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Antioxidants (Vitamin E and Gallic Acid) as Valuable Protective Factors against Myocardial Infarction

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ABSTRACT

Myocardial infarction is acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. It is the leading cause of death in high or middle income countries and second only to lower respiratory infections in lower income countries. Reactive oxygen species participate in normal cell signaling as mediators that regulate vascular function. In the vascular wall, ROS are produced by all layers including endothelium, smooth muscle and adventitia. Under physiological condition, ROS are produced in law concentrations and act as signaling molecule that regulate VSMC contraction and relaxation, and participate in VSMC growth Under pathophysiological conditions, these free radicals play important role in various conditions, including atherosclerosis, ischemic heart diseases, arrhythmias, cardiomyopathy and congestive heart failure.So, pharmacological studies in the present work were done to evaluate the protective effects of different antioxidants (vit. E and gallic acid) on cardiac marker enzymes (AST, ALT, CK and LDH), CTnI, lipid peroxidation and antioxidant parameters in isoprenaline-induced MI in rats.

In this study fifty six adult male albino rats were divided into four groups (14 rats each), Group I: Negative control rats were subcutaneously injected with saline twice at an interval of 24 hours. Group II: positive control rats were subcutaneously injected with ISO (100 mg/kg) twice at an interval of 24 hours, it serves as control for group III and IV. Group III: rats were pretreated with gallic acid (15mg/kg) once daily orally for 10 days and then subcutaneously injected with ISO at an interval of 24 h for 2 days. Group IV: rats were pretreated with vitamin E (100 mg/kg) once daily orally for 30 days and then subcutaneously injected with ISO at an interval of 24 h for 2 days. At the end of the experimental period, blood samples was collected for estimation of cardiac marker enzymes, hearts were homogenized for the assay of MDA, SOD, GSH and histopathological examinations were done. The results of the present study showed that:-

1- Prior treatment with vitamin E and gallic acid significantly decrease the activity of serum cardic marker enzymes in ISO induced MI in rats.

2- Prior treatment with vitamin E and gallic acid significantly increase the levels of antioxidants in ISO induced MI in rats.

3- Prior treatment with vitamin E and gallic acid significantly decrease the level of lipid peroxidation in ISO induced MI in rats.

So, the use of antioxidants (vit. E and gallic acid) have a valuable protective role against MI through overcoming free radicals.

Key words: Myocardial infarction, lipid peroxidation, antioxidants, vitamin E and gallic acid.

INTRODUCTION

Myocardial infarction (MI) is an acute condition of necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand (Bono and Boon 1992). MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart (Petrich et al., 1996).

Pharmacological studies have been undertaken to evaluate the cardioprotective effects of different antioxidants (vitamin E and gallic acid) on cardiac marker enzymes (aspartate transaminase, alanine transaminase, lactate dehydrogenase, creatine kinase) and troponin-I in serum of ISO-treated rats. Lipid peroxidation and antioxidant parameters in the heart tissue of ISO-treated rats have been also evaluated (Bono and Boon 1992).

It is now well recognized that isoprenaline (ISO), a synthetic catecholamine in large doses produce MI. Among the various mechanisms proposed to explain ISO-induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of ISO has been implicated as one of the important causative factors. Excessive formation of free radicals may result in the loss of function and integrity of myocardial membrane (Priscilla and Prince 2009).

Patients with diabetes have a substantially greater risk of atherosclerotic vascular disease in the heart as well as in other vascular beds. Diabetes increases the risk of MI because it increases the rate of atherosclerotic progression and adversely affects the lipid profile. This accelerated form of atherosclerosis occurs regardless of whether a patient has insulin-dependent or non–insulin-dependent diabetes (Graham et al. 2007). Elevated levels of total cholesterol, low density lipoprotein (LDL), or triglycerides are associated with an increased risk of coronary atherosclerosis and MI. Levels of

platelet aggregation (Lüscher and Barton 1997).

Bradykinin stimulates release of NO, prostacyclin, and endothelium-derived hyperpolarizing factor, another vasodilator, which contributes to inhibition of platelet aggregation (Drexler, 1998).

Bradykinin also stimulates production of tissue plasminogen activator and thus may play an important role in fibrinolysis. The endothelium also produces vasoconstrictor substances, such as endothelin and angiotensin II. Angiotensin II not only acts as a vasoconstrictor but is also pro-oxidant and stimulates production of endothelin (Sowers, 2002). Endothelin and angiotensin II promote proliferation of smooth muscle cells and thereby contribute to the formation of plaque (Drexler, 1998).

Damage to the endothelium upsets the balance between vasoconstriction and vasodilation and initiates a number of processes that promote or exacerbate atherosclerosis; these include increased endothelial permeability, platelet aggregation and leukocyte adhesion (Ross, 1999).

Nitric oxide mediates endothelium-dependent vasodilation by opposing the effects of endothelium-derived vasoconstrictors such as angiotensin II and endothelin. It also inhibits platelet adherence and aggregation, leukocyte adhesion and proliferation of vascular smooth muscle cells. Nitric oxide prevents oxidative modification of LDL (Rubbo et al., 2002) . It is one of the main mediators of endothelium-dependent relaxation (EDR). NO release is induced by either vascular stress or by endothelial nitric oxide synthase (eNOS) activation in response to cytokine activation and plays a protective role in suppressing abnormal proliferation of vascular smooth muscle cells (VSMCs) following various high density lipoprotein (HDL) less than 40 mg/dL also portend an increased risk of MI (Smith et al., 2006).

Inflammation is known to be an important step in the process of atherosclerotic plaque formation. C-reactive protein (CRP) is a sensitive but non-specific marker for inflammation. Elevated CRP blood levels, especially measured with high-sensitivity assays, (Wilson et al., 2006).

Inflammation in periodontal disease may be linked to coronary heart disease, (Janket et al., 2003).

Periodontitis tends to increase blood levels of CRP, fibrinogen and cytokines (Scannapieco et al., 2003);

Preclinical research suggests that periodontal bacteria can promote aggregation of platelets and promote the formation of foam cells (Qi et al., 2003).

Calcium deposition is another part of atherosclerotic plaque formation. Atherosclerosis is the gradual buildup of cholesterol and fibrous tissue in plaques in the wall of arteries (Woollard and Geissmann 2010).

Disruption of the endothelial surface can cause the formation of thrombus via platelet-mediated activation of the coagulation cascade. If a thrombus is large enough to occlude coronary blood flow, an MI can result (Tsujita et al., 2010).

The normal healthy endothelium regulates vascular tone and structure and exerts anticoagulant, antiplatelet, and fibrinolytic properties.The maintenance of vascular tone is accomplished by the release of numerous dilator and constrictor substances. A major vasodilator substance released by the endothelium is nitric oxide (NO), originally identified as endothelium-derived relaxing factor (EDRF). Other endothelium-derived vasodilators include prostacyclin and bradykinin (Drexler, 1998) Prostacyclin acts synergistically with NO to inhibit

injections, thoracic outlet syndrome, and pulmonary embolism (Apple et al., 2003).

Troponin is a regulatory complex of 3 protein subunits located on the thin filament of the myocardial contractile apparatus. The 3 subunits are designated troponin CTnC (the calcium-binding component), CTnT (the tropomyosin-binding component), and CTnI (the inhibitory component) (Bertinchant et al., 1996).

Cardiac-specific troponins I and T accurately distinguish skeletal from cardiac muscle damage. The troponins are now considered the preferred biomarker for diagnosing MI (Reichlin et al., 2009). Both troponins increase in serum within 4 to 9 hours after MI, peak at 12 to 24 hours, and remain elevated for up to 14 days (Wu et al., 1999).

Troponins have a number of advantages over other cardiac markers, which has led to their adoption as the new gold standard for myonecrosis (Wu et al., 1999).

It is important to note that cTnT and cTnI are not detected normally in the blood of healthy persons. Consequently, significant elevations of cTnT or cTnI are thought to most likely reflect myocardial necrosis. CK increases 10- to 20-fold above the upper limit of the reference range, cTnT and cTnI typically increase more than 20 times above the reference range. Thus, now Troponins are sensitive and specific markers for acute MI (Thygesen et al., 2007).

The use of aspirin has been shown to reduce mortality from MI. Aspirin in a dose of 325 mg should be administered immediately on recognition of MI signs and symptoms (Andreson et al., 2007).

The nucleus of an occlusive coronary thrombus is the adhesion of a small collection of activated platelets at the site of intimal disruption in an unstable atherosclerotic plaque. Aspirin irreversibly pathological situations (Shinyashiki et al., 2004).

Oxidation of LDL has been proposed as a major mechanism of the atherosclerotic process (Steinberget and Witztum 2002); impaired production or activity of NO leads to events that promote atherosclerosis, such as vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion, and oxidative stress (Endres et al., 1998).

Serum AST, ALT and LDH are well known markers of MI. When myocardial cells are damaged or destroyed due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture which results in leakage of enzymes. These enzymes enter into the blood stream thus increasing their concentration in the serum (Arya et al., 2006).

Lactate dehydrogenase is a sytosolic enzyme, which is present in all the tissues involved in glycolysis hence, detection of elevated concentration of this enzyme released into the blood stream from the damaged tissue has become a definitive diagnostic and prognostic criterion for various diseases and disorders. It can be differentiated from other types of tissue damage, since LDH begins to rise in 12-24h following MI and peaks in 2-3 days gradually dissipating in 5-14 days (Jaffe et al., 1996).

The measurement of CK level has long been used for the diagnosis of MI. CK, an enzyme present in many tissues, including the myocardium and skeletal muscle, following myocardial injury, CK rise occurs 4 to 9 hours after the onset of chest pain, peaks at 24 hours, and returns to baseline at 48 to 72 hours. False-positive results are seen in patients with muscle disease, alcohol intoxication, diabetes mellitus, skeletal muscle trauma, after vigorous exercise, convulsions, intramuscular

to be effective when administered intravenously or subcutaneously according to specific guidelines. The minimum duration of heparin therapy after MI is generally 48 hours, but it may be longer, depending on the individual clinical scenario. Heparin has the added benefit of preventing thrombus through a different mechanism than aspirin (Andreson et al., 2007).

Low-molecular-weight heparin (LMWH) can be administered to MI patients who are not treated with fibrinolytic therapy and who have no contraindications to heparin. The LMWH class of drugs includes several agents that have distinctly different anticoagulant effects. (Andreson et al., 2007).

Glycoprotein IIb/IIIa receptors on platelets bind to fibrinogen in the final common pathway of platelet aggregation. Antagonists to glycoprotein IIb/IIIa receptors are potent inhibitors of platelet aggregation. The use of glycoprotein IIb/IIIa inhibitors during percutaneous coronary intervention (PCI) and in patients with MI and acute coronary syndromes has been shown to reduce the composite end point of death, reinfarction, and the need to revascularize the target lesion at follow-up. The current guidelines recommend the use of a IIb/IIIa inhibitor for patients in whom PCI is planned. For high-risk patients with NSTEMI who do not undergo PCI, a IIb/IIIa inhibitor may be used for 48 to 72 hours (Andreson et al., 2007).

Free radicals are molecules containing one or more unpaired electrons in atomic or molecular orbitals (Gutteridge and Halliwell 2000).

There is increasing evidence that abnormal production of free radicals lead to increased oxidative stress on cellular structures and causes changes in molecular pathways that underpins the pathogenesis of several important interferes with function of cyclooxygenase and inhibits the formation of thromboxane A2. Within minutes, aspirin prevents additional platelet activation and interferes with platelet adhesion. The long-term benefit is sustained, even at doses as low as 75 mg/day (Antman et al., 2007).

Oxygen should be administered to patients with symptoms or signs of pulmonary edema or with pulse oximetry less than 90% saturation MI (Andreson et al., 2007).

The rationale for using oxygen is the assurance that erythrocytes will be saturated to maximum carrying capacity. Because MI impairs the circulatory function of the heart, oxygen extraction by the heart and by other tissues may be diminished. In some cases, elevated pulmonary capillary pressure and pulmonary edema can decrease oxygen uptake as a result of impaired pulmonary alveolar-capillary diffusion. Supplemental oxygen increases the driving gradient for oxygen uptake (Cotran et al., 1994).

Intravenous nitrates should be administered to patients with MI and congestive heart failure, persistent ischemia, hypertension, or large anterior wall MI. The primary benefit of nitrates is derived from its vasodilator effect. Nitrates are metabolized to NO in the vascular endothelium. Nitric oxide relaxes vascular smooth muscle and dilates the blood vessel lumen. Vasodilatation reduces cardiac preload and afterload and decreases the myocardial oxygen requirements needed for circulation at a fixed flow rate. Nitrates can reverse the vasoconstriction associated with thrombosis and coronary occlusion (Andreson et al., 2007).

Unfractionated heparin is beneficial until the thrombotic cause (ruptured plaque) has completely resolved or healed. Unfractionated heparin has been shown

It is suggested that superoxide production by eNOS is important in oxidation of LDL during the formation of atherosclerosis in the setting of hyperlipidemia. Endothelial cells, smooth muscle cells, neutrophils and monocytes all have the potential to oxidatively modify LDL, leading to the generation of ROS and lipid peroxidation products. (Nourooz-Zadeh et al., 2001).

In order to prevent or reduce the ROS-induced oxidative damage, the human body and other organisms have developed an antioxidant defense system that includes enzymatic, metal-chelating, and free radical-scavenging activities to neutralize these radicals after they have formed. In addition, intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body (Geier et al., 2009).

Antioxidants may be molecules that can neutralize free radicals by accepting or donating electron(s) to eliminate the unpaired condition of the radical. The antioxidant molecules may directly react with the reactive radicals and destroy them, while they may become new free radicals which are less active, longer-lived and less dangerous than those radicals they have neutralized. They may be neutralized by other antioxidants or other mechanisms to terminate their radical status (Jian-Ming et al., 2010).

Many antioxidants may directly react with ROS and/or free radical intermediates induced by ROS and terminate the chain reaction, thereby stopping the ROS-induced damage (De Feudis et al., 2003). Small molecules such as vitamin C, vitamin E, uric acid and glutathione play important roles as cellular antioxidants (Rababah et al., 2004).

Synthetic antioxidants such as tert-butylhydroxyl-toluene, tert-butylhydroxyanisole and tert-butylhydroquinone have been widely used in the food industry to retard lipid diseases, including cardiovascular diseases (Pacher and Szabo 2008).

Reactive oxygen or oxidant species (ROS) participate in normal cell signaling as mediators that regulate vascular function. In the vascular wall, ROS are produced by all layers; including endothelium, smooth muscle, and adventitia (Lassegue and Clempus 2003).

ROS include free radicals such as superoxide anion (O2.-), hydroxyl radical (HO.), lipid radicals (ROO-) and NO. Other reactive oxygen species, hydrogen peroxide (H2O2), peroxynitrite (ONOO-) and hypochlorous acid (HOCl), ROS has been implicated in cell damage, necrosis and cell apoptosis due to its direct oxidizing effects on macromolecules such as lipids, proteins and DNA. Production of one free radical can lead to further formation of radicals via sequential chain reactions (Valko et al., 2006).

Under physiological conditions, ROS are produced in low concentrations and act as a signaling molecule that regulate vascular smooth muscle cell (VSMC) contraction and relaxation, and participate in VSMC growth (Touyz and Schiffirin 1999).

Under pathophysiological conditions, these free radicals play important roles in oxidative damage and that they share a common mechanism of molecular and cellular damage (Vassalle et al., 2008).

Several mechanisms or pathways are associated with the production of free radicals within cells under physiological conditions. These include mitochondrial respiration, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, xanthine oxidoreductase and uncoupled NO synthases (Sanjib et al., 2011). Hypercholesterolaemia increases endothelial O2 production and vascular oxidative stress. Hypercholesterolaemia was found to be independently associated with increased NADH-dependent superoxide production (Guzik et al., 2000).

irreversibly (Scandalios, 2005). Thus, methods to reduce the damage induced by oxidative stress are extensively investigated. Intracellular antioxidant enzymes are an important protective mechanism against ROS. These enzymes are produced in the cell and provide an important defense against free radicals. SOD, CAT, GPX, glutathione reductase (GRd), glutathione S-transferase (GST), thioredoxin reductase (TrxR), heme oxygenase and biliverdin reductase are the most important antioxidant enzymes (Scandalios, 2005).

Both GST and GPX are involved in eliminating peroxides that are formed during metabolism. GRd regulates the equivalent of reduced glutathione (GSH) and oxidized glutathione (GSSG), and the ratio of GSH/GSSG is a well known index of oxidative stress (James et al., 2009).

Lipid peroxidation refers to the oxidative deterioration of lipids containing any number of carbon-carbon double bonds, such as unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. ROS attack the unsaturated fatty acids which contain multiple double bonds and the methylene -CH2-groups with especially reactive hydrogen atoms, and initiate the radical peroxidation chain reactions (Jian-Ming et al., 2010). Radical scavengers can directly react and quench peroxide radicals to terminate the chain reaction. Lipid peroxidation and DNA damage are associated with a variety of chronic health problems, such as cancer, ageing and atherosclerosis (Marnett, 2000).

The term vitamin E describes a family of eight antioxidants: four tocopherols (alpha-, beta-, gamma-, and delta-) and four tocotrienols (alpha-, beta-, gamma-, and delta-). Alpha-tocopherol is the only form of vitamin E that is actively maintained in the human body; therefore, it is the form of vitamin E found in the oxidation. However, such synthetic antioxidants are not preferred for pharmacologic use due to toxicological concerns. Thus, more and more interests have focused on identifying plant extracts to use as dietary antioxidant supplements (Rababah et al., 2004).

Most of these natural antioxidants come from fruits, vegetables, spices, grains, and herbs such as ginseng, curcuma, ginkgo, rosemary, green tea, grape, ginger and garlic. They contain a wide variety of antioxidant compounds, such as phenolics (phenol and polyphenols), flavonoids, carotenoids, steroids and thiol compounds (Lotito and Frei 2006).

These antioxidants may help to protect cellular damages from oxidative stress and also lower the risk of chronic diseases. For example, ginseng contains steroid-like compounds, ginsenosides, which show antioxidant activities against free radical damage on the vascular endothelium (Lü et al., 2009). Ginkgo has been reported to have strong antioxidant activities due to flavone glycosides that scavenge free radicals (De Feudis et al., 2003). Flavonoids such as catechin and epicatechin in green tea and grape seed extracts could be responsible for their potent antioxidant activities (Williamson and Manach 2005).

Another important function of antioxidants is to regulate ROS-related enzymes. Antioxidants may decrease the cellular level of free radicals either by inhibiting the activities or expressions of free radical generating enzymes such as xanthine oxidase (XO) or by enhancing the activities and expressions of antioxidant enzymes such as SOD, CAT and GPX (Panchatcharam et al., 2006). These antioxidant enzymes produced in the body provide an important defense against free radicals (Shih et al., 2007).

During metabolism, ROS such as H2O2, O2−•, and ·OH, etc, are produced

preventing the long-term complications of diabetes (Pizzorno, 2000).

Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants (Reynolds and Wilson 1991).

Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid possesses significant anti-inflammatory properties and prevents the expression of inflammatory chemicals including cytokines and histamines (Sang-Hyun, 2006).

Gallic acid possesses significant antioxidant activity and may protect the liver from the harmful effects of free radicals that are formed as a result of various metabolic processes in the body. The unstable free radicals may interact with DNA and proteins of the human cells and damage them (Rasool, 2010).

Gallic acid may also benefit diabetes patients by triggering the release of insulin by the pancreatic cells (Sameermahmood, 2010)

MATERIAL AND METHODS

Adult male albino rats were chosen as an animal model for this study. Rats were brought from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on a balanced diet with free water supply in clean containers. They were kept for two weeks under this condition to adapt the laboratory conditions before the start of the experiment. Fifty six age-matched rats with initial body weights ranging from 150 to 200g were used. The rats were divided into four groups (14 rats each). Eight animals were used for biochemical estimations and six animals for histopathological study:

largest quantities in blood and tissues (Traber, 2006).

Numerous foods provide vitamin E. Nuts, seeds, and vegetable oils are among the best sources of alpha-tocopherol, and significant amounts are available in green leafy vegetables and fortified cereals (U.S. Department of Agriculture 2010).

Most vitamin E in American diets is in the form of gamma-tocopherol from soybean, canola, corn (Dietrich et al., 2006).

Vitamin E is an antioxidant that is important in the prevention of cancer and cardiovascular disease. As an antioxidant, vitamin E prevents cell damage by inhibiting the oxidation of lipids and the formation of free radicals. It protects other fat-soluble vitamins from destruction by oxygen and aids in the utilization of vitamin A. It retards aging and may prevent age spots (Verhagen et al., 2006).

Vitamin E plays an important role in promoting health and preventing and treating diseases (Kowdley et al., 2006).

The mechanisms by which vitamin E might provide this protection include its function as an antioxidant and its roles in anti-inflammatory processes, inhibition of platelet aggregation and immune enhancement (Kowdley et al., 2006).

Vitamin E may offer the greatest protection against the oxidation of LDL cholesterol because of its ability to be easily incorporated into the LDL molecule (Pizzorno et al., 2000) According to several studies, there is a clear correlation between the dosage and effect of vitamin E. Meaning, the higher the dosage of vitamin E, the greater the degree of protection against oxidative damage to LDL cholesterol. (Pizzorno et al., 2000)

Diabetics appear to have an increased requirement for vitamin E. High dose of vitamin E (900IU) not only improves insulin action, but also exerts a number of beneficial effects that may aid in

biochemical estimations. Hearts were excised, weighed and homogenized in tris HCl buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was used for the assay of MDA, SOD and GSH.

1-Assay of cardiac marker enzymes:

A-Estimation of Serum aspartate transaminase :

The activity of AST in serum was determined by enzymatic colorimetric method (Reitman and Frankel 1957).

B-Estimation of Serum alanine aminotransferase (ALT)

Serum alanine aminotransferase level was estimated by enzymatic– colometric method (Reitman and Frankel 1957).

C- Estimation of Serum lactate dehydrogenase (LDH)

Serum lactate dehydrogenase level was estimated by enzymatic– colometric method (Pesce, 1984).

D- Estimation of serum creatine kinase:

Serum level of CK was done by enzymatic– colometric method (Bergmeyer, 1987).

E- Estimation of serum Troponin-I (CTnI):

Serum level estimation of (CTnI) was done by Immunoenzymometric assay (Apple et al., 1999).

2- Estimation of the level of reduced glutathione (GSH) in the heart:

The level of GSH in the heart was estimated by colorimetric method (Beutler et al., 1963).

3- Estimation of the level of superoxide dismutase (SOD) in the heart:

The level of SOD in the heart was estimated by colorimetric method (Nishikimi et al., 1972).

4- Estimation of the level of Malondialdehyde (MDA) in the heart:

The level of (MDA) in the heart was estimated by colorimetric method (Ohkawa et al., 1978).

Group I: Negative control rats were subcutaneously injected with saline twice at an interval of 24 hours.

Group II: Positive control rats were subcutaneously injected with ISO (100 mg/kg) twice at an interval of 24 hours, it serves as control for group III and IV.

Group III: rats were pretreated with gallic acid (15mg/kg) once daily orally for 10 days and then subcutaneously injected with ISO at an interval of 24 hrs for 2 days

Group IV: rats were pretreated with vitamin E (100 mg/kg) once daily orally for 30 days and then subcutaneously injected with ISO at an interval of 24 hrs for 2 days

1-Induction of myocardial infarction (MI)

It is now well recognized that ISO, a synthetic catecholamine in large doses produces MI (Priscilla and Prince 2009). ISO (100 mg/kg) dissolved in saline was subcutaneously injected to rats at an interval of 24 hrs for 2 days. ISO-induced MI was confirmed by elevated levels of serum CK, CTnI, AST and LDH in rats (Kumaran and Prince 2010).

2- Drug administration:

Gallic acid was given in a single dose of 15 mg/kg body weight /day orally for 10 days (Priscilla and Prince 2009). Gallic acid was dissolved in saline and administered to rats orally using intragastric tube (Priscilla and Prince 2009).

Vitamin E was given in a dose of 100 mg/kg body weight /day orally for 30 days (Upaganlawar et al., 2010). It was suspended in saline to be given via gastric tube (Upaganlawar et al., 2010).

3- Collection of blood samples

At the end of the experimental period, after 12 hrs of second ISO injection all the rats were anesthetized with ether and sacrificed by cervical decapitation. Blood was collected into a dry clean graduated glass centrifuge tube. It was rapidly set to centrifuge at 5000 r.p.m for 10 minutes and serum separated for various

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean ± standard error (S.E). Statistical analysis of the difference between groups was performed by using One –way analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means. A value of $P<0.05$ were used as the limit for statistical significance.

Histopathological examination

After decapitation, the hearts were rapidly dissected out and washed immediately with saline and fixed in 10% neutral buffered formalin, embedded in paraffin, prepared as 5-μm-thick sections, stained with Hematoxylin and Eosin (H&E) and viewed under light microscope to assess the histopathological examination (Upaganlawar et al., 2010).

Statistical analysis

RESULTS

Table 1. Effect of pretreatment with vitamin E and gallic acid on the activity of aspartate transaminase (AST) in serum of ISO-induced myocardial infarcted rats.

Groups	AST (IU/l)
Normal control	34.02±2.24
Isoprenaline	60.57±2.81 *
Gallic acid+ Isoprenaline	49.32±2.82 * #
Vitamin E+ Isoprenaline	47.23±2.48 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group #

Table 2. Effect of pretreatment with vitamin E and gallic acid on the activity of (ALT) in serum of ISO-induced myocardial infarcted rats.

Groups	ALT (IU/l)
Normal control	24.48±2.62
Isoprenaline	53.31±2.28 *
Gallic acid + Isoprenaline	40.59±4.19 * #
Vitamin E + Isoprenaline	37.88±2.36 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 3. Effect of pretreatment with vitamin E and gallic acid on the activity of (LDH) in serum of ISO-induced myocardial infarcted rats.

Groups	LDH (IU/l)
Normal control	80.98±3.63
Isoprenaline	163.03±2.20 *
Gallic acid + Isoprenaline	105.07±4.02 * #
Vitamin E + Isoprenaline	95.13±3.26 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 4. Effect of pretreatment with vitamin E and gallic acid on the activity of (CK) in serum of ISO-induced myocardial infarcted rats.

Groups	CK (IU/l)
Normal control	165.76±4.56
Isoprenaline	282.47±8.57 *
Gallic acid + Isoprenaline	197.06±6.96 * #
Vitamin E + Isoprenaline	192.60±3.88 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 5. Effect of pretreatment with vitamin E and gallic acid on the levels of (cTnI) in serum of ISO-induced myocardial infarcted rats.

Groups	cTnI (ng/ml)
Control	0.158± 0.027
Isoprenaline	2.35±0.015*
Gallic acid + Isoprenaline	1.10±0.049* #
Vitamin E + Isoprenaline	0.96±0.048* #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 6. Effect of pretreatment with vitamin E and gallic acid on the levels of (GSH) in the heart of ISO-induced myocardial infarcted rats.

Groups	GSH (mmol/g tissue)
Control	12.62±0.83
Isoprenaline	3.91±0.39 *
Gallic acid + Isoprenaline	8.41±0.79 *#
Vitamin E + Isoprenaline	9.05±0.50 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 7. Effect of pretreatment with vitamin E and gallic acid on the activity of (SOD) in the heart of ISO-induced myocardial infarcted rats.

Groups	SOD (U/g tissue)
Control	17.47±1.34
Isoprenaline	6.03±0.65 *
Gallic acid + Isoprenaline	11.25±1.01 * #
Vitamin E + Isoprenaline	11.96±0.94 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

Table 8. Effect of pretreatment with vitamin E and gallic acid on the level of (MDA) in the heart of ISO-induced myocardial infarcted rats.

Groups	MDA (nmol/g tissue)
Control	1.04±0.16
Isoprenaline	2.65±0.18 *
Gallic acid + Isoprenaline	1.50±0.15 * #
Vitamin E + Isoprenaline	1.68±0.10 * #

Each value represents the mean ± SE (standard error) of 8 animals
Significant result as compared to control group *
Significant result as compared to isoprenaline group#

diagnosing MI (Reichlin et al., 2009). Elevated troponin levels predict the risk of both cardiac death and subsequent infarction (Priscilla and Prince 2009).

In the present study increased levels of CTnI in serum of ISO treated rats was observed. Results of the present study are in agreement with those reported by Chikku and Rajamohan 2012,they found that ISO treated rats showed significant ($P<0.05$) increased activity of LDH, CK, AST, ALT and increased the concentration of CTnI in the serum compared to normal control rats. They also reported that ROS are formed at an accelerated rate in the myocardium due to ISO administration. Myocardial cells contain enzymes like LDH, CK, AST, ALT and structural proteins like troponins which are released in blood

DISSCUISION

Isoprenaline in large doses induces morphological and functional alterations in the heart leading to myocardial necrosis (Bursell and King 1999). ISO also produces excessive free radicals resulting from its oxidative metabolism (Upaganlawar et al., 2009). There are increasing evidences that cardiotoxicity of ISO occurs because of generation of free radicals and oxidative stress (Punithavathi and prince 2009)

The present study revealed that ISO treatment results in marked elevation in the levels of serum cardiac marker enzymes including AST, ALT, LDH and CK.

Cardiac-specific troponins accurately distinguish skeletal from cardiac muscle damage. The troponins are now considered the preferred biomarker for

agreement with the results obtained by researchers; Upaganlawar et al., 2010 reported that Treatment with vitamin E for 30 days showed a significant ($P < 0.01$) reduction in the activities of all serum cardiac marker enzymes including AST, ALT, LDH and CK as compared to the ISO treated group. This protection is due to the effect of vitamin E on the myocardium, which had reduced the extent of myocardial damage induced by ISO and thereby restricting the leakage of these enzymes from the myocardium, suggesting the membrane stabilizing potential of vitamin E. The results of the present study showed that ISO treatment results in an increase in the levels of lipid peroxidation products MDA in the heart. Results of the present study are in agreement with those reported by Chikku and Rajamohan 2012. They found that ISO administration is associated with increased levels of lipid peroxidation as evidenced by increased levels of MDA in the heart. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage and this leads to oxidative damage of cell components like proteins, lipids and nucleic acids.

Kumaran and Prince 2010 reported that Rats treated with ISO showed considerable ($P<0.05$) increased levels of MDA in the heart compared to normal control rats. An increase in heart lipid peroxide level indicates enhanced lipid peroxidation by free radicals generated on ISO administration. Activated lipid peroxidation is an important pathogenic event in MI and the levels of lipid peroxide reflect the major stages of disease and its complications.

The results of this study showed that, Pretreatment with gallic acid (15mg/kg) daily for 10 days significantly ($p<0.01$) decreased the level of MDA compared with ISO alone treated rats. This finding is due to cellular dysfunction and necrosis as a result of oxidative stress during MI. Leakage of these cardiac specific enzymes and troponins leads to their increased activity/concentration in serum and hence they serve as diagnostic markers of cardiac damage.

Kumaran and prince 2010 showed that the activity of serum CK was considerably ($P<0.05$) increased in ISO-treated rats compared to normal control rats. The increased activity of this enzyme in serum might be due to ISO- induced myocardial necrosis.

In the present study the results showed that pretreatment with gallic acid significantly decreased the activity of AST, ALT, LDH and CK and the levels of CTnI in serum of ISO treated rats. These results are in agreement with the results obtained by Priscilla and Prince 2009 who showed that pretreatment with gallic acid decreased the activities of CK, AST, ALT and LDH in serum of ISO-treated rats. The reduction of the activities of these enzymes is probably due to the protective effect of gallic acid on the myocardium, which had reduced the extent of myocardial damage and restricting the leakage of these enzymes. Pretreatment with gallic acid also decreased the levels of CTnI significantly and this could be due to the reduction of the degree of damage in the myocardium by gallic acid thereby preventing their leakage.

Patel and Goyal 2011 found that a significant decrease in serum LDH and CK levels was observed by treatment of gallic acid in diabetes-induced myocardial dysfunction in rats, which indicates beneficial effects in reducing the cardiovascular risk in diabetes mellitus.

In the present study results showed that pretreatment with vitamin E significantly decreased the activity of AST, ALT, LDH, CK and the levels of CTnI in serum of ISO treated rats. These results are in

The results of this study showed that rats treated with ISO showed considerable ($p<0.01$) decrease in the levels of GSH and SOD in the heart compared to normal control rats. This finding is in agreement with the results obtained by Kumaran and Prince 2010 who reported that ISO metabolism produces quinones, which reacts with oxygen to produce O2• and H2O2 leading to oxidative stress and depletion of the endogenous antioxidant system. Depletion of heart GSH level seems to be a major mechanism for inducing an imbalance of myocardial function.

A decrease in the activity of GRd makes heart more susceptible to the ISO-induced damage, which leads to change in its composition and function. The decrease in the activity of GRd observed in the ISO-treated rats is due to the reduced availability of its substrate, GSH. In this context, decreased levels of GSH were observed in the heart of ISO-treated rats (Kumaran and Prince 2010).

Chikku and Rajamohan 2012 found that Decrease in the activities of SOD and GSH were observed in the heart of ISO alone treated rats. Superoxide radicals generated by ISO at the site of damage modulate SOD, resulting in decreased activity of this enzyme. This can lead to the accumulation of O2• which further damages the myocardium. The decreased activities of GSH dependent enzymes such as GPx in the heart of MI induced rats may be due to decreased GSH concentration. Inactivation of GRd leads to the oxidation of GSH leading to the formation of Glutathione disulfide (GSSG), which in turn inactivates the enzymes with sulfhydryl groups.

Filho et al., 2011 reported that the induction of MI led to a reduction of SOD activity in myocardial tissue, as evidenced when comparing the simulated and infarction groups. Free radical scavenger in agreement with the results obtained by Priscilla and Prince 2009 who showed that gallic acid pretreatment decreases the levels of lipid peroxides in ISO treated rats. The antioxidant nature of gallic acid may hinder the ROS which are produced by isoprenaline.

patel and Goyal 2011 found that a significant decrease in lipid peroxidation levels was observed by treatment of gallic acid in diabetes-induced myocardial dysfunction in rats. This study showed that, Pretreatment with vitamin E (100mg/kg) daily for 30 days to ISO-treated rats significantly ($p<0.01$) decreased the level of MDA compared with ISO alone treated rats. This finding is in agreement with the results obtained by Upaganlawar et al., 2010 who reported that Maximum induction of lipid peroxides was observed in ISO treated rats. The change in lipid peroxides was significantly decreased in rats pretreated with vitamin E compared to ISO treated rats.

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like MI, the generation of ROS can dramatically upset this balance with an increased demand on the antioxidant defense system. Free radical scavenging enzymes such as SOD and GRd are the first line of cellular defense against oxidative injury. These enzymes are lowered due to enhanced lipid peroxidation (Priscilla and Prince 2009).

Superoxide radicals generated at the site of damage in MI modulates SOD resulting in the lowered activities of the enzyme and accumulation of superoxide anion, which also damages the myocardium (Priscilla and Prince 2009).

These results are in agreement with the results obtained by Upaganlawar et al., 2010 who reported that rats injected with ISO showed a significant ($P < 0.001$) decrease in the activity of SOD and the level of GSH as compared to control groups. pretreatment with vitamin E significantly ($P < 0.001$) increased the activity of SOD and the level of GSH as compared to ISO intoxicated rats.

These results are in disagreement with the results obtained by The Heart Outcomes Prevention Evaluation Study Investigators 2000. They reported that vitamin E, did not reduce MI, stroke, or death in adults at high risk for cardiovascular events.

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enzymes such as SOD, are the first line of defense against oxidative injury, decomposing H_2O_2 and O_2 before their interactions to form the (OH). During acute MI, superoxide radicals modulate the activity of SOD, resulting in reduced activity of this enzyme and accumulation of superoxide radicals, with consequent damage to the myocardium.

The concentrations of GSH in the myocardial tissue were reduced in the infarcted group compared to the simulated group. ROS are generated in the early stages of acute MI, and glutathione is involved in reducing free radical hydrogen peroxide, with decreased levels of glutathione in this period. Glutathione is important in protecting the myocardium against damage by free radicals and a reduction in its levels can compromise recovery after periods of ischemia.

The results of this study showed that pretreatment with gallic acid (15mg/kg) daily for 10 days to ISO-treated rats significantly ($p<0.01$) increased the levels of GSH and SOD compared with ISO alone treated rats.

The results of this study are in agreement with the results obtained by Priscilla and Prince 2009 who reported that rats pretreated with gallic acid showed increased activities of SOD and GSH which suggest that gallic acid may have the ability to prevent deleterious effects induced by free radicals in ISO treated rats.

Patel and Goyal 2011 found that treatment with gallic acid increased the levels of endogenous antioxidant SOD and GSH in diabetes-induced myocardial dysfunction in rats. The results of this study showed that pretreatment with vitamin E (100mg/kg) daily for 30 days to ISO-treated rats significantly ($p<0.01$) increased the levels of GSH and SOD compared with ISO alone treated rats.

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