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Protective effect of syringaldehyde on biomolecular oxidation, inflammation and histopathological alterations in isoproterenol induced cardiotoxicity in rats

Sumayya Shahzad[a], Somaiya Mateen[a], P.M. Mubeena Mariyath[b], Syed Shariq Naeem[c], Kafil Akhtar[d], Waseem Rizvi[c], Shagufta Moin[a,*]

[a] Department of Biochemistry, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India
[b] Interdisciplinary Brain Research Unit, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India
[c] Department of Pharmacology, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India
[d] Department of Pathology, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, 202002, Uttar Pradesh, India

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ABSTRACT

Background: Ischemic injury during myocardial infarction (MI) is responsible for increased deaths among patients with cardiovascular disorders. Recently, research has been directed for finding treatment using natural compounds. This study was performed to investigate the effects of syrigaldehyde (SYD), a phytochemical against isoproterenol (ISO) induced cardiotoxicity model.

Methods: For induction of MI, rats were intoxicated with two doses of ISO and were treated with SYD at three different concentrations (12.5, 25 & 50 mg/kg) both prior and simultaneous to ISO administration.

Results: ISO group revealed amplified activity of marker enzymes (CKMB, LDH, AST, ALT), increased oxidation of proteins and lipid molecules. Moreover, augmentation in pro-inflammatory markers was also found. The same group also displayed marked changes in histopathology and erythrocyte (RBCs) morphology. SYD treated groups showed diminished levels of serum markers enzymes, lipid peroxidation and protein carbonyl (PC) with increment in antioxidant defense in cardiac tissues of ISO administered rats. Our findings also revealed the modulatory effect of SYD on membrane bound ATPases, showing that SYD significantly improved the ISO induced changes in membrane fluidity. Furthermore, decline in infarct size, alleviation of structural RBC damage and improved myocardial histopathological outcome were observed in treated groups. In addition, mitigation of biochemical and histopathological changes by SYD was found to be dependent on its concentration.

Conclusion: SYD had cardioprotective efficacy owing to its antioxidative and anti-inflammatory properties. Our results support incorporation of SYD in regular diet for prevention of MI.

1. Introduction

Myocardial infarction (MI), is a diseased condition associated with necrosis of cardiac muscle fibers that mainly occurs due to critical disruption of equilibrium between myocardial demand and coronary blood supply [1]. Other deleterious effects that follow the incident of MI are formation of Reactive Oxygen Species (ROS), lipid peroxides, release of inflammatory mediators, destruction of cellular membranes and defects in antioxidant defense system [2]. Though, modern treatment is effective but it is accompanied with certain adverse effects like inability to decrease infarct size and fibrosis. Therefore, there is renewed interest in finding natural products or herbal extracts having cardioprotective potential. Moreover, the plant based drugs are economical and has lesser side effects. The current study was undertaken to explore the role of syringaldehyde (SYD) in preventing MI induced cardiac damage by isoproterenol (ISO), which is a synthetically produced, non-selective β-adrenergic agonist that produces strikingly similar pathophysiological changes that are observed during MI in humans [3]. ISO oxidation generates quinone compounds which form radicals like hydroxyl, superoxide anions, hydrogen peroxide and ultimately results in intense oxidative stress and infarct like necrosis [4].

SYD is a polyphenolic compound (Fig. 1) belonging to the group of flavonoids and is found in different plant species like *Manihot esculenta* and *Magnolia officinalis* [5]. SYD has been found to exert anti-hyperglycemic effect in rat model of diabetes induced by streptozotocin [6]. Apart from antioxidant capability, SYD also has anti-inflammatory

* Corresponding author.
E-mail address: moinshagufta2@gmail.com (S. Moin).

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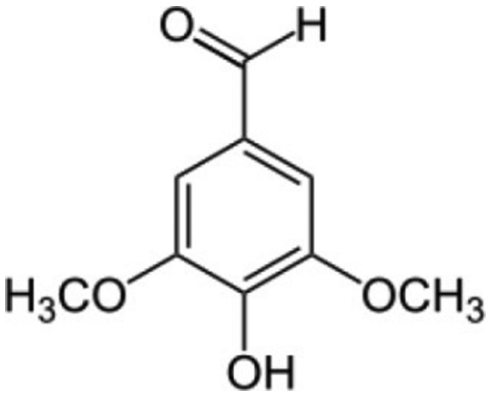

Fig. 1. Chemical structure of syringaldehyde.

activity as it was found to have inhibitory action on cyclo-oxygenase 2 (COX 2) in mouse macrophage cell line [7].

2. Materials and methods

2.1. Chemicals

Syringaldehyde and isoproterenol were purchased from Sigma Aldrich, MO, USA. All chemicals were of analytical grade.

2.2. Animals

Adult male albino wistar rats (weighing 180–220 g) were kept in polypropylene cages. They were given free access to pellet diet and drinking water under hygienic and standard conditions of 23 ± 2 °C temperature along with 12-h light/dark cycle. They were acclimated to their surrounding for a week prior to experimentation.

2.2.1. Animal ethics

The protocols and experiments of the study were approved by the Institutional Animal Ethics Committee (IAEC) of Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, India. The animals were handled according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.3. Animal treatment and experimental design

The animals were randomly divided into six groups (n = 6), first group served as control and received vehicle (orally) for 21 days. Second group was given SYD in saline orally at 50 mg/kg for 21 days. Third group received vehicle for a period of 21 days and then ISO (100 mg/kg, s.c.) on 20th and 21 st day at an interval of 24 h. Fourth group was given SYD for 21 days at 12.5 mg/kg, p.o. and ISO on 20th and 21 st day. Fifth group received SYD at concentration of 25 mg/kg, p.o. for 21 days and ISO on 20th and 21 st day. The sixth group was treated with 50 mg/kg of SYD for 21 days and ISO on 20th and 21 st day. During the experimental procedure body weight of animals were monitored and on 22nd day, 24 h after second injection of ISO, rats were sacrificed by cervical decapitation. After collection of blood sample, rats hearts were dissected out, rinsed and weighed. The blood was centrifuged to separate serum, plasma and RBCs. Heart tissue samples were homogenized in 50 mM phosphate buffered saline (PBS, pH 7.4). The homogenate was centrifuged at 6000 g for 10 min. at 4 °C and supernatant was utilized for various biochemical assays.

2.3.1. Heart weight to tibia length ratio

For each rat, heart weight (H wt), body weight and the ratio of the heart weight (H wt) to the tibia length was calculated [8].

2.3.2. Estimation of cardiac marker enzymes

Serum activity of enzyme Lactate dehydrogenase (LDH) was assayed according to Kornberg method [9]. Creatine kinase-MB (CKMB) isozyme activity was determined by standard commercial kit following manufacturer's instructions (Agappe Diagnostics, India). Alanine transaminase (ALT) and aspartate transaminase (AST) activity were evaluated by using Reitman & Frankel method [10].

2.3.3. Estimation of cardiac troponin I (c Tn I)

The serum level of c Tn I was estimated by Enzyme-Linked Immunosorbent Assay (ELISA) technique using standard kit (Kamiya Biomedical Company, Seattle, WA) following manufacturer's instruction.

2.3.4. Assay for malondialdehyde (MDA) and protein carbonyl (PC)

Cardiac MDA level was determined by following the protocol of Beuge and Aust [11] and PC content was analyzed by utilizing the method of Reznick et al. [12].

2.3.5. Assessment of antioxidant molecules and enzymes

In heart tissue homogenate, the method of Jollow et al was followed for estimation of reduced glutathione (GSH) [13]. Activity of various antioxidant enzymes were also estimated in the homogenate. Superoxide dismutase (SOD) was measured by the method of Marklund and Marklund [14]. Catalase was determined by protocol of Claiborne [15]. Glutathione peroxidase (GPx) was assessed according to Flohe and Gunzler method [16]. Glutathione-S-transferase (GST) and glutathione reductase (GR) activities were evaluated by the methods described by Habig [17] and Carlberg and Mannerick [18], respectively. The amount of protein in the samples were calculated by Lowry et al. method [19].

2.3.6. Determination of total antioxidant activity (TAC) of rat plasma

TAC was done by following the method of Erel [20]. The extent of quenching of radical 2,2′-Azino-di-[3-ethylbenzthiazdine sulphonate] ($ABTS^+$) by plasma samples of rats was taken as a representation of TAC of rats plasma.

2.3.7. Assay for membrane bound ATPases

The activities of membrane-bound enzymes, Na^+/K^+ ATPase and Ca^{2+} ATPase were assayed in the tissue homogenate by making use of the method devised by Bonting [21] and Hjerten and Pan [22], respectively.

2.3.8. Pro-inflammatory cytokines estimation

Serum level of pro-inflammatory cytokines (TNFα and IL 6) were measured by usage of specific ELISA kit as per the instructions detailed by manufacturer (Koma Biotech, Korea).

2.3.9. Estimation of nitric oxide (NO) content

Concentration of NO in heart tissue was determined by using Griess reagent, according to the method of Guevara et al. [23].

2.3.10. Scanning electron microscopy

Erythrocytes (RBCs) of rats from different study groups were washed, fixed and coated to view under scanning electron microscope.

2.3.11. Triphenyl tetrazolium chloride (TTC) staining

TTC assay was done by following the method of Lie et al. [24] to distinguish the area of necrosis from viable tissue. Photographs were taken and percent of infarct size were analyzed using image processing software (ImageJ, Version 1.52a, NIH, USA).

2.3.12. Histopathological studies

A part of the heart tissue was fixed immediately in neutral buffered formalin. Then, the tissues were embedded, sectioned and stained with hematoxylin and eosin. After staining sections were examined under