PROTECTIVE EFFECT OF *Vitis vinifera* AGAINST MYOCARDIAL ISCHEMIA INDUCED BY ISOPROTERENOL IN RATS

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Summary

Sustained high levels of circulating catecholamines may induce cardiotoxicity through oxidative mechanisms. Isoproterenol is a synthetic catecholamine with increasing attention owing to this application in cardiology. The aim of the present study was to investigate the cardioprotective effects of *Vitis vinifera* seed against isoproterenol-induced myocardial ischemia. Normal Wistar strain rats were pretreated with *Vitis vinifera* seed (500mg/kg body weight) for 28 days and the intoxicated with isoproterenol (ISO) (20mg/100g, i.p. for 2 consecutive days). The activities of cardiac marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) were analyzed in heart and plasma. Cardiac protein as troponin T was estimated in serum, the levels of lipid peroxide products (thiobarbituric acid reactive substances) (TBARS), reduced glutathione (GSH) were analyzed in heart and plasma. In ISO-treated group, shrinkage of cardiac markers in plasma and elevated lipid peroxidation where accompanied by decreased content of reduced glutathione in heart and plasma. The prior administration of *Vitis vinifera* significantly prevented the isoproterenol-induced alterations and restored the cardiac markers. These findings indicate the cardioprotective activities of *Vitis vinifera* during isoproterenol-induced myocardial ischemia.

Key words: *Vitis vinifera;* Cardiac markers; Lipid peroxidation; Isoproterenol*:*

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Introduction

Myocardial infarction, the most spectacular and lethal manifestation of cardiovascular disease, is the result of long process of subtle deterioration of the circulatory system and it is the common cause of mortality in all industrialized nations (1). Catecholamines are important regulators of myocardial contractility and metabolism. However, it has been known for a long time that excess catecholamines are responsible for cellular damage, observed in clinical Conditions such as transient myocardial ischemia, angina, acute Coronary insufficiency, and subendocardial infarct (2). Administration of large amount of catecholamines, particularly isoproterenol to experimental animals constitutes a rapid and reproducible means of provoking myocardial ischemia (3). Isoproterenol is reported to induce pathophysiological changes, resulting in infarct like necrosis, comparable to that of human myocardial infarction (4). Till today, it remains a clinical challenge and a problem of great importance. There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the adequate management of cardiovascular diseases.

The World Health organization (WHO) estimates that 80% of the people of developing countries rely on traditional medicines, mostly plant derived drugs, for their primary health needs. Medicinal plants are commonly used in treating and preventing specific ailments and are considered to play a significant role in health care. Traditional medicinal systems use plants as indispensable sources of medicinal preparations. Hundreds of species are recognized as having medicinal value. Indeed, 'Phytomedicines' are beginning to link traditional and modern medicines (5). The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed (6). Recently, pharmacological functions of *Vitis vinifera* seed an active phytochemical proanthocyanidins and natural polyphenolic antioxidant present in seeds of *Vitis vinifera.* These proanthocyanidins have demonstrated a marked spectrum of biological, pharmacological, therapeutic, and chemoprotective properties against oxygen free radicals and oxidative stress (7-9). In the present study, an attempt has been made to assess the protective effects of *Vitis vinifera* seed on cardiac function in isoproterenol-induced myocardial infarction in rats.

Methods

Chemicals

Isoproterenol hydrochloride, thiobarbituric acid, 2,4-Dinitro phenyl hydrazine, and glutathione were purchased from sigma chemical, Mumbai. All other reagents and chemicals used in this study were of analytical grade with high purity.

Animals

Wistar strain male albino rats, weighing 100-120 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28±2ºC, humidity 60-70%, 12 h light/dark cycle).

The animals were allowed a standard feed and water *ad libitum.* They were acclimatized to the environment for 1 week prior to experimental use. The study protocol was carried out as per the rules and regulation of the institutional animal's ethics committee (IAEC).

Preparation of plant extract

The seeds of *Vitis vinifera* were collected from local at Thanjavur, Tamil Nadu, South India. The seed powder was dried and soaked with ethanol (70%) for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The *Vitis vinifera* seed powder extract was dissolved in distilled water just before oral administration.

Induction of myocardial infarction

The myocardial infarction was induced in experimental rats by intraperitoneal (i.p) injection of isoproterenol hydrochloride (ISO, 20mg/ 100 g body weight, dissolved in physiological saline, for two consecutive days (10).

Experimental protocol

The rats were randomly divided onto three groups with six rats each.

Group I: Normal animals received with standard fed and water to allow *ad libitum* throughout the experimental period.

Group II: Rats were orally fed 0.9% normal saline once daily for 28 days and in addition received isoproterenol (20mg /100g b.wt.) on the 29^{th} and 30^{th} day at an interval of 24 hrs.

Group III: Rats were pretreated with *Vitis vinifera* seed (500mg/kg b.wt) for a period of 28 days and in addition received Isoproterenol (20mg/100g b. wt) on the 29^{th} and 30^{th} day at an interval of 24 hrs.

On completion of the experimental period, animals were anaesthetized with Thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as anticoagulant. Plasma and serum were separated by centrifugation. Heart was excised immediately and immersed in physiological saline. Tissue homogenate, plasma and serum were used for the analysis of various biochemical parameters.

Biochemical analysis

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel (11). Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities were determined by the method of King (12) and Okinaka *et al*. (13), respectively. The level of thiobarbutaric acid reactive substances (TNARS) and reduced glutathione (GSH) were estimated by the method of Beuge and Aust (14) and Moron *et al.* (15). Troponin- T was estimated by the method of Bhaskar and Rao (16). The protein content was estimated by the method of Lowry's *et al.* (17)

Statistical analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons (18). Statistical analysis carried out by Ms-Windows based graph pad Instat software (Graph Pad Software, San Diego, CA, USA) 3 version was used and $p< 0.001$ was considered to be significant.

Results

Intraperitoneal administration of isoproterenol caused a significant ($p<0.001$) increase in lipid peroxidation in plasma and heart tissue of group III rats as compared with that of group I rats (Table 1). This was paralleled by significant reduction in the level of reduced glutathione in the heart tissue and plasma as compared with that normal control rats (Table 1.) In-group III rats, the prior administration of *Vitis vinifera* significantly prevented the isoproterenol-induced lipid peroxidation in plasma and heart tissue and maintained the level of reduced glutathione at near normalcy in group I rats. Significant ($p<0.001$) rise observed in the levels of diagnostic marker enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), creatine phosphokinase (CPK)) and Troponin-T in plasma (Table 2) and decreased activities of ALT, AST, LDH and CPK in heart (Table 3) of group III myocardial infarction induced rats as compared to group I control animals. The pretreatment with *Vitis vinifera* significantly reduced the release of these diagnostic marker enzymes and the level of Troponin T into the systemic circulation as compared with group III rats.

Discussion

Myocardial ischaemia results from the reduction of coronary flow to such an extent that supply of oxygen to the myocardium does not meet the oxygen demand of myocardial tissue. When this ischaemia is prolonged and irreversible then myocardial cell death and necrosis occurs which is defined as myocardial infarction (MI) (19). Recent studies suggest that increased free radical formation and subsequent oxidative stress associated with occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanism for the development of heart failure after myocardial infarction (20).

Isoproterenol (ISO), a synthetic catecholamine, has cardio toxic effects on the myocardium. The effects of ISO on heart are mediated through β1- and β2-adrenoceptors. Both β1- and β2- adrenoceptors mediate the positive inotropic and chronotropic effects to β adrenoceptor agonists (21). Thus ISO produces relative ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension (22), and induces myocardial ischemia due to cytosolic Ca^{2+} overload (20). Additionally, ISO causes myocardial ischemia due to excessive production of free radicals resulting from oxidative metabolism of catecholamines (23, 24). Excessive formation of free radicals may result in the loss of function and integrity of myocardial membranes.

The study of lipid peroxidation is attracting much attention in recent years due to its role in diseases process membrane lipids are particularly susceptible to lipid peroxidation due to the presence of polyunsaturated fatty acids. It has been implicated in the pathogenesis of a number of diseases and clinical conditions. These include atherosclerosis, cancer etc., Experimental and clinical evidence suggests that aldehyde products of lipid peroxidation can also act as bioactive molecule in physiological and pathological conditions. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity (25).

Table 1. Level of lipid peroxide (LPO) and reduced glutathione (GSH) in plasma and heart tissue of normal and experimental groups of rats

Groups	Group I	Group II	Group III
Plasma			
Lipid peroxide	1.76 ± 0.15	4.26 ± 0.31[a]	1.58 ± 0.13
Reduced glutathione	12.32 ± 0.62	8.59 ± 0.65 [a]	12.02 ± 0.67
Heart			
Lipid peroxide	0.98 ± 0.09	1.97 ± 0.11 [a]	0.88 ±0.08
Reduced glutathione	5.21 ± 2.17	2.75 ± 0.29 [a]	5.60 ± 2.43

Group I, normal control rats received standard diet. Group II, myocardial infarction was induced by intraperitoneal (i,p,) injection of ISO for 2 days after 28 days of feeding with standard diet. Group III, myocardial infarction was induced by intraperitoneal (i,p,) injection of ISO (20mg/100g body weight)for 2 days after 28 days of feeding with *Vitis vinefera* seed extract (500mg/kg body weight). Results are mean ± SD for 6 animals. Values expressed: Plasma lipid peroxide-nmol/ml, Reduced glutathione- mg/dl; Heart lipid peroxide- nmol/mg protein, Reduced glutathione-µg/g wet tissue; [a]$p< 0.001$ significantly different compared with Group I control animals.

Lipid peroxidation in vivo has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia (26). It is already known that lipids are the most susceptible macromolecules to oxidative stress and our results showed that the level of lipid peroxides, measure in term of TBARS was significantly increased in plasma and heart of ISO treated group. The metabolism of arachidonic acid via the lipioxygenase and cyclooxygenase pathways results in the formation of reactive oxygen species and other free radicals (27). High levels of lipid peroxides injure blood vessels, causing increased adherence and aggregation of platelets to the injured sits (28). *Vitis vinifera* pretreatment in the present decreases the level of plasma and myocardial lipid peroxides by an apparent direct scavenging of superoxide and hydroxyl radicals and by inactivating the enzyme cyclo-oxygenase (8).

GSH status is a highly sensitive indicator of cell functionality and viability. It is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defences against xenobiotics and naturally occurring deleterious compounds such as free radicals and hydroperoxides. GSH depletion is linked to a number of disease states including cancer, neurodegenerative and cardiovascular diseases. Glutathione not only protects cell membranes from oxidative damage, but also helps to maintain the sulphydry groups of many proteins in the reduced form, requirements for their normal function (29).

In the present study, the reduction noticed in the level of GSH in plasma and heart of ISO-induced myocardial infarction was either due to increased degradation or decreased synthesis of glutathione. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study. Pretreatment with *Vitis vinifera* prevented the ISO-induced lipid peroxidation and maintained the level of reduced glutathione near normal level in plasma and heart. This is due to antioxidant activity of *Vitis vinifera.*

The diagnosis of MI is based on clinical symptoms, electrocardiography changes and characteristic pattern of chances in some serum and heart enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), transaminases (SGOT, SGPT) and cardiac specific proteins like troponins (19). Cardiac markers or cardiac enzymes are protein from cardiac tissue found in the blood. The diagnosis of organ disease is aided by measurement of a number of non-functional plasma enzymes characteristics of that tissue or organ. The amount of enzymes released depends on the degrees or cellular damage, the intracellular concentration of the enzyme and the mass of affected tissue. The cause of the damage the enzyme released reflects the severity of the damage (30).

Table 2. Levels of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and troponin T in plasma of normal and experimental groups of rats

Groups	Group I	Group II	Group III
ALT	103 ± 8.90	285 ± 35[a]	111 ± 9.8
AST	86.8 ± 8.5	247 ± 26 [a]	92.5 ± 8.7
LDH	196 ± 14.6	297 ± 31 [a]	205 ± 15.3
CPK	135 ± 9.8	275 ± 24 [a]	147 ± 10.9
Troponin T	5.72 ± 2.24	14.2 ± 3.6 [a]	6.18 ± 2.44

Experimental conditions are the same as those in Table 1. Results are mean ± SD for 6 animals. Values expressed: ALT, AST and LDH-µmol pyruvate liberated/h/liter; CPK-µmol creatine liberated/h/liter. Troponin T-mg/dl. [a]$p<$ 0.001 significantly different compared with Group I control animals.

In this study, significant decline was shown in the activities of cardiac markers such as SGOT, SGPT, LDH and CK in the heart of acute ISO-treated rats, which is consistent with earlier reports (31). Decreased activities of theses enzymes were due to the leakage from the damaged heart tissues into the blood stream as a result of necrosis induced by isoproterenol in rats. Senthil *et al*. (32) observed that these cardio-specific marker enzymes are released from the heart into the blood during myocardial damage due to myofibril degeneration and myocyte necrosis. Significant increase was noticed in the activities of cardiac markers (SGOT, SGPT, LDH and CK) in plasma of ISO-treated rats, which is consistent with earlier reports (31), might be due to enhanced susceptibility of myocardial cell membrane to the isoproterenol mediated peroxidative damage, resulting in increased release of these diagnostic marker enzymes into the systemic circulation.

Table 3. Levels of alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK) and troponin T in heart of normal and experimental groups of rats

Groups	Group I	Group II	Group III
ALT	34.23± 2.90	15.20± 1.12[a]	29.82 ± 1.8
AST	50.08 ± 3.50	32.23 ± 2.6[a]	48.65 ± 2.07
LDH	255 ± 19.6	150 ± 3.91 [a]	229 ± 9.3
CPK	206 ± 13.58	130 ± 4.30 [a]	182 ± 10.9

Experimental conditions are the same as those in Table 1. Results are mean ± SD for 6 animals. Values expressed: ALT, AST and LDH-µmol pyruvate liberated/h/mg protein; CPK-µmol creatine liberated/h/mg protein. [a]$p< 0.001$ significantly different compared with Group I control animals.

In the present study, the prior administration of *Vitis vinifera* was significantly prevented the isopoterenol-induced elevation in the levels of diagnostic marker enzymes in plasma, indicating the cytoprotective activity of *Vitis vinifera* Thus, it is possible that likewise *Vitis vinifera* may also prolong the viability of myocardial cell membrane stabilizing action.

A serum marker that once held promise as cardiac specific marker for MI is the cardiac troponin. Troponin is a protein found in cardiac tissue and located in the thin filament of striated muscles consisting of the three subunits Troponin T, Troponin I and Troponin C. Of the three troponins T and I are being used as the biochemical markers for the diagnosis myocardial injury. When the myocardial damage occurs the

cytosolic troponins reach the blood stream quickly resulting in a rapid peak of serum troponin observed during the first few hours. This is followed by the release of structurally bound troponin resulting in a second peak lasting for several days (19). In this study, significant increased level of Troponin T in serum of ISO-treated rats. Increased level of troponin T was due to the leakage from the damaged heart tissues into the blood stream as a result of necrosis induced by isoproterenol in rats. Pretreatment with *Vitis vinifera* to ISO-treated rats restored the level of troponin T in serum indicates the protective action of *Vitis vinifera* against peroxidative damage.

The present results clearly emphasize the beneficial action of as a cadioprotective agent. *Vitis vinifera* seed proved to be effective in reducing the extent of myocardial damage, associated lipid peroxidation, thus maintaining, as suggested by biochemical indices, the structure and function of the myocardium. The potential cardioprotective activity of *Vitis vinifera* may be due to the presence of therapeutic phytochemicals such as proanthocyanidins and natural polyphenolic.

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