Cardioprotective effect of methanolic extract of *Syzygium cumini* seeds on isoproterenol-induced myocardial infarction in rats

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Abstract

The present study was designed to scientifically evaluate the cardioprotective potential of methanolic extract of *Syzygium cumini* (Family: Myrtaceae) seeds, a medicinal herb, on isoproterenol-induced Myocardial Infarction (MI) in rats. Five groups of albino rats, each comprising six animals, were selected for this study. Group I served as a control, Group II rats were given isoproterenol (20 mg/100 g, subcutaneously), and Group III rats were given methanolic extract of *Syzygium cumini* seeds (SME) of 500 mg/kg. Groups IV and V rats were given SME (250 mg/kg and 500 mg/kg, respectively) and isoproterenol (20 mg/100 g subcutaneously) prior to MI induction. The transaminases (Aspartate Transaminase and Alanine Transaminase), Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK), were estimated in both the serum and heart tissues, and the serum uric acid level was also estimated. Isoproterenol significantly increased the activities of CPK, LDH and the transaminases in serum with a concomitant decrease in these enzymes in tissue. Pretreatment with SME at a dose of 500 mg/kg body weight for 30 days had a more significant effect on the activities of marker enzymes compared to 250 mg/kg treated group. Serum uric acid level, which increased on isoproterenol administration, registered near normal values on treatment with SME under study. The study confirms the cardioprotective potential of methanolic extract of *Syzygium cumini* seeds against isoproterenol-induced biochemical alterations in rats.

Keywords: *Syzygium cumini*, isoproterenol, myocardial infarction

Introduction

Ischemic Heart Disease (IHD) is the leading cause of morbidity and mortality in world wide and according to the World Health Organization it will be the major cause of death in the world by the year 2020 [1]. Due to changing lifestyles in developing countries, such as India, and particularly in urban areas, Myocardial Infarction (MI) is making an increasingly important contribution to mortality statistics [2]. MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart. [3]. MI results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart [4].

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles [5]. ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes [6]. ISO induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction, [7] as the pathophysiological changes following isoproterenol administration are comparable to those taking place in human MI [8]. MI induced by ISO in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia and increase in serum creatine phosphokinase, alanine aminotransferace, aspertate aminotransferase and lactate dehydrogenase activities [9]. The mechanism proposed to explain ISO induced MI involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine to cause cell necrosis and contractile failure in rat heart [10].

Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects [11]. Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side effects and relatively low cost [12]. Now-a- days the usage of herbal drugs is gaining greater acceptance from the medical and public profession due to their positive contribution and influence on health and qualify of life. So search for indigenous cardioprotective herbal drugs is still continuing as part of scientific research.

Syzygium cumini (Syn. Eugenia cumini, Eugenia jambolana, jambul, black plum) is a tree of the family Myrtaceae distributed in Asia. The barks, leaves and seed extracts of *Syzygium cumini* have been reported to possess anti-inflammatory [13], hypoglycemic [14], antibacterial [15] and anti HIV activity [16]. In our recent studies also the methanolic extract of *Syzygium cumini* seeds has been reported to have anti-arthritic [17] and immunomodulatory [18] activities. However, there is lack of information regarding the cardioprotective effect of *Syzygium cumini*. Based on the variable significant biological effects contributed by this plant, the present was conducted to investigate the cardioprotective effect of the methanolic extract of *Syzygium cumini* seeds on myocardial necrosis induced by isoproterenol with reference to marker enzymes in the serum, heart tissues and serum uric acid.

Materials and Methods

Plant material

The fully mature *Syzygium cumini* seeds were collected locally during the month of January of 2009. The plant was botanically identified and authenticated our botanist and voucher specimen was deposited in the department herbarium.

Preparation of methaolic extract

The *Syzygium cumini* fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using cold percolation method [17]. The percentage yield was 10.34% in methanol. The extract was stored at 70^{o}C.

Preliminary Phytochemical Screening

One gram of the methanol extract of *Syzygium cumini* (SME) was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (v/v). The extract thus obtained was subjected to preliminary phytochemical screening [19, 20]

Animals

Albino rats of either sex, weighing between 320-360 g were used in the study. They were procured from National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2^{o}C and 50 ± 15% relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee of our institute. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals

Isoproterenol and Adenosine Triphosphate were obtained from Sigma Chemical Company, St. Louis, MO, USA, and all other chemicals used were of analytical grade.

Pharmacological experiment

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines of OECD [21]. One tenth of the medium lethal dose [LD_{50}] was taken as an effective dose [17]

Experimental animals

The rats were divided into five groups of six animals each. Group I served as a control, Group II rats were administered with isoproterenol (20 mg/100 g administered subcutaneously twice at an interval of 24 h) dissolved in normal saline [22]. Group III

rats were pretreated with SME (500 mg/kg) for a period of 30 days. Groups IV and V animals were pretreated with SME (250 mg/kg and 500 mg/ kg, respectively) for a period of 30 days [5] and isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 hours) at the end of the treatment period on the 29^{th} and 30^{th} days. All the drugs were administered to the respective groups by oral gavage.

Biochemical analysis

After the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected and the serum was separated and used for the assay of marker enzymes. The activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) in serum were determined spectrophotometrically by the method of Mohur and Cook [23]. The Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) were determined by the method of King [24] and by the method of Okinaka et al. [25.], respectively. The serum was also used for the assay of marker enzymes as well as uric acid, and protein was estimated by the methods of Caraway [26] and Lowry et al. [27], respectively. The heart was dissected, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes.

Data and statistical analysis

Data were expressed as mean ± SEM. The data was subjected to Student's 't' test to determine the statistical significance ($p<0.05$).

Result and Discussion

Preliminary phytochemical screening

This investigation showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids in the methanolic extract of Syzygium cumini seeds (SME).

Acute toxicity studies

From the acute toxicity study, the LD_{50} cut-off dose for SME was found to be 5000 mg/kg body weight. Hence one tenth of LD_{50} dose (500 mg/kg body weight) was selected as maximum therapeutic dose and 250 mg/kg body weight was selected as lower dose for this study.

Effect of SME on biochemical parameters

There was a significant elevation in the transaminases (AST and ALT), LDH and CPK profiles in isoproterenol injected animals compared to the controls (Table 1). In the Groups IV and V rats pretreated with SME, there was a significant reduction ($p<0.05$) in the level of uric acid and the activity of marker enzymes compared with the isoproterenol-administered rats (Group II).

Table 1. Effect of SME on different serum biochemical parameters in rats

Group	AST	ALT	LDH	CPK	Uric acid
I	32.66 ± 3.72	15.61 ± 2.46	76.40 ± 2.64	272.32 ± 4.64	3.82 ± 1.38
II	56.34 ± 2.46*	27.64 ± 4.64*	142.64 ± 3.26*	520.44 ± 4.68*	5.26 ± 2.64*
III	33.26 ± 4.56	14.68 ± 3.64	76.28 ± 1.34	270.96 ± 3.64	4.46 ± 2.64
IV	38.64 ± 2.34**	16.32 ± 2.06**	78.46 ± 1.46**	284.46 ± 3.34**	4.50 ± 2.42**
V	34.22 ± 1.28**	15.20 ±1.48**	78.10 ± 1.28**	274.64 ± 2.24**	3.96 ± 1.96**

Values were given as mean ± SEM (N=6)
SME: methanolic extract of *Syzygium cumini* seeds
AST: Aspartate transaminase (nmol of pyruvate liberated/sec/mg protein)
ALT: Alanine transaminase (nmol of pyruvate liberated/sec/mg protein)
LDH: Lactate dehydrogenase (nmol of pyruvate liberated/sec/mg protein)
CPK: Creatine phosphokinase (µmol of phosphorous liberated/sec/mg protein)
*Significance at $p<0.05$ (compared with group I)
** Significance at $p<0.05$ (compared with group II)

Rats in Group II were given isoproterenol subcutaneously. Rats in Groups IV and V were given SME and isoproterenol subcutaneously at the end of the treatment period. Compared to controls, there was a significant reduction in the activity of marker enzymes (AST, ALT, LDH and CPK) on isoproterenol administration (Group II) (Table 2). Pretreatment with SME (Groups IV and V) retained the activity of these enzymes to near normal levels. In all the parameters studied, SME at a dose of 250 mg/kg showed a minor effect, whereas doses of 500 mg/kg showed significant effect, which is found to be the most effective.

Table 2. Effect of SME on different membrane biochemical parameters in rats

Group	AST	ALT	LDH	CPK
I	45.64 ± 2.62	27.64 ± 1.36	115.64 ± 2.02	13.64 ± 3.33
II	28.62 ± 3.02*	18.64 ± 2.34*	78.32 ± 3.36*	9.36 ± 2.64*
III	46.34 ± 4.64	28.44 ± 2.64	113.46 ± 3.46	14.68 ± 2.48
IV	44.68 ± 3.62**	27.46 ± 3.06**	112.34 ± 3.24**	12.38 ± 3.08**
V	47.68 ± 2.34**	29.64 ± 4.02**	114.96 ± 2.64**	14.62 ± 2.68**

Values were given as mean ± SEM (N=6)
SME: methanolic extract of *Syzygium cumini* seeds
AST: Aspartate transaminase (nmol of pyruvate liberated/sec/mg protein)
ALT: Alanine transaminase (nmol of pyruvate liberated/sec/mg protein)
LDH: Lactate dehydrogenase (nmol of pyruvate liberated/sec/mg protein)
CPK: Creatine phosphokinase (µmol of phosphorous liberated/sec/mg protein)
*Significance at $p<0.05$ (compared with group I)
** Significance at $p<0.05$ (compared with group II)

Isoproterenol is well known cardiotoxic agent due to its ability to destruct myocardial cells. As a consequence, cytosolic enzymes such as LDH, ALT, AST and CPK were released into blood stream and serve as the diagnostic markers of myocardial tissue damage [28, 29]. The amount of these cellular enzymes present in blood reflects the

alterations in plasma membrane integrity and/or permeability. In our study, isoproterenol treated rats showed significant elevation in the levels of these diagnostic marker enzymes. Moreover, elevated levels of these enzymes are an indicator of the severity of isoproterenol-induced myocardial membrane necrosis. It is well known that isoproterenol-induced myocardial injury is mediated primarily via the β_1-adrenergic receptor. Acute β-adrenergic receptor stimulation not only rapidly generates reactive oxygen species, but also depresses total cellular antioxidant capacity, down regulates copper-zinc superoxide dismutase enzyme activity, protein and mRNA and reduces glutathione level, leading to the loss of membrane integrity and inducing heart contractile dysfunction and myocyte toxicity finally producing myocardial necrosis [30, 31].

Medicinal plants have long been valued as sources of new compounds with cardioprotective activity. In our study, SME has efficiently protected the myocardium against isoproterenol-induced myocardial infarction. Isoproterenol administration brought about a significant decrease in the activities of cardiac marker enzymes such as AST, ALT, CPK and LDH in the myocardial tissue, with a subsequent increase in the activities of these enzymes in the serum. The significant rise observed in the levels of diagnostic marker enzymes in the serum of isoproterenol administered rats as compared to that of control rats is an indication of the severity of the necrotic damage to the myocardial membrane and which is consistent with the literature [32]. Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue. The release of cellular enzymes reflects non-specific alterations in the membrane integrity and permeability as a response to β-adrenergic stimulation. The significant elevation observed in the level of serum uric acid in the isoproterenol-injected groups could be due to the excessive degradation of purine nucleotides and proteolysis [33]. In our study, we found that SME protected myocardium from isoproterenol-induced myocardial functional and structural injury via normalization levels of diagnostic marker enzymes. The data of the present study clearly showed SME modulated biochemical parameters were maintained to normal status in isoproterenol rats, suggesting the beneficial action of SME as a cardioprotective agent.

Conclusion

These findings might be rational to understand the beneficial effects of methanolic extract of *Syzygium cumini* seeds on cardioprotection against myocardial injury. The cardioprotective effect of methanolic extract of *Syzygium cumini* seeds is probably related to its ability to strengthen the myocardial membrane by its membrane-stabilising action. This cardioprotective activity of *Syzygium cumini* might be due to the presence of multiple chemical constituents in the methanolic seeds extract. However, this study warrants the investigation to isolate and identify the active principles and to elucidate the exact mechanism of action.

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