolic compounds, flavonoids, proteins and saponins were detected in the extract. HPTLC fingerprints of aqueous extract was established using CAMAG HPTLC (WinCAT software, version 2.2) and benzene: ethyl acetate (6:4) as solvent system, which showed presence of 7 spots (R<sub>f</sub> values: 0.32, 0.34, 0.42, 0.45, 0.52, 0.65 and 0.78) at 520 nm wavelength.

**Chemicals and reagents** — Isoproterenol (ISO) used in the study was obtained from Sigma Chemicals (St Louis, MO, USA). It was administered by subcutaneous route in 5.25 and 8.5 mg/kg dose, for two consecutive days to induce myocardial infarction. All other chemicals used were of analytical grade. Double distilled water was used for all biochemical assays.

Experimental animals — The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which was registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India (Registration no. 173/CPCSEA, dated 28 January, 2000). Albino rats of either sex, weighing 200-250 g, procured from the Central Animal House Facility, Hamdard University, New Delhi and were acclimatized under standard laboratory conditions at 25°C ± 2°C, RH 50 ± 15% (12 hr light/dark cycle) for 7 days. Commercial pellet diet (Nav Maharashtra Chakan Oil Mills Ltd, Delhi, India) and water were provided \textit{ad libitum}. After acclimatization, 40 rats were randomly divided into four groups with ten animals in each group and subjected to respective treatment. Group I (normal control) received only Tween 80 (1%) in normal saline. Group II (Pathogenic control) rats were administered with ISO. Group III (ER pretreatment) rats received aqueous ER extract (100 mg/kg body wt, dose based on preliminary study) orally for 40 days followed by ISO administration on 41 and 42 day. Group IV (gliclazide pretreatment) rats received gliclazide (25 mg/kg body wt) orally for 40 days followed by ISO administration on 41 and 42 day.

In all the animals, hemodynamic parameters viz., heart rate and systolic blood pressure (BP) were recorded on day 43, as described previously\textsuperscript{23} using non-invasive method of rat’s tail cuff plethysmography using LE 5001 pressuremeter (LETICA Scientific Instruments, USA). After hemodynamic measurement, blood samples were collected from the retro-orbital plexus using micro-capillary technique\textsuperscript{24} from all the groups of overnight fasted rats and serum was separated for biochemical estimation. After blood collection, all animals were sacrificed by cervical dislocation and hearts were dissected out and immediately frozen in liquid nitrogen for tissue biochemical estimations and histopathological examination.
Biochemical analysis — In serum, lactate dehydrogenase (LDH) and creatine kinase (CK) levels were estimated using the methods of Lum et al.\textsuperscript{25} and Rosalki et al.\textsuperscript{26} respectively. Homogenate (10%) of myocardial tissue in ice cold KCl (0.15 M) was used for the assay of malondialdehyde according to the method of Ohkawa et al.\textsuperscript{27} and in phosphate buffer was used for the assay of superoxide dismutase (SOD) activity,\textsuperscript{28} catalase (CAT) activity,\textsuperscript{29} and glutathione (GSH) content.\textsuperscript{30}

Histopathological studies — At the end of the experiment, myocardial tissues from all the groups were subjected to histopathological studies.\textsuperscript{31} The tissues were fixed in formalin (10%), routinely processed and embedded in paraffin wax. Paraffin section (5μm) were cut on glass slides and stained with hematoxylin and eosin (H & E) after dewaxing, and examined under a light microscope.

Statistical analysis — Statistical analysis was carried out using Graphpad Prism 3.0 (Graphpad software; San Diego, CA). All data were expressed as mean ± SE. Groups of data were compared with an analysis of variance followed by Dunnett’s t test. Values were considered statistically significant at \( P<0.01 \).

Results
A significant increase in the heart rate and systolic blood pressure (BP) was observed in pathogenic control group (Group II), as compared to control rats (Group I). Aqueous ER extract (Group III) and gliclazide treated (Group IV) rats showed a significant decrease in heart rate as compared to pathogenic control group (Group II). However, no significant change in systolic BP was observed in aqueous ER extract and gliclazide treated rats as compared to pathogenic control rats (Table 1).

Serum LDH and CK levels were significantly increased in pathogenic rats (Group II) when compared with control rats (Group I), while aqueous ER extract (Group III) and gliclazide treatment (Group IV) significantly decreased the elevated levels of serum LDH and CK levels as compared to Group II rats (Table 1).

Myocardial LPO level was found to be significantly higher in Group II, as compared to Group I, while pretreatment with aqueous ER extract (Group III) and gliclazide (Group IV) decreased significantly the elevated level of LPO (Table 2).

The levels of endogenous antioxidants (SOD, catalase and tissue GSH) were decreased significantly in the Group II rats, as compared to control group I rats and this reduction was significantly reversed in Group III and Group IV rats (Table 2).

Histopathological studies — Photomicrograph of vehicle control group revealed a normal architecture with regular morphology of myocardial cell membrane (Fig 1a). However, the heart sections of ISO-treated pathogenic rats showed confluent necrosis of cardiac muscle fibre with infiltration of red blood cells leading to impairment of membrane structural and functional integrity (Fig. 1b). In animals, treated with aqueous ER extract pretreatment, the morphology of myocardium was essentially within normal limits. No area of necrosis and cellular infiltration was seen (Fig. 1c) indicating that aqueous ER extract has significant cardioprotective effect and it also, maintained myocardial membrane integrity. Photomicrograph of gliclazide treated group showed normal morphology with absence of inflammation and sign of muscle necrosis (Fig 1d).

| Table 1 — Effect of aqueous extract of E. ribes pretreatment on isoproterenol induced changes in the activities of hemodynamic parameters and serum enzymes |
|-------------------------------|-------------------|-----------------|-------------------|------------------|
| Treatment                      | Hemodynamic parameters | Serum enzymes | |
|                               | HR | Systolic BP | LDH | CK |
| Control                        | 417.290±1.190 | 115.000±1.250 | 191.110±7.400 | 60.740±3.100 |
| Isoproterenol                 | 544.830±5.056\textsuperscript{a} | 122.650±1.090\textsuperscript{a} | 488.900±18.500\textsuperscript{a} | 747.010±20.900\textsuperscript{a} |
| E. ribes + isoproterenol      | 484.500±5.536\textsuperscript{b} | 119.660±1.022 | 283.320±1.870\textsuperscript{b} | 292.320±1.970\textsuperscript{b} |
| Gliclazide + isoproterenol    | 512.670±3.180\textsuperscript{b} | 122.670±1.909 | 392.110±5.810\textsuperscript{b} | 396.050±3.770\textsuperscript{b} |

HR= Heart rate, beats per minute (BPM); BP= blood pressure (mm Hg); LDH= lactate dehydrogenase (IU/L); CK= creatine kinase (IU/L)

\( P \) values <0.01; when compared with \textsuperscript{a}normal control group, \textsuperscript{b}pathogenic control group.

Concentrations were – isoproterenol (5.25 and 8.5 mg/kg, sc, respectively for two consecutive days); E. ribes extract (100 mg/kg, po); and gliclazide (25 mg/kg, po)
Further, no significant biochemical and histopathological changes were observed when rats were treated with aqueous ER extract only (100 mg/kg) for a period of 40 days i.e. ER control group as compared with the normal healthy control group. Therefore, the data is not presented in the present research findings.

**Discussion**

The results of the present study showed the protection elicited by aqueous extract of dried fruits of *E. ribes* to ISO-induced infarction in rats. Experiments were performed inducing myocardial infarction by ISO, selected mainly because it resulted

<table>
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<tr>
<th>Treatment</th>
<th>LPO</th>
<th>GSH</th>
<th>SOD</th>
<th>CAT</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.522 ± 0.044</td>
<td>99.60 ± 6.008</td>
<td>1.410 ± 0.030</td>
<td>1.933 ± 0.610</td>
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<tr>
<td>Isoproterenol</td>
<td>3.940 ± 0.167&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.01 ± 1.750&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.101 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.216 ± 0.031&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>E. ribes</em> + isoproterenol</td>
<td>0.948 ± 0.025&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.60 ± 1.240&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.034 ± 0.018&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.968 ± 0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gliclazide + isoproterenol</td>
<td>2.183 ± 0.060</td>
<td>36.900 ± 0.721&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.629 ± 0.081&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.355 ± 0.026</td>
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LPO = lipid peroxides (nmol MDA/mg protein); GSH = glutathione (µmol of phosphorous liberated/min/mg protein); SOD = superoxide dismutase (IU/mg protein); CAT = catalase (nmol H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein)

P values: <sup>a</sup><0.01, when compared with normal control group, <sup>b</sup><0.01, <sup>c</sup><0.05 when compared with pathogenic control group.

Concentrations of isoproterenol, *E. ribes* extract, and gliclazide used were as indicated in Table 1

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**Fig. 1** — Histological examination of heart in experimental animals. (a) - Vehicle control group (Group I) rat showing normal architecture of heart with regular morphology of myocardial cell membrane (10X); (b) - Pathogenic control group (Group II) rat showing inflammatory infiltrate (A) with oedema (B) in heart section (10X); (c) - *E. ribes* treatment group (Group III) rat showing absence of inflammation and sign of muscle necrosis in heart section (10X); (d) - gliclazide treatment group (Group IV) rat showing normal myocardial fibres (10X).
in less impairment of function. Using this protocol, *E. ribes* significantly improved the recovery of myocardial infarction after ISO administration. In any case, whichever factors induced the heart rate and systolic BP after the ISO administration, heart rate was significantly attenuated by treatment with *E. ribes*. In addition, we could not observe significant decrease in the systolic BP.

The induction of myocardial infarction in experimental animals by ISO is probably due to action on the sarcolemmal membrane, stimulation of adenylyl cyclase, activation of Na⁺ and Ca⁺ channels, exaggerated Ca⁺ inflow and energy consumption leading to cellular death. Further, a significant increase in serum LDH and CK activity was observed in ISO-treated rats. An increase in the levels of serum LDH and CPK indicate cardiac muscular damage which may be due to leakage of enzymes from the heart as a result of ISO-induced myocardial infarction. Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane structural and functional integrity. This concurs with the present findings wherein the levels of TBARS were found to be significantly (*P* < 0.01) increased in animals subjected to ISO exposure. Due to this increased lipid peroxidation, glutathione levels are lowered. Further, the ISO-treated animals showed decreased activity of the key antioxidants SOD, CAT and GSH. The decrease in the activity of these antioxidants can lead to an excess availability of the superoxide anion (O₂⁻) and hydrogen peroxide in biological systems, which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation.

In the present study, pretreatment with *E. ribes*, significantly (*P* < 0.01) reduced the levels of serum LDH and serum CPK suggesting cardioprotective potential of *E. ribes* fruits. Further, significant decrease in TBARS and increase in GSH, SOD and CAT levels were observed, thereby, enhancing the endogenous myocardial antioxidant levels. Furthermore, the results of ER were comparable to glinazide, a standard positive control.

Furthermore, histopathological observations revealed that *E. ribes* prevented the degeneration of myofibrillar tissue and leucocytic infiltration in myocardial infarction. In conclusion, the present results indicated that the rats pretreated with *E. ribes* were significantly protected from myocardial damage caused by ISO. Pharmacological augmentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in disease associated with increased oxidative stress.

Chemically, *Embelia ribes* is reported to contain embelin, quercitol (polyphenol), tannins and alkaloids, which may contributes to its antioxidant activity. Further, embelin has been isolated from the ethanolic extract of *Embelia ribes*. In the present study, on phytochemical analysis of the aqueous extract of *Embelia ribes*, it was found to contain alkaloids, carbohydrates, phenolic compounds, flavonoids, proteins and saponins. It can, thus, be concluded that antioxidant effect of aqueous extract of *Embelia ribes* might be due to polyphenols, flavonoids, saponins and alkaloids.

**Acknowledgment**

The author (U B) acknowledges University Grants Commission, New Delhi, India for financial support.

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