**Terminalia arjuna** (Roxb.) Wight & Arn. augments cardioprotection via antioxidant and antiapoptotic cascade in isoproterenol induced cardiotoxicity in rats

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Worldwide, Ischemic heart disease (IHD) affects a large population. Implication of myocardial infarction (MI) and its multiple pathophysiology in cardiac function is well known. Further, isoproterenol (ISP) is known to induce MI. Today, there is an urgent need for effective drug that could limit the myocardial injury. Therapeutic intervention with antioxidants has been shown useful in preventing the deleterious changes produced by ISP. Here, we investigated the protective effects of oral pre-treatment of hydroalcoholic extract of bark of *Terminalia arjuna* (HETA) on biochemical and apoptotic changes during cardiotoxicity induced by isoproterenol (ISP) in rats. HETA was orally administered at a dose of 100, 200 and 400 mg/kg body wt., for 30 days with concurrent administration of ISP (85 mg/kg body wt.) on days 28th and 29th at an interval of 24 h. ISP caused deleterious changes in the myocardium and significantly increased (*P* <0.05) malondialdehyde, serum glutamate oxaloacetate transaminase, creatine kinase-MB, lactate dehydrogenase and troponin-I. However, it significantly decreased (*P* <0.05) glutathione and superoxide dismutase compared to healthy control. Oral pre-treatment of HETA for 30 days significantly decreased (*P* <0.05) the biochemical parameters of oxidative stress and cardiac markers as compared to ISP control. Histopathological findings also revealed that architecture of the myocardium was restored towards normal in HETA pre-treated group. Overall, the present study has shown that the hydroalcoholic extract of bark of *T. arjuna* (HETA) attenuates oxidative stress, apoptosis and improves antioxidant status in ISP-induced cardiotoxicity in rats.

**Keywords:** Apoptosis, Arjuna tree, Cardiotoxicity, Heart, HETA, Myocardial ischemia, Oxidative stress, ROS.

Ischemic heart disease (IHD) affects a large population in the world¹. Myocardial infarction (MI) is a pathological condition associated with morphological changes and involves an interface of multiple pathophysiology of MI²-⁴. Isoproterenol (ISP) is nonselective β-adrenergic, synthetic agonist, which has been found to induce MI in rats⁵,⁶. Myocardial necrosis induced by ISP is responsible for increase in cardiac marker enzymes, accumulation of lipid peroxides, elevation in pro-inflammatory cytokines and damaged cardiac function²-⁷. There is an urgent need for a drug, which could limit the myocardial injury. It is known that reactive oxygen species (ROS) is involved in the pathophysiology of MI²,³.

Therefore, therapeutic intervention with antioxidant may be useful in preventing the deleterious changes produced by ISP.

*Terminalia* spp. (Combretaceae) are known to exhibit antibacterial⁸ and antidepressant activity⁹ and also used for treatment of various ailments such as cardiovascular disease⁶,¹⁰ and urolithiasis¹¹. The bark of *T. arjuna* has been mentioned in ancient Indian medicinal literature to have beneficial effects in heart diseases¹⁰. The crude bark of *T. arjuna* has strong antioxidant property and it augments endogenous antioxidant compound in rat heart¹². The stem bark of *T. arjuna* has been reported to contain different groups of chemical constituents *viz.*, hydrolysable tannins¹³, triterpene, flavonoids, phenolics, and phytosterols¹⁴,¹⁵. Important triterpenes are arjunetin, arjunic acid, arjunolic acid and arjunigenin¹⁵.

In the present study, we investigated the molecular modulation of ISP related myocardial damage, biochemical and histopathological alterations and
Preventive effect produced by hydro alcoholic extract of *Terminalia arjuna* (HETA). Vitamin E (α-tocopherol), a natural antioxidant with characteristic susceptibility to oxidation was used as a standard reference drug.

**Materials and Methods**

**Animals**— Male wistar albino 10-12 wk old rats weighing 180-230 g (210±20.58 g) were used in the study. The study protocol was reviewed and approved by the Institutional Animal Ethical Committee of University College of Medical Sciences (UCMS), Delhi, India (IAEC/UCMS/DB-11/2008) and confirms to the Indian National Science Academy guidelines for the use and care of experimental animals in research. Animals were obtained from the central animal house facility of UCMS. Rats were housed (4 per cage) under standard laboratory conditions with natural light and dark cycles (approx. 12:12 h) and maintained at humidity of 55±5% and an ambient temperature of 22±2°C. The animals were allowed free access to standard pellet diet (Durga Brothers Pvt. Ltd.) and tap water *ad libitum*.

**Drugs and reagents**— ISP was obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals used were of analytical grade. CK-MB, SGOT and LDH assay kits were procured from Spinreact SA, Spain, and Troponin I ELISA kit from Calbiotech, USA. Immunohisto staining kit based on HRP polymer detection system was purchased from Thermo Fisher Scientific, USA and primary antibodies (Bax mouse monoclonal IgG2 and Bcl-2 mouse monoclonal IgG1) from Santa Cruz Biotechnology, USA, TUNEL assay kit from R&D Systems Minneapolis. α-Tocopherol was purchased from Merck, USA.

**Plant material**— *Terminalia arjuna* was obtained from the campus of Institute of Human Behaviour and Allied Sciences (IHBS), Delhi, India and authenticated by Dr. Sayeed Ahmad, Bioactive Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi, India. The voucher specimen (BNPL/JH/078/2008) has been kept for future reference.

**Preparation of hydro alcoholic extract of *T. arjuna* (HETA)**— *T. arjuna* bark was crushed in a mixer to coarse powder (sieve #60) and then extracted with 50% v/v ethanol thrice by macerating the material in 1:20 drug: solvent ratio for 24 h with occasional shaking at room temperature and sonicating the mixture for 30 min before filtration using 5-6 layers of muslin cloth. The filtrate was first centrifuged and the supernatant was lyophilized and stored at 2-8°C in a tightly closed bottle, protected from light till used. The yield of the HETA was 18.5 % w/w of the dried powder of *T. arjuna*. The HPTLC fingerprint analysis and estimation of arjunolic acid was done for quality control/standardization of extract.

**Quantitative analysis of the HETA by HPTLC**— The sample was prepared by taking 750 mg of hydroalcoholic extract of *T. arjuna* in 25 ml of aqueous acidic solution (HCl 5.0 % v/v in water) and refluxed for one h on water bath. The extract was filtered and taken into a separating funnel. It was then extracted with chloroform by taking same quantity and the process was repeated three times for complete extraction. The chloroform extracts were pooled and evaporated to dryness. The residue obtained was dissolved in 10 ml of HPLC grade methanol. The stock solution of standard arjunolic acid (purity 98%) was prepared in HPLC grade methanol to get 1.0 mg ml⁻¹ solution. The estimation was carried out as per the method described by Singh *et al.*

The samples were spotted in the form of bands of width 4 mm using microlitre syringe on pre-coated silica aluminum sheet 60F₂₅₄ (5.0×10 cm 0.2 µm thickness) using Camag Linomat V sample applicator (Switzerland). The plates were pre-washed with methanol and activated at 60°C for 20 min prior to chromatography. A constant application rate of 120 nL s⁻¹ was employed and space between two bands was 15 mm. The slit dimension was kept at 4×0.30 mm and 20 mm s⁻¹ scanning speed were employed. The mobile phase consisted of chloroform: toluene: ethanol (4:4:1, v/v/v) and 10 ml of mobile phase was used for per chromatography. Linear ascending development was carried out in 10×10 cm twin trough glass chamber, which was previously saturated with mobile phase for 15 min. The length of the chromatogram run was 80 mm. After the development, TLC plates were dried in a current of air with the help of an air dryer and sprayed with anisaldehyde sulphuric acid reagent and again air dried then kept in oven for 10 min at 110°C. The densitometric scanning was performed on Camag TLC scanner III operated by WinCats software using wavelength 600 nm.

**Induction of myocardial ischemia**— ISP was freshly prepared in normal saline and injected subcutaneously (sc) at a dose of 85 mg/kg body wt.
the rats on 28th and 29th day at an interval of 24 h.8

Experimental protocol— Animals were divided into 6 groups, each consisting of eight rats (n=8). Group I, Healthy control (orally administered normal saline); Group II, Ischemic control (normal saline + ISP, 85 mg/kg body wt.); Group III, HETA 100 mg/kg body wt. + ISP (85 mg/kg body wt.); Group IV, HETA 200 mg/kg body wt. + ISP (85 mg/kg body wt.); Group V, HETA 400 mg/kg body wt. + ISP (85 mg/kg body wt.); and Group VI, Vit E 100 mg/kg body wt. + ISP (85 mg/kg body wt.). The treatment was given for 30 consecutive days once in a day using standard orogastric intubation. On 28th and 29th days, rats were subjected to ISP (85 mg/kg body wt.) with 24 h interval. Healthy control group was not subjected to ISP insult. Vit E was dissolved in 5% gum acacia.

Selection of doses of HETA— We have carried out a dose ranging (50-800 mg/kg) study based on the reported pharmacological significance. The dose range 100-400 mg/kg was found effective in a dose dependent manner, and we took this dose range for our study to evaluate the cardioprotective activity.

Sample collection— Blood sample of 12 h fasted rats were collected from retro orbital plexus at 0th, 21st and 30th days to perform biochemical studies. After blood sampling on 30th day, the rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p). For histopathological studies, heart was immediately dissected, washed in ice-cold saline and stored in 10% buffered neutral formalin solution.

Measurement of oxidative stress parameters— Serum MDA levels were measured as an index of lipid peroxidation using the colorimetric method as described by Satoh.19 Lipid peroxides are precipitated from serum with trichloroacetic acid (TCA) and heated with thiobarbituric acid (TBA). The reaction results in formation of a pink chromogen, extracted with n-butyl alcohol. Absorbance of organic phase is determined at 530 nm. Activity of superoxide dismutase (SOD) in erythrocytes was assayed by the method described by Marklund and Marklund and further modified by Nandi and Chatterjee.21 This method is based on the ability of the enzyme SOD to inhibit the auto oxidation of pyrogallol. For the assay of SOD, an extract called Tsuchihasi extract was prepared by adding 3.5 ml of cold distilled water, 1 ml of ethanol and 0.6 ml of chloroform to 0.5 ml of hemolysate and vortexed the mixture for 5 min. The tube was centrifuged for 10 min at 3000 rpm. The clear supernatant was used for the enzyme assay. The absorbance was taken at a wavelength of 530 nm against blank (Drabkin’s reagent). Finally, the results were expressed as unit per g of hemolysate Hb (U/gHb).

Studies on cardiac marker enzymes— The myocardial injury markers such as creatine kinase-MB (CK-MB) and creatine kinase (CK) are enzymes that catalyse the transfer of phosphate from creatine phosphate and thereby ADP to form ATP. The rate of change of NAD is directly proportional to CK-MB activity. The change in NAD can be measured spectrophotometrically at 340 nm. Serum glutamate oxaloacetate transaminase (SGOT) was estimated by UV kinetic method, without pyridoxal phosphate activation, modified by IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)23, Lactate dehydrogenase (LDH) in serum was estimated spectrophotometrically with commercially available kit. Cardiac Troponin I (cTnI) in serum was measured by standard ELISA kit.

Apoptotic studies— Myocardial tissue samples preserved in 10% buffered formalin were carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. Cross sections (5 µm thick) of the fixed myocardial tissues were cut from paraffin-embedded blocks on a microtome and mounted onto poly-lysine coated slides and dried completely to proceed for immunohistostaining and TUNEL assay.24

Immunostaining for the localization of Bax and Bcl-2— A mouse monoclonal anti-Bcl-2 and Bax proteins were used as the primary antibody for Bcl-2/Bax immunohistochemical staining. The Ultravision ONE HRP polymer detection system locates primary antibody by a universal secondary antibody polymer formulation. The amino acid polymer is conjugated to horseradish peroxidase (HRP) and the Fab fragments of secondary antibody. The polymer complex was visualized with an appropriate chromogen/substrate. Briefly, formalin-fixed paraffin-embedded myocardial sections were subjected to the immunohistochemical procedure.
for localization of Bax and Bcl-2 proteins using specific mouse monoclonal primary antibodies. Sections were first blocked and then incubated in primary antibody followed by Ultra Vision One HRP polymer. The target protein (Bax/Bcl-2) was visualized by incubation in peroxidase substrate (H$_2$O$_2$) using 3,3’-diaminobenzidine (DAB) as the chromogen.

**Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL assay)**— Myocardial apoptosis was quantified by detection of DNA fragmentation using the TUNEL technique. Briefly, the enzyme terminal deoxynucleotidyl transferase was used to incorporate residues of digoxigenin nucleotide into 3’ OH ends of DNA fragments. The free end of cellular DNA was labeled by incubating the specimens in streptavidin conjugated to horseradish peroxidase enzyme and peroxidase substrate. The signal of TUNEL was used to identify apoptotic cells using secondary reaction with antibodies and DAB chromogen. The slides were counter stained in methyl green. Total cell counts and TUNEL positive cells in the specimens were determined by light microscope. The cells with clear nuclear labeling were defined as TUNEL positive cells.

**Histopathological studies**— Myocardial tissue fixed in buffered formalin was processed for paraffin embedding, sectioned at 5 µm and mounted onto slides. These sections were stained with haematoxylin and eosin (H & E), and visualized under light microscope to study the histoarchitectural changes of the myocardium.

**Data analysis**— The results were expressed as mean ± SEM and statistical differences between mean values were determined by repeated measure analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

### Results

**Characterization of HETA**— Characterization of HETA was done by HPTLC. The analysis of HETA showed the presence of 0.394 % w/w arjunolic acid (Fig. 1).

**Effect of HETA on oxidative stress parameters**— Table 1 shows the effect of HETA on oxidative stress parameters. ISP control rats exhibited significant rise in serum MDA levels and significant reduction in SOD activity and GSH content as compared to healthy control. HETA (100, 200, 400 mg/kg) produced dose dependent response on antioxidant markers (SOD & GSH). Insignificant effect was observed in SOD&GSH at a dose of 100 mg/kg of the HETA extract as compared to ISP control. However, HETA at 200 and 400 mg/kg dose produced significant improvement in the activity of SOD ($P < 0.05$ and $<0.001$, respectively) and GSH levels ($P < 0.05$ and $<0.01$, respectively). MDA levels were found to be significantly reduced ($P < 0.001$) in all the groups treated with HETA compared to ISP control. The rats treated with the reference drug vitamin E also showed significant decrease ($P < 0.001$) in MDA levels and significant increase in SOD ($P < 0.001$) and GSH ($P < 0.05$) levels.

![HPTLC chromatogram](image1)

**Fig. 1—** HPTLC chromatogram: (A) Arjunolic acid standard; and (B) Hydroalcoholic extract of *Terminalia arjuna* (HETA) showing the presence of arjunolic acid.

### Table 1— Effect of HETA per se on oxidative stress parameters

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>MDA (nmol/mL)</th>
<th>GSH (mg/dL)</th>
<th>SOD (U/gHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>1.59±.166</td>
<td>35.85±5.56</td>
<td>1250±76.07</td>
</tr>
<tr>
<td>ISP control</td>
<td>3.59±.417*</td>
<td>26.80±1.55</td>
<td>940.17±60.29*</td>
</tr>
<tr>
<td>HETA 100</td>
<td>2.10±.284</td>
<td>33.21±3.66</td>
<td>1110.17±64.32</td>
</tr>
<tr>
<td>HETA 200</td>
<td>2.08±.122***</td>
<td>34.62±1.60*</td>
<td>1132.67±55.26*</td>
</tr>
<tr>
<td>HETA 400</td>
<td>1.43±.128***</td>
<td>40.96±1.30**</td>
<td>1316.57±62.12***</td>
</tr>
<tr>
<td>Vit E 100</td>
<td>1.84±.290***</td>
<td>38.54±1.97**</td>
<td>1212±17.16***</td>
</tr>
</tbody>
</table>

ISP was given at a dose of 85 mg/kg body wt. Values are expressed as means ±SEM (n=8); $P ≤ 0.01$; $P ≤ 0.001$ vs. normal rats; $*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001$ vs. ISP control.
Effect of HETA on cardiac markers— Table 2 shows the effect of HETA on cardiac markers. ISP control rats significantly demonstrated \( P < 0.001 \) higher levels of SGOT, CK-MB, Troponin I and LDH as compared to healthy rats. Pre-treatment with HETA (100, 200 & 400 mg/kg) produced significant reduction \( P < 0.001 \) in CK-MB, SGOT and LDH levels compared to ISP control group. Troponin I level was significantly decreased with all the tested doses of the extract. However, statistical difference with 100 mg/kg dose \( P < 0.01 \) was less significant as compared with 200 and 400 mg/kg doses \( P < 0.001 \). Vitamin E treated group showed significant reduction \( P < 0.001 \) in oxidative stress as well as cardiac markers. There was no significant change in antioxidant status and cardiac markers on 21st day of pre-treatment in all the groups, hence excluded.

Effect of HETA on histology of myocardium— Microscopic examination of sections from healthy control, presented normal architecture of the myocardium (Fig. 2A). Sections from ISP control rat showed marked focal myonecrosis, hyper-contracted myofibrils, vacuolar degeneration and lymphocytic infiltration (Fig. 2 B). HETA treated rats (400 mg/kg body wt.) demonstrated structural improvement as it decreased degree of myonecrosis and contraction in myofibrils (Fig. 2 C). In addition, lesser vacuolization and inflammation in the myocytes was observed. Vitamin E treated rats revealed reduction in myocardial abnormalities (Fig. 2 D). The histopathological changes during ischemic episode were graded and summarized (Table 3).

Effect of HETA on myocardial apoptosis myocyte Bax/Bcl2 and TUNEL positivity

Myocyte Bax expression— As shown in Fig. 3 A, slight Bax immunoreactivity \( (3.2\% \pm 0.7\%) \) was observed in myocytes of the healthy control. ISP-induced myocardial injury significantly increased

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>SGOT(U/L)</th>
<th>CK-MB (U/L)</th>
<th>Trop I (µg/L)</th>
<th>LDH(U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>149.13±15.86</td>
<td>299.38±23.97</td>
<td>0.03±0.00</td>
<td>695.3±5.95</td>
</tr>
<tr>
<td>ISP control</td>
<td>1307.50±80.04$</td>
<td>2137.83±82.85$</td>
<td>6.01±0.30$</td>
<td>1426±6.13$</td>
</tr>
<tr>
<td>HETA 100</td>
<td>1072.50±64.18$</td>
<td>1831.50±172.63$</td>
<td>4.77±0.73$</td>
<td>1335±6.17$</td>
</tr>
<tr>
<td>HETA 200</td>
<td>1044.50±88.16$</td>
<td>1707.33±88.96$</td>
<td>4.52±0.52$</td>
<td>1305±5.76$</td>
</tr>
<tr>
<td>HETA 400</td>
<td>908.14±73.54$</td>
<td>1590±94.25$</td>
<td>3.73±3.84$</td>
<td>1210±9.12$</td>
</tr>
<tr>
<td>VitE 100</td>
<td>1032.57±65.49$</td>
<td>1628.43±66.65$</td>
<td>3.89±0.49$</td>
<td>1235±8.67$</td>
</tr>
</tbody>
</table>

ISP was given at a dose of 85 mg/kg body wt. \( [SGOT, \text{serum glutamate oxaloacitate transaminase}; \text{CK-MB, creatine kinase myocardial band; Trop-I Troponin I; LDH, lactate dehydrogenase. Values are expressed as means ±SEM (n=8); $ \text{P} \leq 0.001 \text{ vs. normal rats; **P} \leq 0.01 \text{ vs. ISP control.}] \]

Table 3— Effect of HETA on the degree of myocardial damage

<table>
<thead>
<tr>
<th>Groups</th>
<th>Edema</th>
<th>Necrosis</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>ISP control</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>HETA 100</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>HETA 200</td>
<td>++</td>
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<tr>
<td>HETA 400</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vit E 100</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

ISP was given at a dose of 85 mg/kg body Wt. Photomicrographs were used to evaluate the degree of damage in the heart tissues: (A) no changes; (+) Focal changes; (++) mild changes; (+++) marked changes. ISP, isoproterenol; HETA, hydroalcoholic extract of \( T. \text{arjuna} \). All doses were given in mg/kg body wt.]

Fig. 2— Representative photomicrographs of myocardial section stained with Haematoxylin and Eosin (H & E, 200X) from different experimental groups. (A) Healthy control rats showing normal architecture of myocardium (H & E 200X); (B) ISP control rats, showing myocardial necrosis with excessive inflammatory cell infiltration (H & E 200X); (C) HETA 400 treated rats showing normal myocardium with less inflammation (H & E 200X); and (D) Vitamin E treated rats showing normal myocardium.
However, Bax expression was found to be significantly attenuated ($P < 0.01$) in the HETA (5.05%±0.5%) and in Vit E (5.03%±0.7%) treated groups (Fig. 3 B-D).

**Myocyte Bcl-2 expression**— Bcl-2 was expressed in the myocardium of healthy control as indicated by positive Bcl-2 immunoreactivity in the myocytes (1.75%±0.25%) (Fig. 4 A). ISP administration resulted in a reduction of Bcl-2 expression as decreased Bcl-2 immunoreactivity was demonstrated in light microscopic evaluation of sections from ISP control group (Fig. 4 B). Sections from pre-treated rats with HETA revealed significant up regulation ($P < 0.01$) in the expression of Bcl-2 (8.15%±0.15%) (Fig. 4 C). Vitamin E group also showed improvement in Bcl-2 expression (Fig. 4 D).

**TUNEL assay**— No TUNEL positive cells were observed in the healthy control (0.5%±0.21) (Fig. 5 A). Accordingly, in healthy control, the cells were stained by methyl green. However, TUNEL positive cells, i.e., the diseased ones, were marked by...
significant red to brown staining (P <0.001) in the myocytes of ischemic control group (3.5%±0.5%) (Fig. 5 B). Pre-treatment with HETA 400 mg/kg body wt. significantly reduced (P <0.01) TUNEL positivity (0.7%±0.3%). Pre-treatment with vitamin E also reduced (P <0.01) the number of TUNEL positive cells vs. ischemic control group (0.65%±0.5%) (Fig. 5 C and D).

Discussion
ISP is a known cardio toxic agent and mediates myocardial injury primarily via the β1-adrenergic receptor. Due to stimulation of β-adrenergic receptor, superoxide dismutase enzyme activity was down-regulated and glutathione level was also reduced which further leads to loss of membrane integrity and myocyte toxicity, a remarkable sign of myocardial necrosis. In the present study, ISP administration showed significant elevation in lipid peroxidation and suppression in antioxidant defence cascade as evident by significant reduction in SOD and GSH levels. Prior administration of HETA at 200 and 400 mg/kg doses decreased MDA levels and improved the SOD activity and glutathione content. Since oxidative stress is the major deleterious factor, it may be suggested that antioxidant effect of HETA significantly narrowed down the development of myocardial infarction produced by ISP. T. arjuna attributes antioxidant effect due to its flavonoid and other polyphenolic constituents, leading to radical scavenging of both free radicals and ROS. Crude bark of T. arjuna possess strong antioxidant property due to various phytochemicals and it augments endogenous antioxidant compounds in rats.

In the present study, the quantitative analysis of the HETA revealed the presence of arjunolic acid (0.394% w/w), which has been reported earlier for its beneficial cardioprotective effect in isoproterenol-induced MI in rats. ISP destroys myocardial cells, releases cytosolic enzymes into blood stream, and thus, serves as the diagnostic tool of experimental myocardial tissue damage. Here, the ISP treated rats showed significant elevation in the serum levels of cardiac markers (SGOT, CK-MB, LDH and Troponin I), and HETA (100, 200 and 400 mg/kg) showed significant reduction in cardiac markers. Significant rise observed in the levels of diagnostic markers in the serum following ISP administration indicated severity of the necrotic damage to the myocardial membrane. Amount of these markers appears in serum is proportional to the number of necrotic cells. Thus, reduction in cardiac markers reflects reduced extent of myocardial damage in rats pre-treated with HETA. Two distinct types of cell death in myocardium i.e. necrosis and apoptosis, have been linked with cardiac damage. Although necrotic cell death leads to destruction of a large group of cells, apoptosis may independently contribute to irreversible myocardial damage. Induction of apoptosis is implicated in myocardial injury among various cardiovascular diseases. The fact that apoptosis plays a role in tissue damage observed after myocardial infarction has pathological and therapeutic implications. Cardiomyocyte apoptosis plays an important role in initiation and progression of cardiac disease. Drugs that effectively and specifically inhibit apoptosis might be useful therapeutic agents for attenuating myocardial injury. Hence, screening of HETA for anti-apoptotic activity is of clinical importance. Here, anti-apoptotic evaluation was carried out by means of TUNEL assay and the localization of Bax and Bcl-2 to delineate the involvement of apoptosis in ISP-induced injury. Results have demonstrated that ISP administration triggers apoptotic/necrotic cell death as evident by TUNEL positivity, Bax expression and reduction in Bcl-2 expression in ISP control as compared to healthy control. This observation is in alignment with the earlier studies. Pre-treatment with HETA reduced cell death as evidenced by increased Bcl-2, and attenuated Bax expression. Cook et al. has demonstrated that not only ROS per se, but also their oxidation products and other by-products generated by ROS can trigger programmed cell death. It has also been reported that the antioxidants inhibit programmed cell death. Therefore, it may be suggested that HETA exhibits anti-apoptotic potential due to its strong antioxidant property. Histopathological evaluation further confirms the cardioprotective potential of HETA. Pre-treated rats demonstrated improvement in structural myocardial morphology in contrast to ischemic control. Hence, HETA may have salvaged myocyte and prevented cell loss induced by apoptosis and necrosis.

Conclusion
The above study has revealed that pre-treatment with HETA significantly exhibited cardioprotection in ISP challenged ischemic rats. It has also demonstrated that HETA pre-treatment exerts better antioxidant and
anti-apoptotic activity which further strengthens the cardioprotective potential of HETA. Hence, it can be concluded that HETA may be useful as adjunct therapy along with conventional drugs.

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Conflict of Interest Statement
The authors declare that there is no conflict of interest.

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