

Research Article

## Effect of Ethanolic Extract of *Spondias pinnata* on Ischemia-Reperfusion Injury and Ischemic Preconditioning of Heart

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### ABSTRACT

**Background and Purpose:** Cardiac ischemic preconditioning represents the most powerful endogenous protective mechanism against ischemia. *Spondias pinnata* is rich source of flavonoids and phenolic compounds. The purpose of present study is to investigate the effect of ethanolic extract of stem bark of *Spondias pinnata* (100 mg/kg or 200 mg/kg) on ischemia-reperfusion injury and ischemic preconditioning of heart. **Experimental Approach:** Hearts from albino rats of Wistar strain were isolated and immediately mounted on Langendorff's apparatus for retrograde perfusion. Myocardial ischemia reperfusion injury was produced by mounting isolated rat hearts on Langendorff's apparatus and global ischemia was produced for 30 min followed by reperfusion for 120 min. In ischemic preconditioning group, the hearts were subjected to four episodes of 5 min ischemia and 5 min of reperfusion after 10 minutes of stabilization and then 30 min global ischemia, followed by 120 min of reperfusion. Myocardial infarct size was estimated macroscopically using TTC staining. The magnitude of cardiac injury was measured by Lactate Dehydrogenase (LDH) and Creatine Kinase (CK) concentration in the coronary effluent. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R induced myocardial injury. **Key Results:** *Spondias pinnata* ethanolic extract significantly reduced ischemia reperfusion-induced myocardial injury in vitro, but it does not enhanced the cardioprotective effect of ischemic preconditioning. **Conclusion and Implications:** Administration of *Spondias pinnata* ethanolic extract may prevent ischemic and reperfusion induced myocardial injury probably by its antioxidant activity.

**Keywords:** Ischemia, Ischemia preconditioning, Reperfusion, *Spondias pinnata*

### INTRODUCTION

Ischemic heart disease (IHD) represents a global burden on health care resources and will be the leading cause of morbidity and mortality in the world by 2030<sup>15</sup>. Acute occlusion of major coronary artery, leading to myocardial infarction, represents a staggering economic burden in the industrialized and developed countries<sup>2</sup>. The twentieth century has seen extraordinary advances in cardiovascular medicine from interventional cardiology to coronary artery bypass and heart transplantation<sup>16</sup>. Despite recent advances in the treatment of coronary artery disease, cardiac ailments continue to rank as the most frequent cause of mortality in the world wide<sup>6</sup>. Ischemic preconditioning is a phenomenon in which brief, reversible episodes of myocardial ischemia protect the myocardium against subsequent prolonged ischemic insult<sup>13</sup>. *Spondias pinnata* (Anacardiaceae) is the potential source of β-amyrin, oleanolic acid and amino acids (alanine and leucine)<sup>25,26</sup>. The Plant's fruits are used as astringent, antiscorbutic and in bilious dyspepsia. The bark of plant is astringent, refrigerant and used in diarrhoea and dysentery. Topically, the paste of bark is employed in rheumatic pain. Moreover, roots of plant are employed for regulating menstruation<sup>11</sup>. *Spondias pinnata* is rich source of

flavonoids and phenolic compounds<sup>8</sup>. The administration of plant extract of *Spondias pinnata* (200 mg/kg, p.o) has been reported to have antibacterial and ulcerprotective activity<sup>1</sup>. Additionally, the antioxidant activity of this plant has been reported by using 70% of methanolic extract of stem bark of *Spondias mangnifera*<sup>8</sup>. Moreover, recently the hepatoprotective activity of the stem heart wood of *Spondias pinnata* has also been reported<sup>21</sup>.

### MATERIALS AND METHODS

#### Procurement of plant materials

The bark of *Spondias pinnata* was procured from Dhanlakshami Agro Plantations and Consultancy, Tamilnadu. The bark was authenticated by Dr. B. N. Sridhar, Assistant Director, Regional Research Institute, Bangalore. The plant materials were dried under shade and ground to coarse powder.

#### Chemicals

All chemicals, reagents and solvents used in quantitative analysis and chemical investigation were of analytical grade.

#### Experimental animals

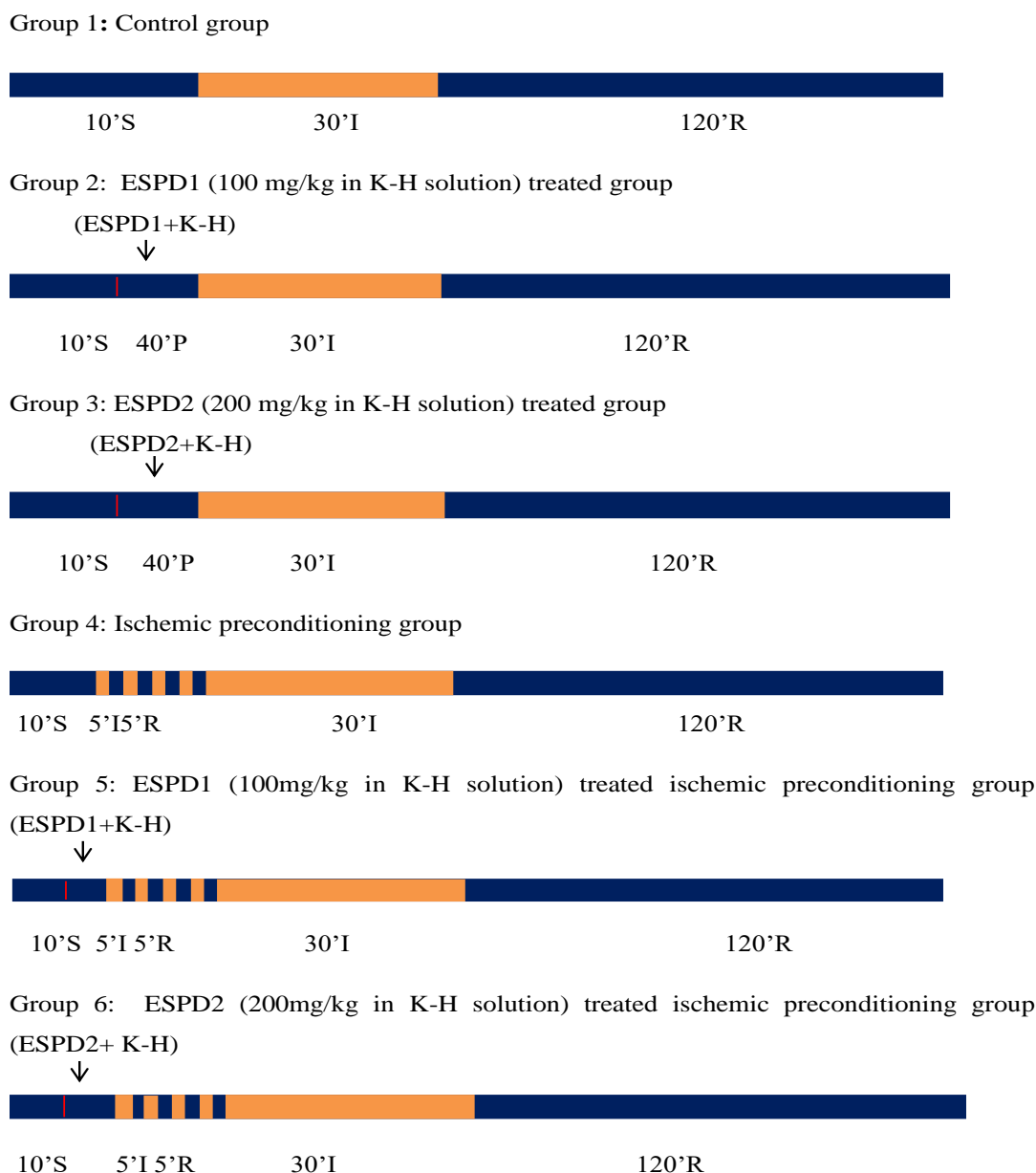


Figure 1: Diagrammatic representation of grouping of animals. Abbreviations: ESPD1 (Ethanolic extract *Spondias pinnata* dose (100 mg/kg), ESPD2 (Ethanolic extract *Spondias pinnata* dose (200 mg/kg), I (Ischemia), R (Reperfusion), P (Perfusion)

Table 1: Allocation of earthworms to various groups

Group no.	Group name	Dose (mg/ml)
I	Control group (vehicle)	-
II	Test group ethanolic extract	100
III	Test group ethanolic extract	200
IV	Preconditioning group	-
V	Preconditioning group ethanolic extract	100
VI	Preconditioning group ethanolic extract	200

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC approval no.954/AC/06/CPCSEA/09/15) of Insitution. Wistar albino rats of either sex weighing (150-200 g) were acclimatized in the Institutional animal house. The animals were kept under natural day and night cycle with temperature 21±2° C. They were fed with the commercial pelleted animal feed (supplied by M/s. Hindustan Lever Ltd. Bangalore, India) and water ad libitum. The study was approved by institutional animal ethical committee.

*Isolated Perfused Rat heart*

Rats were heparinised (500 IU, i.p.) about 20 min before sacrificing them by cervical dislocation. Heart was rapidly excised and immediately mounted on Langendorff's apparatus. Isolated heart was retrogradely perfused at

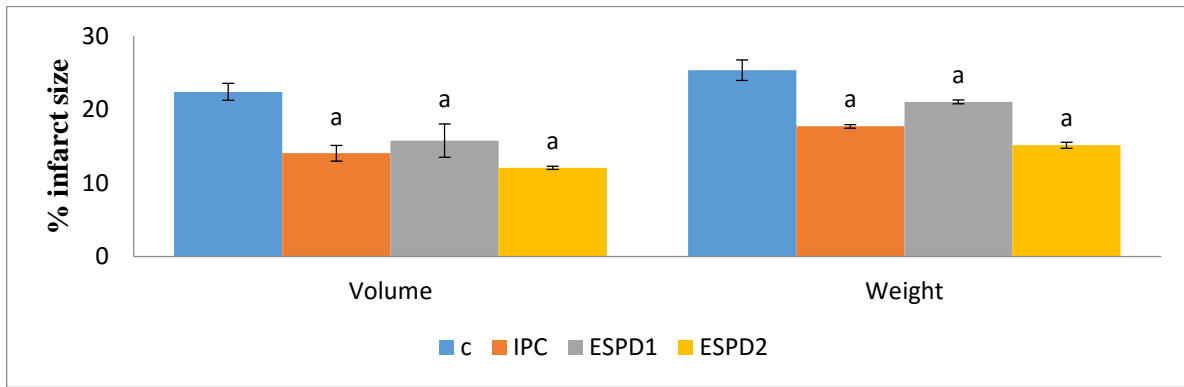


Figure 2: Effect of IPC and *Spondias pinnata* ethanolic extract on I/R-induced infarct size measured by Volume and Weight Method. C, control; IPC, ischemic preconditioning group; ESPD1 ethanolic extract of *Spondias pinnata* (100 mg/kg); ESPD2, ethanolic extract of *Spondias pinnata* (200 mg/kg). Values are mean ± SEM, <sup>a</sup> $p < 0.01$  vs C.

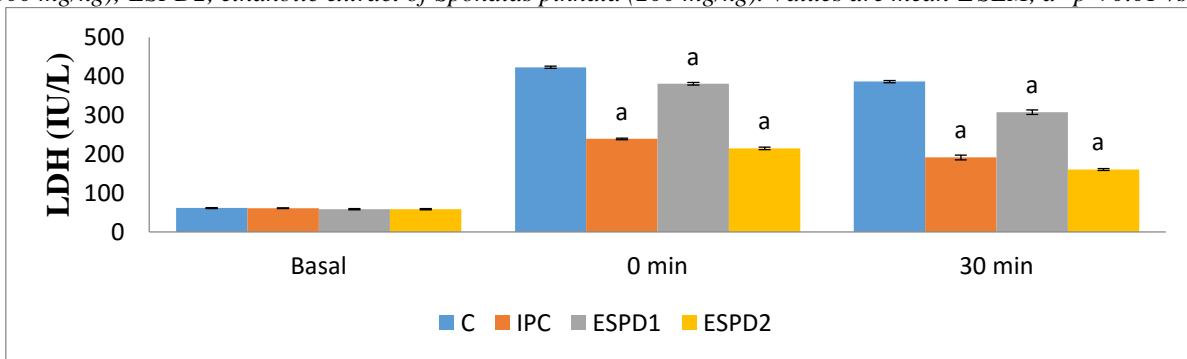


Figure 3: Effect of IPC and *Spondias pinnata* ethanolic extract on I/R-induced LDH release. C, control; IPC, ischemic preconditioning group; ESPD1, ethanolic extract of *Spondias pinnata* (100 mg/kg); ESPD2, ethanolic extract of *Spondias pinnata* (200 mg/kg). Values are mean ± SEM. <sup>a</sup> $P < 0.01$  vs C.

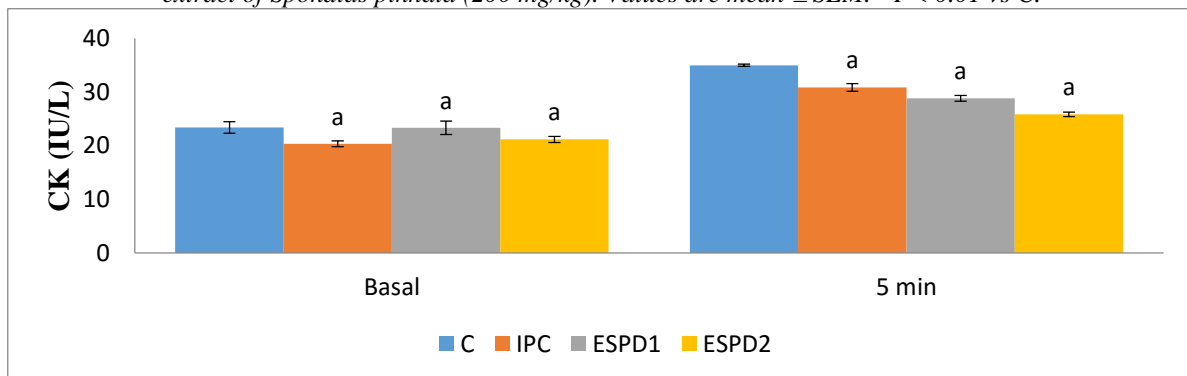


Figure 4: Effect of IPC and *Spondias pinnata* ethanolic extract on I/R-induced CK release. C, control; IPC, ischemic preconditioning group; ESPD1, ethanolic extract of *Spondias pinnata* (100 mg/kg); ESPD2, ethanolic extract of *Spondias pinnata* (200 mg/kg) Values are mean ± SEM,  $n=5$ , <sup>a</sup> $P < 0.01$  vs C.

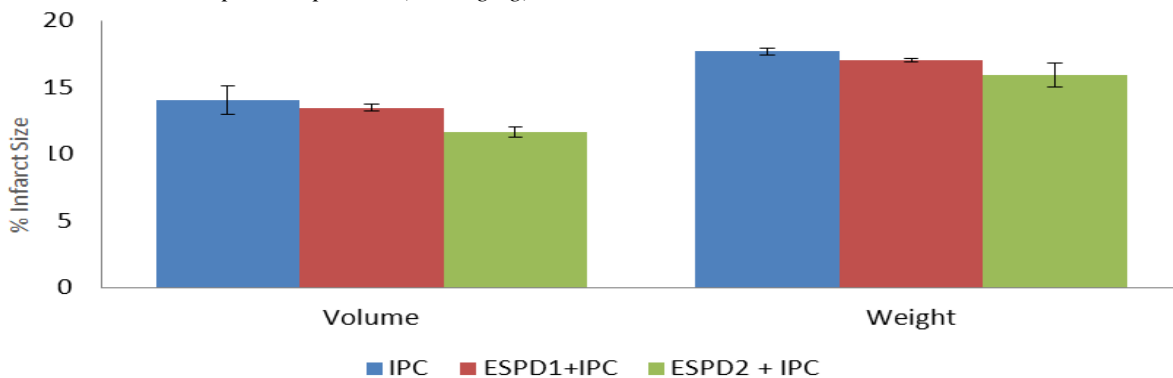


Figure 5: Effect of *Spondias pinnata* ethanolic extract on IPC-mediated infarct size. IPC, ischemic preconditioning group; ESPD1+ IPC, ischemic preconditioned group with infusion of *Spondias pinnata* (100 mg/kg) ethanolic extract; ESPD2 + IPC, ischemic preconditioned group with infusion of *Spondias pinnata* (200 mg/kg) ethanolic extract. Values are mean ± SEM.  $P < 0.01$  vs IPC.

constant pressure of 80 mm Hg with Krebs's Henseleit (KH) buffer (NaCl 118 mM; KCl 4.7 mM; CaCl<sub>2</sub> 2.5 mM; MgSO<sub>4</sub>·7H<sub>2</sub>O 1.2 mM; NaHCO<sub>3</sub> 25 mM; KH<sub>2</sub>PO<sub>4</sub> 1.2 mM; C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> 11 mM), pH 7.4, maintained at 37°C and bubbled with 95% O<sub>2</sub>. Flow rate was maintained at 7- 9 ml/min using Hoffman's screw. The heart was enclosed in a double walled jacket, the temperature of which was maintained by circulating water heated to 37°C. Global ischaemia was produced for 30 min by blocking the inflow of Krebs's Henseleit solution followed by reperfusion for 120 min. Four episodes of ischemia and reperfusion after stabilization, each comprising of 5 min occlusion and 5 min reperfusion, were used to produce ischemic preconditioning (in case of preconditioning groups). Coronary effluent was collected immediately and then after 5 min and 30 min after reperfusion for estimation of LDH and CK

#### Assessment of Myocardial Injury

I/R-induced myocardial injury was assessed by measuring the infarct size in the heart and estimating the release of LDH and creatine kinase CK in the coronary effluent.

#### Size assessment of infarct

Heart was removed from Langendorff's apparatus. Both the auricles and the root of aorta were excised and ventricles were kept overnight at 0°C. Frozen ventricles were sliced into uniform sections of 2-3 mm thickness. The

slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C in 0.2 M Tris buffer (pH 7.4) for 20 min. TTC is converted to red formazone pigment by NADH and dehydrogenase enzyme and therefore, the viable cells stained deep red. The infarcted cells have lost the enzyme and cofactor and thus remained unstained or dull yellow. The ventricular slices were placed between two glass plates. A transparent plastic grid with 100 squares in 1 cm<sup>2</sup> was placed above it. Average area of ventricular slice was calculated by counting the number of squares on either side. Similarly, numbers of square falling over non-stained dull yellow area were counted. Infarct size was expressed as percentage of average ventricular area. All the ventricular slices were weighed. Infarcted dull yellow part was dissected out and weighed. Infarct size was expressed as a percentage total ventricular weight.

#### Enzyme activity assays

Lactate dehydrogenase (LDH) and creatine kinase (CK) activity was measured to assess the extent of myocardial cell injury. Briefly, samples were collected from the coronary effluent at the end of the experiment, and the activities of LDH and CK were assayed using 2, 4-DNPH method.

#### Experimental Protocol

Six groups were used in the present study, and each group comprised five animals. A diagrammatic representation of

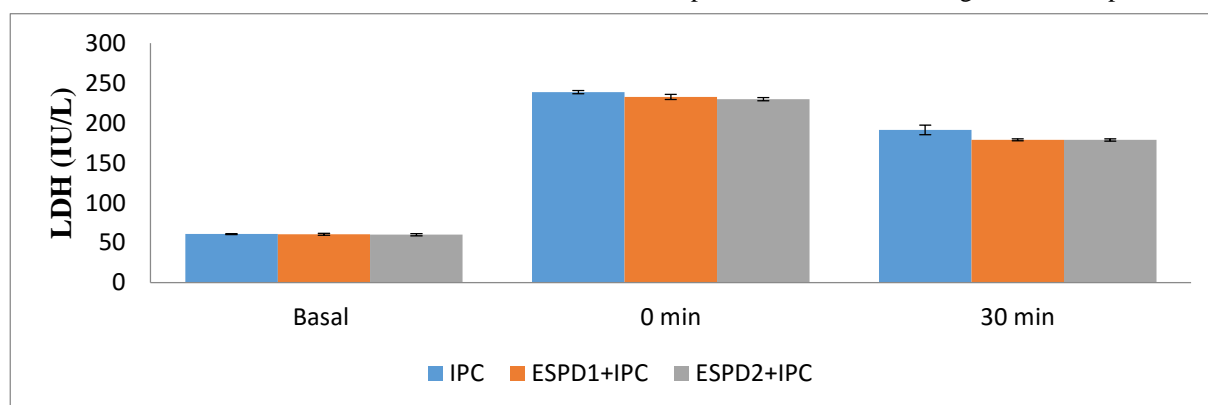


Figure 6: Effect of *Spondias pinnata* ethanolic extract group on IPC-mediated LDH release. *IPC*, ischemic preconditioning group; *ESPD1+ IPC*, ischemic preconditioned group with infusion of *Spondias pinnata* (100 mg/kg) ethanolic extract; *ESPD2 + IPC*, ischemic preconditioned group with infusion of *Spondias pinnata* (200 mg/kg) ethanolic extract.

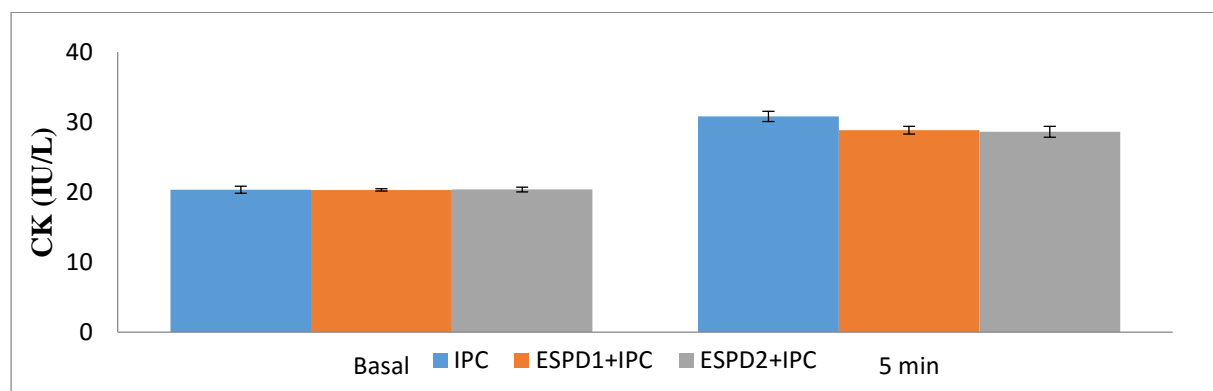


Figure 7: Effect of *Spondias pinnata* ethanolic extract on IPC-mediated CK release. *IPC*, ischemic preconditioning group; *ESPD1+ IPC*, ischemic preconditioned group with infusion of *Spondias pinnata* (100 mg/kg) ethanolic extract; *ESPD2 + IPC*, ischemic preconditioned group with infusion of *Spondias pinnata* (200 mg/kg) ethanolic extract. Values are mean  $\pm$  SEM.  $P < 0.01$  vs IPC

experimental protocol is shown in Figure 1. In all groups, the isolated rat hearts were allowed to stabilize for 10 min by perfusing with KH solution

#### Statistical Analysis

The results were expressed as mean  $\pm$  SEM for five animals per group. The data obtained from various groups was statistically analysed using one way ANOVA followed by 'dunnett test'. A *p* value of less than 0.01 was considered to represent a statistically significant difference.

## RESULTS

*Effect of IPC and Spondias pinnata ethanolic extract on ischemia and reperfusion- induced myocardial infarct size.* Global ischemia for 30 min followed by reperfusion for 120 min significantly increased myocardial infarct size measured by volume and weight method (Figure 2). IPC significantly reduced the ischemia and reperfusion induced increase in myocardial infarct size. Furthermore, *Spondias pinnata* ethanolic extract significantly reduced ischemia and reperfusion- induced increase in myocardial infarct size. However, *Spondias pinnata* (200 mg/kg) ethanolic extract showed a significant decrease in ischemia reperfusion- induced myocardial infarct size (Figure 2).

*Effect of IPC and Spondias pinnata ethanolic extract on ischemia and reperfusion- induced LDH release.*

LDH was estimated in coronary effluent after stabilization of isolated rat heart (Basal), immediately (0 min) and 30 min after reperfusion. Global ischemia followed by reperfusion for 120 min increased the LDH release immediately and 30 min after reperfusion (Figure 3). IPC and *Spondias pinnata* ethanolic extract showed a significant decrease in LDH release immediately and 30 min after reperfusion. However, a significant reduction in LDH level was observed only in 200 mg/kg *Spondias pinnata* ethanolic extract treated group (Figure 3).

*Effect of IPC and Spondias pinnata ethanolic extract on ischemia and reperfusion- induced CK release.*

CK was estimated in coronary effluent after stabilization of isolated rat heart (Basal) and 5 min after reperfusion. Global ischemia followed by reperfusion for 120 min increased the CK level after 5 min of reperfusion injury (Figure 4). IPC and *Spondias pinnata* ethanolic extract showed a significant decrease in CK release after 5 min of reperfusion. However, a significant reduction in CK level was observed only in 200 mg/kg *Spondias pinnata* ethanolic extract treated group (Figure 4).

*Effect of Spondias pinnata ethanolic extract on cardioprotective effect of ischemic preconditioning.*

*Spondias pinnata* ethanolic extract (100 mg/kg or 200 mg/kg) has not been reported to enhance ischemic preconditioning- mediated decrease in myocardial infarct size (Figure 5), LDH release immediately and after 30 min of reperfusion (Figure 6) and CK after 5 min of reperfusion (Figure 7).

## DISCUSSION AND CONCLUSIONS

Langendorff preparation and working heart preparation are haemodynamically comparable to investigate the effects of pharmacological interventions on ischemia and reperfusion induced myocardial injury<sup>18</sup>. The isolated rat

heart preparation perfused retrogradely on Langendorff apparatus has been employed in present study. Global ischemia for 30 min leads to cardiac dysfunction such as myocardial ischemia. Reperfusion of previously ischemic myocardium is often followed by detrimental changes in coronary arteries and cardiac tissue known as I/R injury<sup>24</sup>. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R- induced myocardial injury<sup>10, 20, 22,23</sup>. The peak release of LDH was observed immediately after reperfusion, whereas peak release of CK reported to occur 5 min after reperfusion which are in accordance with the earlier reports<sup>22,23</sup>. The initial release of LDH observed immediately after reperfusion may be due to ischemic injury and delayed released of LDH, observed after 30 min of reperfusion may be due to reperfusion injury<sup>3, 23</sup>. Similarly, CK is also known to increase in cardiac injury<sup>10</sup>. The infarct size has been assessed macroscopically because a good correlation has been reported between macroscopic and microscopic assessment of infarct size<sup>23</sup>. The NADH and dehydrogenase enzyme present in viable myocardium convert triphenyltetrazolium chloride (TTC) to red formazone pigment and stained it deep red in colour<sup>23</sup>. However, infarcted cells lost dehydrogenase enzyme and cofactor NADH and thus remained unstained or dull yellow<sup>19</sup>. Moreover, reperfusion of 120 min employed in present study is sufficient to washout the NADH and dehydrogenase enzyme from infarcted cells<sup>4</sup>. The short occlusion and reperfusion of myocardium have been demonstrated to produce cardioprotection against sustained ischemia and reperfusion. Similarly, in the present study, four episodes of myocardium preconditioning have significantly attenuated ischemia and reperfusion- induced increase infarct size and release of CK and LDH. These observations are consistent with our previous reports<sup>10,23</sup>. The cardioprotective effect of IPC are mediated through the release of vasoactive substance such as adenosine<sup>5</sup>, bradykinin<sup>7</sup>, nitric oxide<sup>17</sup>), opioids<sup>9</sup>, acetylcholine<sup>12</sup>, calcitonin gene related peptide (CGRP)<sup>14</sup>. In the present study, an attempt has been made to examine the effect of *Spondias pinnata* ethanolic extract on ischemia and reperfusion- induced myocardial injury and the effect of *Spondias pinnata* ethanolic extract on the protection offered by ischemic preconditioning. *Spondias pinnata* has been reported to have antioxidant activity<sup>8</sup>. Therefore, it may be possible to postulate that *Spondias pinnata* being an antioxidant may play an important role in cardioprotection. The development of I/R-induced oxidative stress due to 30 min ischaemia and 120 min reperfusion may be responsible for the noted I/R-induced myocardial injury in the present study. A marked increase in infarct size, release of LDH and CK was observed in the control group. It has been investigated in the present study that administration of *Spondias pinnata* (100 mg/kg or 200 mg/kg) ethanolic extract significantly improved the cardioprotection. This contention is further supported by the results obtained in the present study that *Spondias pinnata* ethanolic extract (100 mg/kg or 200 mg/kg) significantly reduce I/R injury assessed in term of infarct size, release of LDH and CK level. *Spondias pinnata* plant

extract contains significant amounts of flavonoids and phenolic compounds. These compounds have good antioxidant potential. The mechanism of phenolic compounds may be through hydroxyl group which confer scavenging activity. However, the mechanism of flavonoids may involve scavenging or chelating activity<sup>8</sup>. It has been investigated that *Spondias pinnata* (100 mg/kg or 200 mg/kg) not enhanced the cardioprotective effect of ischemic preconditioning, thereby indicating that *Spondias pinnata* requires an additional stimulus in the form of ischemia to enhance the cardioprotection offered by preconditioning. On the basis of above discussion, it may be concluded that administration of *Spondias pinnata* ethanolic extract may prevent ischemic and reperfusion induced myocardial injury probably by its antioxidant activity.

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