Cardiovascular disease, particularly ischemic heart disease (IHD), has become a worldwide health problem. IHD is the leading cause of death in developed countries and increasing alarmingly in developing countries (Karthikeyan et al., 2007; Torabian et al., 2009; Subhashini et al., 2011; El-Sayed et al., 2011) and is reaching to pandemic proportion in Indian subcontinent (Goyal and Yusuf, 2006; Backer, 2009). It is caused by an imbalance between myocardial oxygen supply and demand. Several approaches are present as a method of choice to counteract the ill effect of myocardial ischemic injury, such as beta-blockers, ACE inhibitors, antiplatelet agents, thrombolytics, calcium antagonist, nitrates and antioxidants (Verma et al., 2002; Moens et al., 2005).

ISP model is well standardized and most reliable model for assessing the cardioprotective activity of several drugs (Dwivedi et al., 1988; Bhindi et al., 2006; Anandan et al., 2007; Panda and Naik, 2008; Goyal et al., 2010; Parveen et al., 2011; Shukla et al., 2012). The pathophysiological and morphological alterations in ISP induced rat model mimic resemblance with human myocardial infarction (Anandan et al., 2007; Panda and Naik, 2008; Zhou et al., 2008). The pharmacological treatments for myocardial ischemia aimed at re-establishing the balance between oxygen delivery to oxygen demand by the myocardial tissue. This equilibrium is maintained by decreasing the mechanical power consumption of the myocardial tissue through a reduction in heart rate, blood pressure and contractibility or coronary vasodilation (Dhalla et al., 2000; Wang et al., 2002; Bolli et al., 2004).

Currently, a number of pharmacological approaches have been proposed with limited success to delay myocardial necrosis and decrease ischemic injury. As such, novel therapeutic strategies for protecting the heart against ischemic injury are urgently needed to reduce myocardial injury, preserve cardiac function, prevent the
development of heart failure, and improve clinical outcomes in patients with IHD (Yellon and Hausenloy, 2007; Ovize et al., 2010).

There is increasing trend towards the application of herbal medicines to treat the cardiovascular diseases (Nandave et al., 2007; Hina et al., 2010; Ojha et al., 2011). Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties (Shukla et al., 2010; Govind and Sahni, 2011). The traditional medicine all over the world is now days revalued by an extensive activity of research on different plant species and their therapeutic principles (Gupta et al., 2010). Previous studies have demonstrated cardioprotective effects of several herbs against ISP-induced myocardial infarction (Sharma et al., 2001; Gupta et al., 2004; Mohanty et al., 2004; Prabhu et al., 2006; Gauthman et al., 2006; Bansal et al., 2006; Arya et al., 2006). However, there is paucity of information on several medicinal plant species and there is an urgent need to screen their protective activities for development of potential cardioprotective drugs. Keeping these considerations we have taken two useful medicinal plants viz. T. arjuna and E. jambolana for screening of their cardioprotective effects in ISP-induced myocardial ischemia.

In this context, an attempt has been made to investigate the effect of hydroalcoholic extract of T. arjuna and E. jambolana on maintaining the myocardial integrity in animals employing a wide array of biochemical, histopathological, and immuno-histochemical parameters in isoproterenol-induced myocardial ischemia.

The present study entitled “Molecular and immunohistochemical studies on cardioprotective mechenism(S) of Terminalia arjuna and Eugenia jambolana in ischemic model of myocardial infarction- An experimental Study” was undertaken to
achieve following objectives, (i) To study the effect of *T. arjuna* and *E. jambolana* on modulation of antioxidant markers and to delineate the involvement of reactive oxygen species (ROS) in pathophysiology of myocardial infarction (ii) To study the effect of herbal extracts on modulation of apoptotic markers bcl-2 and Bax proteins and the role of apoptosis in ischemic model of myocardial infarction (iii) To study the role of TNF-α, IL-6 and CRP in the pathophysiology of myocardial infarction and the effect of herbal extracts on its expression (iv) To study the effect of herbal extracts (*T. arjuna* and *E. jambolana*) on histological changes in ischemic model of myocardial infarction.

**Effect of *T. arjuna*, *E. jambolana* and their combination on oxidative stress parameters and antioxidant activity in ISP-induced MI**

- To access the extent of oxidative stress during ISP induced MI, oxidative stress parameters (GSH, SOD and MDA) were evaluated by the standardized biochemical methods.

- The oral administration of hydroalcoholic extracts of *T. arjuna* (HETA) and *E. jambolana* (HEEJ) decreased the lipid peroxidation product and increased the antioxidant (GSH & SOD) activity. However the more promising effect was seen with their combination (HETA+HEEJ).

- HETA, HEEJ and combination of HETA and HEEJ exerted a dose dependent effect on oxidative stress parameters and antioxidant activity. When HETA, HEEJ alone and in combination were orally administered at a dose 100, 200 and 400 mg/kg b. w., a dose dependent effect on oxidative stress parameters was observed. However the dose of 400 mg/kg b. w. alone and in combination has highly significant effect (p<0.05) on oxidative stress parameters.
• The antioxidant activity of these herbal extracts is comparable to Vit E pre-treated group.

• It is predicted that the observed antioxidant activities of these herbal extracts are due to their phytoconstituents.

Effects of HETA, HEEJ and combination of HETA and HEEJ on cardiac markers

• HETA and HEEJ alone, and in combination exhibited potential cardioprotective activity.

• Serum SGOT, CPK-MB and Trop-I levels were improved with pre-treatment of HETA and HEEJ at a dose 100, 200 and 400 mg/kg b. w.

• HETA and HEEJ at dose 400 mg/kg b. w. showed significant (p<0.05) reduction in elevated levels of cardiac markers. However, when these extracts were administered in combination, the reduction was highly significant (p<0.05).

Effects of HETA, HEEJ and their combination on pro-inflammatory cytokines

• Hydroalcoholic extracts of T. arjuna (HETA) and E. jambolana (HEEJ) exerted significant effect on pro-inflammatory cytokines.

• When HETA and HEEJ were administered alone at a dose of 100, 200 and 400 mg/kg b. w., the effect was seen in dose dependent manner. HETA and HEEJ at a dose of 100 mg/kg b. w. showed significant effect on pro-inflammatory cytokines. However 200 and 400 mg/kg b. w. dose of both
extracts showed significant (p<0.05) reduction in the elevated level of CRP, IL-6 and TNF-α.

- The combination of HETA and HEEJ at a dose of 400 mg/kg b. w. showed highly significant (p<0.05) effect on pro-inflammatory cytokines.

**Effects of HETA and HEEJ on histopathology**

- The deleterious changes occurred during MI were evaluated histopathologically and it was found that HETA and HEEJ improved the histopathological features.
- Significant improvement was observed with 400 mg/kg b. w. of HETA and HEEJ.
- When these herbal extracts were given in combination (HETA+HEEJ) at different doses, ranging from 100-400 mg/kg b. w., the 400 mg/kg b. w. dose significantly improved the architecture of myocardium.

**Anti-apoptotic effect of pre-treatment with HETA and HEEJ**

- Anti-apoptotic effect of the HETA and HEEJ were quantified by Bcl-2 and Bax protein expression and TUNEL assay.
- Pre-treatment with HETA and HEEJ at a dose 100, 200 and 400 mg/kg b. w. showed better improvement in apoptotic parameters.
- Expression of anti-apoptotic protein Bcl-2 was increased with pre-treatment with combination of HETA and HEEJ at 400 mg/kg dose with concomitant decrease in Bax expression.
- Highly significant increase in expression of apoptotic markers (Bcl-2 and Bax) were observed with combination of HETA and HEEJ at a dose 400 mg/kg b. w.
CONCLUSIONS

- Pre-treatment with hydroalcoholic extract of *T. arjuna* (HETA), *E. jambolana* (HEEJ) exerted significant cardioprotective effects in the experimental myocardial ischemia. However the combination of HETA and HEEJ (400 mg/kg b. w.) is found to have highly significant cardioprotective activity in comparison to that of individual herbal extract.

- The combination of *T. arjuna* and *E. jambolana* significantly ameliorates the deleterious changes of ischemic episode. It included improvement in antioxidant status, reduction in oxidative stress, conferring cardioprotection by reducing cardiac markers, pro-inflammatory cytokines and apoptosis/necrosis. Moreover, pre-treatment with combination of HETA and HEEJ resulted in marked improvement of histopathological features in isoproterenol induced cardiotoxicity.

- The present study provides scientific basis of employing the combination of HETA (hydroalcoholic extract of *T. arjuna*) and HEEJ (hydroalcoholic extract of *E. Jambolana*). It may be used as an adjunct therapy along with conventional drugs for cardioprotection. This herbal combination has the potential for the management of the patients at risk of IHD.

- In view of demonstrable efficacy and traditional acceptability of these medicinal plants used in the present study, a well controlled prospective clinical trial should be contemplated to establish their therapeutic role in cure and prevention of ischemic heart disease (IHD) including long term side effects, if any.