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Research Article

PROTECTIVE EFFECTS OF VANILLIC ACID ON MITOCHONDRIAL ANTIOXIDANT ENZYMES AND LIPIDS IN ISOPROTERENOL INDUCED CARDIO TOXICITY IN RATS

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ABSTRACT

The present study was designed to investigate the preventive potential of vanillic acid (VA) on isoproterenol (ISO)-induced oxidative stress and heart mitochondrial damage in rats. Male albino Wistar rats were pretreated with VA (10mg/kg, body weight) once daily for 10 days. After the pretreatment antioxidant, and oxidative stress parameters in serum and heart tissues were measured. ISO treated rats showed the activity of myoglobin was increased significantly ($p < 0.05$) in the serum of ISO rats. Furthermore, the levels of lipids such as total cholesterol, triglycerides and free fatty acids in the mitochondrial fraction of the heart. TEM findings also correlated with these biochemical parameters. These findings revealed the preventive effects of VA against ISO-induced oxidative stress and cardio toxicity in rats. These observed effects are mediated via antioxidant power and free radical scavenging activity of VA.

INTRODUCTION

MI is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Isoproterenol is known to generate free radicals as well as accumulation of lipid peroxides has been recognized as one of the possible biochemical mechanisms of myocardial damage (Sushmakumari *et al*., 1989).

Mitochondria are the main consumers of molecular oxygen in the cardiac cell, and this process functions as a transuding device to provide the energy required for ATP synthesis in the oxidative phosphorylation [Echtay *et al*., 2002). The altered mitochondrial energy metabolism plays an important role in the mechanism of heart failure and is a topic of much interest [Echtay *et al*., 2002; Echtay *et al*., 2002]. In acute myocardial infarction, reactive oxygen species toxicity shuts down mitochondrial oxidative phosphorylation and TCA cycle, blocking mitochondrial ATP production and leading to cardiac contraction [Kehrer, 1993].

Most of these phenolic compounds are antioxidants *in vitro* and antioxidants may protect against CVDs. Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a phenolic derivative of edible plants and fruits. The highest amount of vanillic acid in plants is found in the root of *Angelica sinensis* (Stanely Mainzen Prince, *et al*., 2011a). Vanillic acid possesses antibacterial (Rai and Maurya, 1996), antifilarial (Varma *et al*., 1993) and antimicrobial (Delaquis *et al*., 2005) properties.

Previously, we reported preventive effects of vanillic acid on serum lipid peroxidation, serum and heart antioxidants, serum lipids and pro inflammatory markers in myocardial infracted rats were reported earlier (Stanely Mainzen Prince 2011b) In continuation of our research work on vanillic acid, the present investigation was undertaken to study the preventive effects of vanillic acid in reducing the extent of mitochondrial damage in the myocardium of isoproterenol induced cardio toxic rats.

MATERIALS AND METHODS

Chemicals

Vanillic acid, isoproterenol hydrochloride, α-N-benzoyl–DL-arginine-p-nitroanilide hydrochloride and Folin's phenol reagent were purchased from Sigma Chemical Co., St, and Louis, MO, USA. All the other chemicals and solvents used were of analytical grade.

Experimental animals

The experiment was carried out according to the guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by

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the Animal Ethical Committee of Annamalai University (Proposal No: 865, Dated; 10 /01 /2012).

Preparation of myocardial infarcted rats

Isoproterenol (100 mg/kg body weight) dissolved in saline was subcutaneously injected into rats at an interval of 24 h for 2 days (Duke4453676)

Experimental design

The animals were grouped into four groups of six rats each. Group I: normal control rats; Group II :rats were orally treated with vanillic acid (10mg/kg body weight) daily for a period of 10 days by an intragastric tube; Group III: rats were subcutaneously injected with ISO (100 mg/kg body weight) at an interval of 24 h for 2 days (on 11th and 12th day); Group IV: rats were orally pretreated with vanillic acid (10mg / kg body weight) daily for a period of 10days by an intragastric tube and were subcutaneously injected with ISO at an interval of 24 h for 2 days (11th and 12th day). Vanillic acid was dissolved in saline and administered to rats orally by an intragastric tube daily for a period of 10 days. Normal control and ISO control rats were given saline alone orally daily for a period of 10 days by an intragastric tube. Twelve hours after the second dose of ISO injection (on 12th day), all the rats were anesthetized by pentobarbital sodium (60 mg / kg body weight) and then sacrificed by cervical decapitation. Blood was collected; serum and plasma were separated by centrifugation and used for the estimation of various biochemical parameters.

Estimation of antioxidants in the mitochondrial fraction of the heart

The activities of mitochondrial antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and were assayed by the methods of Kakkar *et al*. [Kakkar *et al*., 1984], and Rotruck *et al*. [1973], respectively. The concentration of reduced glutathione (GSH) was estimated by the method of Ellman [1959].

Estimation of total cholesterol in the heart mitochondrial fraction

The levels of total cholesterol in the heart mitochondrial fraction were estimated by the method of Zlatkis *et al.,* (1953).

Estimation of TGs in the heart mitochondrial fraction

The levels of TGs in the heart mitochondrial fraction were estimated by the method of Fossati and Lorenzo (1982).

Estimation of free fatty acids (FFAs) in the heart mitochondrial fraction

FFAs levels in the heart mitochondrial fraction were estimated by the method of Falholt *et al.* (1973).

TEM studies on the structure of mitochondria

Small pieces of heart were taken and rinsed in 0.1M phosphate buffer (pH, 7.2). Approximately, 1mm heart pieces were trimmed and immediately fixed into 3% ice-cold glutaraldehyde in 0.1M phosphate buffer (pH, 7.2) and kept at 4 C for 12 h. Then, tissues processing for TEM studies were carried out. The grids containing sections were stained with 2% uranyl acetate and 0.2% lead acetate. Then, the sections were examined under a transmission electron microscope.

Statistical Analysis

Statistical analysis was performed by One-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using Statistical Package for the Social Science software package version 12.00. Results were expressed as mean ± standard deviation for six rats in each group. P values <0.05 were considered significant.

RESULTS

Figure 1 shows the effect of vanillic acid on the level of serum myoglobin in normal and isoproterenol induced myocardial infarcted rats. Isoproterenol induced myocardial infracted rats showed significant ($p<0.05$) increase in the levels of cardiac marker, myoglobin in the serum of isoproterenol induced myocardial infracted rats compared with isoproterenol alone induced myocardial infracted rats.

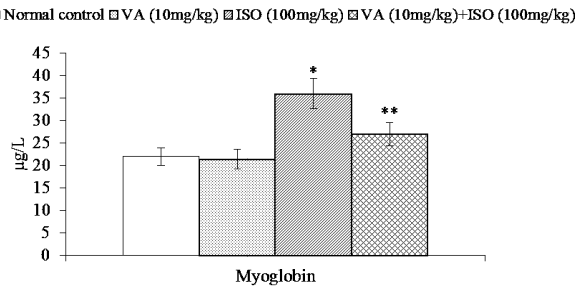

Figure-1 Effect of vanillic acid on lipids in the heart mitochondria

Table 1 shows the activities of antioxidants in the heart mitochondria of normal and experimental rats. Rats induced with isoproterenol showed significant ($P < 0.05$) decrease in the activities of SOD, GPx and GSH in the heart mitochondria compared to normal control rats. Oral pretreatment with VA (10 mg/kg) to ISO-induced rats daily for a period of 10 days significantly ($P < 0.05$) increased the activities of SOD, GPx and GSH in the heart mitochondria compared with ISO alone induced rats.

Table 1 The levels / activities of heart mitochondrial superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) in normal and isoproterenol induced myocardial infarcted rats.

Groups	Normal control	Normal+ Vanillic acid (10mg/kg)	ISO Control (100mg/kg)	Vanillic acid (10mg/kg) +ISO
SOD (*units/100mg protein)	10.8± 0.9 [a]	11.7±1.4 [a]	7.0±0.5 [b]	9.6±0.7 [c]
GPx (nM of GSH oxidized/min/100mg protein)	5.2±0.6 [a]	5.4±0.5 [a]	2.5±0.2 [b]	4.3±0.4 [c]
GSH (Nm/100 mg protein	5.7±0.5 [a]	5.8±0.5 [a]	1.7±0.1 [b]	4.4±0.4 [c]

Each value is mean ± SD for six rats in each group; Values not sharing a common superscript (a,b,c) differ significantly with each other (P<0.05;DMRT) infarcted rats

Figure 2 shows the levels of total cholesterol, TGs and FFAs in the heart mitochondria of normal and experimental rats. Isoproterenol induced myocardial infarcted rats showed

significant ($p<0.05$) increase in the levels of these lipids in the heart mitochondria compared to normal control rats. Pretreatment with vanillic acid revealed significant ($p<0.05$) decrease in the levels of these lipids in the isoproterenol induced myocardial infarcted rats compared to isoproterenol alone induced myocardial infarcted rats.

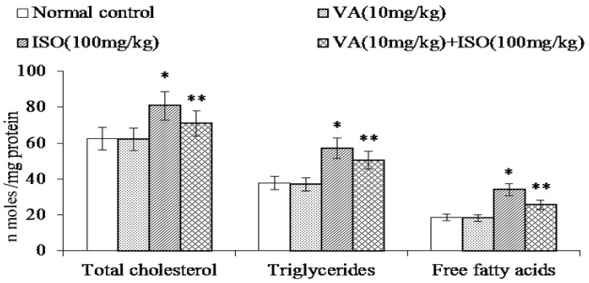

Figure-2 Effect of vanillic acid on lipids in the heart mitochondria

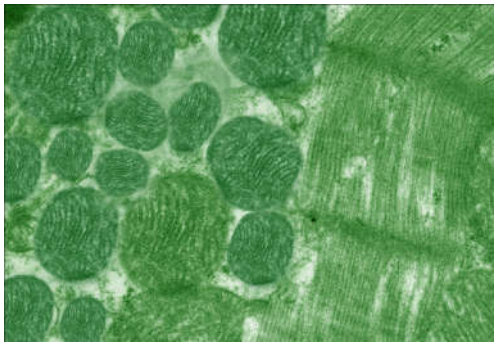

Fig. 3a Normal control rat's heart mitochondria (Group-I) showing normal architecture without any damage

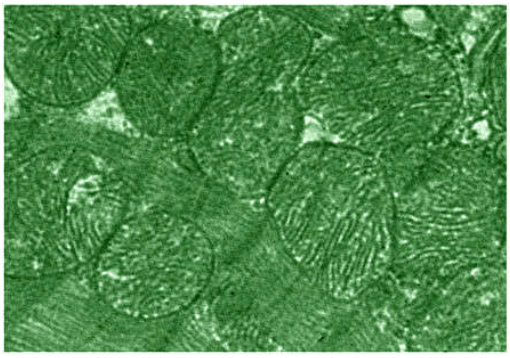

Fig. 3b Vanillic acid (10mg/kg body weight) treated rat's heart mitochondria (Group-II) also revealing normal architecture without any damage

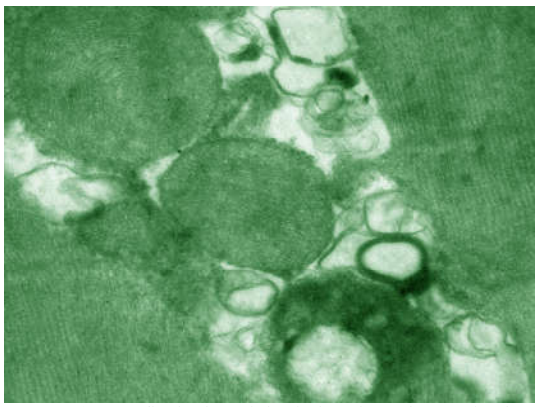

Fig. 3c ISO (100mg/kg body weight) induced rat's heart mitochondria (Group-III) revealing swelling, vacuolation and loss of cristae

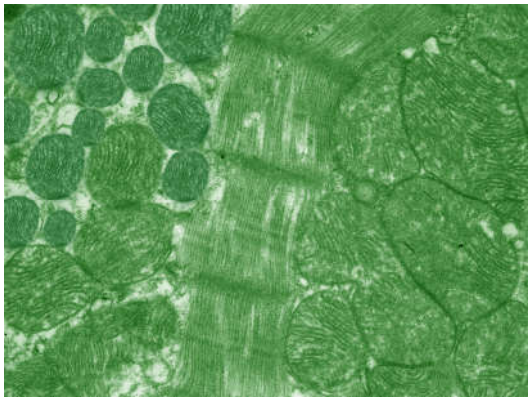

Fig.3d Vanillic acid (10mg/kg body weight) pretreated and then induced MI with ISO (100mg/kg body weight) rat's heart mitochondria (Group-IV) revealing near normal architecture without swelling and vacuolation

Figure 3 (a,b,c) TEM study on heart mitochondria confirmed the protective effect of vanillic acid. Isoproterenol induced rat's mitochondria showed damage with swelling, vaculation, loss of cristae and irregular size and shape (Figure 3c. Group: III). Vanillic acid pretreated ISO-induced myocardial infarcted rats revealed near normal mitochondrial architecture (Figure3.d. Group: IV). Thus, vanillic acid protects rat's heart mitochondria.

Oral treatment with VA daily for a period of 10 days to normal control rats did not show any significant change in all the biochemical parameters studied.

DISCUSSION

Cardiac markers or cardiac enzymes are proteins from cardiac tissue found in the blood. These proteins are released into the blood stream when damage to the heart occurs, as in the case of MI. Myoglobin is a small (17.8kD) cytosolic protein that is among the earliest markers released into the circulation after the onset of myocardial necrosis. This results in superior sensitivity of myoglobin in the ISO-induced myocardial infracted rats. Pretreatment with vanillic acid significantly ($p<0.05$) reduced the levels of serum myoglobin in ISO-induced myocardial infracted both with and without overt cardiovascular diseases.

The oxidative stress may be exerted through quinone metabolites of isoproterenol which react with oxygen to produce superoxide anions and other ROS and interfere with antioxidant. *In -vitro* scavenging effects of VA on superoxide and hydroxyl radicals. Free radical scavenging enzymes such as SOD is the first line of cellular defense against oxidative injury. The observed decrease in the activities of these enzymes in heart mitochondria might be due to increased generation of ROS, such as superoxide and hydrogen peroxide, which in turn leads to the inhibition of these enzymes. GSH protects mitochondrial membrane from the damaging action of lipid peroxidation. Decreased concentration of mitochondrial GSH indicates a major mechanism of inducing an imbalance of mitochondrial function [Raghavendran *et al*., 2005]. GSH is utilized for the inactivation of lipid peroxides through the activity of GPx which generates GSSG as byproducts.[Tappel, 1973] has reported that decrease in the activity of GPx makes mitochondria more susceptible to ISO-induced cardiac damage that leads to mitochondrial dysfunction. A phase II enzyme

such as GST not only catalyzes the conjugation of both hydroquinones and epoxides of polycyclic aromatic hydrocarbons with GSH for their excretion, but also shows low activity towards organic hydroperoxides for their detoxification from cells/tissues [Ketterer *et al*., 1987]. Our results show that VA prevented the decrease in the activities of enzymic antioxidants in ISO-induced rats.

Mitochondrial and cellular damage can be prevented by increasing intracellular GSH content. The increased levels of GSH observed in VA pretreated rats resulted in increased activities of GSH-related enzymes in the mitochondrial fraction of ISO-induced rats. VA may act as an antioxidant by scavenging ROS and also improving the endogenous antioxidant system in ISO-treated rats. Altered levels of lipids are observed in the heart mitochondrial fraction of myocardial infarcted rats. The increased levels of mitochondrial lipids indicate clear evidence for altered cardiac function and ultrastructure in MI. Activation of lipid peroxidation process resulted in changes in lipid composition. Increased levels of mitochondrial cholesterol are well associated with MI (Rouslin *et al*., 1982). Increased levels of FFAs and TGs in the heart mitochondrial fraction are observed in ISO- induced myocardial infarcted rats. When supply of oxygen is reduced as in MI, oxidation of FFAs ceases leading to increased synthesis of triacylglycerol due to FFAs accumulation (Taegtmeyer *et al*., 1985). An increased level of FFAs inhibits respiratory activities and depresses cardiac function in ischemic condition (Jackson *et al*., 1984). Alteration in myocardial metabolism resulted in increased level of TGs in the heart mitochondrial fraction of ISO - induced myocardial infarcted rats. Pretreatment with vanillic acid decreased the levels of FFAs and TGs in the heart mitochondrial fraction of ISO –induced myocardial infarcted rats. These results clearly revealed the antihyperlipidaemic effect of vanillic acid.

Our study reveals that vanillic acid is a potent reductant and the reducing power reveals its antioxidant capacity. In conclusion, the in vivo and in vitro findings obtained from our study indicate that vanillic acid (10 mg / kg body weight) offers protection to the myocardium against ISO-induced oxidative stress in rats. This could be due to inhibition of lipid peroxidation system by its potent antioxidant effect.

CONCLUSION

In conclusion, pretreatment with vanillic acid (10 mg/kg body weight) exhibits preventive effects in isoproterenol induced myocardial infarcted rats by modulating mitochondrial lipids and cardiac markers. *In vivo* studies have shown that VA at the dose of 10 mg/kg protects the mitochondria in ISO-treated rats. Thus, our study reveals that VA protects the mitochondria from damage during ISO-induced cardio toxicity by its free radical scavenging and antioxidant effects. The possible mechanism for the overall observed preventive effects of vanillic acid is due to its antilipidemic and antioxidant property. The antioxidant property of vanillic acid indirectly helps to decrease the levels of lipids, by reducing or inhibiting the lipid peroxidation in myocardial infarcted rats.

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