Research Article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Cardio Protective Effect of *Caesalpinia crista* Linn. on Isoproterenol Induced Myocardial Necrosis in Rats

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ABSTRACT

The alcoholic and aqueous extract of *Caesalpinia crista* Linn (*Caesalpiniaceae*) was evaluated for protection against isoproterenol (85 mg/kg b.w.) induced myocardial infarction in albino rats. The heart damage induced by isoproterenol was indicated by elevated levels of the marker enzymes such as Creatine Kinase-isoenzyme (CK-MB), Lactate dehydrogenase (LDH), Serum Glutamate Oxaloacetic Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) in serum with increased lipid peroxide and reduced glutathione content in heart homogenates. Microscopical examination (histopathology) was also performed on the myocardial tissue. Pretreatment with an ethanolic and aqueous extract of *Caesalpinia crista* Linn at a dose of 400 mg/kg body wt., administered orally for 30 days, reduced significantly ($p < 0.01$) the elevated marker enzyme levels in serum and heart homogenates in isoproterenol-induced myocardial infarction. Histopathological observation revealed a marked protection by the extract in myocardial necrotic damage.

KEYWORDS: *Caesalpinia crista* Linn; myocardial infarction; isoproterenol; Lipid profile

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INTRODUCTION

Myocardial infarction (MI) is caused due to an interruption in blood supply to any part of heart, resulting in death of cardiac tissue (Myocardial necrosis; MN). Consequences of MI include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity[1]. Cardiovascular diseases (CVDs) have a high prevalence in developing and developed countries and MI accounts for majority of deaths and disabilities[2].

Isoproterenol (isoprenaline) (ISO), a synthetic catecholamine and β-adrenergic agonist, has been found to induce myocardial injury in rat resulting in infarct like necrosis of the heart muscles[3]. Some of the mechanisms proposed to explain the Isoproterenol-induced injury to myocardial cells include hypoxia[4], calcium overload[5], depletion of energy reserves[6] and excessive production of free radicals resulting from oxidative metabolism of catecholamines[7]. The pathophysiological and morphologic alterations in the heart of this non-coronary myocardial necrotic rat model are similar to those taking place in human myocardial infarction[8]. Isoproterenolinduced myocardial infarction is considered a well standardized model to study the beneficial effects of many drugs and cardiac functions[9]. By studying the biochemical alterations that take place in an animal model, it is possible to gain more insight into the mechanisms leading to the altered metabolic process in human MI.

The side effects of medicines currently used to treat myocardial infarction, had led to a growing interest in establishing the therapeutic potentials of medicinal plants. The use of plant extracts for medicinal purposes seems to be more natural, cheaper and without side effects. Efforts are now being focused on the prophylactic and therapeutic effect of many plant extracts with antioxidant activity in reducing isoprenaline-induced cardiovascular toxicity[10,11]. Antioxidants not only suppress the formation of ROS but also have a modulatory effect on the survival and death signaling of ROS[12].

Caesalpinia crista Linn. is an important plant that also belongs to the family *Caesalpiniaceae*. It is a popular traditional medicinal plant which is widely distributed throughout the tropical and subtropical regions of Southeast Asia. In hindi, it is known as karanjwa. Seed kernels of this plant have been used as an antimalarial and anthelminthic. Plants of this family have been reported for various activities, e.g., *Caesalpinia crista* is used as tonic for the treatment of rheumatism and backache while *Caesalpinia pulcherrima* is applied as abortifacient and emmenagogue. The active constituents of these two species are mainly diterpenoids, flavonoids and peltogynoids. Some of the constituents of this species are known to possess antitumor, antimicrobial and antimalarial properties. It is also used as an anthelmentic. Ten new diterpenes from the *Caesalpinia crista* from Indonesia and twenty new diterpenes from Myanmar

have been isolated[13]. There is an emerging interest in the use of naturally occurring antioxidants for the management of a number of pathophysiological conditions, most of which involve free radical damage[14].

MATERIALS AND METHODS

Chemicals

Isoproterenol hydrochloride was purchased from Sigma chemical company. Diagnostic kits used for the estimation of marker enzymes CK-MB, LDH, SGOT, SGPT and for plasma profile reagents were procured from Span Diagnostic Ltd. India. All other biochemical reagents and chemicals were of analytical grade.

Animals

Healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study. All the animals were procured from the Central Animal House of the NIMS University. The animals were acclimatized by keeping them in the animal house facility of NIMS Institute of Pharmacy, Jaipur for a week. They were housed in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (25±2^{o}c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee (IAEC) of NIMS Institute of Pharmacy, Jaipur was taken for conducting cardioprotective activities. (Registration No. IAEC /NIMS PH/JPR/12/2011)

Plant

Caesalpinia Crista L seeds were collected from local market of Ropar (Punjab) authenticated by Botanical Department of Rajasthan University, Jaipur, Rajasthan.. The authenticated parts were dried in shade and powdered coarsely.

Extraction:

Extraction was done according to standard procedures using analytical grade solvents. Coarse powder of seed of *Caesalpinia Crista* (150 gm) was soxhlet extracted successively with petroleum ether and absolute alcohol (at 60^{o} C). The aqueous extract was prepared using 600 g coarse powder by maceration process. The extracts obtained were concentrated under reduced pressure to yield ethanolic (13.5%) and the aqueous extracts (18.6 %). Qualitative chemical tests were conducted for ethanolic and

aqueous extracts of *Caesalpinia Crista to* identify the various phytoconstituents. The various tests conducted and observations are recorded.

Cardioprotective Activity-

Isoproterenol Induced Myocardial Infarction[15-17]

Albino Wistar rats either sex were divided into four groups (n = 6). Plant extracts were treated for 30 days. At the end of the treatment period, groups II, III and IV were administered with isoproterenol at a dose of 85 mg/kg body wt., subcutaneously, twice, at an interval of 24 h.

Groups	**Treatment**
Group I	Normal Control
Group II	Isoproterenol (IP)
Group III	IP + CCAE
Group IV	IP + CCEE

Estimation of Plasma lipid profile in rats

Plasma total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were analyzed using commercially available kits .Very low density lipoproteins (VLDL) and low density lipoprotein (LDL) were measured.

Estimation of serum enzyme levels in rats

After experimental period blood was withdrawn from the retro orbital sinus, the serum was separated by centrifugation and was used for the estimation of marker enzymes CK-MB, LDH, SGOT and SGPT using AGAPPE diagnostic kits.

Animals were sacrificed and heart was isolated and subjected to histopathological studies.

Estimation Cardiac endogenous antioxidants in heart homogenates

Hearts of the animal of each group were removed by dissection, and three hearts from each group were chosen for lipid peroxide content and glutathione content measurements. Cardiac tissue pieces from control and treated groups were Weighed and homogenized (10%w/v) in chilled Tris buffer (10 mM, pH7.4), centrifugedat10,000 g for 20 min in high speed cooling centrifuge. Clear supernatant was used for assaying lipid peroxidation, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH).

Histopathological studies

Hearts of the animal of each group were removed by dissection. All the groups were subjected to histopathological studies. The tissues were fixed using a 10% formalin solution in a phosphate buffer. All hearts were sent to NIMS hospital pathology laboratory for observation of histopathological changes in hearts

RESULT

Table 1 shows control group recorded the plasma TC (62.84±1.91), TG (46.44±0.98), LDL (31.99±1.27), VLDL (31.99±1.27), and HDL level (27.8±1.12). Isoproterenol (IP) group recorded significant increment ($P<0.05$) in plasma TC (106.31±2.03), TG (116.7±3.29), LDL (91.16±2.04), VLDL (14.6±0.94), along with a significant decrement in HDL level (13.46±0.78) compared to control group. CCAE group showed significantly decrement plasma TC (87.45 ±1.5), TG (91.59±2.12), LDL (67.79±1.80), VLDL (12.46±0.68), along with a significant increased in HDL level (18.67±0.72) when compared to untreated isoproterenol group. Ethanolic extract of Caesalpinia Crista (CCAE) + Isoproterenol treated group showed decrease lipoproteins level except HDL of plasma. CCAE group showed significantly decrement plasma TC (81.23±1.99), TG (73.82±1.34), LDL (60.34±1.56), VLDL (10.53±0.54), along with a significant ($P<0.01$) increased in HDL level (19.38±1.25) when compared to untreated isoproterenol group.

Table 1: Effect of different extracts on plasma lipid profile in Isoproterenol induced myocardial infarction in rats.

Group	Total Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control	62.84±1.91	46.44±0.98	27.8±1.12	31.99±1.27	6.98±0.87
Isoproterenol	106.31±2.03^{C}	116.7±3.29^{C}	13.46±0.78^{C}	91.16±2.04^{C}	14.6±0.94^{C}
CCAE	87.45 ±1.5^{c}	91.59±2.12^{c}	18.67±0.72^{a}	67.79±1.80^{c}	12.46±0.68^{b}
CCEE	81.23±1.99^{c}	73.82±1.34^{c}	19.38±1.25 ^{b}	60.34±1.56^{c}	10.53±0.54^{c}

Data were expressed as mean±S.D. (n=6) and analyzed by one way ANOVA followed by dunnets comparison test.
a-($P<0.05$), b-($P<0.01$), c-($P<0.001$) when ISO v/s extracts,
A-($P<0.05$), B-($P<0.01$), C-($P<0.001$) when Normal v/s ISO

Cardiac Antioxidants

Table 2 shows Isoproterenol treated group recorded significant (P<.001) decrement in cardiac SOD content (3.22±0.34) and lowered activity level of GSH (2.92±0.13), CAT (2.52±0.24) and GST (528.6±13.34) when compared to control group.

CCAE treated group showed increased in cardiac SOD content (6.93±0.22) and lowered activity level of GSH (6.93±0.43), CAT (6.45±0.36) and GST (723.6±14.65) when compared to isoproterenol group. There was also significant prevention in the reduction of the levels of enzymatic antioxidants SOD (6.88±0.16), GSH (6.53±0.42), CAT (6.72±0.16), GST (746.2±12.45) by pretreatment of CCEE when compared with isoproterenol group.

Table 2: Effect of Plants extracts on Cardiac antioxidants levels in Isoproterenol induced myocardial infarction in rats.

Group	SOD	GSH	CAT	GST
Normal Control	8.51±0.18	8.76±0.35	7.23±0.37	812.3±11.32
Isoproterenol	3.22±0.34 ^{C}	2.92±0.13 ^{C}	2.52±0.24 ^{C}	528.6±13.34 ^{C}
CCAE	5.92±0.42 ^{c}	6.21±0.67 ^{b}	5.23±0.21 ^{b}	696.3±11.42^{c}
CCEE	6.88±0.16 ^{c}	6.53±0.42 ^{b}	6.72±0.16 ^{c}	746.2±12.45^{c}

Data were expressed as mean±S.D. (n=6) and analyzed by one way ANOVA followed by dunnets comparison test.
Unit for SOD: Units/mg protein, GSH: µg/mg protein, CAT: µmol of H_2O_2/min/mgpr, GST: µmol/min/mgpr
a-(P<0.05), b-(P<0.01), c-(P<0.001) when ISO v/s extracts,
A-(P<0.05), B-(P<0.01), C-(P<0.001) when Normal v/s ISO

Plasma Marker of Cardiac Damage

Table 3 represents the effects of isoproterenol and plants treatment on cardiac marker enzyme including CK-MB, LDH, AST, ALT and uric acid level in plasma. Plasma marker CK-MB (97.67±8.76), ALT (52.12±5.45), AST (48.45±6.32), LDH (96.32±8.61) and uric acid (1.44±0.12) in normal control were recorded in normal control group. The activities of these enzyme marker CK-MB (176.45±10.76), ALT (155.23±9.32), AST (120.62±5.21), LDH (185.56±4.54) and uric acid (5.12±0.45) were increased significantly (P<0.001) in isoproterenol treated rats as compared to normal control group rats. CCAE pretreatment in isoproterenol treated animals significantly (P<0.01) for CK-MB (162.66±9.2) and ALT (165.12±9.23), (P<0.001) for (AST (78.98±3.52), LDH (156.42±6.87) and uric acid (3.96±0.34)

decreased cardiac marker enzyme including CK-MB, LDH, AST, ALT and uric acid level when compared to untreated isoproterenol group. The activities of these enzyme marker CK-MB (144.87±5.32), ALT (75.56±9.12 2), AST (68.32±6.54), LDH (126.65±6.76) and uric acid (2.54±0.31) were decreased significantly (P<0.001) in CCEE pretreatment in isoproterenol treated group when compared to untreated isoproterenol group.

Table 3: Effect of plants extracts on plasma markers of cardiac damage in Isoproterenol induced myocardial infarction in rats.

Group	CK-MB (IU/L)	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	Uric acid (mg/dl)
Normal Control	97.67±8.76	52.12±5.45	48.45±6.32	96.32±8.61	1.44±0.12
Isoproterenol	176.45±10.76^{C}	155.23±9.32^{C}	120.62±5.21^{C}	185.56±4.54^{C}	5.12±0.45^{C}
CCAE	162.66±9.21^{b}	165.12±9.23^{b}	78.98±3.52^{c}	156.42±6.87^{c}	3.96±0.34^{c}
CCEE	144.87±5.32^{c}	75.56±9.12^{c}	68.32±6.54^{c}	126.65±6.76^{c}	2.54±0.31^{c}

Data were expressed as mean±S.D. (n=6) and analyzed by one way ANOVA followed by dunnets comparison test.
a-(P<0.05), b-(P<0.01), c-(P<0.001) when ISO v/s extracts,
A-(P<0.05), B-(P<0.01), C-(P<0.001) when Normal v/s ISO

Histopathological Study of Cardiac Tissue

HE staining of cardiac tissue from normal control rats showed histoarchitecture of myofibers that characteristically multinucleated and there is no visible necrotic damage to the myocytes. However, extensive myocytes membrane damage, myonecrosis, fibroblastic proliferation and infiltration of inflammatory cells was observed in isoproterenol group. IP treated rats showed large pale yellow coloration was suggestive of necrosis. However, IP+ aqueous extract of *Caesalpinia Crista* and ethanolic extract of *Caesalpinia Crista* showed relatively less disruption of myofibers and mild muscle separation with few inflammatory cells.

DISCUSSION

Myocardial Infarction (MI) is a major public health concern and common presentation of ischemic heart disease. It is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in necrosis of the myocardium. Isoproterenol-induced myocardial infarction is a

standardized model to study the beneficial effects of many drugs and antioxidants. ISO produced reactive oxygen species (ROS) *via* its auto-oxidation and subsequently produced oxidative stress. Higher level of catecholamines depletes the energy reserve of cardiac muscle cells, leading to complex biochemical and structural changes that cause irreversible cellular damage and ultimately necrosis[18].

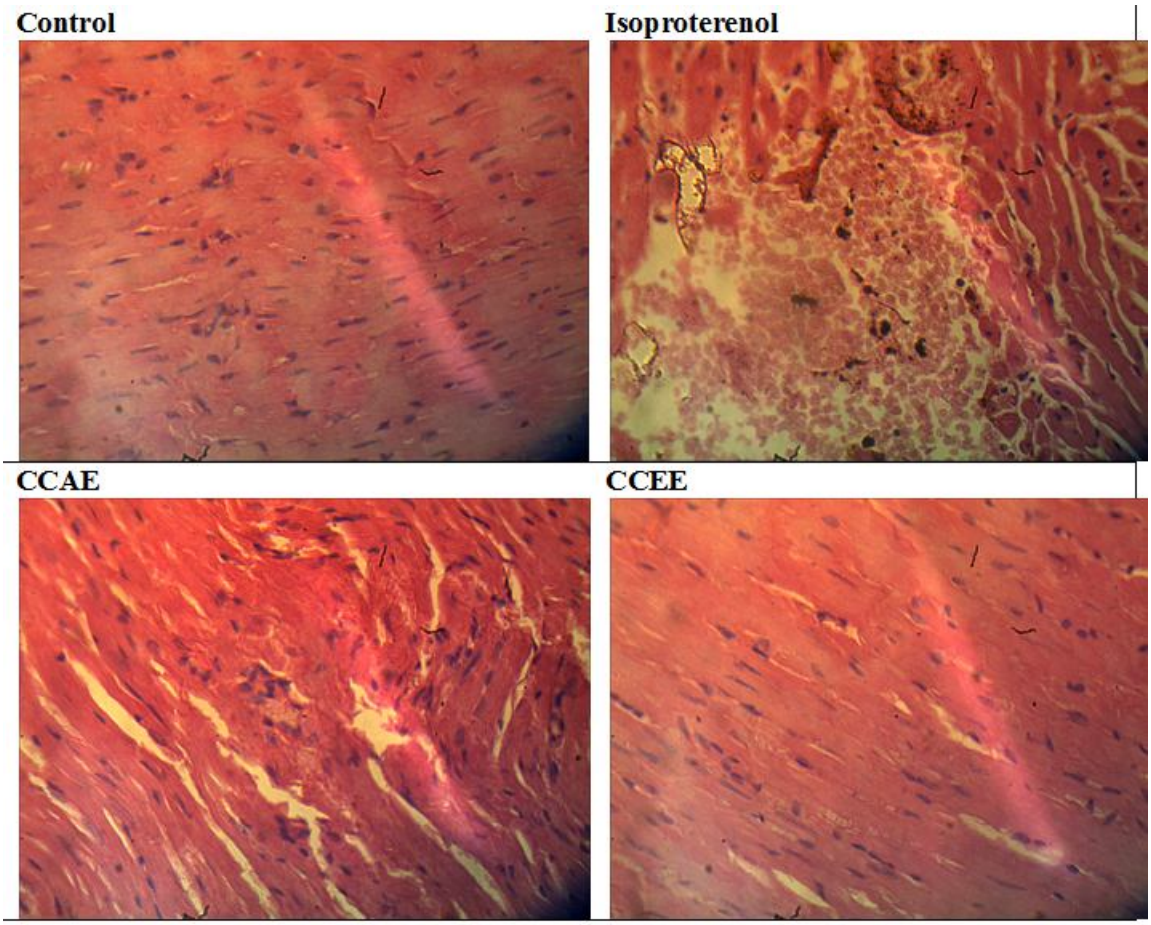

Fig.1. (A) Photomicrograph showing normal architecture of normal rat heart. (B) Photomicrograph of rat heart to isoproterenol induced focal myonecrosis with myopahgocytosis and lymphatic infiltration. Vacuolar changes and oedema are prominent with chronic inflammatory cells visible. (C) Photomicrograph of rats heart of aqueous extract *Caesalpinia Crista* treated groups showing lesser degree of myonecrosis and infiltration of inflammatory cells. (D) Photomicrograph of rats heart of ethanolic extract *Caesalpinia Crista* respectively treated groups showing also lesser degree of myonecrosis and infiltration of inflammatory cells and showed healthy myofibers. (H X E).

Several mechanisms of isoproterenol induced myocardial infarction have been reported. Isoproterenol acts both on β1 and β2 adrenoceptors, activation of which leads to positive inotropic and chronotropic effects. Thus, isoproterenol produces relative ischemia due to myocardial hyperactivity and coronary hypotension[19]. Other probable mechanisms include increased cyclic adenosine monophosphate , increased intracellular Ca++ overload, depletion of high energy phosphate stores and oxidative stress.

Increased generation of cytotoxic free radicals, due to the auto-oxidation metabolic products of isoproterenol, is one of the well recognized mechanisms of isoproterenol induced myocardial necrosis. Isoproterenol, upon auto-oxidation produces quinones which react with oxygen to produce superoxide anion (O2 −) and H2O2. The production of superoxide radical results in secondary formation of H2O2 and hydroxyl radical (•OH)[20].

In the present study, with the focus on the protective effects of aqueous and ethanolic extracts of Caesalpinia Crista pre-treatment improve against isoproterenol induced myocardial infarction in rats. It showed that pre-treatment with of these indigenous plants could prevent myocardial infarction induced by ISO in the rats. Free radical scavenging enzymes such as SOD, catalase and GPx are the first line of cellular defense system against oxidative stress, eliminating reactive oxygen radicals such as superoxide and hydrogen peroxide and preventing the formation of more deteriorating hydroxyl radicals.

The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like myocardial infarction, the generation of reactive oxygen species can dramatically disturb this balance with an increased demand of the antioxidant defense system [21]. As discussed earlier, isoproterenol auto-oxidation leads to generation of enormous amounts of reactive oxygen species. These reactive oxygen species may attack any type of molecules, but their main target appears to be polyunsaturated fatty acids (PUFAs) within membranes forming peroxyl radicals. These radicals then attack adjacent fatty acids within membranes causing a chain reaction of lipid peroxidation [22].

In the present study isoproterenol administration resulted in marked elevation in SOD, catalase and GPx and GST. Activities of antiperoxidative enzymes (SOD and catalase) were decreased significantly in the heart tissue of isoproterenol injected animals. SOD is a class of enzymes, which catalyses the dismutation of two superoxide radicals to form hydrogen peroxide and molecular oxygen. In the present study, It was observed a decreased concentration of GSH in the heart and decreased activities of glutathione dependant enzymes such as GPx and GST in the heart of isoproterenol injected rats. GSH, a tripeptide, is one of the most abundant non-enzymatic antioxidant bio molecules present in the body[23]

Cytosolic enzymes CK-MB, LDH, AST, ALT and uric acid which serve as the diagnostic markers leak out from the damages tissue to blood stream when cell membrane becomes permeable or rupture. The amount of these cellular enzymes in serum reflects the alterations in plasma membrane integrity and/or permeability [24,25].

Lipids play an important role in cardiovascular diseases, not only by way of hyperlipidemia and the development of atherosclerosis, but also by modifying the composition, structure and stability of the

cellular membranes. Isoproterenol administration raised total cholesterol, TG, LDL cholesterol, VLDL and decreased HDL cholesterol level in the serum of Group II animals. Interestingly, treatment with aqueous and ethanolic extracts of *Caesalpinia Crista* reversed the effects of isoproterenol.

Further, histopathological findings confirmed the induction of myocardial infarction by isoproterenol and the protection rendered by extracts treatment to the cardiac muscle (Fig. 1). Histopathological examination of myocardial tissue in control illustrated clear integrity of the myocardial cell membrane and no inflammatory cell infiltration was observed. Isoproterenol injected rats showed coagulative necrosis, separation of cardiac muscle fibers and infiltration of inflammatory cells. The reduced inflammatory cell infiltration and normal cardiac muscle fiber architecture further confirmed the cardioprotective effect of plant extracts. The active constituents of *Caesalpinia Crista* mainly diterpenoids, flavonoids, peltogynoids and other polyphenolic compound could be beneficial due to their antioxidant properties .

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