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Medicinal Herbs can Play Significant Role in Attenuation of Ischemia and Reperfusion Injury

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Abstract

Nature has been a source of medicinal treatments for thousands of years and plant-derived products continue to play an essential role in the primary health care of about 80-85% of the world's population. Medicinal herbs are widely used in Ayurveda, the Indian System of Medicine and have been observed to possess numerous activities with regard to cardiovascular system viz. antiplatelet, hypolipidemic, anti-inflammatory, hypoglycemic and hypotensive actions. Hence, these herbal extracts traditionally used have been evaluated scientifically in the present study with an aim to define the role of these agents in limiting the deleterious effects of myocardial ischemia and reperfusion (IR) injury by providing scientific data to validate their use as prophylactic approaches or as an adjunct to standard treatment (synthetic compounds employed in conventional treatment protocols) of ischemic heart disease. The efficacy of *Withania somnifera (Ws), Curcuma longa* (Cl) and *Ocimum sanctum (Os)*, and herbal combination (HCB) including {*Ws* (50 mg/kg) + *Cl* (100 mg/kg) + *Os* (75 mg/kg} to limit injury in the setting of myocardial ischemia and reperfusion was explored in the present study. An open chest left anterior descending coronary artery (LAD) occlusion and reperfusion induced myocardial injury was used as the experimental model. Wistar albino rats were divided into ten groups and orally fed saline once daily (sham, control IR) or medicinal herbs (Ws/Cl/Os/HCB; Ws-IR, Cl-IR/Os-IR/HCB-IR) respectively for 1 month. On the 31st day in the rats of the Control IR and Ws-IR, Cl-IR/Os-IR/HCB-IR groups, LAD was occluded for 45 min, and reperfused for 1 h. Hemodynamic parameters were recorded at preset points and subsequently sacrificed for biochemical, immunohistochemical and pathological studies. In the control IR group, significant ventricular dysfunction, cardiac necrosis, apoptosis; decline in antioxidant status and elevation in lipid peroxidation was observed. Chronic oral treatment with HCB *per se* for 1 month resulted in significant enhancement of the myocardial endogenous antioxidant enzymes. Pretreatment with *Ws, Cl* and the herbal combination exerted significant cardioprotective effects in the experimental model of myocardial injury. The most remarkable observation of the present study was that cardioprotective effect exerted by HCB treatment was found to be superior to that shown by singular treatment with individual herbal extracts. The combination of herbal extracts was found to significantly ameliorate the ischemia and reperfusion cardiomyocyte apoptosis, cardiac dysfunction, compromised antioxidant status and histopathologic alterations as compared to control IR group. Cardioprotection by HCB treatment may be attributed to its favorable hemodynamic effects, myocardial adaptogenic properties, and significant antioxidant and antiapoptotic properties. Furthermore, HCB decreased the severity of pathological changes and significantly preserved the myocardial creatinine phosphokinase confirming its myocardial salvaging effects. Results clearly demonstrated the therapeutic potential of the herbal drugs in the treatment of myocardial ischemia and reperfusion injury. If the beneficial effects can be established in-patients, these findings may represent a novel adjunctive therapy of ischemic heart disease and Myocardial Infarction.

Keywords: Herbs; Ischemia; Reperfusion; Apoptosis; *Withania somnifera*; *Curcuma longa*; *Ocimum sactum*

Introduction

Myocardial ischemia initiated by occlusion or blockade of a major coronary artery leads to myocardial cell death.Thrombolytic therapy (i.e streptokinase, tissue plasminogen activator) by producing prompt reperfusion of ischemic myocardium relieves or reduces ischemia and the morbidity and mortality associated with an acute myocardialinfarction [1]. It has been well documented that early reperfusion of viable but ischemic jeopardized myocardium is essential to prevent cardiac damage. However, reperfusion itself has been shown to enhance myocardial injury and leads to further complications such as diminished cardiac contractile function and metabolic derangements. Myocardial injury associated with restoration of blood flow into previously ischemic tissue has been termed '**reperfusion injury'**. Reperfusion injury is defined as 'those metabolic, functional and structural consequences of restoring coronary artery flow that can be avoided or reversed by modification of the condition of reperfusion' [2].

The deleterious consequences of myocardial reperfusion include the hastening of the necrotic process of irreversibly injured myocytes, cell swelling, the no-reflow phenomenon, hemorrhagic myocardial infarction, the calcium and oxygen paradox, the production of oxygen derived free radicals which may further damage the ischemic myocytes, depletion of the antioxidant network of the myocardium and the prolonged post ischemic depression of ventricular function or the so called 'stunning' of the myocardium. Reperfusion of the ischemic tissue may also induce a number of important cardiac electrophysiological changes, which in turn can cause a variety of arrhythmias, some benign, others potentially lethal [3,4].

Thus, myocardial reperfusion may be viewed as a 'double-edged sword', although it clearly exerts deleterious effects on the severely ischemic cells, when reperfusion is carried out relatively early in the course of ischemia its net effects are usually beneficial [5]. Thus, given the enormous potential clinical importance of early reperfusion in limiting infarct size, preserving antioxidant status, left ventricular function, and thus ensuring a significant decrease in patient morbidity and mortality, the development and identification of safe and effective interventions to reduce myocardial ischemia and reperfusion induced injury and/or optimize the benefit/risk ratio remain fertile areas for clinical and experimental investigation.

In the present investigation, modification of the condition of

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reperfusion has been achieved with the use of plant derived agents giving new insight to advanced therapeutic targets and strategies for the treatment of myocardial ischemic and reperfusion injury. The review summarizes the work undertaken in the laboratory to evaluate the cardioprotective potential of certain herbs {*Withania somnifera (Ashwagandha)*, *Curcuma longa (Turmeric)*, *Ocimum sactum* (Tulsi), the herbal combination including *Withania somnifera* (50 mg/kg) + *Curcuma longa* (100 mg/kg) + *Ocimum sanctum* (75 mg/kg)} and to elucidate their possible mechanisms of action on the basis of hemodynamic, biochemical and histopathological studies. The cardioprotective effects were evaluated in the in-vivo (coronary artery occlusion and reperfusion) and in the isoproterenol model of myocardial necrosis. In addition, the anti-apoptotic properties of the herbal extracts were studied using a combination of techniques of TUNEL positivity and immunohistochemical localization of Bax and Bcl-2 proteins.

The present study reveals that severe myocardial ischemia and reperfusion results in the biochemical derangements, deterioration of myocardial function and leads to the development of infarction. Although reperfusion is essential for the salvage of myocardium, it may enhance myocardial injury. Results clearly demonstrated the therapeutic potential of the herbal drugs in the treatment of acute Myocardial Infarction (MI). If the beneficial effects can be established in-patients, these findings may represent a novel therapy of ischemic heart disease and MI.

Materials and Methods

Experimental animals

Adult male Wistar rats, 10 to 12 weeks old, weighing 150 to 200 g were used in the study. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee and conforms to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research. Animals were obtained from the Central Animal Facility of All India Institute of Medical Sciences, New Delhi, India and were maintained under standard laboratory conditions in the department animal house. Rats were housed in polyacrylic cages (38×23×10 cm) with not more than four animals per cage. They were housed in an air-conditioned room and were kept in standard laboratory conditions under natural light and dark cycles (approximately 14 h light/10 h dark) and maintained at humidity 60 ± 5% and an ambient temperature of 25 ± 2°C. All experiments were performed between 9.0 and 16.0 h. The animals were allowed free excess to standard diet (Ashirwad; Chandigarh) and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 24% protein, 5% fat, 4% fiber, 55% carbohydrates, 0.6% calcium, 0.3% phosphorous, 10% moisture and 9% ash w/w.

Chemicals

All Chemicals were of analytical grade, purchased from Sigma Chemical Co., St Louis, USA. The ABC staining kit and primary (Bax mouse monoclonal IgG_{2b} and Bcl-2 mouse monoclonal IgGI) & secondary antibodies (Anti mouse IgG) were procured from Santa Cruz Biotechnology, USA. TUNEL assay kit was purchased from Roche Diagnostics, USA. Double distilled water was used in all biochemical assays.

Test drugs

Hydro-alcoholic lyophilized extracts of *Withania somnifera and Ocimum sanctum* was procured from Dabur Research Foundation, India. Aqueous extract of *Curcuma longa* was purchased from Sanat Research Laboratories, India. Standard Drugs: Vitamin E was procured from Sigma Chemical, Co., USA and Lisinopril from Cadila Pharmaceuticals, India.

Experimental Models of Myocardial Infarction

Dose selection studies (Isoproterenol model of myocardial necrosis)

The ISP (85 mg/kg) model of myocardial necrosis was used for the evaluation of therapeutic intervention of herbal extracts on the extent of jeopardized myocardium and evolution of infarction in ISP administered rats and select the optimum cardioprotective dose of the herbal extracts for further studies in the ischemia and reperfusion model of myocardial injury.

Experimental myocardial ischemia and reperfusion (IR) model: coronary artery occlusion and reperfusion

An open chest left anterior descending coronary artery (LAD) occlusion and reperfusion induced myocardial injury was used in the present study. In anesthetized rats, LAD occlusion was undertaken for 45 min, thereafter, the occlusion was released and reperfusion of the ischemic myocardium was allowed for a period of 1h. Baseline readings of all the hemodynamic variables were monitored and recorded after 15 min stabilization period, immediately before LAD ligation (time 0 min); thereafter, continuously throughout the experimental period (1 h 45 min) at preset time points. The hemodynamic variables were recorded at 5, 15, 25, 35, 45 min after ligation and at 5, 15, 30, 45, 60 min following reperfusion. At the end of the reperfusion period, animals were sacrificed by an overdose of anesthesia. Myocardial tissue was fixed in accordance to the specific methodology for biochemical, immunohistochemical and histopathological studies.

Experimental parameters evaluated

Cardiac function: The time-course of changes in {mean arterial pressure (MAP), heart rate (HR), left ventricular end diastolic pressure (LVEDP), left ventricular (LV) peak positive (+) dP/dt (rate of pressure development) and negative (-) dP/dt (rate of pressure decline)} were monitored and recorded during coronary artery ligation and reperfusion in different experimental groups.

Cardiac oxidant-antioxidant balance: In the present study, the biochemical indicators of myocardial oxidative damage, lipid peroxidation product; TBARS (thiobarbituric acid reactive substances), endogenous antioxidant: glutathione (GSH), antioxidant enzymes {superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx)} and the myocardial enzyme creatine phosphokinase (CPK) were determined in the different experimental groups of the study.

Histopathologic evaluation: The Hematoxylin and eosin stained sections of the left ventricle were used to study the light microscopic architecture of the myocardial tissue and the degree of necrosis, edema and inflammation was quantified in the hearts of animals from the different experimental groups.

Apoptotic parameters: TUNEL positivity and the immunohistochemical localization of Bax and Bcl-2 proteins were studied to delineate the involvement of apoptosis in ischemia and reperfusion induced myocardial injury in the different experimental groups

Results

Pilot study (Isoproterenol model of myocardial necrosis)

In the present study 25, 50 &100 mg/kg doses of Ws; 25, 50, 100 & 200 mg/kg doses of Cl and 25, 75 & 150 mg/kg of Os were screened in the ISP model of myocardial necrosis in rats. Ws (50 mg/kg), Cl (100 mg/kg) and Os (75 mg/kg) were found to be most effective for functional recovery of the myocardium and the favorable restoration of biochemical and histopathological alterations. Hence, these doses were selected for further evaluation in the ischemia and reperfusion model of myocardial injury in rats.

Experimental myocardial Ischemia and Reperfusion (IR) model

Post-ischemic reperfusion injury resulted in significant cardiac necrosis, apoptosis; depression of left ventricular dynamics, peripheral hemodynamics (mean blood pressure) and heart rate; and decline in antioxidant status and elevation in lipid peroxidation. In addition, consistent with the increase in TUNEL staining in the control IR group, ischemia and reperfusion slightly reduced Bcl-2 expression and significantly increased Bax expression ($p<0.01$) compared with that observed in the sham group, demonstrating the phenomenon of ischemia and reperfusion induced enhanced myocardial apoptotic cell death.

***Withania Somnifera*:** Augmentation of myocardial endogenous antioxidant reserve {SOD, CAT, GSHPx ($p<0.01$)} following chronic oral administration of Ws to healthy controls (study group of animals without any experimental challenge to the myocardium; via ISP administration or inducing ischemia and reperfusion injury) significantly improved defense against oxidative stress as compared to the sham group (oral administration of saline for one month to healthy experimental animals). Further, Ws treatment favorably reestablished the ischemia-reperfusion-induced abnormality of left ventricular functions {(+)LVdP/dt, (-)LVdP/dt and LVEDP}, restored the myocardial oxidant-antioxidant balance, corrected the metabolic derangements (reduced levels of TBARS and enhanced SOD, CAT, GSHPx and CPK activity); exerted marked antiapoptotic effects {(upregulated Bcl-2 ($p<0.001$) protein, decreased Bax ($p<0.01$) protein, and attenuated TUNEL positivity ($p<0.01$)}; and reduced myocardial damage as evidenced by histopathologic evaluation in the ischemia and reperfusion model of myocardial injury; emphasizing its beneficial action as a cardioprotective agent. Thus, favorable modulation of cardiac function by Ws and its myocardial adaptogenic, antioxidant and anti-apoptotic properties may contribute to the cardioprotective effects observed in the present study.

***Curcuma longa*:** Chronic oral administration of Cl to healthy controls for one month significantly enhanced the myocardial activity of CAT ($p<0.05$) as compared to the sham group. The present investigation demonstrates that Cl has significant cardioprotective activity as shown by its mitigating effects on several myocardial injury induced hemodynamic {(+) LVdP/dt, (-) LVdP/dt & LVEDP}, biochemical {GSH ($p<0.001$), TBARS ($p<0.01$), CPK ($p<0.05$)} and histopathological perturbations. In addition, Cl administration significantly reduced the percent of TUNEL positive cells ($p<0.05$) as compared to the control IR group; demonstrating its significant anti-apoptotic activity. Treatment with Cl was associated with significantly increased expression of Bcl-2 ($p<0.001$) and attenuated Bax expression ($p<0.01$) in comparison to the control IR group.

***Ocimum sanctum*:** In the present study, chronic oral administration of Os *per se* to healthy experimental animals for 30 days resulted in significantly enhanced myocardial antioxidant status of basal SOD ($p<0.05$), CAT ($p<0.05$) as compared to the healthy sham control groups. Chronic administration of Os resulted in significant correction of oxidant-antioxidant balance {GSH ($p<0.001$), TBARS ($p<0.05$)} and modulation of the hemodynamic alterations (MAP, LVEDP) as compared to control IR group. However, this drug failed to significantly prevent leakage of myocardial CPK and prevent the histopathological alterations as compared to control IR group. Moreover, Os treatment did not demonstrate any significant anti-apoptotic activity as determined by TUNEL staining and immunohistochemical results. No significant change in the expression of Bax and Bcl-2 proteins was observed with Os treatment as compared to control IR group.

Herbal combination: To investigate whether a combination of the herbal extracts under investigation would offer any added advantage over treatment with individual herbal agents *per se*; the effects of chronic oral administration (30 days) of an herbal combination of (HCB) of Ws (50 mg/kg) + Cl (100 mg/kg) + Os (75 mg/kg) were evaluated. The effects of HCB were investigated in healthy experimental rats and compared with normal healthy sham control group and those of the HCB treated group submitted to myocardial ischemia-reperfusion protocol were compared to the control IR group. Furthermore, the efficacy of HCB was compared to that of both vitamin E and lisinopril used as standard reference drugs.

The results of the present study provide substantial evidence that HCB has significant cardioprotective potential. Although, the precise underlying mechanism of its cardioprotective effects is presently not fully understood; some explanations and likely mechanism(s) are being proposed based on the obtained data: Chronic oral treatment with HCB *per se* resulted in significant enhancement of the myocardial endogenous antioxidants; SOD, CAT, GSHPx ($p<0.01$) and reduction in myocardial TBARS level ($p<0.05$) as compared to the sham control group. Furthermore, HCB was effective enough to beneficially modify the ischemia-reperfusion-induced hemodynamic alterations. It significantly reduced the surrogate preload marker LVEDP as compared to control IR. It has been shown that interventions that decrease LVEDP improve myocardial blood flow in the deeper (endocardium) regions. Furthermore, HCB treatment significantly improved both inotropic and lusitropic function of the heart as evidenced by increased (+) and (-) LVdP/dt. HCB significantly restored the myocardial antioxidant network, as assessed by the increased levels of GSH content and SOD, CAT, GSHPx ($p<0.001$) activity, decreased level of TBARS ($p<0.01$)} as compared to control IR group. Most importantly, HCB demonstrated significant anti-apoptotic effects i.e. decreased Bax ($p<0.01$), upregulated Bcl-2 ($p<0.001$) expression and attenuated TUNEL positivity ($p<0.001$). Loss of contractile cells in the heart poses an additional workload on the remaining viable myocytes that may be unbearable, resulting in pathological stimuli and death signals. Hence, it is likely that chronic treatment with HCB might salvage these viable myocytes, which are at risk of irreversible injury and prevent cell loss induced by apoptosis and necrosis. Preserved myocardial CPK activity ($p<0.01$) and histopathologic evaluation further confirm its myocardial salvaging effects.

Discussion

Myocardial consequences of Ischemia and Reperfusion

Hemodynamic changes: Myocardial ischemia occurs when myocardial oxygen supply is insufficient to meet the demand. Hence, the evaluation of hemodynamic variables associated with myocardial

oxygen demand and supply was done in the present study to assess this critical balance and the functional status of the heart specifically the left ventricular dynamics. Furthermore, the extent of myocardial injury couples with the degree of left ventricular dysfunction. Therefore, hemodynamic monitoring is essential to assess response to therapy. Because no single hemodynamic variable can reliably predict the outcome of myocardial ischemia and the effectiveness of a therapeutic approach, a combination of hemodynamic indices has been used to improve the value of hemodynamic monitoring. Alterations in MAP, HR, LVEDP, (+) LVdP/dt and negative (-) LVdP/dt during coronary artery ligation and reperfusion have been studied in different experimental groups.

In the present study, on occlusion of the LAD coronary artery there was a significant fall in MAP in conjunction with a non- significant change in HR. However fall in both these parameters progressed with the duration of ischemia and reached statistical significance at 45 min of ischemia. Coronary artery occlusion for 45 min and subsequent reperfusion for 60 min, resulted in a marked depression in myocardial contractility and diastolic function as evidenced by a fall in (+) and (-) LVdP/dt in concert with a significant elevation of LVEDP. Similar observations have been reported by other workers [6].

Following reperfusion, a further fall in MAP and HR was observed, which was sustained till the end of the reperfusion period. Fall in MAP ideally elicits reflex sympathetic activation, which should have increased HR. However, the significant fall in HR observed in the present study may be due to:

ι) Anesthesia induced blunting of the reflex neural activity

ιι) Fall in MAP might not be biologically adequate for a reflex neural activation

ιιι) Impairment of conduction (A-V block) of the heart following ischemia and reperfusion induced injury

Reperfusion of the ischemic myocardium also caused a further significant decrease in both (+) and (-) LVdP/dt, which failed to recover over the entire period of reperfusion. Such deterioration in the hemodynamic functions during reperfusion as observed in the present study is suggestive of an injury occurring following reinstitution of blood flow into the regionally ischemic myocardium. Similar observations (popularly known as ischemia and reperfusion injury) have also been made by several investigators in different experimental models [7]. Hasan and McDonough [6], have reported that reperfusion of the ischemic myocardium resulted in a significant and prolonged depression of contractile function in a rat model. Depression of (+) LVdP/dt has also been reported in a rat model subjected to ischemia and reperfusion. Reperfusion of the ischemic myocardium was effective in lowering LVEDP, which was ultimately corrected to near baseline levels after 1 hr. of reperfusion. These observations suggest that though the impaired contractility in reperfused myocardium is dependent on the preceding ischemic insult, yet reperfusion carriers with itself an injurious component. This is in accordance with the theory of post-ischemic dysfunction or stunned myocardium.

Biochemical changes: Several recent studies have demonstrated that altered oxygen utilization and/or increased formation of reactive oxygen species (ROS) contribute to myocardial infarction and its progression. While direct evidence of ROS-induced cardiac injury during hypoxia or ischemia and reperfusion in humans is lacking (due to inadequate methodology), many studies have shown increase in biomarkers of oxidant production and/or decrease in antioxidant capacity during myocardial infarction [8]. In the present study biochemical indicators of oxidative damage, via the lipid peroxidation product, TBARS [9], endogenous antioxidant: GSH [10], antioxidant enzymes: SOD [11], CAT [12], GSHPx [13] and myocardial enzymes: CPK [14] and LDH [14] have been evaluated.

Lipid peroxidation marker: The level of TBARS, a biological marker of oxidative stress was elevated in the myocardium following ischemia and reperfusion. It is well established that a burst of oxygen free radicals (OFR) generation occurs immediately after reinstitution of blood flow to the previously ischemic myocardium. Due to reactions involving OFR and lipid component of cells, more stable lipid peroxidation components like malondialdehyde (MDA) are formed. MDA is measured in biological fluids by forming an adduct with thiobartituric acid, known as TBARS. Increase in TBARS as a marker of lipid peroxidation in conditions of myocardial ischemia and reperfusion is well documented both in clinical and experimental studies [15]. The results of the present study concur with earlier findings.

Myocardial antioxidants: In the present study, along with significant myocardial lipid peroxidation, myocardial GSH content, SOD and CAT activities were also depleted significantly following ischemia and reperfusion induced injury. However, the activity of the antioxidant enzyme, GSHPx was not significantly reduced. Superoxides are the major and the first formed OFRs and SOD is the enzyme, which dismutates superoxides to form H_2O_2 and O_2. The function of SOD has often been termed as primary defense against OFR, because this enzyme prevents further generation of free radicals. Catalase is also a major primary antioxidant enzyme that catalyses this function through the GSH system. Furthermore, cellular defense mechanisms rely on autolysis as well as inactivation of H_2O_2 by CAT to produce water and oxygen. It appears that, in the present study the major burden of neutralizing the ischemia and reperfusion induced oxidative stress, was borne out of GSH, SOD and CAT and to a lesser extent by GSHPx. This is reflected by the extent of depletion of the respective antioxidants. There are several studies, which have documented the evidence of depletion of different antioxidant compounds along with the increase of TBARS in different *in vitro* and *in vivo* models [16]. Thus, the increased TBARS production and the reduced levels of endogenous antioxidants provide strong evidence for the occurrence of oxidative stress during ischemia and reperfusion injury.

Histopathological changes: Morphologically, reperfusion after a certain period of ischemia can accelerate necrosis in irreversibly injured myocytes because of an increase in cell swelling, disruption of cell ultrastructure, formation of contraction bands, and deposition of intra-mitochondrial calcium phosphate granules. Sarcolemmal damage may also occur, leading to impairment of fluid regulation and ion flux balance [1]. The hematoxylin and eosin staining was used to study the light microscopic architecture of the myocardium and the degree of necrosis, edema and inflammation was quantified. On histopathological examination, control IR rat heart subjected to ischemia and reperfusion showed marked edema, confluent areas of myonecrosis, loss of myofiber and mild inflammation as compared to those in the sham group. Histopathological findings confirmed that ischemia and reperfusion resulted in significant myocardial damage.

Apoptotic changes: The recognition of a different cell death phenomenon, 'apoptosis', has recently become a major clinical interest. It accounts for a great proportion of cell death associated with myocardial infarction and/or myocardial ischemia and reperfusion. Cell loss through apoptosis contributes to the impairment of cardiac performance, and also plays an important role in myocardial and

vascular remodeling processes. Induction of apoptosis is implicated in cardiac dysfunction. Not only ROS *per se*, but also their oxidative products and their secondary messenger molecules generated by ROS can trigger the programmed cell death. TUNEL positivity and the immunohistochemical localization of Bax, an inducer of apoptosis and Bcl-2 proteins, inhibitors of apoptosis were studied to delineate the involvement of apoptosis in ischemia and reperfusion induced injury. The TUNEL assay identifies single strand DNA breaks with free 3'-OH terminals. Several studies have raised doubts about the specificity of TUNEL staining. Collective evidence suggests that the TUNEL assay is useful in identifying apoptosis but should be complemented by additional evidence of apoptosis, such as the up-regulation of pro- or anti-apoptotic gene products or structural criterion [17].

TUNEL positive cells were expressed as percentage of total normal nuclei. In the sham myocardium, few cells stained TUNEL positive. In contrast, TUNEL positive nuclei were significantly increased in the control IR group as compared to non-ischemic sham group demonstrating the presence of enhanced apoptotic cell death. The process of apoptosis is regulated by the Bcl-2 family of proteins, which suppress (e.g. Bcl-2, Bcl-X_L) or promote (eg. Bax, Bad) apoptosis. The ratio of the anti-apoptotic proteins and pro-apoptotic proteins is critical in determining whether the cell survives or dies. Consistent with increase in TUNEL staining in the control IR group, ischemia and reperfusion slightly reduced Bcl-2 expression and significantly increased Bax expression as compared with sham group, suggesting a role of apoptosis in contribution of myocardial injury after ischemia and reperfusion. This observation receives support from earlier studies [18].

Pilot study (Isoproterenol model) for dose selection of herbal extracts: Isoproterenol (ISP), a synthetic β-adrenergic agent, causes ischemic necrosis in rats, which closely resembles human myocardial infarction. Rona et al. first described the production of myocardial necrosis and hypertrophy in the rat heart by intermittent subcutaneous administration of ISP. The pathological features observed in ISP treated rat concurs with earlier reports and consisted of myofiber degeneration, interstitial edema, subcutaneous congestion associated with infiltration of both neutrophils and lymphocytes [19].

A number of patho-physiogenic mechanisms have been outlined to explain the experimental lesions produced by ISP via altered membrane permeability, increased turnover of nor-adrenaline, generation of cytotoxic free radicals and marked inotropic and chronotropic actions resulting in greater oxygen demand. In addition to these, the reduction of blood pressure that is observed on administration of ISP, by means of peripheral vasodilatation is also suggested to cause myocardial necrosis [19].

Various studies have shown that oxidative stress results in the reduction of the efficacy of the beta-adrenoceptor agonists probably due to reduction in cAMP formation, caused by an impaired coupling between the receptor and adenylate cyclase. The reduction in maximal beta-adrenoceptor-mediated response might be the result of cytotoxic aldehydes that are produced during the oxidative stress. This beta-adrenoceptor hyperstimulation leads to cardiotoxicity. Oxidative stress may also impair the sarcolemmal Ca^{2+} transport and result in the development of intracellular Ca^{2+} overload and ventricular dysfunction. Hence, therapeutic interventions having antioxidant activity may be useful in preventing these deleterious changes [20].

In the present investigation, the biochemical and histopathological confirmation of the cardiotoxic effect produced by ISP (85 mg/kg), has established the suitability of this model for studying the cardioprotective effect of the herbal extracts. This experimental protocol was used for the evaluation of the prophylactic and/or therapeutic intervention with the herbal extracts on the extent of the jeopardized myocardium and evolution of the irreversible tissue injury in ISP administered rats and select the optimum dose of the herbal extracts exhibiting maximum cardioprotective effects for further studies in the ischemia and reperfusion model of myocardial injury. In addition, hemodynamic, biochemical and histopathological parameters were incorporated in the study design to investigate the underlying mechanisms of their myocardial salvaging effects.

Withania somnifera

i) Dose selection studies with *Withania somnifera*: *Withania somnifera* (Ws) most commonly known as Ashwagandha, belongs to the natural order Solanaceae. The roots of Ws have been extensively employed as a valuable drug in Ayurveda, the ancient Indian system of medicine. Although its therapeutic potential on account of its immunomodulatory, adaptogenic, antioxidant, hypoglycemic and anticancer activities are reported [21], very few studies assessing its cardioprotective potential are presently available [22]. In the present study 25, 50 & 100 mg/kg doses of Ws were screened in the murine model of ISP-induced myocardial necrosis and the optimum dose exhibiting maximum cardioprotective effect was evaluated.

Myocardial injury was evident by leakage of myocardial CPK and a significant rise in TBARS. Superoxide radical generation and hydrogen peroxide formation after ISP administration may be the reason for lipid peroxidation in cell membrane. The metabolism of ISP produces quinones, which react with oxygen to produce superoxide anions and hydrogen peroxide leading to oxidative stress and depletion of the antioxidant system. The present data are in concurrence with the concept that the generation of highly cytotoxic free radicals through the auto-oxidation of catecholamines is one of the important causative factors for ISP induced myocardial necrosis. In the present study, ISP injection resulted in reduced GSH content as well as lowered activities of antioxidant enzymes; SOD, CAT and GSHPx in the cardiac tissue. These findings corroborate earlier reports [23]. In the present study, the fall in the activity of GSHPx in the ISP group might be correlated to the decreased availability of its substrate; that is, reduced GSH. Moreover, due to impairment in both enzymatic and non-enzymatic antioxidant defense mechanism, it is quite likely that the free radicals are not effectively neutralized and hence myocardium shows enhanced susceptibility to lipid peroxidation. The observation that Vitamin E (100 mg/kg) and Ws (50 & 100 mg/kg) treatment significantly restored LDH and CPK activity compared to ISP control group evidences their cardioprotective effect. Furthermore, both these drugs treatment restored the myocardial antioxidant status and maintained membrane integrity as evidenced by a decline in TBARS levels. Furthermore, histopathological examination confirmed the cardioprotective effects of Ws (50 & 100 mg/kg) and Vit E.

Various studies have shown that Ws possesses characteristic ginseng-like adaptogenic properties, which enhance myocardial tolerance subsequent to stress, a phenomenon known as 'adaptation' [24]. Although the exact mechanism of such an adaptation is not fully understood, but it may work through the induction of antioxidant enzymes such as SOD, CAT, GSHPx and antioxidants such as GSH and proteins like heat shock protein (HSP). In the present study, there was a concomitant increase in CAT and GSHPx along with SOD activity in the Ws (50 & 100 mg/kg) per se treated control groups. Vit E, however did not exhibit any such adaptogenic property as no significant increase

in the levels of the endogenous antioxidants was observed in the Vit E treat group.

Measurement of the hemodynamic variables was also incorporated into the experimental design for better understanding and more precise information of the co-relation between biochemical and functional changes in the myocardium subjected to ISP induced damage. Previous studies have reported that exposure of the hearts to an oxidative stress depresses the ventricular functions and these changes are significantly prevented by antioxidants [24,25]. The results of the present study are consistent with these observations. In the ISP control group, myocardial dysfunction was clearly evident by a significant fall in MAP, HR, (+) & (-) LVdP/dt and a rise in LVEDP, which might be due to ISP induced myocardial necrosis. The (-) LVdP/dt was more markedly depressed indicating an increased diastolic dysfunction *per se* which may result in the persistence of the increase in LVEDP. Although Ws (25, 50 & 100 mg/kg) and Vit E (100 mg/kg) treatment did not significantly increase MAP, an increase in heart rate was observed as compared to ISP control group. Moreover, both the drugs appeared to preserve left ventricular function as evidenced by significant restoration of (+) and (-) LVdP/dt and correction of elevated LVEDP. Hence, it is suggested that preservation of cardiac reflexes resulting in improved ventricular dynamics may be on account of the myocardial protection exerted by Ws (50 & 100 mg/kg) and Vit E.

In summary, the present study strongly suggests that multiple mechanisms may be responsible for the cardioprotective effect of Ws. It produced myocardial adaptive changes (augmentation of endogenous antioxidants) on chronic administration to healthy experimental animals (that is animals without any myocardial pathologic challenge). In addition, it restored the antioxidant status of the myocardium, subsequent to ISP induced oxidative stress. These beneficial effects also translated into functional recovery of the myocardium as evidenced by favorable modulation of hemodynamic variables. Histopathological assessment further confirmed the protective effect of Ws on the myocardium. Taken together, the results of the present study demonstrate that although Ws (100 mg/kg) displayed modest protective effects, maximum cardioprotective effects were observed by pre- and co-treatment with Ws at the dose of 50 mg/kg. Ws (50 mg/kg) dose was found to be the most effective in functional recovery of the heart and favorable restoration of biochemical and histopathological alterations. Hence, Ws (50 mg/kg) dose was selected for further evaluation in the ischemia and reperfusion model of MI [26].

ii) Myocardial Consequences of Intervention with *Withania somnifera* during ischemia and reperfusion: It is well established that myocardial injury leads to loss of structural integrity and increased permeability. As described earlier, a good correlation between the histopathological evidence of myocardial necrosis and the enzymatic activity of enzyme CPK elucidates that the degree of CPK leakage from myocardium corresponds well with the myocardial injury [27]. In the present study, Ws (50 mg/kg) treatment significantly prevented leakage of myocardial enzyme CPK and preserved the myofiber architecture as compared to the IR control group.

As discussed earlier, a concept is now emerging of 'adaptogenic drugs', first time reported by Brekhman and associates in *Eleuthrococcus* and *Panax ginseng*, these are agents that increase non-specific resistance of the users to a variety of stresses. The definition of an adaptogen according to Brekhman (1969) is based on the following

i. Safety of the adaptogen's action on the organism

ii. A wide range of regulatory activity, but manifesting its action only against the actual challenge to the system.

iii. Act through a nonspecific mechanism to increase the non-specific resistance (NSR) to harmful influences of an extremely wide spectrum of physical, chemical and biological factors causing stress and has a normalizing action irrespective of the direction of forgoing pathological changes [28].

Adaptogenic property of various herbs like *Ocimum sanctum*, *Bacopa monniera* and *Withania somnifera* has already been reported in various experimental studies (Bhattacharya et al., Rege et al., Gupta et al.). These herbs allow one to adapt to a variety of heightened stressful circumstances. Although the exact mechanism of such adaptation is presently not known, it has been proposed that these drugs may act by inducing a number of antioxidant enzymes such as SOD, CAT, GSHPx and antioxidants such as GSH, proteins like heat shock protein (HSP) in the heart [25]. The present study demonstrated the adaptogenic property of Ws. Chronic oral administration of Ws per se to experimental animals; resulted in a significant increase in myocardial GSHPx, CAT along with SOD activity as compared to sham control group. Increase in antioxidant levels following chronic Ws treatment might considerably improve the myocardium's defense against oxidative stress and account for the cardioprotective effect of Ws. Any increase in SOD activity is beneficial in the event of increased free radical generation. However, it has been reported that an augmented SOD activity, without a concomitant rise in the activity of CAT and/or GSHPx might be detrimental, since S8D activity, generated hydrogen peroxide as a metabolite, which is more cytotoxic than oxygen radicals and must be scavenged by CAT or GSHPx. A simultaneous increase in CAT and/or GSHPx activity is essential for an overall beneficial effect of an increased SOD activity [16]. Thus, simultaneous increase in myocardial SOD, GSHPx and CAT activities observed in the present study with chronic administration of Ws underscores the distinct importance of enhanced beneficial effects of this herbal extract.

In addition, subsequent to ischemia and reperfusion induced myocardial injury, Ws treatment demonstrated significant antioxidant activity. It decreased the level of TBARS compared to control IR group, which could be imparted due to reduced formation of TBARS from fatty acids. Furthermore, protection against ischemia reperfusion induced oxidative stress in Ws treated rat hearts was evidenced by preservation of endogenous antioxidants enzyme SOD and GSHPx.

Monitoring of key hemodynamic variables was incorporated into the experimental design to demonstrate the close relationship between functional and biochemical changes in the myocardium subjected to ischemia and reperfusion induced injury. Exposure of the heart to an oxidative stress has been shown to depress left ventricular function and lower blood pressure and the use of antioxidants has been shown to reverse these hemodynamic alterations [19].

However, in the present study, Ws treatment appeared to preserve left ventricular function as evidenced by significant improvement of the inotropic and lusitropic state viz, (+) LVdP/dt and (-) LVdP/dt and correction of elevated LVEDP at various time points during the entire experimental period. However, these drugs at the doses used did not have significant effect on MAP and HR during the ischemic period. Cardioprotection afforded by Ws cannot be attributed to a reduction of myocardial oxygen demand as this drug did not significantly influence HR and MAP [29].

In addition, in the present study, Ws demonstrated significant anti-apoptotic property. In association with a reduction in the percentage

TUNEL positive cells in the ischemic myocardium, Ws treatment upregulated the expression of anti-apoptotic protein, Bcl-2 and down regulated the expression of pro-apoptotic protein, Bax. These effects may significantly reduce the marked apoptotic cell death in the myocardium of the control IR group. The anti-apoptotic property of Ws has been reported earlier in a rat model of stroke [30].

On the basis of the obtained hemodynamic, biochemical and histopathological data, it was concluded that Ws is a highly effective cardioprotective agent. The favorable modulation of ventricular function, significant antioxidant and anti-apoptotic properties may contribute to the beneficial effects of Ws [31]. The study provides scientific rationale for the use of Ws in Ayurveda, the ancient Indian system of medicine known as Maharasayana [24].

Curcuma longa

i) Dose selection studies with *Curcuma longa*: The cardioprotective effects of 25, 50, 100 and 200 mg/kg *Curcuma longa* (Cl), a perennial herb used in Ayurveda, the Indian System of Medicine, as a general health tonic and healing agent, were studied in the ISP model of MI. Cl (Turmeric), common Indian dietary pigment and spice has been shown to possess a wide range of therapeutic utilities in the traditional Indian medicine. It's role in wound healing, urinary tract infections, liver ailments are well-documented [32]. The active component of turmeric identified as curcumin exhibits a variety of pharmacological effects including antioxidant, adaptogenic, anti-inflammatory and anti-infectious activities [33]. However, only few studies are presently available that documents its cardioprotective potential.

In the present study, chronic oral administration of Cl *per se* to healthy experimental animals resulted in a significant increase in CAT activity with Cl-50, 100 and 200 mg/kg doses and inhibition of lipid peroxidation with Cl (50 & 100 mg/kg) doses. This adaptogenic property may contribute to its cardioprotective effect and strengthen the antioxidant defense mechanisms of the heart. However, none of the doses of Cl evaluated in this study resulted in significant augmentation of myocardial GSH content and SOD and GSHPx activity. Furthermore, the depressed myocardial antioxidant enzyme levels subsequent to ISP induced myocardial necrosis, were significantly restored by Cl treatment. However Cl failed to significantly prevent the loss of GSH subsequent to ISP challenge. In the present study, enhanced lipid peroxidation, as indicated by elevated myocardial levels of TBARS was observed in the ISP control group. However, Cl treatment significantly decreased the myocardial level of TBARS; this may be due to inhibitory action on the formation of lipid peroxides from fatty acids. It has been reported that curcumin, the active constituent of Cl inhibits the metabolism of arachidonic acid via the cyclo-oxygenase and lipo-oxygenase pathways that generates reactive oxygen species; resulting in a decrease in the levels of lipid peroxides [34]. Further, Cl (50, 100 & 200 mg/kg) treatment restored the activity of the marker enzyme CPK, demonstrating the biochemical basis of protective action of Cl against ISP induced myocardial injury.

Among the different doses of Cl studied, only 100 mg/kg dose of Cl appeared to preserve left ventricular function as evidenced by significant restoration of (+) LVdP/dt and (-) LVdP/dt and correction of elevated LVEDP subsequent to ISP challenge. Increase in (+) LVdP/dt and (-) LVdP/dt reflects an overall enhancement of myocardial contractility and relaxation respectively. Another consequence of reduced LVEDP is the increase in blood flow through the sub-endocardial region of the ventricular muscle that bears the maximum brunt of the ischemic insult. Under ischemic conditions, there is a disproportionate reduction in blood flow to the subendocardial regions of the heart, which is subjected to the greatest extra-vascular compression during systole [35]. Cl (100 & 200 mg/kg) may indirectly tend to restore blood flow in these regions towards normal by correcting the elevated LVEDP. Correction of the altered hemodynamic variables observed with Cl treatment may be due to myocardial salvage exerted by the agent.

Thus, the present study demonstrates a significant protective effect of Cl in the ISP model of myocardial necrosis. It may be concluded that the cardioprotective effect of Cl is the result of the combination of its antioxidant and adaptogenic properties as well as its favorable modulation of the hemodynamic variables. Histopathologic evaluation further confirmed the protective effects of Cl on the myocardium.

Cl (100 & 200 mg/kg) doses significantly reversed myonecrosis via augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant correction of the altered hemodynamic variables. Among the various doses evaluated in the present study, Cl at 100 mg/kg exhibited maximum cardioprotective activity [37].

ii) Myocardial consequences of intervention with *Curcuma longa* during ischemia and reperfusion: Augmentation of endogenous antioxidants is an adaptive mechanism against oxidative stress. As oxidative stress plays a significant etiopathological role in ischemic heart disease, such adaptive changes are supposed to be beneficial in combating ischemia-induced oxidative stress and its consequences. In the present study, chronic administration of Cl per se to healthy experimental animals (i.e without any experimentally induced ischemic insult), resulted in a significant increase in myocardial CAT activity along with a significant reduction in TBARS levels. It is proposed that this adaptogenic property may contribute to its antioxidant effect and its ability to protect the myocardium against the severity of ischemic damage.

Cl treatment significantly prevented the rise in TBARS as compared to control IR group suggesting that the antioxidant effect of Cl is responsible for lower TBARS generation in this group. However, Cl treatment showed modest corrective effects on the myocardial endogenous antioxidants enzyme: SOD, CAT and GSHPx. Nonetheless, myocardial GSH levels were significantly preserved by Cl, which might be responsible for lower TBARS generation in this group. This suggests that the direct antioxidant effect of Cl rather than a better antioxidant milieu was responsible for its cardioprotective effects.

The observation that Cl (100 mg/kg) treatment significantly restores the activity of the marker enzyme CPK as compared to control IR group evidences the protective effect of Cl on the myocardium. Histopathological evaluation confirmed its myocardial salvaging effects.

There are several reports supporting or refuting a direct antioxidant property of Cl [34]. However, till date there are no in vivo studies, which have explored the relationship between the putative antioxidant effects of Cl on the functional recovery of ischemic-reperfused myocardium. In the present study chronic (30 days) oral administration of Cl prevented the ischemia and reperfusion associated deterioration in the hemodynamic functions. Cl treatment resulted in preserved left ventricular function as reflected by a significant increase in the indices of contractility (+) LVdP/dt, relaxation (-) LVdP/dt and decrease in preload (LVEDP). It is speculated that, Cl treatment may have indirectly restored blood flow in the ischemic regions towards normal as assessed by its efficacy in improving cardiac performance, especially correcting

the ischemia and reperfusion-induced increase in LVEDP. However, Cl did not significantly affect MAP and HR.

The present investigation indicates that Cl has significant cardioprotective activity as shown by its mitigating effects on several myocardial injury induced biochemical, hemodynamic and histopathologic perturbations. In addition, Cl administration significantly reduced the percent of TUNEL positive cells vs. the control IR group; demonstrating its significant anti-apoptotic activity. Treatment with Cl was associated with greater Bcl-2 and attenuated Bax expression as compared to the control IR group. Curcumin, the active ingredient of the rhizome of the turmeric plant (*Curcuma longa*), a commonly used spice, has been reported to prevent cancer in animal tumor models possibly by its apoptosis-inducing and [37] antiproliferative influences. However, in the present study, in contrast to earlier reports, marked anti-apoptotic activity of Cl was observed [38].

The anti-apoptotic effect of Cl, improved ventricular functions, restored endogenous antioxidant network along with improved histologic features suggests that treatment with this agent may exert cardioprotective effects following coronary ligation and reperfusion.

The data from the present study confirm the observations of earlier reports with respect to the antioxidant effects of Cl. The antioxidant activity of Cl is probably mediated through a mixture of curcuminoids such as curcumin, demothoxycurcumin, bis-demothoxycurcumin, and the active ingredients of the Cl rhizome. Curcumin is reported to inhibit nitrite radical induced oxidation of hemoglobin, prostaglandin biosynthesis, scavenge free radicals, inhibit lipid peroxidation, protect SH group of GSH and activate glutathione-s-transferase [34].

Ocimum sanctum

i) Dose selection studies on *Ocimum sanctum*: *Ocimum sanctum* (Os), commonly known, as Tulsi in India is a local herb containing potent antioxidants flavanoids (orientin, vicenin) and phenolic compounds (eugenol, cirsilineol, apigenin). The ancient systems of medicine including Ayurveda, Greek, Roman, Siddha and Unani, have mentioned its therapeutic applications in cardiovascular disorders, diabetes and asthma [39-41]. However, its potential as a cardioprotective agent has not been extensively studied. The cardioprotective potentials of Os at the doses of 25, 75 and 150 mg/kg were studied in the present investigation.

Augmentation of endogenous antioxidants may enhance the myocardial antioxidant reserve and strengthen the defense mechanisms operating in the myocardium. In the present study, chronic oral treatment with Os per se to healthy experimental animals resulted in a significant increase in the activities of GSHPx and SOD as compared to sham control group. This myocardial adaptation seems to be one of the likely mechanisms contributing to its cardioprotective effects. Furthermore, chronic oral treatment with Os significantly preserved the activity of the antioxidant enzymes SOD and CAT and exerted modest preservation of myocardial GSH content and GSHPx activity in the ISP-challenged group. Enhanced lipid peroxidation, as indicated by elevated TBARS level was observed in the ISP control group and Os treatment significantly decreased its levels by preventing the formation of lipid peroxides from fatty acids.

Administration of Os (75 and 150 mg/kg) significantly increased HR as compared to ISP control. Os at all the doses studied, significantly reduced the surrogate preload marker LVEDP as compared to ISP control. However, it failed to significantly improve both inotropic and lusitropic functions of the heart.

In summary, the present study demonstrated that Os (75 mg/kg) significantly reduced ISP induced myocardial injury. Histopathologic examination further confirmed its cardioprotective effects. Most importantly, the study demonstrated that chronic oral treatment with Os augments the endogenous antioxidant status of the heart (myocardial adaptation), decreased myocardial necrosis, edema and inflammation and improved cardiac function. Decreased myocardial necrosis as evidenced by reduced CPK release, improved histologic picture, favorable hemodynamic and antioxidant effects; all contribute to its cardioprotective potential. In this context, it is critical to mention that Os at 25 and 150 mg/kg doses failed to demonstrate any significant myocardial salvaging effects [42].

ii) Myocardial consequences of intervention with *Ocimum sanctum* in ischemia and reperfusion: In the present study, oral administration of Os per se extract for a month resulted in a significant increase in the activities of CAT and SOD as compared to the baseline values of these biochemical parameters in the sham operated group. This is suggestive of the cardiac adaptogenic property of Os.

Chronic oral treatment with Os for one month significantly prevented lipid peroxidation and the loss of GSH content during myocardial ischemia and reperfusion; however, it exerted modest effects on myocardial antioxidant enzyme activity, which was comparable to that observed in the control IR group. Furthermore, it appears that the major burden of neutralizing the ischemia and reperfusion induced oxidative stress was borne by GSH and not by the antioxidant enzymes. Os treatment significantly prevented formation of lipid peroxides from fatty acids evidenced by reduced level of myocardial TBARS as compared to the control IR group.

Os markedly increased MAP, HR and significantly reduced the surrogate preload marker LVEDP as compared to control IR. However, it failed to significantly improve the left ventricular contractility and relaxation.

Although Os treatment demonstrated modest antioxidant effects and modified some of the hemodynamic changes, it failed to significantly prevent leakage of myocardial CPK and modulate the histopathologic alterations compared to control IR group. Moreover Os treatment did not demonstrate any significant anti-apoptotic activity as determined by TUNEL staining and immunohistochemical results. No significant change in the expression of Bax and Bcl-2 proteins was observed with Os treatment as compared to control IR in the present study [38].

In conclusion, Os treatment exerted modest cardioprotective effects; viz decreased preload and enhanced antioxidant status. Nonetheless, it failed to preserve the myocardial cellular integrity as evidenced by histopathologic evaluation and decreased myocardial CPK activity.

iii) The science of herb combining and processing: Although single Ayurvedic herbs and spices such as Brahmi, Turmeric and Ashwagandha are popular, one of the most significant contributions offered by Ayurveda is the science of herbal combination -- formulations that personify 'sanyoga', the fortuitous blending of a variety of herbs that results in a formulation offering the dual benefits of synergy and balance. An Ayurvedic formulation can often contain one or more herbs and spices -- primary herbs that target the area of imbalance, supporting herbs to enhance the benefits of the primary herbs, balancing herbs to counter any possible side-effects from the actions of the main herbs,

and bio-availability enhancers to expedite the transfer of the benefits of the formulation to the parts of the physiology. The most complex of the traditional Ayurvedic herbal combinations are an elite group called rasayanas, extolled at length in the Ayurvedic texts for their positive impact on the physiology [43].

The second principle, 'sanskar', refers to the way the herbs are harvested, used and processed. Ayurvedic formulations traditionally use the whole herb instead of extracting the active ingredient from the plant. Nature's healing wisdom is perceived to reside best in the plant in its entirety. Using the whole herb rather than the isolated ingredient also contributes to a balanced formula less likely to have side-effects, because according to Ayurveda, each medicinal plant has both the primary effect and the antidote present in it in its natural state.

iv) Cumulative benefits: The Ayurvedic approach to health is gentle and comprehensive. The concepts of instant cures and pill-popping for immediate relief are foreign to Ayurveda. Because the endeavor is to seek and correct the source of problems -- imbalances in the physiology -- the best results from Ayurveda come to those who are patient and persistent, who diligently adopt the associated dietary and lifestyle changes needed, and take a degree of responsibility for their own well-being. For those who do make this commitment, Ayurveda offers rich, cumulative health benefits that can help you enjoy a long, healthy and blissful life [44].

Myocardial consequences of intervention with the herbal combination (HCB): *Withania somnifera* (50 mg/kg) + *Curcuma longa* (100 mg/kg) + *Ocimum sanctum* (75 mg/kg) vs. lisinopril and Vitamin E during ischemia and reperfusion : The HCB was effective enough to significantly ameliorate myocardial ischemic injury following LAD coronary occlusion and reperfusion when compared to control IR group. A significant finding of the present study is that the cardioprotection extended by the herbal combination was superior compared to that extended by the herbal extracts administered alone.

HCB did not significantly affect MAP and HR during the ischemic period; however during the latter half of the reperfusion period, HCB and Lsp, significantly restored MAP and HR. However, the modest beneficial hemodynamic effects on HR and MAP exerted by HCB do not explain the marked cardioprotection observed during ischemic and reperfusion injury. In contrast, Vit E did not exhibit any significant effect on these parameters.

In the present study, HCB exerted beneficial effects on left ventricular dynamics as evidenced by (a) the correction of the ischemia-reperfusion induced enhanced level of LVEDP and (b) by significant improvement in myocardial contractility and relaxation. It appears that the herbal extracts are more effective when administered together in preventing the hemodynamic deteriorations observed in the control IR group. However, Lsp treatment demonstrated superior recovery in left ventricular function as compared to HCB and Vit E. In the Vit E treated group, measurement of left ventricular diastolic function and systolic function revealed correction of LVEDP and partial prevention of the diastolic dysfunction; that is an increased (-) LVdP/dt. However, a parallel protective effect on the contractile status of the heart (+) LVdP/dt was not observed.

It is well known that one of the major causes of myocardial infarction is an imbalance between oxidants and antioxidant defenses. Hence, it is possible to prevent or ameliorate disease progression by favoring the balance towards lower oxidative stress. Potential antioxidant therapy should, therefore, include exogenous supplementation of natural antioxidants that affect augmentation of endogenous antioxidants [45].

In the present study, chronic treatment with HCB augmented basal endogenous antioxidants and inhibited the increase in TBARS levels i.e enhanced the antioxidant reserve, favorably modulating the antioxidant defense mechanisms of the myocardium in the healthy experimental animals. However, chronic treatment (30 days) with Lsp and Vit E *per se* did not show any marked effect on the baseline oxidant-antioxidant parameters. However, a key question, which remains unanswered in the present study, is the mechanism by which HCB augments basal endogenous antioxidants. Although the precise mechanisms of such an effect are not clear from the present protocol, several factors might be playing contributing roles. In this regard it has been reported earlier that both Ws and Os possess adaptogenic properties; hence, it is speculated that they may contribute to the myocardial adaptogenic activity observed in the HCB control group. Subsequent to ischemia and reperfusion induced oxidative stress it was observed that the HCB, Lsp and Vit E group demonstrated significant antioxidant property, which might contribute to the observed cardioprotective effect of these interventions.

In addition, in the present study, HCB and Lsp demonstrated significant anti-apoptotic potential as it upregulated the expression of anti-apoptotic protein, Bcl-2 and downregulated the expression of pro-apoptotic protein, Bax; in association with a reduction in the percentage of TUNEL positive cells in the ischemic -reperfused myocardium. The exact mechanism by which the herbal combination may reduce myocardial ischemia and reperfusion induced myocardial apoptosis is far from clear presently; and may not be answered fully by the present study. However it can be speculated that it may attenuate apoptosis via a number of mechanisms: Upregulation of Bcl-2 may result in formation of heterodimers with Bax, resulting in no/fewer free Bax protein available for homodimerization. If Bax homodimers predominate cell death will occur, but when Bcl-2 and Bax heterodimererization prevails cells can survive. Substantial evidence indicates that the mitochondria play a critical regulatory role in the signal transduction pathway leading to apoptosis. HCB may attenuate mitochondrial injury resulting from ischemia and reperfusion and preserve mitochondrial function. By this mechanism, it may prevent the formation of the permeability transition pore in the mitochondrial membrane, inhibit the release of pro-apoptotic molecules such as apaf-1 and apaf-2 (cytochrome c) from the mitochondria, and reduce myocardial apoptosis. HCB may also attenuate myocardial apoptosis through prevention of the dephosphorylation of Bad, a pro-apoptotic protein of Bcl-2 family, by calceneurin (a calcium/calmodulin dependent protein serine/thereonine phosphate). Preventing the activation of calcineurin keeps Bad in its phosphorlylated state and inhibits its translocation to the mitochondrial surface, preventing subsequent cytochrome c release. Moreover, free radicals have been demonstrated to directly activate calcium and magnesium dependent endonuclease (DNase I), thus resulting in DNA fragmentation and cell apoptosis [46]. The herbal combination treatment through its antioxidant mechanism may prevent this DNAase activation and reduce myocardial apoptosis. In contrast, Vit E did not exhibit any significant anti-apoptotic effect.

The protective effects of the HCB, Lsp and Vit E were supported by histopathologic examination and in concert with preserved myocardial CPK content. However, the cardioprotection afforded by Lsp was found to be superior as compared to that exerted by HCB treatment.

Finally, the potential of HCB as a novel therapeutic strategy for cardioprotection during ischemia and reperfusion injury has been well elucidated by the dosing protocol of the present study. The data of the current study evidence that the beneficial effects of chronic

administration of the combination of the herbs under investigation were superior to those exerted by the respective agents administered singularly. Although the precise mechanism of the cardioprotective effects of HCB in this model of ischemia and reperfusion induced myocardial injury is not fully understood, however some of the likely mechanisms of its myocardial salvaging effects are being proposed on the basis of the obtained data: To summarize, administration of the HCB significantly augmented the levels of endogenous antioxidants in the healthy HCB control group (treated with HCB per se without any pathological challenge). Furthermore, HCB reduced the surrogate preload marker LVEDP as compared to control IR. The major consequence of the reduction in LVEDP is to increase blood flow through the sub-endocardial region of the left ventricular muscle that bears the maximum brunt of the ischemic insult. Under ischemic conditions, there is a disproportionate reduction in microcirculation and blood flow to the subendocardial regions of the heart, which is subjected to the greatest extra-vascular compression during systole. In addition, HCB significantly improved both inotropic and lusitropic function of the heart and significantly restored the antioxidant defense capacity of the myocardium subjected to ischemic and reperfusion injury. This effect maybe due to the free radical scavenging properties of the herbs under investigation. Most importantly, treatment with HCB demonstrated significant anti-apoptotic effects. Loss of contractile cells in the heart poses an additional workload on the remaining viable myocytes that may be unbearable, resulting in pathologic stimuli and death signals. In the present study, HCB treatment may have salvaged these myocytes and prevented cell loss induced by apoptosis. Preserved myocardial CPK activity and histopathologic evaluation further confirm the cardioprotective potential of such a combination [47].

Conclusions

Chronic oral pretreatment with *Withania somnifera*, *Curcuma longa* and the herbal combination (HCB) including *Withania somnifera* (50 mg/kg) + *Curcuma longa* (100 mg/kg) + *Ocimum sanctum* (75 mg/kg) exerted significant cardioprotective effects in the experimental models of myocardial injury. The most remarkable observation of the present study is that cardioprotective effect exerted by HCB treatment was found to be superior to that shown by singular treatment with individual herbal extracts. The combination of herbal extracts was found to significantly ameliorate the ischemia and reperfusion cardiomyocyte apoptosis, cardiac dysfunction, compromised antioxidant status and histopathologic alterations as compared to control IR group. Cardioprotection by HCB treatment may be attributed to its favorable hemodynamic effects, myocardial adaptogenic properties, and significant antioxidant and antiapoptotic properties. Furthermore, HCB decreased the severity of pathological changes and significantly preserved the myocardial CPK confirming its myocardial salvaging effects.

The present study provides scientific rationale of the employment of herbs/herbal extracts for cardioprotection, as described in Ayurveda, the ancient Indian system of medicine. These herbal extracts have the potential for the management of patients at risk of myocardial infarction. In view of the safety, efficacy and traditional acceptability of these agents, well-controlled prospective clinical trials should be contemplated to establish their efficacy in the treatment of ischemic heart disease.

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