International Journal of Cardiology 165 (2013) 494–498

Contents lists available at SciVerse ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Cardioprotective activity of *Amaranthus viridis* Linn: Effect on serum marker enzymes, cardiac troponin and antioxidant system in experimental myocardial infarcted rats

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ARTICLE INFO

Article history:
Received 30 March 2011
Received in revised form 6 July 2011
Accepted 5 September 2011
Available online 29 September 2011

Keywords:
Antioxidants
Amaranthus viridis
Isoproterenol
Lipid peroxides
Myocardial infarction
Troponin

ABSTRACT

Background: Cardiovascular diseases (CVDs) have a high prevalence in developing and developed countries and myocardial infarction accounts for majority of deaths and disabilities. The current study dealt with the protective role of *Amaranthus viridis* Linn on isoproterenol (ISO)-induced myocardial infarction (MI) in rats.
Methods: Subcutaneous injection of ISO (20 mg/kg body weight in 1 ml saline) to rats for two consecutive days offered significant alteration in cardiac marker enzymes (AST, ALT, LDH and CPK), cardiac troponin, lipid peroxidation products (TBARS and hydroperoxide) and antioxidant system (CAT, SOD, GPx, GST, GSH and GSSG). ISO-induced myocardial damage was indicated by increased activities of marker enzymes in serum and the levels of cardiac troponin in the serum. In addition to these diagnostic markers, the levels of lipid peroxidation products in the heart were significantly ($p<0.05$) increased and the activities of enzymic antioxidants and non-enzymic antioxidant such as glutathione in the heart was significantly ($p<0.05$) decreased and GSSG in the heart was increased in ISO-induced rats.
Results: Effect of *Amaranthus viridis* oral treatment (100, 200 and 300 mg/kg body weight) for 45 days elicited a significant cardio protective activity by lowering the levels of serum marker enzymes, cardiac troponin, GSSG and lipid peroxidation and elevated the levels of antioxidant enzymes and GSH. The effect at a dose of 300 mg/kg of *A. viridis* was more pronounced than that of the dose 100 mg/kg and 200 mg/kg and brought back all the parameters to near normal. The effect produced by *A. viridis* was compared with α-tocopherol.
Conclusions: 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 of membrane.

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1. Background

Myocardial infarction (MI) remains the major cause of death in the developed world and a major pathological issue worldwide despite the rapid advances that have been made in the treatment of coronary artery disease [1]. Myocardial ischemia may occur as a result of increased myocardial metabolic demand and decreased supply of oxygen and nutrients to the myocardium *via* the coronary circulation and as a result it causes cell injury known as myocardial infarction, which is one of the most lethal manifestations of cardiovascular diseases [2]. MI continues to be a major public health problem, not only in western countries but also increasingly in developing countries and makes significant contribution to the mortality statistics [3].

Isoproterenol (ISO), a synthetic catecholamine and an important regulator of myocardial contractility and metabolism serves as a standard model to study the beneficial effect of many drugs on cardiac function [4]. ISO induced cardiac necrosis include increased oxygen consumption, insufficient oxygen utilization, increased calcium overload and accumulation, changes in myocardial cell metabolism, increased myocardial cAMP levels, deranged electrolyte milieu, alterations of membrane permeability, intracellular acidosis and increase in lipid peroxides [5]. The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial alterations [4].

Medicinal plants constitute an important source of active natural products which differ widely in terms of structure and biological properties and play an important role in the development of various human diseases, including cardiovascular diseases. *Amaranthus viridis* L. (Amaranthaceae) has been used in Indian traditional system to

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doi:10.1016/j.ijcard.2011.09.005

reduce labor pain and as antipyretic [6]. *A. viridis* has been reported to have a high concentration of antioxidant components [7].

2. Study aims

A wide variety of pharmacological actions of *A. viridis* were reported—antidiabetic–antihyperlipidemic and antioxidant activities. However, no research has been carried out to investigate the efficacy of methonolic extract of *A. viridis* in ISO-induced myocardial infarcted rats. The present study has been designed to evaluate the cardioprotective activity of *A. viridis* in ISO-induced cardiac damage in rats and attempts to understand the mechanism of its therapeutic effect with reference to biochemical markers and lipid peroxidation.

3. Methods

3.1. Animals

Male Wistar rats (weighing 150–180 g) were used for the study. 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 ± 2 °C, and humidity of 45–64%. The protocol of this study was approved by Institutional Ethical Committee of Nandha College of Pharmacy, Erode, India.

3.2. Drugs and chemicals

Isoproterenol and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade.

3.3. Plant material and extraction

The fresh plants of *A.viridis* were collected from local market in ERODE, Tamil Nadu, India and was identified by Dr. P. Ponmurugan, Department of Biotechnology, K.S.R. College of Technology, India. A voucher specimen (AMA03) was deposited in Department Herbarium. The extraction of *A.viridis* was done based on the previous method (7). The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and extract was concentrated to dryness in vacuum. The greenish brown extract was obtained and is dissolved in Tween 80 for pharmacological studies.

3.4. 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 [8].

3.5. Experimental design

The experimental animals were divided into six 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 [4].
Group III: Animals were orally treated with *A. viridis* extract (100 mg/kg/day, for a period of 45 days and isoproterenol (20 mg/100 g) was administered subcutaneously once a day for 2 days.
Group IV: Animals were orally treated with *A. viridis* extract (200 mg/kg/day, for a period of 45 days and isoproterenol (20 mg/100 g) was administered subcutaneously once a day for 2 days.
Group V: Animals were orally treated with *A. viridis* extract (300 mg/kg/day, for a period of 45 days and isoproterenol (20 mg/100 g) was administered subcutaneously once a day for 2 days.
Group VI: Animals were orally treated with α-tocopherol (60 mg/kg) orally for a period of 45 days and isoproterenol (20 mg/100 g) was administered subcutaneously once a day for 2 days.

After the experimental period, all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected and serum and plasma were separated by centrifugation. Heart tissue was excised immediately and rinsed in ice-chilled normal saline. A known weight of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

3.6. Assay of cardiac marker enzymes

Activities of CK were measured in the serum using commercial kits purchased from Span Diagnostics (Chennai, India). The activities of LDH, AST and ALT were assayed in serum using commercial kits purchased from Qualigens Diagnostics (Mumbai, India).

3.7. Estimation of cardiac troponin

The levels of cardiac troponin in serum were estimated using standard kit by chemiluminescence immunoassay (Roche Diagnostics, Switzerland).

3.8. Assay of biochemical parameters

3.8.1. Estimation of TBARS

The level of tissue thiobarbituric acid reactive substances (TBARs) was estimated by the method of Yagi [9]. In this method, TBARS were measured by their reactivity with TBA in acidic conditions to generate a pink colored chromophore, which was read at 535 nm.

3.8.2. Estimation of lipid hydroperoxides

Tissue lipid hydroperoxides (LOOH) was estimated by the method of Jiang et al. [10]. In this method, oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore, which has an optical density at 560 nm.

3.8.3. Assay of SOD

Superoxide dismutase activity (SOD) was determined by the method of Misra and Fridovich [11]. Superoxide radicals react with nitro blue tetrazolium in the presence of NADH and produce formazon blue. SOD removes the superoxide radicals and inhibits the formation of formazon blue. The intensity of the color is inversely proportional to the activity of the enzyme and read at 560 nm.

3.8.4. Assay of catalase

Catalase activity was assayed by the method of Sinha [12]. In this method, dichromate in acetic acid was converted to perchromic acid and then to chromic acetate when heated in the presence of hydrogen peroxide. The chromic acetate formed was measured at 620 nm.

3.8.5. Assay of glutathione peroxidase

Glutathione peroxidase activity was assayed by the method of Rotruck et al. [13]. A known amount of enzyme preparation was allowed to react with hydrogen peroxide and GSH for a specified time period. The GSH content remaining after the reaction was measured by Ellman's reaction.

3.8.6. Assay of glutathione-S-transferase

Glutathione-S-transferase activity was assayed by the method of Habig and Jackoby [14]. In this method, the reaction mixture was incubated at 37 °C for 5 min then, 0.1 ml of reduced glutathione was added and change in the absorbance was measured at 340 nm for 3 min at 30 s interval in a Systronics UV–visible spectrophotometer.

3.8.7. Determination of reduced glutathione

Estimation of GSH in cardiac tissue was carried out by the method of Ellman [15]. This method is based on the development of yellow color when dithionitrobenzoic acid (DTNB) is added to compounds containing sulfhydryl groups.

3.8.8. Determination of oxidized glutathione

Oxidized glutathione was measured according to the method described by Aseni et al. [16]. It is based on the principle of glutathione reductase enzyme reducing GSSG to GSH with the concomitant oxidation of NADPH to NADP+. To 0.9 ml of 1.75 mol/l K_3PO_4 buffer (pH 7.0) containing 20 mmol/l NEM were added 0.05 ml of sample extract and 0.025 ml of 10 mg/ml of NADPH–Na solution. Absorbance at 340 nm was measured for 30 s immediately after addition of 0.025 ml of (10 mg/ml) glutathione reductase (GR) to the assay mixture.

3.9. 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, and significance level at $p<0.05$, 0.01, and 0.001 was considered to indicate statistical significance.

4. Results

Preliminary phytochemical analysis of *A. viridis* showed the presence of flavonoids, saponins, glycosides, terpenoids, amino acids, alkaloids, carbohydrates, phenolic compounds and proteins.

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