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CARDIOPROTECTIVE EFFECT OF *CASSIA AURICULATA LINN.,* PETAL EXTRACT ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN MALE ALBINO RATS

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ABSTRACT

Protective effect of *Cassia auriculata Linn.,* floral extract was examined against Isoproterenol induced Myocardial infarction in male albino rats. The oral administration of aqueous extract of *Cassia auriculata,* afforded protection against Isoproterenol induced alterations in Cholesterol, LDL, HDL, TG, Protein, AST, ALT, LDH, Catalase and GPx. The protective effect was further supported by the histological observations. The results clearly demonstrate that *Cassia auriculata* flowers have potent cardioprotective effect.

INTRODUCTION: Heart is an incredible organ, which determines survival of an individual. The basic physiological function of heart is to make blood circulation, although we speak of heart as seat of emotions, affections, mind, soul, etc. The branching muscle fibers of myocardium connected to each other in the form of network allowing contraction to begin at one point in the heart and spread outwards in all directions.

Myocardium contracts and relaxes rhythmically. The natural rhythm is generated with in the muscles itself, not by impulses from the nervous system, hence human heart is called myogenic. It does not fatigue despite rapid rhythmic action over many years.

The rhythm of heart lasts until the end of life, in certain cases the rhythm of heart shows abnormalities with in short period to end the life. Hence it is our responsibility to protect heart and ensure its functioning for a longer period in each individual's life.

Heart disease is the leading cause of death. Many of the risk factors for heart disease are influenced by life style. For example, smoking, lack of exercise and consumption of a high fat diet all contribute to risk. A healthy diet is important for both prevention and treatment of cardiovascular disease. It is essentially a clinical syndrome of characteristic chest pain produced by increased work of the heart.

The major underlying cause of cardiovascular disease has been associated with atherosclerosis. Arteries that supply the heart with blood may become narrowed due to atherosclerosis. This condition is called coronary artery disease. Prolonged high blood pressure, heart attack and other cardiovascular diseases can cause congestive heart failure. In coronary heart disease these arteries become narrowed and the heart is starved of blood and oxygen. Ischemic heart disease is caused by an imbalance between the myocardial blood flow and the metabolic demand of the myocardium.

Isoproterenol (ISO), a synthetic catecholomine causes myocardial cell damage when administered in large doses [1, 2].

Because of its cardiac stimulatory action it may increase systolic blood pressure slightly, but it reduces mean arterial and diastolic blood pressure.[3]. Isoproterenol (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac damage. ISO generates highly cyto toxic free radicals through auto oxidation of catecholamine. Medicinal plants are used as a major source of drugs for the treatment of various health disorders.

The present study was undertaken to evaluate the effect of aqueous *Cassia auriculata* petal extract on isoproterenol induced myocardial damage, in male albino rats.

MATERIALS AND METHODS:

Collection of Plant Materials: The flowers of *Cassia auriculata* were collected from near by villages of Trichy, were carefully identified with the help of available literature and a botanist.

Animals: Male albino rats of average body weight (150-200g) were taken and acclimatized in the laboratory conditions for 7 days and they were randomly grouped into four groups with three animals each. They were maintained at room temperature under standard laboratory condition, and fed with commercial pellet diet, with an unlimited supply of drinking water.

Chemicals: Isoproterenol was purchased from Nice chemicals Pvt. Ltd., Cochin. All other chemicals and reagents used in this study were procured from Qualigen and Ranbaxy fine chemicals Pvt. Ltd., Mumbai. All chemicals were used of analytical grade.

Extraction of Plant Material: The petals of flowers were carefully separated, dried at room temperature for two weeks. Aqueous and alcoholic extracts were prepared according to the methodology of Indian pharmacopoeia. The shade dried flower petals were subjected to pulverization to get coarse powder. The powdered material was subjected to soxhlet extraction with distilled water. It was concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). The aqueous extract was put in airtight container, stored in a refrigerator.

Induction of myocardial ischemia: Myocardial infarction was induced by subcutaneous injection of isoproterenol hydrochloride (ISO, 85 $mgkg^{-1}$ bw), dissolved in physiological saline, for 2 consecutive days.

Experimental design:

Group I → Animals served as normal control

Group II → Animals served as experimental control

Group III→ Animals fed with *Cassia auriculata* Petal extract alone (250 mg/ kg b.w.,)

Group IV → Animals fed with Cassia *auriculata* Petal extract (250mg/Kg b.w.,) and Isoproterenol induced MI (85 mg kg−1 b.w.,).

Phytochemical Analysis: Phytochemical analysis for major phytoconstituents of the plant extract was undertaken using standard methods as described by. [4] The plant extracts were screened for the presence of biologically active compounds like sugars, aminoacids, proteins, phenols, terpenoids, etc .

Biochemical parameters such as cholesterol, HDL, LDL, TG, protein, ALT, AST, LDH, catalase, glutathione peroxidase were evaluated using standard procedures.

RESULTS: In the present study, the effect of aqueous extract of *Cassia auriculata* on isoproterenol induced myocardial infraction was evaluated through various biochemical parameters and the results were summarized as follows.

Table 1 shows the results of qualitative phytochemical analysis, which shows the presence of alkaloids, flavonoids, volatile oil, quinones and coumarins. **Table 2** shows the results of Cholesterol and Triglycerides levels. **Table 3** shows the results of LDL and HDL levels. **Table 4** shows the results of AST and ALT levels. The results of LDH and Catalase levels are presented in **Table 5**. **Table 6** shows the results of Protein and Glutathione peroxidase levels.

TABLE 1: PRELIMINARY PHYTO CHEMICAL ANALYSIS OF THE AQUEOUS EXTRACT OF *CASSIA AURICULATA* LINN.

Phytochemical Compound	Result of Qualitative test
Sugar	-
Terpenoids	-
Alakloids	+
Phenolic Compounds	+
Tannins	+
Flavonoids	+
Volatile Oil	+
Quinones	+
Steroids	-
Coumarins	+

+ = Presence, - = Absence

TABLE 2: LEVELS OF CHOLESTEROL AND TRIGLYCERIDES IN THE SERUM OF NORMAL AND EXPERIMENTAL GROUPS

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)
Group – I	75.8 ± 0.4	5.25 ± 0.60
Group – II	88.9 ± 1.3*	8.15 ± 0.91*
Group – III	76.3 ± 0.91 **	5.73 ± 0.83 **
Group - IV	80.3 ± 0.74 **	6.21 ± 1.0 **

Values are given as mean ± SD (n=6); * Significant (P<0.05) when compared with group I. ** Significant (P<0.05) when compared with group II.

TABLE 3: LEVELS OF HDL AND LDL IN THE SERUM OF NORMAL AND EXPERIMENTAL GROUPS

Groups	HDL (mg/dL)	LDL (mg/dL)
Group – I	58.3 ± 0.4	27.5 ± 1.8
Group – II	31.2 ± 1.1*	42.3 ± 0.31*
Group – III	56.4 ± 0.71 **	29.5 ± 0.96 **
Group - IV	50.3 ± 1.51 **	30.2 ± 1.15 **

Values are given as mean ± SD (n=6). * Significant (P<0.05) when compared with group I. ** Significant (P<0.05) when compared with group II

TABLE 4: LEVELS OF AST AND ALT IN THE SERUM OF NORMAL AND EXPERIMENTAL GROUPS

Groups	AST (U/L)	ALT (U/L)
Group – I	85.53 ± 0.971	58.43 ± 1.01
Group – II	100.64 ± 0.73*	83.64 ± 1.27*
Group – III	87.21 ± 1.21 **	60.13 ± 1.15 **
Group - IV	93.45 ± 0.56 **	72.18 ± 1.96 **

Values are given as mean ± SD (n=6). * Significant (P<0.05) when compared with group I. ** Significant (P<0.05) when compared with group II.

TABLE 5: LEVELS OF LDH AND CATALASE IN THE SERUM OF NORMAL AND EXPERIMENTAL GROUPS

Groups	LDH (U/L)	Catalase (U/L)
Group – I	206.72 ± 0.67	4.5 ± 1.5
Group – II	320.10 ± 1.31*	2.6 ± 0.8 *
Group – III	208.01 ± 1.08 **	4.9 ± 0.01**
Group - IV	265.55 ± 0.98 **	4.2 ± 0.8 **

Values are given as mean ± SD (n=6). * Significant (P<0.05) when compared with group I. ** Significant (P<0.05) when compared with group II.

TABLE 6: LEVELS OF PROTEIN AND GLUTATHIONE PEROXIDASE IN THE SERUM OF NORMAL AND EXPERIMENTAL GROUPS

Groups	Protein (g/dL)	GPx (U/L)
Group – I	4.2 ± 0.3	3.2 ± 0.21
Group – II	4.54 ± 1.2 *	1.3 ± 1.03*
Group – III	4.1 ± 0.8**	3.0 ± 0.10 **
Group - IV	4.44 ± 0.5 *	2.5 ± 0.18 **

Values are given as mean ± SD (n=6). * Significant (P<0.05) when compared with group I. ** Significant (P<0.05) when compared with group II.

DISCUSSION: Cardiovascular disease is the most frequent cause of adult death in industrialized societies and is increasingly important in developing countries. The most common cardiovascular disease is myocardial infarction and is commonly known as the heart attack, is a disease state that occurs when the blood supply to a part of the heart is interrupted [5].

Patients at increased risk of developing myocardial infraction include those with multiple coronary risk factors and those with unstable angina, less common underlying medical conditions. Predisposing conditions to myocardial infarction include hypercoagulability, collagen vascular disease, coronary emboli etc [6].,

Plasma contains many functional enzymes which are secreted into plasma. But some few nonfunctional enzymes in plasma, which comes out from cell due to normal wear and tear. Their normal level in blood are very low, but are drastically increased during cell death or disease. Therefore assay of these enzymes are very useful in diagnosis of diseases.

A significant increase in serum protein was observed in isoproterenol treated rats (Table 6). Treatment with the flower extract produced remarkable changes to bring back the normal protein levels.

Usually the concentration of cholesterol, triglycerides and phospholipids increase during the period of peak infarction in animals injected with isoproterenol. In the present study also Isoproterenol induction resulted in increased levels of cholesterol and triglycerides (Table 2). Treatment with the petal extract brought back the values to normal.

The HDL level was markedly decreased in group II when compared to group I and group III (Table 3) . Group I and III had similar results with no significant difference, where as the group IV animals showed increased HDL level than the animals infarcted with isproterenol. The increase in HDL level in the animals pretreated with *Cassia auriculata* petal extract before infarction was very encourage since HDL is termed as good cholesterol. This HDL is capable of scavenging the accumulated cholesterol.

It is important to note that LDL cholesterol increases significantly at peak period during myocardial infarction. This is due to increased direct uptake of LDL from the blood by these tissues. Hence the decrease in HDL and increase in LDL levels (Table 3) indicated the extent of infarction, where as the increase in HDL level and decrease in LDL levels exhibit the hypolipidemic nature of the plant extract which has a positive feed back on its cardioprotectivity.

Lactate dehydrogenase catalyses the oxidation of L-lactate to pyruvate with NAD^+ as the hydrogen acceptor. LDH is widely distributed with high concentration is the heart, skeletal muscle, liver, kidney, brain and erythrocytes. The enzyme is localized in the cytoplasm. Marked increase in total LDH occurs in myocardial infarction. The enzyme activity rises 12-24 h after the onset of chest pain, reaches a peak 48-72 h later and remains elevated for 7-12 days [7].

In the present study, the cardioprotective effect of *Cassia auriculata* was recognized by determination of the activity of LDH. Increase in LDH level indicated damage of heart in group II (Table 5). But in group IV isoproterenol with aqueous extract of *Cassia auriculata* treatment produced considerable reduction in LDH. This cardioprotective activity of *Cassia auriculata* may be due to oligomeric tannin compounds present in it.

Due to its high concentration in the cardiac muscle, plasma AST levels increase following myocardial infarction. The enzyme activity which increase 6-8 h after the onset of chest pain, reaches peak values between 24-42h and falls to the normal range by 4-6 days. The estimation of plasma AST is now widely used to confirm myocardial infarction [8].

In the experimental animals, the level of AST is increased two fold because of isoproterenol induction (Table 4). In group IV the level was maintained normally, as it is pretreated with *Cassia auriculata.* Some of the phenolic compounds present in *Cassia auriculata* accounts for maintaining the normal level.ALT is found in high amounts in the liver with smaller quantities in the heart, kidney and skeletal muscle. ALT level is not significantly altered in complicated myocardial infarction.

In the group 2 animals, the level of ALT is increased because of isoproterenol induction (Table 4). Treatment with *Cassia auriculata* flower extract brought back the values to normal. Phenolic compounds present in *Cassia auriculata* accounts for maintaining the normal level.

CAT is one of the important enzymes in the supportive team of defense against ROS. CAT is a hemoprotein containing four heme groups. The enzyme catalyses the decomposition of hydrogen peroxide to water and oxygen thus protecting the cell from oxidative damage by hydrogen peroxide and OH [9]. This study revels that the activity of CAT was significantly lowered in mice treated with isoproterenol (Table 5), due to oxidative injury. However, the normal expression of the antioxidant enzyme with *Cassia auriculata* is indicative of its ability to activate antioxidant defense during myocardial infarction.

Glutathione Peroxidase is selenium containing tetrameric glycoprotein. The antioxidative protective system of GPx depends heavily on the presence of selenium. It plays a significant role in the peroxyl scavenging mechanism and maintaining functional integrity of the cell membrane.

In this study, a decrease in GPx activity was observed in mice administered with isoproterenol which could be due to oxidative stress (Table 6). *Cassia auriculata*. Restored GPx level back to normal. Hence it can be concluded that *C.auriculata* flowers have cardioprotective potential.

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