



## RESEARCH ARTICLE

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## Cardioprotective Effect of Methanolic extract of *Syzygium Aromaticum* on Isoproterenol Induced Myocardial Infarction in Rat

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**ABSTRACT**

Myocardial infarction (MI) is the interruption of blood supply to part of the heart, causing heart cells to die, commonly due to occlusion (blockage) of a coronary artery. Herbal drugs are known to exhibit creditable medicinal properties for the treatment of heart ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. Considering this aspect, the study aimed to elucidate the cardio protective activity of *Syzygium Aromaticum*. Myocardial infarction was induced by a subcutaneous administration of isoproterenol. The positive inotropic and chronotropic response of isoproterenol caused a severe oxidative stress in the myocardium through increased lipid peroxidation. Extract of *Syzygium Aromaticum* was administered at a dose of 250, 500 and 750 mg/kg for 30 days. At the end of treatment period Isoproterenol 5.25 and 8.5 mg/kg s.c. was administered on two consecutive days (31<sup>th</sup> & 32<sup>nd</sup> day). haemodynamic parameters were recorded and the hearts were subsequently removed and processed for histopathological and biochemical studies. Histological examination of rat's heart section confirmed myocardial injury with isoproterenol. Heart tissue enzyme analysis in albino (Wistar) male rats, such as LPO, GSH, GPX, GST, SOD, CAT, CK-MB, MDA and biochemical analysis in serum viz., ALT, AST, LDH, CPK were performed. Methanolic extract of *Syzygium Aromaticum* (CPE) at the dose of (250, 500 & 750 mg/kg) produced significant salvages the heart from isoproterenol induced myocardial ischemic injury. *Syzygium Aromaticum* contain eugenol, eugenol acetate, caryophyllene, sesquiterpene ester are having antioxidant, antilipid peroxidative, free radical scavenging properties and anti-ischemic activity justify its potential therapeutic value in the treatment of ischemic heart diseases in albino rats.

**Keywords** : *S. aromaticum* ( *Syzygium Aromaticum* ), MI ( Myocardial Infarction ), Extract of *Syzygium Aromaticum* ( SAE )

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## 1. INTRODUCTION

Myocardial infarction (MI) means that part of the heart muscle suddenly loses its blood supply. Without prompt treatment, this can lead to damage to the affected part of the heart. An MI is called a heart attack or a coronary thrombosis. There are different types of MI. The two main types are called ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI)<sup>1</sup>.

In a STEMI, the artery supplying an area of the heart muscle is completely blocked. In a NSTEMI, the artery is only partly blocked. The common cause of an MI is a blood clot (thrombosis) that forms inside a coronary artery, or one of its branches. This blocks the blood flow to a part of the heart<sup>2</sup>. The onset of symptoms in myocardial infarction (MI) is usually gradual, over several minutes, and rarely instantaneous<sup>3</sup>.

Classical symptoms of myocardial infarction include acute coronary syndrome, chest pain, shortness of breath, nausea, vomiting, palpitations, sweating, anxiety or a feeling of impending doom<sup>4</sup>. Risk factors for myocardial infarction includes smoking, hypercholesterolemia, hyperlipoproteinemia, high low density lipoprotein and low high density lipoprotein, Diabetes, High blood pressure, Older age, Obesity. Complications of myocardial infarction (MI) include Arrhythmias, Congestive Heart Failure, Cardiogenic Shock, Ventricular Aneurysm, Pericarditis, Dressler Syndrome and Pulmonary Embolism<sup>5</sup>.

Myocardial infarction (MI) and the resultant complication in cardiac function represent the leading cause of morbidity and mortality in developed countries. Moreover, with advanced life style in developing countries, like India, particularly in metropolitan cities, MI is making increasingly important contribution to mortality statistics of such countries<sup>6</sup>. It is well established that MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the cardiac system<sup>7</sup>.

Myocardial infarction, also known as "Heart Attack" is the death of cardiac muscle resulting from ischemia. It is by far the most important form of IHD and alone is the leading cause of death in the India, US and industrialized nations. A new look at the cardiovascular crisis in india : Vardiovascular diseases in india have quadropoled in the last 40 years and WHO estimates that by 2020 close to 60 % of cardiac patients worldwide would be Indians. The field of environmental cardiology is relatively new.

A lot of research is under way at the moment mainly focusing on environmental factors and possible correlation with cardiovascular diseases and the moving on to second hand smoke as well. These factors will cause the generation of reactive free radicals thus causing the damage to the myocytes of cardiac muscles

resulting in tissue necrosis and lipid peroxidation that results in increase in lipid profile<sup>8</sup>.

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The medicinal plants have immensely contributed to the health needs of humans throughout their existence.

Even today, almost one quarter of prescribed medicines in the world control ingredients from plant origin. These are used as a major source of drugs for the treatments of various health disorders. Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects. Nowadays, it is being realized that herbs can protect the heart from heart diseases by their cardioprotective action by providing an integrated structure of nutritional substances mainly phytochemicals which help in restoring and maintaining balanced body systems<sup>9</sup>.

Isoproterenol (ISP), a synthetic catecholamine and  $\beta$ -adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles<sup>10</sup>. ISP induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes<sup>11</sup>. ISP induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction.

Several medicinal plants have been found to possess antioxidant properties and have beneficial effects in pathological conditions like cancer, liver diseases, cataract and myocardial ischemia<sup>12</sup>.

The use of herbal medicines has been steadily increasing over the past decade. A considerable number of these plants/plant based products have been widely used. Therefore, interest in the examination of plants as potential sources of new drugs is increasing.

The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed as they are inexpensive, efficacious and safe<sup>13,14</sup>.

As few systematic scientific studies are currently available, these medicinal plants need to be investigated scientifically. The study is an effort in the same direction thus the present investigation was undertaken to evaluate the cardioprotective effects of the ethanolic extract of whole plants of *Syzygium aromaticum* in isoproterenol induced myocardial infarction in *albino (Wistar)* male rats.

Clove is the dried flower bud of *Syzygium aromaticum* (L.) (Family: Myrtaceae) an evergreen tree 10–20 m in height indigenous to India, Indonesia, Zanzibar, Mauritius and Sri Lanka<sup>15</sup>. It is one of the most

important drugs used in indigenous medicine in India, especially in Unani medicine. Clove is reported as aphrodisiac<sup>16</sup>, stomachic<sup>17</sup>, carminative, antispasmodic<sup>18</sup>.

It is reported to be useful in conceiving in high doses and act as a contraceptive in low doses<sup>19</sup> and useful in cataract<sup>20</sup>. Clove is also reported to have anticarcinogenic property<sup>21</sup>. It inhibits platelet aggregation and alters arachidonic acid metabolism in human platelets<sup>22</sup>.

It possesses antiviral activity against *Herpes simplex*<sup>23</sup>. Phytochemical studies indicate that the clove contains free eugenol, eugenol acetate, caryophyllene, sesquiterpene ester, phenyl propanoid,  $\beta$  caryophyllene, eugenol and acetylene eugenol<sup>24</sup>.

Eugenol, the major constituent, inhibits lipid peroxidation and maintains activities of enzyme superoxide dismutase, catalase, glutathione peroxidase-6 phosphate dehydrogenase, and has also been reported to have vasodilatory and smooth muscle relaxant property<sup>25</sup>. Phytochemical study of the test drug that was carried out according to the methods described by Jenkin et al<sup>26</sup>, showed that it contains alkaloids, amino acids, flavonoids, proteins, sterols, reducing sugar, tannins and phenols. However, clove, or its known compound had not been scientifically studied for their effect on sexual function.

## 2. MATERIALS AND METHODS

*S. aromaticum* was collected from local market of Bangalore, India. Flower buds were powdered with the help of grinder at Karavali College of Pharmacy, Mangalore, India. The whole powdered substance was weighed and packed in Soxhlet extractor. Solvent used for extraction was mixture of methanol and water in the ratio of 50:50. Extraction was continued at the temperature of 50 °C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40 °C. Concentrated extract was dried at 40 °C in hot air oven. Dried extract was packed in an air tight container<sup>27</sup>.

### 2.1 ANIMAL GROUPING AND *SYZYGIUM AROMATICUM*

#### TREATMENT :

The animals were divided into 5 groups (6 rats in each group):

Group I : Control 0.9 % Normal Saline

Group II : Negative Control : Isoproterenol 5.25 and 8.5 mg/kg s.c.

Group III : Standard ( Propranolol 10 mg/kg )

Group IV : Extract of *S.aromaticum* 250 mg/kg s.c

Group V : Extract of *S.aromaticum* 500 mg/kg s.c

Group VI : Extract of *S.aromaticum* 750 mg/kg s.c

## 3. EXPERIMENTAL PROTOCOL:

Male Wistar rats weighing 150–200 g were pretreated with the oral dose of 250, 500 and 750 mg/kg *S.aromaticum* for 30 days. At the end of treatment period, animals of all groups excluding group I received 5.25 and 8.5 mg/kg isoproterenol s.c. on two consecutive days (31<sup>th</sup> & 32<sup>nd</sup> day). Group III served as standard, the animals are fed with normal diet and water for 15 days and from 16<sup>th</sup> day it was given Propranolol ( 10 mg/kg I.P ) and on 29<sup>th</sup> & 30<sup>th</sup> day Isoproterenol (8.5 mg/kg ) is administered at 24 hr interval. Symptoms and mortality in each group were recorded and compared with those of rats given isoproterenol alone. 48 h after the first dose of ISP administration, rats were sacrificed by cervical decapitation method under Xylazine + Ketamine (16 + 100 mg/kg i.m.), blood samples were collected via abdominal aorta puncture using sodium citrate (3.8%w/v) as anticoagulant and the serum separated were used for the determination of diagnostic marker enzymes<sup>28</sup>.

The marker enzymes ALT, AST, LDH and CPK were assayed in serum using standard kits supplied from Swemed diagnostics, Bangalore, India. The heart tissue was excised immediately, washed with chilled isotonic saline, tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer (pH 7.2), used for the assay of clinical marker enzymes LPO & MDA<sup>29</sup>, GSH<sup>30</sup>, GPX<sup>31</sup>, GST<sup>32</sup>, SOD<sup>33</sup>, CAT<sup>34</sup> and CK-MB<sup>35</sup>.

**Values expressed:** Levels of lipid peroxides (LPO) - nmol malondialdehyde released/mg protein; Reduced glutathione (GSH) -  $\mu$ mol (oxidized min<sup>-1</sup> mg<sup>-1</sup> protein); Glutathione peroxidase (GPx) - nmol (oxidized min<sup>-1</sup> mg<sup>-1</sup> protein); Glutathione-S transferase (GST)-  $\mu$ mol (1-chloro-2, 4-dinitrobenzene conjugate formed min<sup>-1</sup> mg<sup>-1</sup> protein); Catalase (CAT) - nmol (H<sub>2</sub>O<sub>2</sub> decomposed min<sup>-1</sup> mg<sup>-1</sup>); Superoxide dismutase (SOD) - (one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation). MDA (nmol/g tissue), CK-MB (IU/mg protein) MDA: Malonaldehyde; CK-MB: Creatine phosphokinase-MB isoenzyme. One unit of CK-MB transfers 1mmol of phosphate from phosphor creatine to ADP per min at pH 7.4 at 30°C.

## 4. RESULTS:

GROUPS	ALT ( $\mu\text{mol/L}$ )	AST ( $\mu\text{mol/L}$ )	LDH ( $\mu\text{mol/L}$ )	CPK ( $\mu\text{mol/L}$ )
Control	86.67 $\pm$ 0.667	95.33 $\pm$ 1.202	120.20 $\pm$ 1.222	112.30 $\pm$ 0.843
Negative Control (Isoproterenol)	320.00 $\pm$ 1.390***	287.00 $\pm$ 1.095***	270.50 $\pm$ 1.384***	290.50 $\pm$ 1.335***
Standard (Propranolol)	106.20 $\pm$ 1.249***	109.70 $\pm$ 0.421***	133.00 $\pm$ 0.730***	128.20 $\pm$ 0.792***
SAE (250 mg/kg)	190.00 $\pm$ 1.390***	183.20 $\pm$ 1.078***	191.80 $\pm$ 0.909***	183.20 $\pm$ 1.078***
SAE (500 mg/kg)	171.50 $\pm$ 1.176***	164.20 $\pm$ 1.579***	175.70 $\pm$ 1.358***	162.50 $\pm$ 0.763***
SAE (750 mg/kg)	151.80 $\pm$ 1.014***	137.00 $\pm$ 1.065***	145.50 $\pm$ 1.335***	144.70 $\pm$ 1.626***

Table.1. Results of Biochemical Analysis in Serum.

Values were mean  $\pm$  S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test.

\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. control

GROUPS	LPO	GSH	GPX	GST	SOD	CAT	CK-MB	MDA
Control	1.16 $\pm$ 0.004	4.16 $\pm$ 0.008	2.77 $\pm$ 0.006	1228 $\pm$ 0.703	5.70 $\pm$ 0.005	8.15 $\pm$ 0.005	159.70 $\pm$ 1.338	70.22 $\pm$ 0.579
Negative Control (Isoproterenol)	2.39 $\pm$ 0.002***	2.17 $\pm$ 0.006***	1.90 $\pm$ 0.003***	734 $\pm$ 1.414***	2.07 $\pm$ 0.003***	3.34 $\pm$ 0.014***	58.40 $\pm$ 0.786***	98.30 $\pm$ 0.657***
Standard (Propranolol)	1.21 $\pm$ 0.003***	4.21 $\pm$ 0.004***	2.71 $\pm$ 0.015***	1206 $\pm$ 1.249***	5.43 $\pm$ 0.068***	7.92 $\pm$ 0.010***	150.30 $\pm$ 0.697***	65.37 $\pm$ 0.463***
SAE (250 mg/kg)	1.96 $\pm$ 0.006***	3.97 $\pm$ 0.004***	2.18 $\pm$ 0.008***	937.3 $\pm$ 4.232***	3.07 $\pm$ 0.016***	5.61 $\pm$ 0.026***	117.40 $\pm$ 0.662***	60.35 $\pm$ 0.635***
SAE (500 mg/kg)	1.77 $\pm$ 0.007***	4.11 $\pm$ 0.004***	2.38 $\pm$ 0.008***	1117 $\pm$ 2.460***	4.61 $\pm$ 0.074***	6.32 $\pm$ 0.050***	132.30 $\pm$ 0.846***	63.00 $\pm$ 0.063***
SAE (750 mg/kg)	1.48 $\pm$ 0.016***	4.18 $\pm$ 0.003**	2.54 $\pm$ 0.007***	1187 $\pm$ 2.120***	5.25 $\pm$ 0.022***	7.42 $\pm$ 0.020***	143.20 $\pm$ 0.617***	64.12 $\pm$ 0.164***

Table.2. Results of Tissue Enzyme Analysis – Heart.

Values were mean  $\pm$  S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnett's test. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. control

## 5. DISCUSSION:

Isoprenaline [1-(3,4-dihydroxyphenyl)-2-isopropyl amino ethanol hydrochloride] is a synthetic catecholamine and beta adrenergic agonist that induces severe stress in the cardiac muscle leading to development of MI. The MI is produced due to its action on the cardiac  $\beta_1$ -receptors. ISP-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membrane. A number of studies are available that suggest the crucial role of free radicals in pathogenesis of ISP-induced myocardial damage. The pathophysiological changes following ISP administration are comparable to those taking place in human myocardial alterations. Hence ISP induced myocardial infarction model was used in this study<sup>36,37,38</sup>.

ALT, AST, LDH and CPK were present in cardiac muscle, injury to these tissues results in the release of the enzyme of the blood stream. Increased levels are found in myocardial infarction. Increased levels of ALT, AST, LDH and CPK in serum 'the diagnostic markers', were due to the leakage of these enzymes as a result of necrosis induced by ISP in rats<sup>39</sup>.

ISP treated rats showed extensive necrosis due to lipid peroxidation the leakage of enzymes from the heart. Reduced necrotic changes in CPE treated animals could be the reason for the decreased activities of the marker enzymes in Group IV, V & VI animals. In the present study, ISP administration in rats resulted increased generation of cytotoxic free radicals is one among the several mechanisms proposed to explain the ISP-induced myocardial necrosis. Large number of studies has demonstrated that free radicals initiate

lipid peroxidation resulting in alteration of membrane integrity, fluidity and permeability<sup>40</sup>.

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase are the first line cellular defence against oxidative injury, decomposing O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> before interacting to form the more reactive hydroxyl radical (OH·). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. Glutathione plays an important role in the regulation of variety of cell function and in cell protection from oxidative injury. In the present study, significant reduction in the activities of glutathione-dependent antioxidant enzymes (GPX and GST) and antiperoxidative enzymes (SOD and CAT) with a concomitant decline in the level of reduced glutathione was observed in the heart tissue of Group II myocardial infarcted rats as compared to Group I normal control animals, reflecting an increased oxidative stress in isoprenaline induced myocardial injury. This is in accordance with previous investigations, which indicated that the tissue antioxidant status was being operated at diminished level in isoprenaline induced myocardial infarction condition. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study. Lowered activities of these prime antioxidant enzymes may lead to the formation of O<sub>2</sub>· and H<sub>2</sub>O<sub>2</sub>, which in turn can form hydroxyl radical (OH·) and bring about a number of reactions harmful to the cellular and subcellular membranes in the heart tissue. Reduction noticed in the activities of the anti peroxidative enzymes in isoprenaline-induced myocardial infarction might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn lead to the inactivation of these enzyme activities<sup>41</sup>.

Phytochemical studies indicate that the clove contains free eugenol, eugenol acetate, caryophyllene, sesquiterpene ester, phenyl propanoid, β caryophyllene, eugenol and acetylene eugenol.

Pre-treatment with orally administered Extract of *S.aromaticum* led to the retention of near normal activities of the clinical marker enzymes in the serum and cardiac tissue. Pretreatment with extract of *S.aromaticum* was associated with a decreased release of enzymes from the cardiac cell fractions, which could be due to the membrane stabilizing effect of extract of *S.aromaticum* on the cardiac cell membrane.

The extract of *S.aromaticum* has been reported to possess phenolic compound and flavanoids which

exhibit lipid peroxidation, antioxidant and free radical scavenging properties.

In the present study, we found that the extract of *S.aromaticum* protected myocardium from isoproterenol-induced myocardial functional and structural injury. The data of the present study clearly showed that extract of *S.aromaticum* modulated most of the biochemical analysis and tissue enzyme analysis parameters were maintained to normal status in isoproterenol rats, suggesting the beneficial action of the extract of *S.aromaticum* as a cardioprotective agent.

These findings might be rational to understand the beneficial effects of extract of *S.aromaticum* on cardioprotection against myocardial injury. The flower bud was found to be most effective in the functional recovery of the heart and restoration of biochemical and tissue enzyme alterations. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of the extract of *S.aromaticum*.

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