

Research Article



Cardioprotective Activity of *Pithecellobium Dulce* Fruit Peel on Isoproterenol-Induced Myocardial Infarction in Rats

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ABSTRACT

The present study is undertaken to evaluate the effect of *Pithecellobium dulce* fruit peel in isoproterenol (ISO) induced myocardial infarction (MI) in adult male Wistar rats. Myocardial infarction was induced by intraperitoneal injection of isoproterenol (ISO) 2 mg/kg in experimental rats. Aqueous and ethanolic extract of *Pithecellobium dulce* fruit peel was administered intraperitoneally at a dose of 200 mg/kg for a period of 14 days. On 14th day the rats were induced with ISO. Serum lipid profile, liver marker enzymes such as serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT), cardiac marker enzymes such as creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were measured in the experimental rats along with histopathological studies of heart tissues. ISO-induced rats showed a significant increase in the activities of marker enzymes such as SGOT, SGPT, CPK and LDH. Pretreatment with aqueous and ethanolic extract of *Pithecellobium dulce* fruit peel, positively altered the activities of marker enzymes and the biochemical parameters in ISO-induced rats. Thus, our study showed that *Pithecellobium dulce* possess cardioprotective effect in ISO induced MI in rats. Results obtained from histopathological studies also supported that *Pithecellobium dulce* has protective effect on ISO induced MI in rats.

Keywords: Myocardial Infarction, *Pithecellobium dulce*, CPK, LDH, Marker enzymes, Isoproterenol.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death in both developed and developing countries. Among these, acute myocardial infarction (MI), is one of the most alarming values¹. Myocardial infarction is a condition in which an imbalance between myocardial oxygen supply and demand occurs². ISO-induced myocardial infarction (MI) serves as a well standardized model to study the beneficial property of numerous drugs and cardiac function. Isoproterenol [ISP], a synthetic catecholamine and β -adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles. ISP induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes^{3,4}.

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. *Pithecellobium dulce* Benth (Family: Leguminosae) is a small to medium-sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andaman. It is known as 'vilayati babul' in Hindi and 'kodukkapuli' in Tamil⁵.

In the traditional system of medicine, roots have been reported to possess estrogenic activity⁶, the fruits have been shown to have anti-inflammatory activity⁷ and leaves have been reported to be a folk remedy for ear

ache, leprosy, peptic ulcer, tooth ache, and venereal disease. It also acts as astringent, emollient, antidiabetic, anodyne and larvicidal in folk medicines. The bark of the plant is reported to be used as an astringent for dysentery, febrifuge and it is also useful in dermatitis and eye inflammation and also possesses antivenomous activity⁸.

The present study was designed to evaluate the cardioprotective effect of ethanol and aqueous extract of *Pithecellobium dulce* (*P. dulce*) fruit pulp in isoproterenol-induced biochemical and histopathological changes using rats.

MATERIALS AND METHODS

Drugs and Chemicals

Isoproterenol (ISP) was obtained from Samarth Life Sciences Pvt. Ltd., Mumbai, and verapamil was obtained from Celon Laboratories Ltd., Gajularamaram, AP. All other chemicals used were of Analytical grade and obtained from Loba-chemie.

Plant Materials

The *P. dulce* fruits were collected from Hosur, Krishnagiri District, Tamilnadu, India. The samples (fruit peel) were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered material was then extracted using ethanol and water in the ratio 1:10 using soxhlet apparatus. After extraction, the solvents were removed by evaporating on water bath which gives rise to a solid mass of the extract. The extracts were stored at 4°C for further use.



Experimental Module

Toxicity studies were performed using male Wister rats with an average weight of 120-150 g. The rats were housed in large spacious cages, which were environmentally controlled (25 ±2°C, 12-h light-dark cycle), with free access to food and water ad libitum. Rats were fed with standard laboratory chow diet during the study. Rats were divided into six groups of six animals each. Group I were control rats. Group II received ISP (2 mg / kg body weight) as a single dose by i.v. on 14th day only. Group III treated with standard drug verapamil (5 μmole/kg body wt) i.v. for a period of 14 days and on the 14th day, ISP (2 mg/kg body wt) is administered after 10 mins of administration of verapamil. Group IV (PDFPE) and Group V (PDFPA) received aqueous and ethanolic extract of *P.dulce* fruit peel (200 mg / kg body weight in saline) for a period of 14 days, followed by administration of ISP (2 mg/kg body wt) on 14th day.

At the end of the experimental period, the rats were sacrificed via cervical decapitation. Blood was collected, allowed to clot, and serum was separated at 2500 rpm for 15 minutes for further biochemical investigations.

Biochemical Parameters

The activities of creatine phosphokinase⁹, lactate dehydrogenase, serum glutamate oxaloacetate transaminase and serum of glutamate pyruvate transaminase were assayed^{10,11}. The enzyme activity was expressed as U/L. The level of cholesterol and triglycerides were assayed^{12,13} and their units were expressed as mg/dl.

Histopathological Examination

After the experimental period, animals were decapitated, and their hearts were removed immediately, then sliced, and washed in saline. For histopathological analysis, heart specimens fixed in 10% formalin were embedded in paraffin, sliced at 5-μm thickness, and stained with hematoxylin and eosin (100X) for detection of cardiac damage. The pathological changes were assessed and photographed¹⁴.

Statistical analysis

All values are expressed as Mean ± SD. The significance of differences between the means of the tests and controls were calculated by one way ANOVA, and p < 0.05 were considered significant.

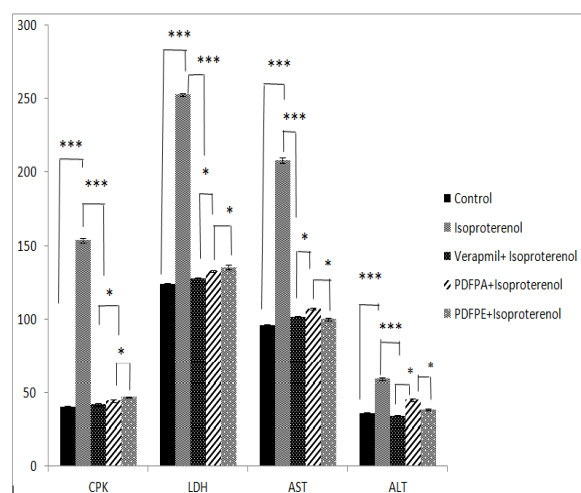
RESULTS AND DISCUSSION

Isoproterenol is well known cardiotoxic agent due to its ability to destruct myocardial cells. As a result of this, cytosolic enzymes such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), transaminases (ALT, AST) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage^{15,16}. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability. Drug treatments such as naringenin,

silibinin and squalene evidenced by a decline in lactate dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase levels indicated their membrane stabilizing action^{15,17}. In accordance to this, many plant-derived drugs also used in modern medicine. These are developed by ethnomedical leads and subsequent ethnopharmacological studies.

Fig. 1 presents the changes in the activity of serum creatine phosphokinase (CPK). Here, isoproterenol-induced cardiac damage was characterized by increased activity of this enzyme when compared with normal control rats. This elevation may be due to necrosis in the heart. Pretreatment with aqueous and ethanolic extracts of *P. dulce* fruit peel (PDFPA and PDFPE) for a period of 14 days of treatment significantly reduced the activity of this enzyme to near normal levels when compared to group II rats given isoproterenol alone.

Fig. 1 presents the changes in the activity of serum lactate dehydrogenase (LDH). Here, isoproterenol-induced cardiac damage was characterized by increased activity of LDH and the same returns to normal level in rats pretreated with aqueous and ethanolic extracts of *P. dulce* fruit peel for a period of 14 days. LDH is localized in the cytoplasm of cells and thus is extruded into the serum when cells are damaged or become necrotic. The measurement of total LDH can be useful when only a specific organ, such as the heart, is known to be involved¹⁸. Pretreatment with aqueous and ethanolic of *P. dulce* fruit peel significantly decreased infarction size which might be due to their potent antioxidant activity which prevents leakage of LDH enzymes.



Each value is expressed as mean ± SEM (n=6); P < 0.05 (*), P < 0.01 (**), P < 0.001 (***)

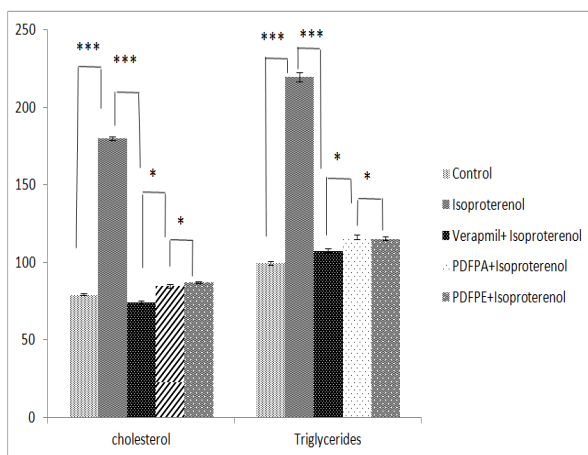
Figure 1: Effect of PDFPA and PDFPE on marker enzymes in control and experimental rats

Fig. 1 presents the changes in the activity of serum aspartate transaminase (AST) and alanine transaminase (ALT). Here, isoproterenol-induced cardiac damage was characterized by increased activity of these enzymes when compared with normal control rats. Pretreatment with aqueous and ethanolic extracts of *P. dulce* fruit peel

(PDFPA and PDFPE) for a period of 14 days of treatment significantly reduced the activity of these enzymes to near normal levels when compared to group II rats given isoproterenol alone.

AST and ALT serve as sensitive indices to assess the severity of myocardial damage¹⁹. The aqueous and ethanolic extracts of *P. dulce* fruit peel seems to preserve the structural integrity of the cardiocellular membrane as evident from the significant reduction in isoproterenol-induced rise in serum AST and ALT in rats. The decreased serum enzymes level in isoproterenol-induced cardiac damage by PDFPA and PDFPE may be due to the prevention of leakage of the intracellular enzymes by their membrane stabilizing activity, which was supported by the limited extent of histological changes.

Fig. 2 shows the changes in the level of serum cholesterol and triglycerides. Here, isoproterenol-induced cardiac damage was characterized by increased level of cholesterol and triglycerides and the same returns to normal level with aqueous and ethanolic extracts of *P. dulce* fruit peel pretreatment.



Each values is expressed as mean \pm SEM (n=6); P <0.05 (*), P <0.01 (**), P <0.001 (***)

Figure 2: Changes in level of total cholesterol and triglycerides in control and experimental rats

The increased concentration of cholesterol in rats administered with isoproterenol may be due to an increase in biosynthesis and decrease in its utilization. Isoproterenol induces free radical formation, which may cause cellular cholesterol accumulation by increasing cholesterol biosynthesis, by decreasing cholesterol ester hydrolysis and by reducing cholesterol efflux. Pretreatment with the plant extract restored the level of cholesterol²⁰.

In histopathological studies (Fig. 3), the control rats showed normal appearance of heart without any histological alterations. Isoproterenol-induced rats showed pathological changes in heart including several congestions and occasional necrosis. The heart was having near normal appearance with mild changes in congestions and necrosis, in rats treated with verapamil+isoproterenol. Same pattern was obtained rats

treated with aqueous and ethanolic extracts of *P. dulce* peel (PDFPA and PDFPE).

The results of histopathological studies suggest that ISP toxicity leads to serious changes in the histology of heart. The increased formation of lipid peroxidation products and associated reactive oxygen species lead to collapse in membrane integrity and other pathological changes in heart. The efficiency of any protective drug is essentially dependent on its capacity of either reducing the harmful effects, or maintaining the normal physiology of cells and tissues, which have been attributed by toxins. The membrane-protective properties and antioxidant nature of aqueous and ethanolic extracts of *P. dulce* fruit peel might be helpful in alleviating the pathological changes caused by isoproterenol in heart.

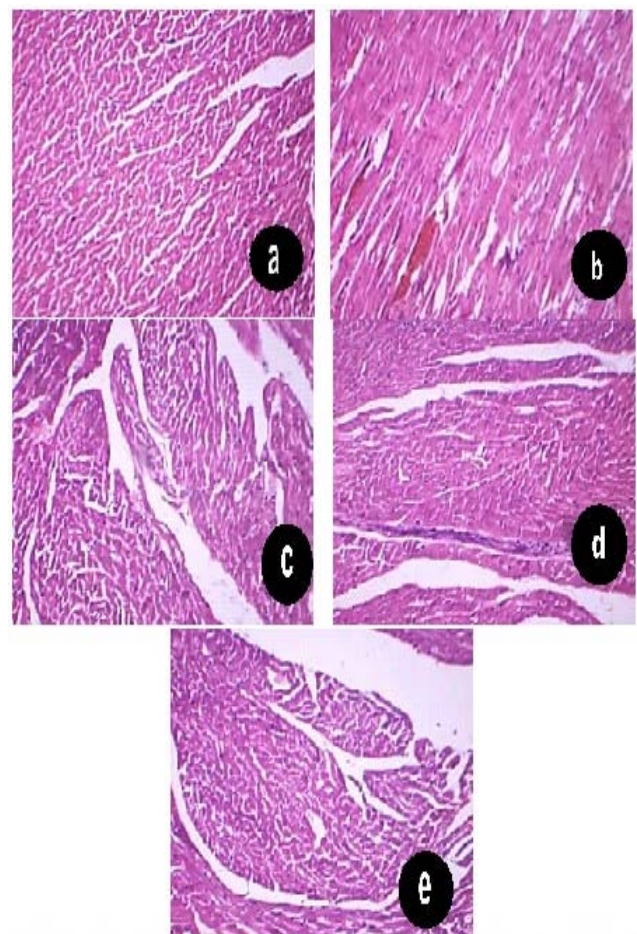


Figure 3 histoarchitecture (magnification of H & E x 100) of heart tissue (a-Normal control showing normal histology of myocardial fibres, b- ISP control showing marked necrosis of myocardial fibres with mild congestion and inflammatory infiltrates, c- ISP with Verapamil showing near normal appearance of myocytes; myofibres with mild edema; no necrotic fibres evident, d- ISP with PDFPA showing near normal appearance of myocytes; normal myofibres with scant

Figure 3: Histological examinations of heart tissue sections in control and experimental animals

CONCLUSION

It can be concluded that the aqueous and ethanolic extracts of *P. dulce* fruit peel possesses cardioprotective activity against isoproterenol-induced heart damage in

rats. According to the results obtained in this study, it may be inferred that, in general, the aqueous and ethanolic extracts of *P. dulce* fruit peel reverses the cardiac damage induced by isoproterenol. Certainly, further studies need to be carried out with other cardiotoxic compounds to prove the cardio protective efficacy of the aqueous and ethanolic extracts of *P. dulce* fruit peel.

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