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Studies on curative efficacy of monoterpene eugenol on anti- leukemic drug arsenic trioxide induced cardiotoxicity

Prakash Binu, Nellikunnath Priya, Surendran Abhilash, Radhakrishnan Chandraprabha Vineetha, Raveendran Harikumaran Nair*

Physiology Research Laboratory, School of Biosciences, Mahatma Gandhi University, Kerala 686 560, India

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ABSTRACT

Background: Arsenic trioxide (As_2O_3) is emerging as a frontline agent for the treatment of acute promyelocytic leukemia (APL) but the therapeutic application is limited by its toxicity. QT prolongation, torsades de pointes and sudden cardiac death have been implicated in the As_2O_3 therapy. So eugenol is a monoterpene compound is well known for its antioxidant properties and protective effect on the cardiovascular system.
Objective: In this study, the cardioprotective effect of eugenol on cardiac electrical conductivity, tissue electrolytes, myocardial markers, antioxidant system, lipid peroxidation and nitric oxide production was investigated in male Wistar rats treated with arsenic trioxide.
Results: The Inductively coupled plasma emission spectroscopic (ICP-OES) analysis pointed out the accumulation of arsenic in heart tissue. The rats administered with arsenic trioxide (4 mg/kg body wt) exhibited myocardial damage that was manifested by the elevation of cardiac markers (LDH, CK-MB) enzymes and deterioration in the antioxidant enzymes (GSH, GST, GPx). Combination treatment with eugenol (5 mg/kg of body wt) upholds the tissue antioxidant level, Na^+/K^+ – ATPase and Ca^{2+}– ATPase activity and brings the cytosolic Ca^{2+}, K^+ and Na^+ levels near to normal value. Conjoined therapy with eugenol ameliorated the membrane peroxidation, restored the normal heart rate and rectified the prolongation of QT interval in the electrocardiogram. Histological examination of cardiac segments also supported the beneficial role of eugenol against arsenic-induced oxidative damages.
Conclusion: Our in vivo experimental findings suggest that monoterpenoid eugenol could be a potent and novel cytoprotective agent of clinical application against As_2O_3 induced cardiotoxicity.

1. Introduction

Arsenic is a metalloid that generates various biological effects. In Rome and Greece arsenic and its derivatives were used for the treatment of psoriasis and other skin related diseases [1]. The clinical trial of As_2O_3 was started in 1971 in Harbin Medical University, China. Therapeutic effects were observed in several cancer types, including lymphoma, esophageal cancer and particularly acute promyelocytic leukemia (APL). In September 2000 the US Food and Drug Administration (FDA) approved As_2O_3 (Trisenox) for the treatment of relapsed/refractory APL [2]. It was found to induce complete remissions in 80–90% of newly diagnosed patients. Promyelocytic leukemia/retinoic acid receptor (PML-RAR) fusion protein blocks the expression of genes required for normal differentiation in APL cells by interacting with cysteine-rich region present in PML protein [3]. Arsenic degrades the oncoprotein, PML-RAR and thereby induces differentiation [4]. All chemotherapeutic drugs have some kind of side effects. Cardiac adverse effects revealed during As_2O_3 therapy is a serious issue faced by clinicians and oncologists. Mathews et al., [5,6] reported the severe hepatic and cardiac stress by arsenic trioxide in rats. Well known effects of outright arsenic poisoning include ECG abnormalities, such as QRS widening, ST depression, T-wave flattening, QT prolongation and torsade de Pointes (TdP), a life-threatening polymorphic ventricular tachycardia [7]. The generation of ROS and the induction of calcium overload by anti-arsenic trioxide result in apoptosis of cancer cells but at the same time cause problems in cardiomyocytes [8]. Combination therapy is a frequently used strategy to pacify the detrimental effects by various drugs. Enhancing the antioxidant status is one of the most effective mechanisms for protecting biological molecules and ameliorating the adverse effects of toxic compounds. In the present

* Corresponding author.
E-mail addresses: harinair@mgu.ac.in, harikumarsbs@gmail.com (R.H. Nair).

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study, we have screened the compound eugenol (4-allyl-2-methoxy-phenol). Eugenol is a pale yellow oily liquid extracted from clove and a member of the allylbenzene class of chemical compounds [9]. The antioxidant action of eugenol compounds is attributed to its methoxy phenolic structure [10]. Previous studies clearly demonstrated that antioxidant activity of eugenol significantly prevents oxidative tissue damage in different experimental models [11,12]. Eugenol is non-toxic, non-mutagenic, noncarcinogenic and recognized as safe by the Food and Drug Administration [13]. Based on these potentials of this antioxidant molecule, in the present study, we hypothesized that monoterpenoid eugenol has the capacity to insulate the cardiotoxicity during APL therapy by arsenic trioxide.

2. Materials and methods

2.1. Chemicals and reagents

Arsenic trioxide, Sodium pyruvate, Reduced glutathione (GSH), Oxidized glutathione (GSSG), Phenazine methosulphate (PMS) and Nitroblue tetrazolium (NBT) were obtained from Sigma-Aldrich, Banglore, India. Eugenol was purchased from Hi-Media Laboratories Pvt. Ltd, Mumbai. 2,4-dinitro bis Nitrobenzoic acid (DTMB), Nicotinamide adenine dinucleotide (NADH), Thiobarbituric acid (TBA), Nicotinamide adenine dinucleotide phosphate (NADPH), 1-chlor, 2,4 dinitrobenzene (CDNB), Potassium chloride (KCl), Ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide (H2O2), Trichloroacetic acid (TCA), Magnesium sulfate (MgSO4), Adenosine triphosphate (ATP), Ammonium molybdate, 8-anilino-1-naphthalesulfonic acid (ANSA), sodium nitrite, Orthophosphoric acid, Naphthyl ethylene diamine dichloride were purchased from Merk Specialities Pvt. Ltd, Mumbai, India. Other chemicals and solvents of analytical grade were purchased from a local retailer.

2.2. Animals and treatment

Twenty-eight male Wistar rats weighing 180–250 g were purchased from Pharmacology Unit, Nagarjuna Herbal Concentrates Ltd, Thodupuzha, Idukki, Kerala, India and acclimatized for six days. All the animals were maintained under standard laboratory conditions of temperature (25 °C) and 12 h light and dark cycles throughout the experiment period. The rats were provided with laboratory chow (Hindustan Lever Ltd. India) and tap water ad libitum. Experiments were conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study protocol was approved by Institutional Animal Ethical committee (IAEC), School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India (Approval No: B29122014/3).

The rats were divided into four groups of seven rats each, a normal control group, a eugenol control which received 5 mg/kg b. wt of eugenol, a group administered with 4 mg/kg b.wt of arsenic trioxide and a combination group treated with 4 mg/kg b.wt of arsenic trioxide and 5 mg/kg b.wt of eugenol orally for a period of 30 days.

2.3. Electrocardiographic analysis (ECG)

Electrocardiography is a transthoracic interpretation of the electrical activity of the heart over a period of time, as detected by electrodes attached to the surface of the skin and recorded by a device external to the body. At the end of the experimental period, rats were anesthetized with Ketamine (65 mg/kg body weight) and Xylazine (5 mg/kg body weight). ECG recording was performed using Power Lab data acquisition system and analyzed with ECG Analysis Module, AD Instruments, Australia.

On the 31 st day, both the experimental and control rats were decapitated and sacrificed. The heart tissue was dissected out and washed in ice-cold saline. The tissue was minced and homogenized accordance with the experimental procedures.

2.4. Determination of tissue electrolytes

0.5 g Of heart tissue was digested by thermal acid microwave digestion at 180 °C for 20 min with Conc. HCl and Conc. HNO_3 followed by dilution with double distilled water. Then the levels of sodium, potassium and calcium ions in heart tissue were analyzed by standard inductively coupled plasma-optical emission spectroscopy (Optima 2000 DV ICP-OES, Perkin Elmer, Inc., Waltham, Massachusetts, USA).

2.5. Assay of Ca^{2+} ATPase

Ca^{2+} ATP ase activity was estimated by the method described by Hjertan and Pan [14]. Tissue homogenate (0.1 mL) was mixed with 0.1 mL of 125 mM Tris-HCl buffer (pH = 8), 0.1 mL of 50 mM $CaCl_2$, 0.1 mL of 10 mM ATP. It was incubated at 37 °C for 15 min mixed with 1 mL of ice-cold 10% TCA and centrifuged at 2000 rpm. To1 mL of the supernatant, 1 mL 2.5% ammonium molybdate, and 0.1 mL 8-anilino −1-naphthalesulfonic acid (ANSA) were added and absorbance was read at 660 nm within 10 min.

2.6. Assay of Na^+/K^+ ATPase

Na^+/K^+ ATPase activity was estimated by the method of Bonting [15]. Tissue homogenate was mixed with 1 mL of 184 mM Tris-HCl buffer, 0.2 mL of $MgSO_4$, 0.2 mL 600 mM NaCl, 0.2 mL of 1 mM EDTA and 0.2 L of 10 mM ATP. After incubation at 37 °C for 15 min, added 1 mL 10% TCA was added and centrifuged at 2000 rpm 1 mL supernatant was taken, added 1 mL of 2.5% ammonium molybdate and 0.1 mL 8-anilino-1-naphthalesulfonic acid (ANSA) were added and absorbance was read at 660 nm within 10 min.

2.7. Assay of tissue GSH

GSH was measured in tissue homogenate according to the method described by Ellman [16]. The assay mixture contained 0.1 mL of sample, 0.85 mL of PBS (0.3 M, pH 7.4), and 0.5 mL of DTNB (10 mM). The reaction was read at 412 nm, and results were expressed as μmoles of GSH/g protein.

2.8. Assay of tissue GST

GST level was assayed by the method of Habig et al. [17]. Tissue was washed in 1.15% KCl and homogenized in phosphate buffer (pH = 7.4) and centrifuged at 9000 rpm for 20 min. After centrifugation, the supernatant was mixed with 3 mL of the reaction mixture (1.7 mL Phosphate buffer + 0.1 mL of CDNB + 1.2 mL GSH) and change in absorbance was read at 340 nm for 5 min.

2.9. Assay of glutathione peroxidase (GPx)

The activity of GPx was determined by the method of Rotruck et al. [18]. Briefly, the reaction mixture contained 0.2 mL of 0.4 M of Tris-HCl buffer (pH 7.0), 0.1 mL of 10 mM of sodium azide, 0.2 mL of homogenate (homogenized in 0.4 M of Tris-HCl buffer; pH 7.0), 0.2 mL of glutathione, and 0.1 mL of 0.2 mM of H_2O_2. The tubes were incubated at 370C for 3 min, and the reaction was terminated by the addition of 0.5 mL of 10% trichloroacetic acid (TCA). To determine the residual glutathione content, the supernatant was removed after centrifugation and to this 1 mL of DTNB reagent was added. The color that developed was read at 412 nm against a