World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:2, No:6, 2008

Alcoholic Extract of *Terminalia Arjuna* Protects Rabbit Heart against Ischemic-Reperfusion Injury: Role of Antioxidant Enzymes and Heat Shock Protein

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***Abstract*—**The present study was designed to investigate the cardio protective role of chronic oral administration of alcoholic extract of *Terminalia arjuna* in in-vivo ischemic reperfusion injury and the induction of HSP72. Rabbits, divided into three groups, and were administered with the alcoholic extract of the bark powder of *Terminalia arjuna* (TAAE) by oral gavage [6.75mg/kg: (T1) and 9.75mg/kg: (T2), 6 days /week for 12 weeks]. In open-chest Ketamine pentobarbitone anaesthetized rabbits, the left anterior descending coronary artery was occluded for 15 min of ischemia followed by 60 min of reperfusion. In the vehicle-treated group, ischemic-reperfusion injury (IRI) was evidenced by depression of global hemodynamic function (MAP, HR, LVEDP, peak LV (+) & (-) (dP/dt) along with depletion of HEP compounds. Oxidative stress in IRI was evidenced by, raised levels of myocardial TBARS and depletion of endogenous myocardial antioxidants GSH, SOD and catalase. Western blot analysis showed a single band corresponding to 72 kDa in homogenates of hearts from rabbits treated with both the doses. In the alcoholic extract of the bark powder of *Terminalia arjuna* treatment groups, both the doses had better recovery of myocardial hemodynamic function, with significant reduction in TBARS, and rise in SOD, GSH, catalase were observed. The results of the present study suggest that the alcoholic extract of the bark powder of *Terminalia arjuna* in rabbit induces myocardial HSP 72 and augments myocardial endogenous antioxidants, without causing any cellular injury and offered better cardioprotection against oxidative stress associated with myocardial IR injury.

***Keywords*—**Antioxidants, HSP72, Ischemic reperfusion injury, *Terminalia arjuna.*

I. Introduction

REPERFUSION of the ischemic myocardium exacerbates myocardial damage, a phenomenon known as myocardial ischemic-reperfusion (IR) injury and is associated with a variety of clinical conditions [1]. Although the etiopathology of this phenomenon is multifactorial [2], [3], oxidative stress is reported to play a central role [4]. Oxidative stress occurs due to overwhelming generation of oxygen free radicals (OFR) at the time of reperfusion with concomitant depletion of certain key endogenous antioxidants e.g. superoxide dismutase (SOD), reduced glutathione(GSH) and catalase (CAT). Pharmacological interventions designed to prevent or correct IR injury is a useful means to reduce complications, secondary to it. [5]- [11].

Heat shock proteins (HSP) are a group of inducible or constitutive proteins with well established cardioprotective properties, although the mechanism of which is not clear. Induction of heat shock protein 72 (HSP72) occurs under a variety of stressful conditions such as heat-shock, hypoxia, hyperthermia and tissue injury [12]. It has been shown that induction of HSP72 results in significant cardioprotection in pathological conditions like, I-R injury of heart [9], [13]-[15], mostly by preventing denaturation of proteins and by inducing endogenous antioxidants. Pharmacological induction of HSP72 has recently been identified as a promising therapeutic tool in IHD [9], [11], [16], as the traditional methods of induction are not conducive to therapeutic application.

The bark of *Terminalia arjuna* (TA), a deciduous tree of Combretaceae family, has been widely used in Indian System of Medicine in cardiac ailments [17], [18]. In recent times, both experimental and clinical studies have claimed that the dried bark powder of TA has significant beneficial effects in ischemic heart disease (IHD) [7]-[9], [11], [15], [19], [20]. Moreover, an antioxidant effect of the *Terminalia* species has also been documented in in-vitro conditions [7], [8], [15], [21]. Our earlier study shows that chronic oral administration of crude bark of *Terminalia arjuna* augments endogenous antioxidants of rat heart and also prevents oxidant stress associated with in-vitro IR injury of the heart [7], [9], [11], [15].

Therefore the present study was designed to investigate the effects of chronic administration of the alcoholic extract of TA in rabbits on (i) the hemodynamic recovery from in vivo ischemic reperfusion injury of heart, (ii) the endogenous myocardial antioxidant status, (iii) the oxidant stress arising out of ischemic reperfusion injury of heart and (iv) the induction of HSP72.

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II. MATERIALS AND METHODS

A. Plant material

The bark of *Terminalia arjuna,*(TA) obtained from the Southern part of India (Madurai district, Tamil Nadu), was authenticated by the Department of Pharmacognosy, K.M.College of Pharmacy, Madurai, India (vide voucher specimen no. 53).

B. Extraction procedure

The dried and pulverized bark powder was extracted with ethyl alcohol (90%) by hot continuous percolation over 72 hours by using Soxhlet apparatus. The alcoholic extract was filtered and concentrated to a dry mass by using vacuum distillation and evaporation. A dark brownish red shiny crystal like residue of 13.9% w/w yield was obtained. The chemical constituents of the alcoholic extract (TAAE) were identified by quantitative analysis and confirmed by thin-layer chromatography [i.e., hRf values] for the presence of flavonoids, alkaloids, glycosides and tannins. The extract was stored in a vacuum dessicator and the weighed dose was dissolved in the distilled water for the present study.

C. Selection of doses

Doses were selected on the basis of the effective dose of the crude bark powder (500 & 750 mg/kg) [7], [9] and percentage yield of alcoholic extract (13%).

D. Experimental procedure

NZW albino rabbits (1.5-3 kg body weight) of either sex were obtained from the Institute's Experimental Animal Facility and were kept at 25° C± 5°C in a well ventilated animal house under 12 hour light and dark cycle, maintained in compliance with the Guidelines for Animal Experimentation (IAEC No.007/2001). Rabbits, divided into three groups (12 in each group), and fed either with the alcoholic extract of *Terminalia arjuna* bark in two doses (6.75 mg/kg [T1], 9.75 mg/kg [T2]) or with vehicle (distilled water), by gavage once a day for 12 weeks [6days/week], along with standard rat chow [Hindustan Lever Co., India (contains protein**:** 24%, fat**:** 5%, fiber**:** 4%, carbohydrate**:** 55%, calcium**:** 0.6%, phosphorous**:** 0.3%, moisture**:** 10% and ash**:** 9% w/w.)] or water ad libitum. There was no significant difference in body weight of the treated rabbits, when compared with control, either at the beginning or at end of the study period. The treatment schedule did not cause any change in food and water intake pattern. At the end of 12 weeks, the rabbits were randomly selected for the following groups of experiments.

E. Baseline changes in the myocardial endogenous antioxidant profile:

Six rabbits from each of the following groups were heparinised (375 units/200gms i.p), after one hour the animals were sacrificed and hearts were harvested and stored in liquid nitrogen for the estimation of endogenous antioxidants and HSP72.

Group **BL** Vehicle treated rabbits
Group **T1BL** Rabbits treated with 6.75mg/kg of TAAE
Group **T2 BL** Rabbits treated with 9.75mg/kg of TAAE

F. Production of in vivo ischemic-reperfusion injury

1. *Experimental Preparation:* Wistar albino rabbits (1.5-3.0 kg of either sex), were anesthetized with Ketamine hydrochloride 35mg/kg (i.v) followed by Sodium pentobarbitone (Sigma, USA) 20 mg/kg, (i.v) and immediately put under artificial respiration with room air. The rate and volume of the respirator was adjusted to maintain normal blood pO_2 and pCO_2. Polyethylene catheters were placed one each in right femoral vein (for drug/vehicle administration) and right femoral artery [for measurement of mean arterial pressure (MAP)].

A left lateral thoracotomy was done in the fifth intercostal space as described earlier [22]. The heart was gently suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated just proximal to its first branch and a silk thread was passed through a plastic snare around this part of the artery. Tightening the snare occluded the LAD and reperfusion was instituted by releasing it. Mean arterial pressure (MAP) and heart rate (HR) were measured on a computerized hemodynamic monitor (Cardiosys, Experimetria, Hungary; Model 101) and left ventricular end-diastolic pressure (LVEDP) along with peak positive and negative dP/dt were measured on a Grass polygraph (model 7D) by Stathum P231D transducers. Standard limb lead II ECG was recorded.

G. Experimental Protocol

After 30 min of post-surgical recovery period, LAD was occluded for 15 min to produce regional left ventricular ischemia, which was evidenced by bluish discoloration of the epi cardium. Thereafter, reperfusion was instituted and continued for 60 min.

The hemodynamic parameters were monitored throughout the experiment, starting from 10 min before LAD occlusion, at 5 min intervals, up to 15 min of reperfusion and thereafter, at 15 min intervals, till the end of the experiment.

Animals were euthanized at the end of 60min reperfusion and transmural myocardial tissue samples were punched out immediately and dipped into liquid N_2 for storage until analysis for i) high energy phosphate (HEP) compounds, *i.e*., adenosine triphosphate (ATP) [23], lactate [23], and creatine phosphate (CP) [23], and ii) myocardial antioxidants, *i.e.*, superoxide dismutase (SOD) [24], catalase (CAT) [25], reduced glutathione (GSH) [26], thiobarbituric acid reactive substance (TBARS) [27], and HSP 72. All chemicals used for biochemical analysis were obtained from Sigma Co., USA. The biochemical parameters were estimated in duplicate.

The groups studied

Group **C -** Sham-operated rabbits

Group **I** - The animals were sacrificed after 15 min of ischemia

Group **V** - Vehicle-treated rabbits subjected to 15 min of ischemia and 60 min reperfusion

Group **T1 IR -** TAEE 6.75 mg/kg treated rabbits subjected to 15 min of ischemia and 60 min of reperfusion.

Group **T2 IR** - TAEE 9.75 mg/kg treated rabbits subjected to 15 min of ischemia and 60 min of reperfusion.

H. Isolation and characterization of HSP72

Induction of myocardial HSP72 in left ventricular tissues was

determined as follows.

1. Whole-Body Hyperthermia [28]: Wistar albino male rabbits [body-weight 2.5-3 kg] were anesthetized with Ketamine hydrochloride 35mg/kg (i.v) followed by sodium pentobarbitone (Sigma USA) 20 mg/kg, (i.v) and an intravenous catheter was inserted into the tail vein for normal saline administration. A rectal thermometer was inserted and animals were placed on a warming blanket and gradually heated to 42°C for 20 minutes under a heating lamp. They were then removed from the heating area to a cool surface and allowed to recover for 48 hours. The animals received normal saline [10-12ml, I.V.] during heat stress and recovery periods. Control animals were anesthetized and left at room temperature. The majority of the animals had a satisfactory recovery. Any animal that appeared morbid after the recovery period was euthanized. After 48-hours, myocardial HSP72 was estimated as described below in the surviving animals [n=5] and the control animals.

2. Western blot protein analysis of HSP72 [28]: Rabbits which had undergone whole body hyperthermia were anesthetized with Ketamine hydrochloride 35mg/kg (i.v) followed by Sodium pentobarbitone (Sigma, USA) 20 mg/kg, (i.v) and hearts were quickly harvested and rinsed in cold saline. After removal of atrial and other connective tissues, the ventricular samples were immediately frozen in liquid nitrogen and stored at -70° C. The heart tissues were homogenized in 0.1 mM phosphate buffer containing 5% SDS, 1% mercaptoethanol and 0.1 mM phenylmethylsulfonyl fluoride [PMSF] for 4s using a Polytron tissue homogenizer at 4°C. The homogenate was then strained through a 27 Gauge needle, followed by centrifugation at 14000×g for 10 min. The supernatant, representing protein lysate, was divided into small aliquots and stored at -70°C until use.

Protein concentration was measured using the protein assay based on the Bradford dye binding procedure with bovine serum albumin as standard [29]. At the time of analysis, samples were thawed and volumes were pipetted to allow loading of approximately 100μg of protein per lane on a slab gel. Proteins were separated by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis on 1mm thick, 12.5% acrylamide gels. After electrophoresis, proteins on the gel were transferred to nitrocellulose membranes by electroelution. Protein transfer was confirmed by employing pre-stained molecular weight markers [Biorad Laboratories U.S.A.]. Following transfer, the membrane was blocked with non-fat dry milk, and the nitrocellulose membranes were incubated with a goat monoclonal antibody cross-reacting to the HSP72 [Santa Cruz Biotechnology USA] at a dilution of 1:1000. The secondary antibody was conjugated with anti-goat IgG [Santa Cruz Biotechnology USA 1:200 dilution] and subsequently the membrane was incubated with avidin-biotin HRP complex [Santa Cruz Biotechnology USA] and signal was detected using DAB [Diamino benzidine]. The membrane was developed, and densitometry was performed to quantify the levels of HSP72 [Microtek Scanmaker U.S.A.]

İ. Statistical Analysis

All values are expressed as mean ± SEM. Significance was set at $p < 0.05$. Two Way Analysis of Variance followed by Multiple Range Test was carried out to test the significance of hemodynamic data. One way ANOVA was used to test the significance of biochemical data of the different groups in myocardial tissue samples. Chi-square test was used to determine survival/mortality significance.

III. RESULTS

A. Survival and mortality

No mortality was observed in groups **N** (n=6), and **I** (n=6). In **V**, out of 12 rabbits, one died during ischemia, three died on reperfusion and six animals survived the whole period of ischemia and reperfusion. In group **T1 IR**, out of nine rabbits, three died immediately following reperfusion and six survived. In group **T2 IR**, out of ten rabbits, four died following reperfusion and six survived. Ventricular fibrillation was the cause of mortality in all these cases.

B. Hemodynamic parameters

1. ***Mean arterial pressure (MAP)*** (Table 1): MAP in the group T1 IR, before ischemia (-10) was 92.3 ± 6.8 mmHg. After 5 min of post-ischemia, there was no significant fall in MAP (89.3 ± 7.5 mmHg). MAP values after 10 (I_{10}) and 15 (I_{15}) min of post ischemic period were 88.1 ± 7.5 mmHg, 86.8 ± 7.8 mmHg, respectively. After 30 min of reperfusion there was no further change in MAP (85.6 ± 8.4 mmHg) and remained unchanged upto 60 min of reperfusion (85.8 ± 8.2 mm Hg) in comparison to (-10).

MAP in the group T2 IR, before ischemia (-10) was 92.5 ± 7.4 mmHg. After 5 min of post-ischemia, there was no significant fall in MAP (91.0 ± 8.5 mmHg). MAP values after 10 (I_{10}) and 15 (I_{15}) min of post ischemic period were 90.8 ± 9.2 mmHg, 91.5 ± 9.5 mmHg, respectively. After 30 min of reperfusion there was no further change in MAP (90.5 ± 6.2 mmHg) and remained unchanged upto 60 min of reperfusion (90.5 ± 6.0 mm Hg) in comparison to (-10).

2. ***Heart rate (HR)*** (Table 2): Heart rate in the group T1 IR rabbits before ischemia was 224.3 ± 10.9 beats/min. There was no significant change in HR during the entire period of reperfusion (226.6 ± 14.2 beats/min).

Heart rate in the group T2 IR rabbits before ischemia was 243.3 ± 19.1 beats/min. There was no significant change in HR during the entire period of reperfusion (212.2 ± 8.9 beats/min).

3. ***Left ventricular end diastolic pressure (LVEDP)*** (Table 3): In group T1 IR, LVEDP before ischemia was 4.2 ± 0.3 mmHg. After 5 min of ischemia, there was a significant ($p < 0.05$) rise in LVEDP to (5.9 ± 0.9 mm Hg) and to (5.4 ± 0.3 mmHg) after 15 min of ischemic period. LVEDP was normalized after 5 min of reperfusion, (5.3 ± 0.9 mm Hg) and it was maintained till the end of reperfusion (4.7 ± 0.9 mmHg).

In group T2 IR, LVEDP before ischemia was 4.9 ± 0.5 mmHg. After 5 min of ischemia, there was a significant ($p < 0.05$) rise in LVEDP to (7.0 ± 0.1 mm Hg) and to (7.0 ± 1.0 mmHg) after 15 min of ischemic period. LVEDP was normalized after 5 min of reperfusion, (5.9 ± 0.7 mm Hg) and it was maintained till the end of reperfusion (4.9 ± 0.4 mmHg).

4. ***LV peak (+) dP/dt*** (Table 4): There was no significant change in LV peak (+) dP/dt, in either T1 IR or T2 IR groups, during 15 min of ischemic period (from 2666.7 ± 186.3 to 2465.3 ± 269.4 mmHg/sec and from 2541.6 ± 332.9 to 2875.0 ± 412.2 mmHg/sec, respectively) or at the end of 60 min reperfusion (2252.3 ± 347.5 and 2375.0 ± 344.6 mmHg/sec, respectively).

5. ***LV peak (-) dP/dt*** (Table 5): There was no significant change in LV peak (+) dP/dt, in either T1 IR or T2 IR groups, during 15 min of ischemic period (from 2302.6 ± 382.3 to 2347.4 ± 408.2 mmHg/sec and from 2500.0 ± 418.3 to 2791.6 ± 571.1 mmHg/sec, respectively) or at the end of 60 min reperfusion (2348.9 ± 389.9 and 2208.3 ± 367.9 mmHg/sec, respectively).

C. Biochemical parameters

1. Basal Changes

1.1 High energy phosphate (Table 6)

1.1.1. Myocardial ATP: Myocardial ATP in the group C (sham operated) was 5.6 ± 0.7 µ mole/g wet wt. In T2 BL group, there was a significant ($p < 0.001$) change in myocardial ATP (9.1 ± 0.8 µ mole/g wet wt) but not in T1 BL group (6.5 ±1.0 µ mole/g wet wt).

1.1.2. Myocardial CP: Myocardial CP level in group C (sham operated) was 4.6 ± 0.8 µ mole/g wet wt. In both T1 BL and T2 BL groups, there was no significant change in myocardial CP levels (4.6 ± 0.7 µ mole/g wet wt and 5.9 ± 1.1 µ mole/g wet wt, respectively).

1.1.3. Myocardial Lactate: Myocardial lactate level in group C (sham operated) was 14.9 ± 0.9 µ mole/g wet wt. In both T1 BL and T2 BL groups, there was no significant ($p < 0.001$) change in myocardial lactate levels (15.3 ± 3.8 µ mole/g wet wt and 18.5 ± 1.8 µ mole/g wet wt, respectively).

1.2. Myocardial antioxidant status (Table 7)

1.2.1. Myocardial TBARS: Myocardial TBARS levels in group C (sham operated) was 108.1 ± 5.3 nmole/g wet wt. In T2 BL group there was significant ($p < 0.001$) elevation of myocardial TBARS (243.4 ± 6.8 nmole/g wet wt) and in T1 BL there was no significant elevation in myocardial levels (187.7 ± 10.7 nmole/g wet wt).

1.2.2. Myocardial GSH: Myocardial GSH level in group C (sham operated) was 389.3 ± 9.2 µ mole/g wet wt. In both T1 BL and T2 BL groups, there was significant ($p < 0.001$) increase in the myocardial GSH levels (412.5 ± 6.4 µ mole/g wet wt and 467.1 ± 8.4 µ mole/g wet wt, respectively).

1.2.3. Myocardial SOD: Myocardial SOD levels were in group C (sham operated) was 14.7 ± 1.6 I.U/mg protein. In both T1 BL and T2 BL groups, there was significant ($p < 0.001$) elevation of myocardial SOD levels (46.3 ± 2.9 I.U/mg protein and 65.1 ± 2.8 I.U/mg protein) respectively.

1.2.4. Myocardial Catalase: Myocardial catalase level in group C was (sham operated) was 65.8 ± 2.5 I.U/mg protein. In both T1 BL and T2 BL groups, there was significant ($p < 0.001$) rise in myocardial catalase levels (95.2 ± 4.3 I.U/mg protein and 118.6 ± 7.8 I.U/mg protein, respectively).

2. Ischemic Reperfusion Injury changes

2.1. High energy phosphates (Table 6)

2.1.1 Myocardial ATP: Myocardial ATP in the group V was 2.5 ± 0.3 µ mole/g wet wt. In both T1 IR and T2 IR groups, there was significant ($p < 0.001$) regeneration of myocardial ATP (8.3 ± 0.3 µ mole/g wet wt and 11.4 ± 1.4 µ mole/g wet wt, respectively) in comparison to group V. In the above groups, significant regeneration of myocardial ATP beyond that of group C (5.6 ± 0.7 µ mole/g wet wt) was seen.

2.1.2. Myocardial CP: Myocardial CP level in group V was 4.7 ± 0.2 µ mole/g wet wt. In both T1 IR and T2 IR groups, there was no significant change in myocardial CP levels (4.8 ± 0.9 µ mole/g wet wt and 5.7 ± 1.3 µ mole/g wet wt, respectively).

2.1.3. Myocardial Lactate: Myocardial lactate level in group V was 16.5 ± 3.1 µ mole/g wet wt. In both T1 IR and T2 IR groups, there was no significant change in myocardial lactate levels (15.6 ± 1.9 µ mole/g wet wt and 15.7 ± 1.2 µ mole/g wet wt, respectively).

2.2. Myocardial antioxidant status (Table 7)

2.2.1. Myocardial TBARS: Myocardial TBARS level in group V was 188.9 ± 13.3 nmole/g wet wt). In both T1 IR and T2 IR, there was significant ($p < 0.001$) depletion of myocardial TBARS (110.9 ± 3.7 nmole/g wet wt and 117.3 ± 8.1 nmole/g wet wt, respectively) when compared with group V.

2.2.2. Myocardial GSH: Myocardial GSH level in group V was 272.1 ± 12.9 µ mole/g wet wt). In both T1 IR and T2 IR groups, there was significant ($p < 0.001$) preservation of myocardial GSH levels (376.9 ± 5.7 µ mole/g wet wt and 359.8 ± 9.2 µ mole/g wet wt, respectively) when compared with group V.

2.2.3. Myocardial SOD: Myocardial SOD level in group and V was 8.2 ± 0.5 I.U/mg protein. In both T1 IR and T2 IR groups, there was significant ($p < 0.001$) elevation of myocardial SOD levels (18.4 ± 0.9 I.U/mg protein and 17.5 ± 1.1 I.U/mg protein, respectively) when compared with group V.

2.2.4. Myocardial Catalase: Myocardial catalase level in group V was 36.4 ± 4.3 I.U/mg protein. In both T1 IR and T2 IR groups, there was significant ($p < 0.001$) preservation in myocardial catalase levels (64.1 ± 3.3 I.U/mg protein and 66.2 ± 1.9 I.U/mg protein, respectively) when compared with group V.

D. Histological study

1. Group C [Fig. 1]: Light microscopy of the tissue sections of control group showed normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils.

2. Group I [Fig. 2]: Light microscopy of the tissue sections of group I showed edema, focal hemorrhage and leukocyte infiltration. The muscle fibres showed vascular changes with fragmentation and hydrophobic changes were seen in the myofibrillar structures.

3. Group V [Fig. 3]: Light microscopy of the tissue sections of group V showed edema, with contraction bands, disruption of myofibrillar structures, hemorrhage and ploymorphonuclear leukocyte infiltration. The cardiac muscle fibres showed vascular changes with fragmentation suggestive of focal necrosis.

4. Group T1 BL and T2 BL [Fig. 4 and 6]: Light microscopy of the tissue sections of groups T1 BL and T2 BL showed normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils.

5. Group T1 IR and T2 IR [Fig. 5 and 7]: Light microscopy of the tissue sections of groups T1 IR and T2 IR showed normal architecture of myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils. The morphology of cardiac muscle fibres was relatively well preserved in both the doses

E. Western blot analysis

Western blot analysis showed a single band corresponding to 72 kDa in homogenates of hearts from rabbits treated with both the doses of *Terminalia arjuna* and in group V rabbits subjected to whole body hyperthermia. HSP 72 expression was undetectable in group C rabbits, which were not fed with *Terminalia arjuna*. Densitometric scanning results showed a significant [$p < 0.001$] increase in the expression of HSP 72 in rabbit hearts of T1 BL and T2 BL groups (5018.7 ± 145.8 and 6718.3 ± 141.1, respectively). Expression of the HSP 72 in the hearts of the group V was 837.8 ± 212.8. Heat-shocked rabbits were taken as positive controls, which showed a comparable expression [6192.5 ± 202.8] of HSP 72. The HSP 72 expression in T1 IR (4018.7 ± 106.8) and T2 IR group (4602.0 ± 129.7) were also significantly ($p < 0.05$) more in comparison to that of group V. Even 50 μg of total protein was enough to detect the signal. [Fig. 8]

IV. DISCUSSION

In the present study, the mean arterial pressure was significantly changed and a better recovery profile was observed in both the doses of alcoholic extract of *Terminalia arjuna* in comparison to vehicle treated group. The heart rate was significantly not changed in both T1 IR & T2 IR treated groups and in the vehicle treated groups at the end of reperfusion. In the T1 IR & T2 IR treated groups the LVEDP was significantly elevated during ischemia and it was corrected after 10 minutes of reperfusion. In the T1 IR & T2 IR treated groups there was no change in either LV peak (+) dP/dt or LV peak (-) dP/dt. Over all recovery of global hemodynamic functions of the regionally ischemic reperfused myocardium was observed in both treatment groups.

Repletion of high energy phosphate (ATP) and normalization of CP along with the prevention of CK leakage into plasma indicated favorable metabolic effect in both the doses of T1 IR and T2 IR. The preserved CK inside the cell could have allowed greater conversion of CP to ATP thereby raising ATP levels. This suggests that some of the compounds present in the alcoholic extract of *Terminalia arjuna* (substances that stimulate vasodilatory effect) play a significant detrimental role in the causation of IR injury.

In the present study, chronic oral administration of alcoholic extract of *Terminalia arjuna* bark caused significant rise in the endogenous antioxidants (SOD, GSH and catalase) in the T1 BL and T2 BL groups. Similar type of observation was seen in the *in-vitro* study and also shown by others also [7], [8], [11], [30], [31]. Similarly, the bark of *Terminalia arjuna* contains flavones and anthocyanins, which may be responsible for its antioxidant effects [32]- [34]. In the present study, IR injury was associated with oxidative stress, as evidenced by enhanced lipid peroxidation and deterioration of myocardial endogenous antioxidant status (SOD, GSH and catalase). Similar observations were made earlier in other studies [22], [35], [36]. The present study also demonstrated that free radical injury begins during the ischemic phase itself, and as a consequence, if an antioxidant is to be effective, it should be administered before the onset of ischemia. So the pre-treatment of alcoholic extract of *Terminalia arjuna bark* gave better protection against the oxidative stress and the structural changes associated with IRI. The mechanism of such protection by alcoholic extract of *Terminalia arjuna bark* pretreatment may be due to myocardial adaptation. Myocardial adaptation against oxidative stress is mediated through augmentation of cellular antioxidants such as glutathione, SOD, catalase [37]. Protection against oxidative stress through myocardial adaptation may be one of the effective therapeutic approaches. This proposed mechanism was discussed in the *in-vitro* section.

In *in-vivo* IR injury of the T1 IR and T2 IR treated groups, there was a significant decrease in the myocardial TBARS levels in comparison to group V. Significant rise in the levels of GSH, SOD and catalase was observed in both T1 IR and T2 IR treatment groups. Better recovery profile along with histological improvement was seen in both T1 IR and T2 IR treated group subjected to *in-vivo* IR injury. This indicates that these dosages withstand the oxidative stress associated with *in-vivo* IR injury.

The results of the present study showed enhanced expression of HSP 72 with both the doses of alcoholic extract of *Terminalia arjuna*. However, in the T2 BL group a significantly high increase in HSP expression in comparison to T1 BL group. In group V expression of the HSP was detectable. The HSP 72 expression was observed in both T1 IR and T2 IR groups was also significantly more in comparison to that of control V group. In this study, we have shown that the free radical scavenging ability was observed with both T1 BL and T2 BL groups. Better recovery profile along with histological improvement was seen in the T1 IR and T2 IR treated groups subjected to *in-vivo* IR injury. Although the mechanism of the cytoprotective effect of HSP is not clearly known, it has been reported that it enhances the myocardial endogenous free radical scavenging ability by increasing cardiac catalase activity [38]. It might be possible that reduced expression of HSP in T1 BL treated group resulted in reduced protection. However, the cause for the relatively lower HSP expression is not immediately clear from the present study. In this respect, the present study showed for the first time that the bark of *Terminalia arjuna* is particularly useful, as it could enhance myocardial endogenous antioxidants and HSP 72 expression without producing any cytotoxic effects.

In conclusion, these results demonstrate that pretreatment with both the doses of *Terminalia arjuna* alcoholic extract can provide post-ischemic myocardial preservation. The induction and expression of HSP 72 suggest an important role for the *Terminalia arjuna* alcoholic extract in myocardial cell protection after regional myocardial IR injury.

V. CONCLUSION

The chronic oral administration of the alcoholic extract of the

bark of *Terminalia arjuna* in rabbit induces myocardial HSP72 and augments myocardial endogenous antioxidants, without causing any cellular injury. This offered protection against oxidative stress associated with myocardial ischemic reperfusion injury. The study reveals an important molecular mechanism of action of the bark of *Terminalia arjuna,* a widely used Indian medicinal plant in ischemic heart disease.

REFERENCES

[1] E. Braunwald and R. A. Kloner, "The stunned myocardium prolonged post ischemic ventricular dysfunction," *Circulation*., 1982, vol. 66, pp. 1146-1149.

[2] J. L. Park and B. R. Lucchest, "Mechanism of Myocardial ischemic reperfusion injury," *Ann Thorac Surg.,* 1999, vol. 68, pp. 1905-1912.

[3] G. Ambrosio, J. T. Flaherty, C. Dullio, I. Tritto, G. Sanoro, P. P. Ellia, M. Condorelli, and M. Chiariello, "Oxygen radicals generated at reflow induce peroxidation of membrane lipids in reperfused hearts," *J Clin Invest*., 1991, Vol. 87, pp. 2056-2066.

[4] R. Ferrari, C. Ceconi, S. Curello, A. Cargnoni, E. Pasini and O. Visioli, "The occurrence of oxidative stress during reperfusion in experimental animals and men". *Cardiovascular Drugs and Therapeutics*., 1991, Vol. 5, pp. 277-288.

[5] J. G. Gross, R. K. Judy, and C. W. David, "Mechanism of post ischemic contractile dysfunction," *Ann Thoracic Surge.,* 1999, vol. 68, pp. 1898-1904.

[6] R. Bolli, "The Late Phase of Preconditioning," *Circ. Res.,* 2000, vol. 87, pp. 972-983.

[7] K. Gauthaman, M. Maulik, R. Kumari, S. C. Manchanda, A. K. Dinda and S.K. Maulik, "Effect of chronic treatment with bark of *Terminalia arjuna*: A study on the isolated ischemic reperfused rat heart," *Journal of Ethnopharmacology*., 2001, vol. 75 (2-3), pp. 197-201.

[8] K. Karthikeyan, B. R. Bai, K. Gauthaman, K. S. Sathish and S. N. Devaraj, "Cardioprotective effect of the alcoholic extract of *Terminalia arjuna* bark in an in vivo model of myocardial ischemic reperfusion injury," *Life Sci.*, 2003, vol. 73(21), pp. 2727-2739.

[9] K. Gauthaman, S. K. Banerjee, A. K. Dinda, C. C. Ghosh and S. K. Maulik, *"Terminalia arjuna* (Roxb.) protects rabbit heart against ischemic-reperfusion injury: role of antioxidant enzymes and heat shock protein," *Journal of Ethnopharmacology.,* 2005, vol. 96, pp. 403–409.

[10] D. M. Yellon, and D. J. Hausenloy, "Myocardial reperfusion injury," *N Engl J Med*., 2007, vol. 357, pp. 1121-1135.

[11] K. Gauthaman, T. S. Mohamed Saleem, V. Ravi and S. Niranjali Devaraj, "Cardioprotective properties of Methanolic extract of *Terminalia arjuna* Linn Bark in an *in vitro* model of Myocardial ischemic reperfusion injury in rats," *Int. J.Pharmacol.Biol.Sci.*, vol. 2 (1), 2008, pp. 13-28.

[12] A. A. Knowlton, "The role of heat shock protein in heart," *Journal of Molecular & Cellular cardiology*., 1995, vol. 27, pp. 121-31.

[13] E. K. Hiodromitis, G. K. Karavolias, E. Bofilis, D. M. Yellon and D.T. Kremastinos, "Enhanced protection of heat shock in myocardial infarction: inhibition of detrimental effect of systemic hyperthermia," *Cardiovascular Drugs Therapy*., 1999, vol. 13 (3), pp. 223-231.

[14] S. K. Powers, M. Locke and H. A. Demirel, "Exercise, heat shock proteins, and myocardial protection from I-R injury," *Med Sci Sports Exerc.,* Mar 2001, vol 33 (3), pp. 386-392.

[15] K. Gauthaman and S. Niranjali Devaraj, *"Terminalia arjuna* barks protects rat hearts with induction of the 72 kDa Heat shock protein (HSP 72)," *Biomedicine*., 2003, (3&4), pp. 26-30.

[16] C. G. Caroline, A. Mohamed and H. Y. Magdi, "Heat stress proteins and myocardial protection: Experimental model or potential clinical tool?" *The International Journal of Biochemistry and Cell Biology*, 1999, vol 31, pp. 559-573.

[17] A. K. Nadkarni, Indian Materia Medica vol.1, Bombay: Popular Prakashan (Pvt.) Ltd., India. 1976.

[18] A. B. Vaidya, "*Terminalia arjuna* in cardiovascular therapy," *J Assoc Physicians India* ., April 1994 , vol. 42 (4), pp. 281-282.

[19] Y. B. Tripathi, "*Terminalia arjuna* extract modulates the contraction of rat aorta induced by KCl and norepinephrine," *Phytotherapy Research.,* 1993, vol. 7, pp. 320-322.

[20] S. Dwivedi, "Antianginal and cardioprotective effects of *Terminalia arjuna*, an indigenous drug, in coronary heart disease," *Journal Association Physician India.,* 1994, vol. 42 (4), pp. 287-289.

[21] M. Gautham, M. Sunit, and K. D. Dipak, "Evaluation of Antioxidant Effectiveness of a few selected Vegetables," *Enviromental & Nutrional Interactions*., 1997, vol. 1, pp. 287-297.

[22] S. K. Maulik, R. Kumari, M. Maulik., S. C. Manchanda and S. K. Gupta, "Captopril and its time of administration in myocardial ischemic-reperfusion injury," *Pharmacol Res*., 2001, vol. 44 (2), pp. 123-127.

[23] W. Lamprecht, F. Stan, H. Weisser, and F. Heinz, *"Determination of creatine phosphate and adenosine triphosphate with creatine kinase," In: Methods of Enzymatic Analysis.,* Bergmayer, H.U. (Ed.). Academic Press: New York 1974, pp. 1776-1778.

[24] P. Kakkar, B. Das and P. N. Viswanathan, "A modified spectrophotometric assay of superoxide dismutase" *Indian Journal Biochemistry Biophysics.,* 1984, vol. 21, pp. 130-132.

[25] H. Aebi, *Catalase*, In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Verlag: Chemic Academic Press Inc., 1974, pp. 673-85.

[26] G. L. Ellman, "Tissue sulphydryl groups," *Archive Biochemistry Biophysics.,* 1959, vol. 82, pp. 70-77.

[27] H. Okhawa, N. Oohishi and K. Yagi, "Assay for Lipid peroxides in animal tissues by thiobarbituric acid reaction," *Annals of Biochemistry.,* 1979, vol. 95, pp. 351-358.

[28] M. M. Hutter, R. E. Sievers, V. Barbose and C.L. Wolfe, "Heat shock protein induction in rat hearts. A direct correlation between the amount of heat shock protein induced and the degree of myocardial protection" *Circulation*., 1994, vol. 89, pp. 355-360.

[29] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry.,* 1976, vol. 7 (72), pp. 248-254.

[30] V Pathania, N. Syal, M. H. Hundal, and K. L. Khanduja, "Geriforte stimulates antioxidant defense system," *Indian Journal of Experimental Biology*., 1998, vol. 36, pp. 414-417.

[31] S. K. Banerjee, A. K. Dinda, S. C. Manchanda and S. K. Maulik, "Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury," *BMC Pharmacology.,* 2002, vol. 2 (1), 16.

[32] A. L. Miller, "Botanical influences on cardiovascular disease," *Alternative Medical Review.,* 1998, vol. 3 (6), pp. 422-431.

[33] H. Yamasaki, H. Uefuji, and Y. Sakihama, *Arch Biochem. Biophys.,* 1996; 332(1), 183-186.

[34] J. C. Tilak, T. P. A. Devasagayam, T. Kon, Y. Naito and T. Yoshikawa, "Protection by an medicinal plant, *Terminalia arjuna*, and its active component, Baicalein against superoxide and singlet oxygen," *In International conference on natural products*, free radicals and padioprotectors in health (NFR – 200) and III annual meeting of SFRR – India., Jan17- 19, 2004, pp. 21-22.

[35] P. K. Singal, A. K. Dhalla, M. Hill, and T. P. Thomas, "Endogenous antioxidant changes in the myocardium in response to acute and chronic stress conditions," *Molecular cell biochemistry,* 1993, vol. 129, pp. 179-186.

[36] S. D. Seth, M. Maulik, C. K. Katiyar, and S. K. Mauilk, "Role of lipistat in protection against isoproterenol induced myocardial necrosis in rats: A biochemical and histopathological study," *Indian Journal of Physiology and Pharmacology.,* 1998, vol. 42 (1), pp. 101-106.

[37] D. K. Das, and N. Maulik, In *"Cell Biology of Trauma*", eds. By Lemasters J.L., Oliver C., Boca Raton CRC press 1995, pp 193-211.

[38] S. E. Steare and D. M. Yellon, "The potential for endogenous myocardial antioxidant to protect the myocardium against ischemic-reperfusion injury; refreshing the parts exogenous antioxidants can not reach," *J. Mol. Cell. Cardiol.,* 1995, vol. 27, pp. 65-74.

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TABLE I
TIME COURSE OF CHANGES IN MEAN ARTERIAL PRESSURE (MMHG) IN VEHICLE AND *TERMINALIA ARJUNA* ALCOHOLIC EXTRACT (TAAE) PRE TREATMENT GROUPS

Time (Min)	A. V **(n=10)**	VI. T1 IR **(n=6)**	A. T2 IR **(n=6)**
-10	87.0 ± 3.1	92.3 ± 6.8	92.5 ± 7.4
0	87.0 ± 3.1	92.3 ± 6.8	92.5 ± 7.4
I_5	79.5 ± 5.0*	89.3 ± 7.5	91.0 ± 8.5
I_{10}	79.2 ± 3.1*	88.1 ± 7.5	90.8 ± 9.2
I_{15}	80.0 ± 3.7*	86.8 ± 7.8	91.5 ± 9.5
R_5	80.8 ± 4.4*	86.2 ± 7.9	90.8 ± 7.7
R_{10}	72.3 ± 2.5*	85.8 ± 8.0	91.8 ± 6.5
R_{15}	71.8 ± 2.6*	85.6 ± 8.3	91.0 ± 5.3
R_{30}	74.3 ± 5.7*	85.6 ± 8.4	90.5 ± 6.2
R_{45}	72.5 ± 2.5*	85.7 ± 8.4	90.5 ± 6.2
R_{60}	68.5 ± 5.5*	85.8 ± 8.2	90.5 ± 6.0

I: Ischemia, R: Reperfusion

*$p<0.05$ vs –10

TABLE II
TIME COURSE OF CHANGES IN HEART RATE (BEATS/MINS) IN VEHICLE AND *TERMINALIA ARJUNA* EXTRACT (TAAE) PRE TREATMENT GROUPS

Time (Min)	A. V (n=10)	VII. T1 IR (n=6)	A. T2 IR (n=6)
-10	267.8 ± 19.7	224.3 ± 10.9	243.3 ±19.1
0	267.8 ± 19.7	224.3 ± 10.9	243.3 ±19.1
I_5	275.5 ± 12.8	224.6 ± 12.6	211.8 ± 10.0
I_{10}	271.6 ± 14.6	225.8 ± 13.2	209.6 ± 10.4
I_{15}	265.2 ± 12.4	229.5 ± 17.3	205.0 ± 9.7
R_5	268.8 ±13.1	229.6 ± 16.5	205.8 ± 10.1
R_{10}	266.6 ± 13.0	229.4 ± 16.0	204.6 ± 9.9
R_{15}	264.0 ± 10.1	228.9 ± 15.2	206.6 ± 11.4
R_{30}	246.3 ± 4.3	228.4 ± 14.5	205.5 ± 9.9
R_{45}	247.0 ± 6.0	227.6 ± 14.1	209.6 ± 8.9
R_{60}	242.1 ± 9.1	226.6 ± 14.2	212.2 ±8.9

I: Ischemia, R: Reperfusion
*p<0.05 vs –10

TABLE III
TIME COURSE OF CHANGES IN LEFT VENTRICULAR END DIASTOLIC PRESSURE (MMHG) IN VEHICLE AND *TERMINALIA ARJUNA* (TAAE) PRE TREATMENT GROUPS

Time (Min)	A. V (n=10)	VIII. T1 IR (n=6)	A. T2 IR (n=6)
-10	3.0 ± 0.3	4.2 ± 0.3	4.9 ± 0.5
0	3.0 ± 0.3	4.2 ± 0.3	4.9 ± 0.5
I_5	6.4 ± 0.4*	5.9 ± 0.9*	7.0 ± 0.1*
I_{10}	5.0 ± 0.8*	5.6 ± 0.7*	7.0 ± 0.3*
I_{15}	5.0 ± 0.6*	5.4 ± 0.3*	7.0 ± 1.0*
R_5	4.7 ± 0.7*	5.3 ± 0.9	5.9 ± 0.7
R_{10}	3.4 ± 0.4	5.2 ± 0.8	5.6 ± 0.4
R_{15}	3.1 ± 0.1	5.1 ± 0.8	5.5 ± 0.4
R_{30}	3.9 ± 0.1*	4.9 ± 0.8	4.5 ± 1.7
R_{45}	4.5 ± 0.3*	4.8 ± 0.9	4.9 ± 0.4
R_{60}	4.6 ± 0.3*	4.7 ± 0.9	4.9 ± 0.4

I: Ischemia, R: Reperfusion

*p<0.05 vs –10

TABLE IV
TIME COURSE OF CHANGES IN LEFT VENTRICULAR PEAK (+) DP/DT (MMHG/SEC) IN VEHICLE AND *TERMINALIA ARJUNA* (TAAE) PRE TREATMENT GROUPS

Time (Min)	A. V (n=10)	IX. T1 IR (n=6)	A. T2 IR (n=6)
-10	2208.3 ± 188.1	2666.7 ± 186.3	2541.6 ± 332.9
0	2208.3 ± 188.1	2666.7 ± 186.3	2541.6 ± 332.9
I_5	2125.0 ± 262.2*	2666.7 ± 186.3	2937.5 ± 445.4
I_{10}	2166.6 ± 141.5*	2562.5 ± 231.1	2770.8 ± 514.8
I_{15}	2050.0 ± 158.1*	2465.3 ± 269.4	2875.0 ± 412.2
R_5	2000.0 ± 105.0*	2406.3 ± 284.7	2750.0 ± 191.6
R_{10}	1808.3 ± 145.7*	2358.3 ± 298.8	2666.6 ± 445.4
R_{15}	1891.6 ± 188.1*	2295.1 ± 332.2	2625.0 ± 447.1
R_{30}	1841.6 ± 188.1*	2255.9 ± 345.0	2291.6 ± 292.3
R_{45}	1866.0 ± 129.0*	2237.0 ± 344.4	2291.6 ± 292.2
R_{60}	1808.3 ± 188.1*	2252.3 ± 347.5	2375.0 ± 344.6

I: Ischemia, R: Reperfusion

*p<0.05 vs –10

TABLE V
TIME COURSE OF CHANGES IN LEFT VENTRICULAR PEAK (-) DP/DT (MMHG/SEC) IN VEHICLE AND *TERMINALIA ARJUNA* (TAAE) PRE TREATMENT GROUPS

Time (Min)	A. V (n=10)	X. T1 IR (n=6)	A. T2 IR (n=6)
-10	2333.3 ± 204.1	2302.6 ±382.3	2500.0 ± 418.3
0	2333.3 ± 204.1	2302.6 ± 382.3	2500.0 ± 418.3
I_5	2083.3 ± 129.1*	2329.1 ± 397.1	2791.6 ± 519.1
I_{10}	2011.6 ± 102.1*	2338.9 ± 401.5	2666.6 ± 683.1
I_{15}	2011.6 ± 102.1*	2347.4 ± 408.7	2791.6 ± 571.1
R_5	2008.1 ± 188.1*	2358.1 ± 408.9	2583.3 ± 408.2
R_{10}	2003.3 ± 204.1*	2357.6 ± 411.0	2458.3 ± 367.9
R_{15}	2008.7 ± 102.2*	2346.8 ± 410.3	2541.6 ± 367.9
R_{30}	1833.3 ± 129.0*	2343.2 ± 404.1	2333.3 ± 341.6
R_{45}	1625.0 ± 137.0*	2344.5 ± 397.4	2291.6 ± 332.2
R_{60}	1708.3 ± 102.1*	2348.9 ± 389.9	2208.3 ± 367.9

I: Ischemia, R: Reperfusion

*p<0.05 vs –10

TABLE VI
MYOCARDIAL HIGH ENERGY PHOSPHATES STATUS OF ALCOHOLIC EXTRACT OF *TERMINALIA ARJUNA* (TAAE) PRE TREATMENT GROUPS

Groups	Parameters		
	ATP µmole/g wet wt	CP µmole/g wet wt	LACTATEµmole/g wet wt
C	5.6 ± 0.7	4.6 ± 0.8	14.9 ± 0.9
I	2.4 ± 0.8*	1.5 ± 0.6*	28.6 ± 3.4*
V	2.5 ± 0.3*	4.7 ± 0.2	16.5 ± 3.1
TA 1 BL	6.5± 1.0*	4.6 ± 0.7	15.3 ± 3.8
TA 1 IR	8.3 ± 0.3♣	4.8 ± 0.9	15.6 ± 1.9
TA 2 BL	9.1 ± 0.8*	5.9 ± 1.1	18.5 ± 1.8
TA 2 IR	11.4 ± 1.4♣	5.7 ± 1.3	15.7 ± 1.2

All values are expressed as Mean ± SE (n=6).
p < 0.001 vs C ***♣p < 0.001 vs V*** (One way ANOVA)

C Sham operated rabbits
I Vehicle-treated rabbits hearts subjected to 15 min ischemia
V Vehicle-treated rabbits hearts subjected to 15 min ischemia + 60 min reperfusion
TA 1 BL Rabbits treated with 6.75 mg/kg of *Terminalia arjuna* alcoholic extract
TA 1 IR 6.75 mg/kg of *Terminalia arjuna alcoholic* extract pre treated rabbit hearts subjected to V
TA 2 BL Rabbits treated with 9.75 mg/kg of *Terminalia arjuna alcoholic* extract
TA 2 IR 9.75 mg/kg of *Terminalia arjuna* alcoholic extract pre treated rabbit hearts subjected to V

TABLE VII
MYOCARDIAL ANTIOXIDANT STATUS OF ALCOHOLIC EXTRACT OF *TERMINALIA ARJUNA* (TAAE) PRE TREATMENT GROUPS

Groups	TBARS nmole/g wet wt	GSH µg/g wet wt	SOD I.U/mg protein	CATALASE I.U/mg protein
C	108.1 ± 5.3	389.3 ± 9.2	14.7 ± 1.6	65.8 ± 2.5
I	129.8 ± 7.6*	231.2 ± 7.5*	6.5 ± 0.6*	25.6 ± 1.8*
V	188.9 ± 13.3*	272.1 ±12.9*	8.2 ± 0.5*	36.4 ± 4.3*
TA 1 BL	187.7 ± 10.7*	412.5 ± 6.4*	46.3 ± 2.9*	95.2 ± 4.3*
TA 1 IR	110.9 ± 3.7♣	376.9 ± 5.7♣	18.4 ± 0.9♣	64.1 ± 3.3♣
TA 2 BL	243.4 ± 6.8*	467.1 ± 8.4*	65.1 ± 2.8*	118.6 ± 7.8*
TA 2 IR	117.3 ± 8.1♣	359.8 ± 9.2♣	17.5 ± 1.1♣	66.2 ± 1.9♣

All values are expressed as Mean ± SE (n=6).
p < 0.001 vs C ***♣p < 0.001 vs V*** (One way ANOVA)

C Sham operated rabbits
I Vehicle-treated rabbits hearts subjected to 15 min ischemia
V Vehicle-treated rabbits hearts subjected to 15 min ischemia + 60 min reperfusion
TA 1 BL Rabbits treated with 6.75 mg/kg of *Terminalia arjuna* alcoholic extract
TA 1 IR 6.75 mg/kg of *Terminalia arjuna alcoholic* extract pre treated rabbit hearts subjected to V
TA 2 BL Rabbits treated with 9.75 mg/kg of *Terminalia arjuna alcoholic* extract
TA 2 IR 9.75 mg/kg of *Terminalia arjuna* alcoholic extract pre treated rabbit hearts subjected to V

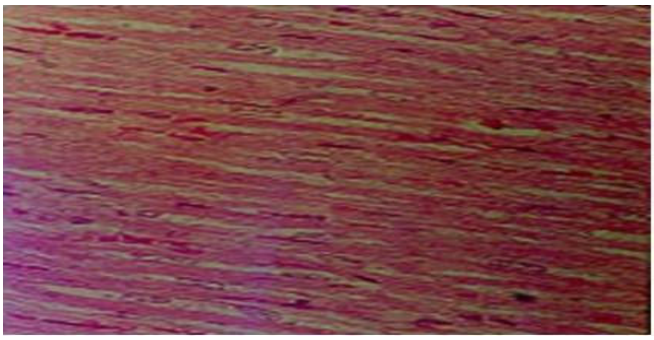

Fig. 1 H & E X 400 stained light microscopy section of group C rabbit myocardium showing well maintained myofibrillar structure.

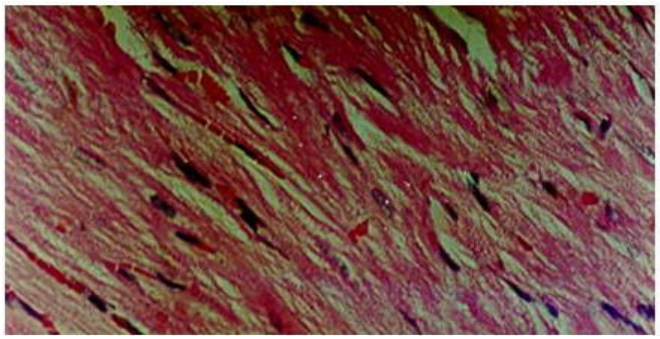

Fig. 2 H & E X 400 stained light microscopy section of group I rabbit myocardium showing extensive degeneration of myofibrils with leukocytic accumulation, edema and vacuolization.

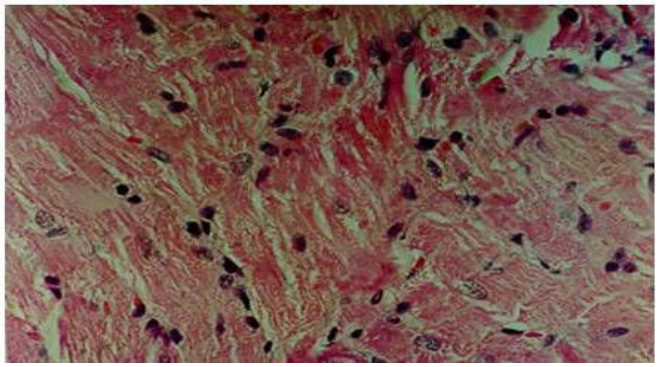

Fig: 3 H & E X 400 stained light microscopy section of group V rabbit myocardium showing disruption in the arrangement of myofibrils with leukocytic accumulation, hydrophobic edema and vacuolization.

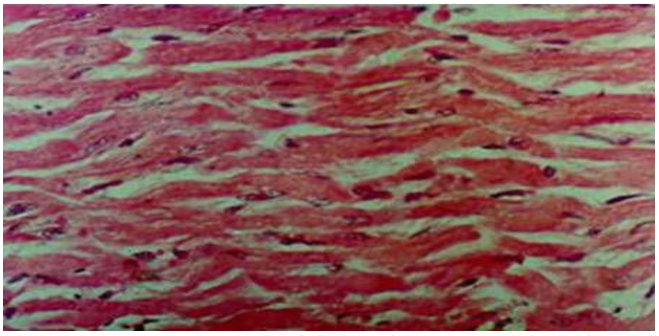

Fig. 4 H & E X 400 stained light microscopy section of group TA1BL treated rabbit myocardium.

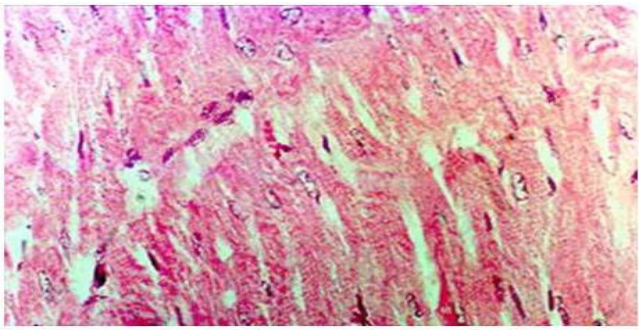

Fig. 5 H & E X 400 stained light microscopy section of group TA1IR treated rabbit myocardium.

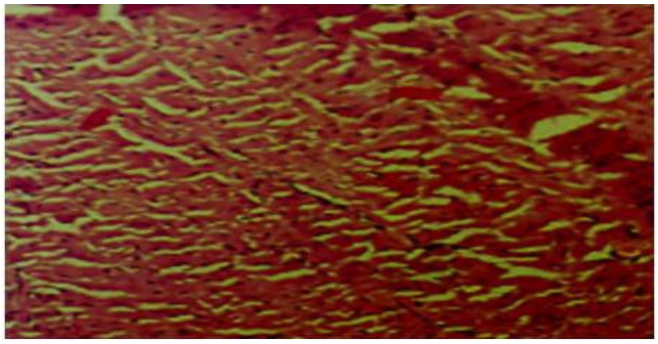

Fig. 6 H & E X 400 stained light microscopy section of group TA2BL treated rabbit myocardium.

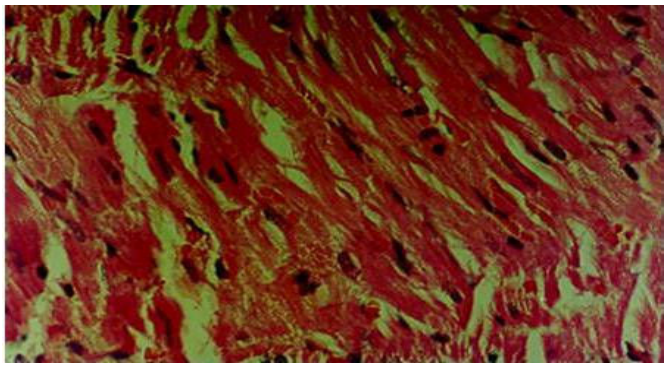

Fig. 7 H & E X 400 stained light microscopy section of group TA2IR treated rabbit myocardium.

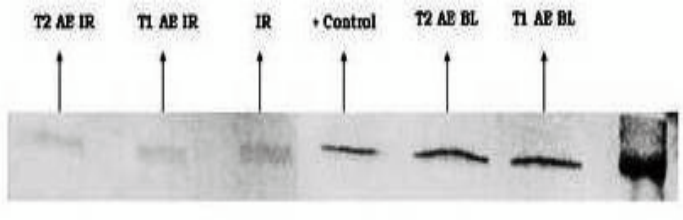

Fig. 8 HSP 72 induction in alcoholic extract of *Terminalia arjuna* treated groups.