COMPARATIVE STUDY OF CHLOROFORM FRACTION OF RUBIA CORDIFOLIA AND CARVEDILOL AGAINST IN VIVO MODEL OF MYOCARDIAL ISCHEMIA-REPERFUSION INJURY IN RATS

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ABSTRACT

Present study evaluated the cardioprotective effect of chloroform fraction of root of Rubiacordifolia (CFRRC) against ischemia-reperfusion (I-R) and Cardioprotective effect of CFRRC was evaluated. The antioxidant and free radical scavenging activity of CFRRC was supported by its in vitro DPPH scavenging activity. Serum estimations revealed a significant decrease in activities of cardiac biomarkers (LDH and CK-MB) in the groups pre-treated with CFRRC. Pre-treatment with CFRRC prevented GSH depletion and also inhibited lipid peroxidation in heart tissue. in vitro and in vivo studies of CFRRC and comparative study with Carvedilol indicate that pretreatment with CFRRC at the doses (100 and 200 mg/kg, i.p.) showed significant therapeutically changes in a dose dependent manner against myocardial ischemia-reperfusion injury. Further clinical research is needed to confirm the beneficial effects of Rubiacordifolia in various cardiovascular disorders.

Keywords: Ischemia-reperfusion, chloroform fraction of root of Rubiacordifolia (CFRRC), Carvedilol

INTRODUCTION

Restoration of coronary blood flow (reperfusion) after an ischemic episode contributes to myocardial ischemia-reperfusion (I-R) injury. Myocardial I-R injury represents a clinically relevant problem associated with thrombolytic therapy, coronary angioplasty, coronary bypass surgery and heart transplantation[1]. Although timely reperfusion is essential for the salvage of dying myocardium, however, sudden restoration of blood may exaggerate myocardial injury paradoxically. Approximately 3.8 million men and 3.4 million women die of myocardial I-R injury each year[2]. I-R injury is associated with increased generation of reactive oxygen species (ROS) leading to oxidative stress, intracellular calcium overload, rapid restoration of physiologic pH and inflammation, which may result in hemodynamic impairment, contractile dysfunction, arrhythmias, endogenous antioxidants depletion and biochemical alterations[3,4].

Development of novel therapeutics to reduce or prevent reperfusion injury is of major importance. Although modern drugs are effective but their use is often limited because of their side effect and adverse reaction[5]. Many of plants have been investigated to contain active substances that are medically useful in myocardial I-R injury and therefore, ethno-botanical and ethno-pharmacological studies of such plants continue to attract investigators for research work globally. In vivo small rodent model of myocardial I-R injury in which surgical occlusion of coronary artery followed by reperfusion have been developed.
to mimic more closely the real clinical setting and is the suitable experimental model for evaluating cardio protective molecular entities and plants origin remedy.

Rubiacordifolia (Rubiacae) commonly known as Manjistha, has been reported for the presence of glycosides, anthraquinones, tannins, hexapeptides, quinones, triterpenoids[7]. Rubiacordifolia role in supporting heart health is evidenced by traditional and reported activities which show that it act as potent blood purifier and diuretic[8], antioxidant[9, 10], calcium channel blocker[11], antiplatelet[12], ACE inhibitor[13], anti-inflammatory[7], antistress[14], hypolipidemic and hypoglycemic[15], wound healing[16], immunomodulator activity[17].

The roots of Rubiacordifolia having high medicinal value and are recognized as official for the treatment of various disorders[17]. Chloroform fraction of root of Rubiacordifolia (CFRRC) contains triterpenoids in major concentration[18]. Oleananes, rubicoumaric acid, rubifolic acid, and various other types of triterpenoids were isolated from CFRRC[18, 19]. Rubiatriol, a new triterpenoid with two known anthraquinones, isolated from the CFRRC, which has inhibitory activity on the angiotensin converting enzyme (ACE)[12]. Isolation of mollugin, furomollugin and dehydro-alpha-laphchone were done from the CFRRC, in which mollugin has been reported for their antiplatelet activity[20]. Rubiadin isolated from chloroform fraction has been known for their potent antioxidant property.

Rubiacordifolia is an individual plant with multiple activities that is essential to support heart health could become a new approach in the management I-R induced myocardial injury and therefore, need to the exploration of this activity. However, so far no scientific study has been reported regarding the cardioprotective activity of CFRRC. Thus, the present study aims to investigate the preventive effect of CFRRC on biochemical, infarct size, histopathology and hemodynamic alterations in myocardial I-R induced cardiotoxicity in rats.

**MATERIALS AND METHODS**

**Experimental animals**: Sprague-Dawley rats (250 ± 30 g) of either sex procured from Central Animal House Facility, NIPER, Mohali, India. The animal study protocol was approved by the Institution Animals Ethics Committee (IAEC) of ASBASJSM College of Pharmacy, BELA (Ropar), Punjab with approval no. ASCB/IAEC/05/12/067. Animals were housed in polypropylene cages under standard laboratory conditions (23± 1°C, 55±10% RH, 12/12h light/dark cycle) and fed with standard rodent pellet diet (M/s Ashirwad Industry, Mohali, India) and purified water ad libitum.

**Chemicals**: All the chemicals were of analytical grade and purchased from Sigma Aldrich, Lobachemie and Hi Media. Lactate dehydrogenase (LDH), creatine kinase-MB and alkaline phosphatase (ALP) kits were purchased from Reckon Diagnostics. Triple deionized water was used for all biochemical estimations.

**Plant material**: Roots of Rubiacordifolia L. were collected and authenticated from Dr. Y.S. Parmar University of Horticulture and Forestry, Solan (Nauni), Himachal Pradesh, India. A voucher specimen of the collected sample was deposited in UHF-herbarium with Field Book No. 12570 for future reference.

**Preparation of plant extract**: Roots of Rubiacordifolia were dried in shade and coarsely powdered in a mechanical grinder by passing with sieve no. 14. Defatted by petroleum ether at 40-60 °C in the soxhlet apparatus and extracted with 80% (v/v) methanol by cold percolation at room temperature. Methanolic extract with three successive extractions, concentrated to a sticky gum in a rotary vacuum evaporator at 45 °C (Heidolph, Germany) under reduced pressure. Methanolic extract was successively fractioned with chloroform in a separating funnel. Then, chloroform fraction of root of Rubiacordifolia (CFRRC) was concentrated under reduced pressure, dried in a vacuum desiccator and weighed.

**Preparation of drugs**

**CFRRC (test drug)**: For in vivo experiments, CFRRC with two dose levels 100 mg/kg and 200 mg/kg, suspended with DMSO (10% v/v) in normal saline. Filtered through 0.45 µ Millipore membrane filter and administered by intraperitoneal route (i.p.) in experimental animals for seven days.

**Carvedilol (Standard drug)**: Carvedilol (1 mg/kg) was dissolve in normal saline and administered by i.p. route in experimental animals for 7 days.

**Experimental design**: A total of 60 rats were used for this study and randomly divided into five experimental groups, each containing 12 rats. Group I (Sham): Rats were administered 0.9% normal saline (i.p.) for 7 days and on the 7th day subjected to the thoracotomy and thread was passed beneath the

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left anterior descending coronary artery (LADCA) but no ligation was performed.

Group II (Control-R): Rats were administered 0.9% normal saline (i.p.) for 7 days and on the 7th day subjected to left anterior descending coronary artery LADCA ligation for 45 min and reperfusion for 60 min to induce myocardial I-R injury.

Group III (Carvedilol+ I-R): Rats were administered Carvedilol (1 mg/kg, i.p.) for 7 days and on the 7th day subjected to left anterior descending coronary artery LADCA ligation for 45 min and reperfusion for 60 min.

Group IV (CFRRC 100 mg/kg + I-R): Rats were administered CFRRC (100 mg/kg, i.p.) for 7 days and on the 7th day subjected to left anterior descending coronary artery LADCA ligation for 45 min and reperfusion for 60 min.

Group V (CFRRC200 mg/kg + I-R): Rats were administered CFRRC (200 mg/kg, i.p.) for 7 days and on the 7th day subjected to LADCA ligation for 45 min and reperfusion for 60 min.

**Myocardium ischemia-reperfusion (I-R) injury:** Rat was anaesthetized with urethane (1.5 g/kg, i.p.) and body temperature was maintained at 35 °C by using heating pad throughout the experimental protocol. Neck was opened and tracheostomy was performed to ventilate with room air from a positive pressure ventilator (Inco, India) using compressed air at the rate of 70 strokes/min and tidal volume of 1.5 ml/100 g. Left jugular vein was cannulated with polyethylene tube for continuous infusion of saline solution (0.9% NaCl). A left thoracotomy was performed at the fifth intercostal space by using rib cutter and retractor. Péricardium was opened to expose the heart and a 5/0 surgical suture (monofilament polyamide) attached to a 16 mm needle (circle cutting) was quickly placed under the LADCA. The animal was then stabilized for 10-15 min before LADCA ligation. LADCA was ligated 2-3 mm from its origin between the pulmonary artery conus and the left atrium with a needle suture (monofilament polyamide) by pressing the polyethylene tubing against the ventricular wall. The animal then underwent 45 min of ischemia confirmed by appearance of epicardial cyanosis. Further, reperfusion of myocardium was done by releasing the snare gently for a period of 60 min. The thoracic cavity was covered with saline-soaked gauze to prevent the heart from drying. At the time of sacrifice, blood was subsequently collected from rat carotid artery and the heart was excised under anesthetic situations and a syringe (with polyethylene tubing fixed on needle) containing PBS (phosphate buffer solution) directly inserted into aorta to retrogradely perfuse rinse the heart to remove any blood that remains and processed for biochemical, triphenyltetrazolium chloride (TTC) and histopathological studies.

**Hemodynamic measurement:** Firstly, separation of carotid artery attached with vagus nerve was done by pointed curve forceps and then, carotid artery cannulated with polyethylene tube (internal diameter 0.30 mm; outer diameter 0.40 mm) attached to a three-way cannula. The cannula was heparinized (Heparin 300 IU/ml) and connected to Power Lab 4/30 (AD Instruments, NSW, Australia) system using a pressure transducer for the measurement of mean arterial pressure (MAP) and heart rate (HR).

**Biochemical estimations in serum:** Blood was subsequently collected from rat carotid artery at the time of sacrifice and serum was prepared by centrifugation after clotting for lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) measurements by using assay kits (Reckon diagnostics) on autoanalyser (Microlab 300).

**Biochemical estimations in tissue**

**Thiobarbituric acid reactive substances (TBARS) estimation:** Lipid peroxidation was determined by the method\(^\text{[21]}\). Heart was homogenized in 0.1 M phosphate buffer- pH 7.4 (10% w/v) using Teflon homogenizer. The clear supernatant, obtained after centrifugation at 6000 for 25 min was used to estimate TBARS. The method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically. 0.2 ml supernatant was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 30% acetic acid and 1.5 ml of 0.8% thiobarbituric acid (TBA) and made volume up to 4 ml by adding distilled water. The tubes were covered with aluminum foils and kept in a shaking water bath for 1 h at 95 °C. After 1 h, the tubes were taken out and kept in ice-cold water for 10 min. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol:pyridine (15:1 v/v) were added and centrifuged at 6000 rpm for 25 min. The absorbance of the supernatant was read at 532 nm at room temperature against a reagent blank with no homogenate. The TBARS value was calculated by using an extinction coefficient of 1.56 × 10^5 M\(^{-1}\) cm\(^{-1}\).

**Tissue glutathione estimation:** Myocardial GSH was estimated by the method\(^\text{[22]}\). Heart was homogenized in 0.1 M phosphate buffer- pH 7.4 (10% w/v) using Teflon homogenizer. The clear supernatant, obtained after centrifugation at 6000 for 25 min was used to
measure the reduced glutathione (GSH) level. In this method, the supernatant was mixed with equal volume of trichloroacetic acid (10% w/v) containing 0.02 M EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 10 min. After 10 min, the contents were transferred to centrifuge tubes and centrifuged at 6000 rpm for 25 min. Following centrifugation, 1 ml of the supernatant was mixed with 4.0 ml of phosphate buffer. The whole solution was mixed well and 0.1 ml of 0.01M DTNB (5, 5'-dithio bis-2-nitrobenzoic acid) was added to it. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. The concentration of reduced glutathione was obtained from the regression equation of standard curve of GSH.

Evaluation of myocardial infarct size: The myocardial infarct size was measured by using triphenyltetrazolium chloride (TTC) staining. Heart tissues were frozen by storing at -20°C (deep freezer) for 30 min. The sections were then incubated for 20 min at 37°C with 1% TTC in PBS (pH 7.4) and fixed in 10% formalin. The image of TTC slices was captured with a digital camera and analyzed by the Image J (software).

Histological examination: The apex portion of heart tissue obtained from all the experimental groups were fixed in buffered formalin solution (40%) and embedded in paraffin, sectioned at 5µm, stained with hematoxylin and eosin (H&E). All this procedure was done in Histopathology department of Sandhu pathology labs, Chandigarh and pictures were obtained from photomicroscope.

Statistical analysis: The data were expressed as mean ± SEM. Results were statistically analyzed by one-way ANOVA followed by Turkey Multiple Comparison Test using Graph Pad Prism. P< 0.05 was considered as statistically significant. The IC₅₀ values were calculated graphically by linear regression analysis.

RESULTS

Phytochemical screening of CFRRC reported positive for cardiac and anthraquinone glycosides, volatile oils, naphthoquinones, steroids and triterpenoids.

Infract size and mortality: A total of 60 rats were selected for the study and divided in to 5 groups out of which 4 groups has undergone the coronary artery ligation. The ligation procedure was not in the sham test however a thread has been passed beneath the artery. The infarct size increased to 38.18% in the control when the ligation was performed on 7th day and the size was significantly reduced to 14.40% after ligation in the rats pretreated with Carvedilol for 7 days and slight reduced to 23.89% after ligation on 7th day in CFRRC 100 mg/kg group and 20.25% after ligation on 7th day in 200 mg/kg group compared to the Carvedilol treated group. Mortality during the study was also in accordance with the infarct size (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Infarct size (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>3.07</td>
<td>—</td>
</tr>
<tr>
<td>Control + I-R</td>
<td>38.18</td>
<td>30</td>
</tr>
<tr>
<td>Carvedilol + I-R</td>
<td>14.40</td>
<td>10</td>
</tr>
<tr>
<td>CFRRC 100 mg/kg + I-R</td>
<td>23.89</td>
<td>20</td>
</tr>
<tr>
<td>CFRRC 200 mg/kg + I-R</td>
<td>20.25</td>
<td>20</td>
</tr>
</tbody>
</table>

Cardiac haemodynamics

The perfused rat heart injured by a 45 min period of global ischemia followed by 60 min of reperfusion exhibits recovery. The MAP at the baseline was 131.77 ± 2.99 mm Hg in the control period and decreased (27.78%) to 95.16 ± 4.26## mm Hg after 60 min of reperfusion and a similar pattern of shift can be observed in case of Carvedilol (1 mg/kg), CFRRC (100 mg/kg) and CFRRC (200 mg/kg). The base line value gradually decreased(7.34%) from 131.77 ± 2.99 mm Hg in the control group to 122.09 ± 3.29 mm Hg in the Carvedilol (1 mg/kg) treated group However it was slightlydecreased in the case of CFRRC (100 mg/kg) and CFRRC (200 mg/kg) pretreated group when compared to Control. At the end of Ischemia and reperfusion the carvedilol treated group shows increased MAP compared to the Control group whereas the CFRRC (100 mg/kg) and CFRRC (200 mg/kg) treated groups shows slight increase in the MAP.A similar trend and shifts can be observed in the case Heart rate in the Baseline, end of ischemia and end of reperfusion (Table 2).
Table 2: Cardiac haemodynamics

<table>
<thead>
<tr>
<th>Group</th>
<th>Base line (B= 10 min)</th>
<th>End of ischemia (I= 45 min)</th>
<th>End of reperfusion (R= 60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>134.75 ± 2.84</td>
<td>126.55 ± 3.89▲</td>
<td>122.35 ± 3.17▲</td>
</tr>
<tr>
<td>Control I-R</td>
<td>131.77 ± 2.99</td>
<td>93.97 ± 3.26###</td>
<td>95.16 ± 4.26##</td>
</tr>
<tr>
<td>Carvedilol (1 mg/kg) + I-R</td>
<td>122.09 ± 3.29</td>
<td>116.10 ± 3.11***</td>
<td>120.43 ± 5.63**</td>
</tr>
<tr>
<td>CFRRRC (100 mg/kg) + I-R</td>
<td>129.85 ± 2.37</td>
<td>103.62 ± 4.19</td>
<td>111.62 ± 4.43</td>
</tr>
<tr>
<td>CFRRRC (200 mg/kg) + I-R</td>
<td>129.50 ± 1.99</td>
<td>108.87 ± 3.10</td>
<td>113.99 ± 4.39*</td>
</tr>
<tr>
<td><strong>HR (beats min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>409.21 ± 6.87</td>
<td>385.87 ± 3.50▲</td>
<td>356.90 ± 5.87▲</td>
</tr>
<tr>
<td>Control I-R</td>
<td>405.68 ± 4.91</td>
<td>234.97 ± 3.15###</td>
<td>267.40 ± 5.19###</td>
</tr>
<tr>
<td>Carvedilol (1 mg/kg) + I-R</td>
<td>384.76 ± 3.79</td>
<td>280.13 ± 4.33***</td>
<td>301.31 ± 5.10**</td>
</tr>
<tr>
<td>CFRRRC (100 mg/kg) + I-R</td>
<td>401.61 ± 4.15</td>
<td>246.73 ± 4.83</td>
<td>272.80 ± 4.92</td>
</tr>
<tr>
<td>CFRRRC (200 mg/kg) + I-R</td>
<td>399.58 ± 5.43</td>
<td>251.20 ± 5.34</td>
<td>288.20 ± 3.89</td>
</tr>
</tbody>
</table>

(n= 12), Values are expressed as mean ± SEM
### (p< 0.001) vs sham and *** (p< 0.001), **(p< 0.01), *(p< 0.05) vs control I-R
(One way analysis of ANOVA followed by Turkey’s test)

**Biochemical estimations in serum:** The carvedilol pretreated rats showed a significant increase in the level of LDH and CK-MB when compared to normal rats. The group CFRRRC (100 mg/kg) and CFRRRC (200 mg/kg) showed decrease in the level of cardiac marker enzymes when compared with Control rats. The administration of CFRRRC extract alone treated group showed an increase in the level of cardiac marker enzymes when compared with Carvedilol treated group (Table 3). Tissue MDA levels of rats were found to be decreased in carvedilol group compared to control treated group (p<0.001). Tissue levels of MDA were recovered in CFRRRC (100 mg/kg) and CFRRRC (200 mg/kg) group when compared to carvedilol administered group (p<0.001).

Tissue GSH levels of rats were found to be increased in carvedilol group compared to control treated group (p<0.001). Tissue levels of GSH were recovered in CFRRRC (100 mg/kg) and CFRRRC (200 mg/kg) group when compared to carvedilol administered group (p<0.001) (Table 4).

Table 3: Biochemical estimations in serum

<table>
<thead>
<tr>
<th>Group</th>
<th>CK-MB (IU/L)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>31.76 ± 2.76</td>
<td>785.88 ± 50.76</td>
</tr>
<tr>
<td>Control I-R</td>
<td>105.88 ± 4.21###</td>
<td>1680.94 ± 58.60###</td>
</tr>
<tr>
<td>Carvedilol (1 mg/kg) + I-R</td>
<td>80.80 ± 4.19**</td>
<td>1143.94 ± 59.70***</td>
</tr>
<tr>
<td>CFRRRC (100 mg/kg) + I-R</td>
<td>89.28 ± 3.92*</td>
<td>1427.98 ± 48.37*</td>
</tr>
<tr>
<td>CFRRRC (200 mg/kg) + I-R</td>
<td>85.68 ± 3.62**</td>
<td>1385.94 ± 60.47*</td>
</tr>
</tbody>
</table>

(n= 12), Values are expressed as mean ± SEM
### (p< 0.001) vs sham and *** (p< 0.001), **(p< 0.01), *(p< 0.05) versus control I-R
(One way analysis of ANOVA followed by Turkey’s test)

Table 4: Tissue estimation

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (µmol/g tissue)</th>
<th>MDA (n mol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10.62 ± 0.29</td>
<td>17.82 ± 1.63</td>
</tr>
<tr>
<td>Control I-R</td>
<td>3.46 ± 0.12###</td>
<td>48.97 ± 2.59##</td>
</tr>
<tr>
<td>Carvedilol (1 mg/kg) + I-R</td>
<td>7.59 ± 0.33***</td>
<td>29.87 ± 1.35***</td>
</tr>
<tr>
<td>CFRRRC (100 mg/kg) + I-R</td>
<td>4.89 ± 0.29*</td>
<td>37.69 ± 1.86**</td>
</tr>
<tr>
<td>CFRRRC (200 mg/kg) + I-R</td>
<td>5.21 ± 0.34**</td>
<td>35.76 ± 2.23**</td>
</tr>
</tbody>
</table>

(n= 12), Values are expressed as mean ± SEM
### (p< 0.001) vs sham and *** (p< 0.001), **(p< 0.01), *(p< 0.05) vs control I-R
(One way analysis of ANOVA followed by Turkey’s test)
Effect of CFRRC on histopathological alterations in rat cardiac apex: The control group has shown highly severe inflammation, muscle separation, edema and myonecrosis when compared to the normal rats. The carvedilol treated rats has shown less inflammation, muscle separation and did not show any show edema and myonecrosis due to is cardioprotective activity. The CFRRC treated rats shown less histopathological alterations in rat cardiac apex as the concentrations increases from 100 mg/kg to 200 mg/kg (Figure 1, Table 5).

Table 5: Effect of CFRRC on histopathological alterations in rat cardiac apex

<table>
<thead>
<tr>
<th>Group</th>
<th>Inflammation</th>
<th>Muscle separation</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Control I-R</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Carvedilol (1 mg/kg) + I-R</td>
<td>++</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>CFRRC (100 mg/kg) + I-R</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>CFRRC (200 mg/kg) + I-R</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

(−): no damage; (+): mild; (++): moderate; (+++): severe; (++++): highly severe.
DISCUSSION

Reperfusion through thrombolysis, coronary angioplasty or bypass surgery is the standard treatment for acute myocardial infarction, meaning that reperfusion injury will be a major clinical problem in the near future because approximately 3.8 million men and 3.4 million women die of myocardial ischemia-reperfusion (I-R) injury each year. Various studies have reported that I-R sequence results in the generation of free radicals in the myocardium [8]. Ischemia reduced the activity of cellular defense systems against free radicals and reperfusion or restoration of oxygen further disturbs the delicate balance of oxidants/antioxidants and generates a burst of free radicals in the tissue. Experimental data obtained from studies using mannitol, ACE inhibitors, calcium-channel blockers, allopurinol and vitamin E suggest that antioxidant therapy may prevent or attenuate I-R induced myocardial injury.

Roots of \textit{Rubia cordifolia} having high medicinal value and are recognized as official. Anti-platelet activity[9] and calcium channel blocker(s) like constituents in this plant[11] are therapeutically important in management of myocardial I-R injury. Rubiatriol (ACE inhibitor), mollugin (antiplatelet) and Rubiadiin (antioxidant) biomarkers have been isolated from chloroform fraction of \textit{Rubia cordifolia} root (CFRRC) [9,11,20] indicates its possibility to treat I-R induced myocardial injury and therefore, CFRRC was selected for this study.

The antioxidant and free radical scavenging activity of CFRRC was supported by its \textit{in vitro} DPPH scavenging activity. [27] The effect of antioxidants on DPPH is due to their hydrogen donating ability. The estimation of total phenolic content (125.5 mg/g GAE) present in CFRRC also showed its powerful antioxidant property due to its ability to donate hydrogen or electron and to form stable radical intermediates. \textit{Rubia cordifolia} known for its antioxidant, anti-inflammatory and potent blood purifier[8] activities, potentiate its therapeutic role in detoxification of I-R induced free radical generation.

\textit{In vivo} small rodent model of myocardial I-R injury in which surgical occlusion of coronary artery followed by reperfusion have been developed to mimic more closely the real clinical setting[6] and therefore, a rat model of myocardial I-R was employed in order to evaluate cardioprotective effect of CFRRC and also to compare the relative ability of Carvedilol and CFRRC to blunt the I-R induced myocardial injury.

Myocardial I-R injury is associated with reduction in cellular GSH content and increased level of lipid peroxidation as evidenced by increased levels of TBARS. In present study increased level of GSH as well as decreased levels of lipid peroxides in heart tissue clearly indicates the antioxidant property of CFRRC may be due to presence of triterpenoids[19] and anthraquinones constituents in CFRRC which neutralizes the cytotoxic free radicals generated during I-R injury, thereby protecting myocardium against the loss of membrane integrity.

In the control I-R group, a continuous and significant fall in mean arterial pressure (MAP) and heart rate (HR) were observed as compared with sham group. ACE inhibition, calcium channel blockers[11], diuretic[8] and anti-stress[13] activities of CFRRC preserved the cardiac function in ischemic-reperfusion rats. Pretreatment with CFRRC significantly restored MAP at the end of the reperfusion period in a dose-dependent manner compared with the control I-R group. Similarly, the HR was significantly decreased throughout the experiment duration in the control I-R group compared with sham group. A significantly activity of CFRRC was not observed in case of HR but at some level HR was restored which may be or at least partially involved in the cardioprotective mechanism against myocardial I-R injury.

When myocardial cells are damaged, leakage of myocardial enzymes such as Lactate dehydrogenase (LDH) and Creatine kinase (CK-MB) occurs which accounts for the increased activities of serum biomarker enzymes and therefore, serve as a sensitive index to assess the severity of I-R induced irreversible myocardial injury. LDH converts pyruvate, the final product of glycolysis to lactate when oxygen it absent or in short supply and the activity of LDH will increase when ischemia lasts. CK catalyses the conversion of creatine and consumes ATP to create phosphocreatine and ADP. Elevation of CK-MB in serum is an indication of muscle damage. As expected, a significant decrease (23.68 %) in CK-MB levels was observed in Carvedilol (1 mg/kg, \textit{i.p.}) treatment and marked reduction (19.07 %) also found in CFRRC (200 mg/kg, \textit{i.p.}) treated groups as compared with control I-R group. The reduced activities of serum biomarker enzymes may be due to antioxidant, anti-inflammatory and presence of calcium channel blocker(s) like constituents in this plant[11], which could be responsible for attenuating reactive oxygen species (ROS) induced oxidative stress, intracellular Ca$^{2+}$ overload and inflammation.
ACE inhibitor\cite{24}, platelet inhibitors\cite{25} and antioxidants\cite{26} have been evaluated for reduction in myocardial infarct size in I-R injury. CFRRC reported with similar activities showed marked reduction in myocardial infarct size.

Histopathological examination of the myocardium of sham group showed clear integrity of myocardial cell membrane. Marked myonecrosis, edema, muscle separation and infiltration of inflammatory cells were observed in control I-R group. The reduction of histopathological scores after CFRRC pretreatment has demonstrated its myocardial salvaging effect could be result of potent antioxidant and anti-inflammatory activity. Reduction in membrane lipid peroxidation by CFRRC was ascribed to morphological recoup and histological protection of the myocardium against I-R injury.

*In vitro and in vivo* studies showed that CFRRC is a potent antioxidant which can effectively scavenge free radicals and thereby reduce the risk of oxidative stress and myocardial infarction induced mortality. Comparative study of Carvedilol and CFRRC also indicates that pretreatment with CFRRC has significant cardioprotective activity as revealed by its mitigating effects on several ischemia-reperfusion induced biochemical, histopathological and hemodynamic alterations.

**CONCLUSION**

The *Rubia cordifolia* roots were subjected to extractions in chloroform. From the experiment it was observed that these plants have certain important constituents which were responsible for Radical scavenging activity. The cardioprotective effect of chloroform fraction of root of *Rubia cordifolia* (CFRRC) against ischemia-reperfusion (I-R) induced biochemical, antioxidant potential, infarct size, hemodynamic, histopathological alterations indicates its possibility to treat I-R induced myocardial injury when compared to the carvedilol.

**REFERENCES**