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Original Research

Amaranthus viridis Linn extract ameliorates isoproterenol-induced cardiac toxicity in rats by stabilizing circulatory antioxidant system

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Abstract
The present study was conducted to evaluate the antioxidant role of *Amaranthus viridis* Linn against isoproterenol (ISO) induced oxidative damage in plasma and erythrocytes of rats. Subcutaneous injection of ISO to rats for 2 consecutive days offered significant increase in the level of lipid peroxidation products, decreased the activities of antioxidant enzymes and vitamin C, vitamin E and reduced glutathione. The administration of *Amaranthus viridis* Linn (300 mg/kg body weight) increased the activities of antioxidant enzymes and reduced the concentration of lipid peroxidation products. Concentration of vitamin C, vitamin E and reduced glutathione was also high in *A.viridis* pretreated rats. The effect produced by *A.viridis* was compared with α-tocopherol. The present findings have demonstrated that the cardioprotective effects of *A.viridis* in ISO-induced oxidative damage may be due to an augmentation of the endogenous antioxidants and inhibition of lipid peroxidation.

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INTRODUCTION

Myocardial infarction (MI) is forecast to be the most common cause of death globally [1]; MI, a common accompaniment and a cause of morbidity in ischemic heart disease (IHD), characterized by necrosis of cardiac muscle induced by decreased supply of blood to a portion of the myocardium [2]. It is well known that IHD are directly or indirectly related to oxidative damage that shares a common mechanism of molecular and cellular damage [3]. Ischemic heart disease is a leading cause of death in developing countries. Recently, India has been declared as the country with an estimated 3 million deaths per year due to IHD [4]. The World Health Organization predicts that deaths due to circulatory system diseases are projected to double by 2020 [5].

Isoproterenol (ISO), a catecholamine, serves as a standard model to study the beneficial effect of many drugs on cardiac function [6]. Catecholamines rapidly undergo autooxidation and it has been suggested that the oxidative products of catecholamines are responsible for the changes in the myocardium [7]. Administration of isoproterenol depletes the energy reserve of cardiac muscle cells and cause complex biochemical and structural changes leading to cell damage [8]. MI induced by ISO has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human MI [9].

At present herbal drugs are acquiring much attention as potential source of antioxidants. They serve as excellent candidates against reactive oxygen species

(ROS) induced pathologies and also it has been found to have certain preventive measures in the treatment of IHD [10-12]. *Amaranthus viridis* L (Amaranthaceae) has been used in Indian traditional system to reduce labour pain and as antipyretic [13]. *A.viridis* has been reported to have a high concentration of antioxidant components [14, 15] It also possess antidiabetic and antioxidant activities in experimental induced diabetes [16]. We previously reported the preventive effect of *A.viridis* on serum marker enzymes, cardiac troponin in ISO induced MI in rats [17]. However, no research has been carried out to investigate the efficacy of *A.viridis* on circulatory antioxidant system in ISO-induced myocardial infarcted rats. Therefore, this study addresses the cardioprotective effect of *A.viridis* on lipid peroxidation and plasma antioxidants system in isoproterenol-induced myocardial infarction in rats.

MATERIALS AND METHODS

Animals

All the experiments were carried out with male albino Wistar rats weighing 150-180 g, obtained from Nandha College of Pharmacy, Tamil Nadu, India. They were fed with commercial pellet diet (M/S AMRUT, Pune, India) and given water *ad libitum*. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 22 ± 2ºC, and humidity of 45-64%. The protocol of this study was approved by institutional ethical committee of Nandha College of Pharmacy, Erode, India.

Drugs and chemicals

Isoproterenol and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO, USA). α-Tocopherol was purchased from Himedia, Mumbai, India, All other chemicals used were of analytical grade.

Plant material and extraction

The fresh plant of *A.viridis* was collected from local market. It was authenticated at the herbarium of Botany, Department of Biotechnology, K.S.R. College of Technology, Tiruchengode and the extraction was done based on previous method with slight modification [16]. The leaves of the plant were dried in air. 25 g of air-dried powder was added to methanol and percolated for 2 h. It was then filtered through 4 layers of muslin cloth and centrifuged at 2500g for 10 min. The supernatants were collected, pooled together and concentrated to make the final volume one-fourth of the original volume. It was then stored at 4ºC. The greenish brown extract was obtained and is dissolved in Tween (polysorbate) 80 for pharmacological studies.

Preliminary phytochemical screening

The methanol extract of *A.viridis* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins and phenolic compounds.

Experimental design

The experimental animals were divided into four groups of six rats each:

-Group I; control rats.

-Group II: normal animals were administered isoproterenol (20 mg/100 g b.w, subcutaneously twice at an interval of 24 h) in saline [6].

-Group III: animals were orally treated with *A.viridis* extract (300 mg/kg per day, for a period of 45 days and isoproterenol (20 mg/100 g) was administered subcutaneously twice at an interval of 24 h [17].

-Group IV: Animals were treated with α-tocopherol (Vitamin E, 60 mg/kg) orally using an intragastric tube daily for a period of 45 days and isoproterenol (20 mg/100 g) was administered as described above.

After the last treatment (45 days), rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected and plasma was obtained after centrifugation.

Preparation of hemolysate

From 2ml of blood, erythrocytes were separated by centrifugation at 1000×g for 10 min at 4 ◦C. The erythrocyte layer was washed three times with 10 volumes of 10 mmol/L PBS. The washed erythrocytes were suspended in phosphate buffer saline (PBS) and adjusted to a hematocrit (HCT) of 5 or 10%. An aliquot of 0.5 ml washed RBC was lysed with 4.5 ml of ice cold distilled water to prepare hemolysate.

Biochemical analysis

Plasma was used for the assay of lipid peroxidation products. The level of thiobarbituric acid reactive substances (TBARs) and lipid hydroperoxides were estimated by the methods of Yagi [18] and Jiang *et al* [19], respectively. The antioxidant enzymes such as superoxide dismutase (SOD)[20], catalase (CAT)[21] and glutathione peroxidase (GPx)[22] were assayed in erythrocytes and non-enzymatic antioxidant such as Vitamin C [23], Vitamin E [24] and glutathione (GSH)[25] were measured in plasma.

Statistical analysis

All the results were expressed as the Mean ± S.D. for six animals in each group. All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test; significance level at $p < 0.05$ was considered to indicate statistical significance.

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RESULTS

Preliminary phytochemical analysis of *A.viridis* showed the presence of flavonoids, saponins, glycosides, terpenoids amino acids, alkaloids, carbohydrates, phenolic compounds and proteins. The qualitative analysis of extract confirmed the presence of quercetin, a potent antioxidant.

Table 1 summarized the levels of TBARS and hydroperoxides in plasma of control and experimental groups of rats. The levels of TBARS and hydroperoxides were significantly increased ($p < 0.05$) in isoproterenol treated rats compared with the control rats. Oral administration of *A.Viridis* and α-tocopherol to isoproterenol treated rats significantly reversed all these changes to near normal levels.

The activities of enzymic antioxidants such as SOD, CAT and GPx in the erythrocytes of control and experimental groups of rats are shown in Table 2. The antioxidant enzyme activities were decreased significantly ($p < 0.05$) in isoproterenol treated rats when compared with those of control rats. Pretreatment with *A.Viridis* and α-tocopherol to isoproterenol-induced rats significantly increased the activities of these enzymes compared with isoproterenol alone induced rats

Table 3 reveals the levels of GSH, vitamin C and E in plasma of control and experimental groups of rats. The levels of GSH, vitamin C and E were significantly decreased ($p < 0.05$) in isoproterenol treated rats compared with the control rats. Oral administration of *A.Viridis* and α-tocopherol to isoproterenol treated rats significantly increased the levels of GSH, vitamin C and E.

Table1. Levels of TBARS and Hydroperoxides in plasma of control and experimental groups of rats

Groups	TBARS (nmol/mL)	Hydroperoxides (mM/dL)
Normal (Group I)	4.28 ± 1.09	15.32 ± 1.65
Isoproterenol (Group II)	8.23 ± 3.26[a]**	23.14 ± 4.85[a]***
Isoproterenol+ *A.viridis* (Group III)	4.98 ± 1.47[b]*	16.87 ± 2.62[b]**
Isoproterenol + Vitamin E (GroupIV)	4.77 ± 1.89[b]*	16.04 ± 2.59[b]**

Values are given as Mean ± S.D. for groups of six animals in each group. p < *0.05, **0.01, and ***0.001 for [a]compared with Group I, and [b]compared with Group II.

Table 2. Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in erythrocytes of control and experimental groups of rats.

Groups	SOD (U[a]/mg Hb)	CAT ((U[b]/mg Hb)	GPx (U[c]/mg Hb)
Normal (Group I)	14.36 ± 0.59	10.15 ± 0.79	5.39 ± 0.16
Isoproterenol (Group II)	9.25 ± 2.81[a]***	6.22 ± 2.06[a]***	2.19 ± 1.89[a]***
Isoproterenol+ *A.viridis* (Group III)	13.12 ± 0.35[b]***	9.69 ± 0.28[b]***	4.12 ± 0.36[b]**
Isoproterenol + Vitamin E (Group IV)	13.98 ± 0.22[b]***	10.03 ± 0.16[b]***	4.95 ± 0.29[b]**

Values are given as Mean ± S.D. for groups of six animals in each group. p < **0.01, and ***0.001 for [a]compared with Group I, and [b]compared with Group II. U[a], the amount of enzyme required to inhibit 50% nitroblue tetrazolium (NBT) reduction; U[b], micromoles of H_2O_2 utilized per min; U[c], micromoles of glutathione utilized per min.

Table 3. Levels of Vitamin C, Vitamin E and reduced glutathione (GSH) in plasma of control and experimental groups of rats.

Groups	Vitamin C (mg/dL)	Vitamin E (mg/dL)	GSH (mg/dL)
Normal (Group I)	4.17 ± 1.28	6.12 ± 2.84	32.65 ± 4.28
Isoproterenol (Group II)	2.55 ± 0.23[a]**	3.18 ± 1.57[a]*	24.64 ± 2.59[a]**
Isoproterenol+ *A.viridis* (Group III)	3.91 ± 0.72[b]***	5.89 ± 0.42 [b]**	30.42 ± 1.15[b]***
Isoproterenol + Vitamin E (Group IV)	4.02 ± 0.83[b]***	5.97± 0.83[b]**	31.16 ± 1.92[b]***

Values are given as Mean ± S.D. for groups of six animals in each group. p < *0.05, **0.01, and ***0.001 for [a]compared with Group I, and [b]compared with Group II.

DISCUSSION

Studies on the antioxidant changes and their significance during heart failure have provided a new insight about the pathogenesis of heart disease. During myocardial infarction, reactive oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals are produced in enormous amount [26] which contributes to myocardial tissue injury. The formation of free radicals and accumulation of lipid peroxides is one of the possible biochemical mechanisms for the myocardial damage caused by isoproterenol [27]. The results of these investigations support this study.

Excessive formation of free radicals and lipid peroxidation by auto-oxidation of isoproterenol results in irreversible damage to heart [28]. Lipid peroxidation is an indication of the severity of isoproterenol induced necrotic damage of the heart and has been linked with altered membrane structure and enzyme inactivation [10, 29]. Isoproterenol treatment showed an increase in the levels of TBARS and hydroperoxides. Elevated TBARS levels may contribute to excess generation of free radicals and/or decreased activities of antioxidant system [30]. *A.Viridis* administration decreases the levels of lipid peroxides in isoproterenol-treated rats. The antioxidant nature of quercetin, a flavonoid present in *A.Viridis* [15], may hinder the reactive oxygen species which are produced by isoproterenol.

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals [31, 32]. Free radical scavenging enzymes such as SOD, CAT and GPx are the first line cellular defense enzymes. SOD, CAT and GPx activities were decreased on isoproterenol administration. These enzymes are lowered due to enhanced lipid peroxidation [33]. Oral treatment with *A.Viridis* in isoproterenol-induced animals showed an increase in the activities of SOD, CAT and GPx suggesting that quercetin, a flavonoid present in *A.Viridis*, acts as an antioxidant that reinforces functioning of the system that scavenges endogenous oxygen free radicals so as to inhibit oxidative stress (such as lipid peroxidation) and consequently protect cardiomyocytes from oxidative damage.

Reduced glutathione is one of the non-enzymatic antioxidants present in the body [34] and efficiently scavenges free radical species. Decreased GSH levels in the present study may be due to its increased utilization in protecting SH containing proteins from lipid peroxides [6]. Isoproterenol-intoxicated rats showed a significant decrease in GSH levels in serum [32]. Treatment with *A.Viridis* to isoproterenol intoxicated rats maintained the concentration of GSH at near control levels. Since *A.Viridis* possesses quercetin, it could have been attributed to maintenance of the endogenous GSH antioxidant balance against isoproterenol mediated cellular oxidation which inhibited the oxidation of GSH.

Vitamin C is a water-soluble vitamin that can directly scavenge singlet oxygen and superoxide radicals [35, 36]. It has been suggested that Vitamin C present in aqueous environment has multiple antioxidant properties [37]. In the present study, decreased concentration of vitamin C in plasma was observed in ISO-treaded rats. The lowered level of Vitamin C might be due to increased lipid peroxidation. Pretreatment with *A.Viridis* to ISO-induced rats significantly increased the level of Vitamin C. Increase in the levels of vitamin C is due to the antioxidant potential of *A.Viridis* against injury caused by free radicals.

Data from human studies have suggested that an inverse correlation exists between serum levels of Vitamin E and mortality from IHD [38]. In our study, decreased concentration of Vitamin E in plasma was observed in ISO-treated rats. Vitamin E has been utilized more for the neutralization of ISO mediated free radicals and lipid peroxidation process [39]. Pretreatment with *A.Viridis* to ISO-treated rats significantly increased the level of vitamin E in plasma. The increased levels of Vitamin E might be due to decreased lipid peroxidation.

In conclusion, the present study depicted experimental evidence that *A.Viridis* maintained the antioxidant enzyme levels and improved cardiac performance following high-dose isoproterenol administration. This finding might be rational to understand the beneficial and therapheutic effects of *A.Viridis* on cardioprotection against myocardial injury, in which oxidative stress was long known to contribute to the pathogenesis.

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CONFLICT OF INTEREST

None

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