Molecular and immunohistochemical studies on cardioprotective mechanism(s) of *Terminalia arjuna* (TA) and *Eugenia jambolana* (EJ) in ischemic model of myocardial infarction—An Experimental Study

ABSTRACT OF THE THESIS SUBMITTED TO FACULTY OF MEDICAL SCIENCES UNIVERSITY OF DELHI FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL BIOCHEMISTRY

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Cardiovascular disease, particularly ischemic heart disease (IHD), has become a worldwide health problem. IHD is the leading cause of morbidity and mortality globally, reaching to the pandemic proportion. It is predicted that IHD will be the most important cause of mortality in India by 2020. Myocardial infarction (MI) is the most lethal manifestation and is a subject of intense investigation for clinicians and basic medical scientists. It is caused by an imbalance between myocardial oxygen supply and demand, resulting in myocardial hypoxia and accumulation of waste metabolites, which occurs oftenly due to atherosclerotic disease of the coronary arteries. Myocardial infarction (MI), the most dreaded sequel among IHD, invariably followed by several biochemical alterations, such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia, elevation in cardiac markers and pro-inflammatory cytokines leading to qualitative and quantitative alterations of myocardium. The pathogenesis of MI involves a complex process including several mechanisms such as oxidative stress due to oxygen free radical formation, calcium overload, neutrophil-mediated myocardial and endothelial injury, contractile dysfunction, metabolic changes and cell death either by necrosis or apoptosis or both.

Isoproterenol (ISP) is a synthetic catecholamine & β-adrenergic agonist, which causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscle. Experimental induction of MI with ISP in animals is a well established model to study the protective effects of different cardio protective agents.

Despite the large number of studies on medicinal plants no, study has yet addressed cardioprotective/antiapoptotic effect of hydroalcoholic extract of bark of T. arjuna and E. jambolana in experimental myocardial ischemia.
**Aims:** This study was aimed to evaluate the preventive effects of *Terminalia arjuna* (TA) and *Eugenia jambolana* (EJ) alone and in combination in ISP-induced ischemic rats, by evaluating oxidative stress parameters, cardiac markers, pro-inflammatory cytokines, apoptotic parameters and histopathological alterations.

**Methods:** Male Wistar rats (150-220g) were pretreated with HETA, HEEJ and their combination and Vit E daily for 30 days. The rats were divided into 6 groups consisting of 8 rats/group. **Gr.1-** Healthy control; **Gr.2-** ISP control; **Gr.3-** HETA (100, 200 & 400 mg/kg b. w); **Gr.4-** HEEJ (100, 200 & 400 mg/kg); **Gr.5-** HETA+HEEJ (100, 200 & 400 mg/kg); **Gr.6-** Vit E (100 mg/kg b. w). The above pre-treatment were given daily for 30 days. ISP (85 mg/kg, s. c) was injected to rats at an interval of 24 h for two days (28th & 29th day) to induce myocardial ischemia. Oxidative stress parameters (GSH, SOD & MDA), cardiac markers (SGOT, CPK-MB & Troponin-I), pro-inflammatory cytokines (IL-6 & CRP) were evaluated in serum on 0th, 21st and 30th day of the study period. After evaluation of biochemical markers rats were sacrificed for histopathological study. The histopathological alterations were evaluated by H&E staining. Further apoptotic parameters (Bcl-2 & Bax) and pro-inflammatory cytokine (TNF-α) were evaluated by immunohistochemistry (IHC) and western blot. Apoptosis was further evaluated by means of TUNEL assay. The results were presented as mean ± SEM for 8 animals in each group. The data was analyzed by analysis of covariance (ANCOVA) taking baseline as a co-variant. Multiple comparisons among the groups were done by Bonferroni adjustment method and statistical differences between mean values were determined using SPSS software version 17.0. A value of p<0.05 was considered statistically significant.
Results:

- **Effect of HETA on oxidative stress parameters, cardiac markers and pro-inflammatory cytokines in ISP-induced myocardial ischemia** -
  
  - Graded dose of HETA (100, 200 & 400 mg/kg b. w) caused dose dependent effect on these parameters. However the significant (p<0.05) effect on oxidative stress parameters, cardiac markers and pro-inflammatory cytokines were observed with HETA 400 mg/kg b. w dose.
  
  - **Effect of HETA on histopathological alterations and apoptosis** - Histopathological alterations were studied with H&E staining and it was observed that HETA 400 dose significantly improved the myocardial architecture. Further apoptosis/ necrosis was reduced significantly with this treatment.

- **Effect of HEEJ on oxidative stress parameters, cardiac markers and pro-inflammatory cytokines in ISP-induced myocardial ischemia** - HEEJ at a dose of 100, 200 and 400 mg/kg b. w produced dose dependent effect on the oxidative stress parameters. HEEJ 400 mg/kg b. w dose was found most effective and the significant effect (p<0.05) was observed with this dose.

  - **Effect of HEEJ on histopathological alterations and apoptosis** - ISP altered the histology of myocardium, which was significantly improved with HEEJ 400 mg/kg b. w dose. HEEJ 400 significantly improved the apoptotic/necrotic changes in the myocardium.

- **Effect of combination of HETA and HEEJ on oxidative stress parameters, cardiac markers and pro-inflammatory cytokines in ISP-induced myocardial ischemia** - Among pre-treated groups, the combination of HETA and HEEJ was found to exert highly
significant effect on oxidative stress parameters, cardiac markers and pro-inflammatory cytokines in dose dependent manner. HETA+HEEJ 400 mg/ kg b. w dose was found most effective and this combination produced significant (p<0.05) effect on these markers.

- **Effect of HETA on histopathological alterations and apoptosis**- Combination of HETA and HEEJ 400 dose was found most effective in reduction of histopathological alteration of myocardium. Therefore pre-treatment with combination of HETA & HEEJ counter-regulated the apoptotic/necrotic changes significantly.

**Key findings**: Isoproterenol treated rats showed increased levels of oxidative stress parameters, cardiac markers and pro-inflammatory cytokines.

Individual dose of HETA and HEEJ (400 mg/ kg b. w) was found effective in the improvement of ISP-induced biochemical and histopathological changes. However the combination dose of HETA and HEEJ (400 mg/ kg b. w) was found most effective in improvement of overall alterations (biochemical and histopathological) produced by isoproterenol.

**Significance**: Our study demonstrated that combination of extracts is better than individual extract in providing the cardioprotection.