

Effects of *Muntingia calabura* L. on isoproterenol-induced myocardial infarction

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ABSTRACT

Introduction: This study was designed to scientifically evaluate the effects of an aqueous extract of *Muntingia calabura* L. (*M. calabura*), a medicinal herb, on isoproterenol-induced myocardial infarction (MI) in rat models.

Methods: Six groups of Wistar 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/100g, subcutaneously), and Group III rats were given *M. calabura* leaf extract (300 mg/kg). Groups IV, V and VI rats were given *M. calabura* leaf extract (100 mg/kg, 200 mg/kg and 300 mg/kg, respectively) and isoproterenol (20 mg/100g subcutaneously) prior to MI induction. The transaminases (aspartate transaminase and alanine transaminase), lactate dehydrogenase (LDH) and creatine phosphokinase (CK), were estimated in both the serum and heart tissues, and the serum uric acid level was also estimated.

Results: Isoproterenol significantly increased the activities of CK, LDH and the transaminases in serum with a concomitant decrease in these enzymes in tissue. Pretreatment with the aqueous leaf extract of *M. calabura* at a dose of 300 mg/kg body weight for 30 days had a significant effect on the activities of marker enzymes compared to the other groups. Serum uric acid level, which increased on isoproterenol administration, registered near normal values on treatment with the leaf extract under study.

Conclusion: The study confirms the protective effects of *M. calabura* leaf extract against isoproterenol-induced biochemical alterations in rats.

Keywords: isoproterenol, leaf extract, *Muntingia calabura*, myocardial infarction, uric acid

INTRODUCTION

The entire world population is turning towards natural

drugs because of the widespread belief that “green medicines” are healthier and safer than synthetic ones.⁽¹⁾ It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life.⁽²⁾ Globally, myocardial infarction (MI) is one of the leading causes of death for both men and women.⁽³⁾ Due to changing lifestyles in developing countries, such as India, and particularly in urban areas, MI is making an increasingly important contribution to mortality statistics.⁽⁴⁾ MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart.⁽⁵⁾ Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects.⁽⁶⁾ Many plant sources such as *Commiphora mukil*, *Terminalia arjuna*, *Inula racemosa*, *Coleus forskohlii* and their extracts are being used extensively to treat heart ailments. *Muntingia calabura* L. (*M. calabura*), which belongs to the family Elaeocarpaceae, is popularly known for its antiseptic and antispasmodic properties besides being a proven hypotensive drug.⁽⁷⁾

Several methods have been used to study the beneficial effects of many drugs on cardiac function.⁽⁸⁾ The administration of isoproterenol, a β -adrenergic agonist, has been found to cause severe stress in the myocardium, resulting in the infarct-like necrosis of the heart muscle.⁽⁹⁾ Isoproterenol-induced MI serves as a well-standardised model because the pathophysiological changes following isoproterenol administration are comparable to those taking place in human MI.⁽¹⁰⁾ The present communication thus embodies the cardioprotective effect of the *M. calabura* leaf extract on myocardial necrosis induced by isoproterenol with reference to marker enzymes in the serum, heart tissues and serum uric acid.

METHODS

Adult male albino rats of the Wistar strain weighing 120–150 g were selected for the study. The rats were fed with commercial pelleted rat chow bought from Sai Durga Feeds and Foods, Bangalore, India and water *ad libitum*. The animals were housed in clean polypropylene cages lined with husk, changed every 24 hours under a 12-hour light/dark cycle at 25°C. The study was carried out after obtaining

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Table I. Effect of *M. calabura* pretreatment on isoproterenol-induced changes in the activities of serum AST, ALT, CK, LDH and uric acid.

Group	Aspartate transaminase [†]	Alanine transaminase [†]	Creatine kinase ^{**}	Lactate dehydrogenase [†]	Uric acid
I	31.77 ± 0.32	14.62 ± 0.16	274.75 ± 0.21	78.8 ± 0.21	3.87 ± 0.09
II	52.63 ± 0.38*	25.68 ± 0.23*	530.52 ± 0.31*	145.43 ± 0.17*	5.55 ± 0.15*
III	32.33 ± 0.33	13.75 ± 0.16	272.43 ± 0.28	78.23 ± 0.10	4.03 ± 0.07
IV	36.77 ± 0.18 [†]	15.67 ± 0.2 [†]	283.45 ± 0.28 [†]	81.0 ± 0.22 [†]	4.5 ± 0.13 [†]
V	35.37 ± 0.18 [‡]	15.42 ± 0.17 [‡]	279.70 ± 0.22 [‡]	80.95 ± 0.15 [‡]	4.33 ± 0.20 [‡]
VI	33.65 ± 0.15 [§]	14.27 ± 0.19 [§]	275.57 ± 0.18 [§]	80.23 ± 0.43 [§]	3.93 ± 0.13 [§]

Data is expressed as mean ± SEM for the six animals in each group.

*p < 0.001 compared with Group I. [†]‡§ p < 0.001 compared with Group II.

[†]nmol of pyruvate liberated/sec/mg protein.

** μmol of phosphorous liberated/sec/mg protein.

Table II. Effect of *M. calabura* pretreatment on isoproterenol-induced changes in the activities of membrane AST, ALT, CK and LDH.

Group	Aspartate transaminase [†]	Alanine transaminase [†]	Creatine kinase ^{**}	Lactate dehydrogenase [†]
I	43.51 ± 0.20	25.28 ± 0.15	13.13 ± 0.10	117.22 ± 0.15
II	27.9 ± 0.13*	16.45 ± 0.16*	9.45 ± 0.13*	80.43 ± 0.18*
III	44.46 ± 0.14	26.18 ± 0.16	14.55 ± 0.13	115.43 ± 0.20
IV	42.18 ± 0.30 [†]	25.35 ± 0.15 [†]	12.36 ± 0.12 [†]	114.78 ± 0.21 [†]
V	44.48 ± 0.15 [‡]	25.92 ± 0.17 [‡]	13.48 ± 0.12 [‡]	115.38 ± 0.14 [‡]
VI	45.45 ± 0.23 [§]	27.25 ± 0.20 [§]	14.16 ± 0.07 [§]	116.85 ± 0.26 [§]

Data is expressed as mean ± SEM for the six animals in each group.

*p < 0.001 compared with Group I. [†]‡§ p < 0.001 compared with Group II.

[†]nmol of pyruvate liberated/sec/mg protein.

** μmol of phosphorous liberated/sec/mg protein.

the necessary approval from the Institutional Animal Ethics Committee. Isoproterenol and adenosine triphosphate were obtained from Sigma Chemical Company, St. Louis, MO, USA, and all other chemicals used were of analytical grade. The plant *M. calabura* L. was identified using the flora of Gamble and authenticated with a voucher specimen deposited in the RAPINET Herbarium, St. Joseph's College, Tiruchirappalli. An aqueous extract was prepared and suspended in 0.9% saline and used for the study.

The rats were divided into six groups of six animals each. Group I served as a control, Group II rats were administered with isoproterenol (20 mg/100g administered subcutaneously twice at an interval of 24 h) dissolved in normal saline.⁽⁶⁾ Group III rats were pretreated with *M. calabura* leaf extract (300 mg/kg) for a period of 30 days. Groups IV, V and VI animals were pretreated with *M. calabura* leaf extract (100 mg/kg, 200 mg/kg and 300 mg/kg, respectively) for a period of 30 days⁽¹¹⁾ and isoproterenol (20 mg/100g subcutaneously twice at an interval of 24 hours) at the end of the treatment period on the 29th and 30th days.

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 lactate dehydrogenase (LDH) by the method of King,⁽¹²⁾ aspartate transaminase (AST) and alanine transaminase (ALT) were estimated according to the method of Mohun

and Cooke⁽¹³⁾ and creatine kinase (CK) by the method of Okinaka et al.⁽¹⁴⁾ 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⁽¹⁵⁾ and Lowry et al,⁽¹⁶⁾ 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. Student's *t*-test was used for statistical analysis. Values are expressed as the mean ± standard error of the mean (SEM) for the six animals in each group. A value of p < 0.001 was considered statistically significant.

RESULTS

There was a significant elevation in the transaminases (AST and ALT), CK and LDH activities in isoproterenol-injected animals compared to the controls (Table I). In the Groups IV, V and VI rats pretreated with the *M. calabura* leaf extract, there was a significant reduction in the level of uric acid and the activity of marker enzymes compared with the isoproterenol-administered rats (Group II).

Rats in Group II were given isoproterenol subcutaneously. Rats in Groups IV, V and VI were given *M. calabura* leaf extract 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, CK and LDH) on isoproterenol

administration (Group II) (Table II). Pretreatment with *M. calabura* extract (Groups IV, V and VI) retained the activity of these enzymes to near normal levels. In all the parameters studied, *M. calabura* extract at a dose of 100 mg/kg showed a minor effect, whereas doses of 200 mg/kg and 300 mg/kg showed significant effects, with the dose of 300 mg/kg found to be the most effective.

DISCUSSION

Medicinal plants have long been valued as sources of new compounds with cardioprotective activity. The present study demonstrated that the *M. calabura* leaf extract has efficiently protected the myocardium against isoproterenol-induced MI. Isoproterenol administration brought about a significant decrease in the activities of cardiac marker enzymes such as AST, ALT, CK and LDH in the myocardial tissue (Table II), with a subsequent increase in the activities of these enzymes in the serum. This might be due to the damage in the heart muscle, rendering the leakage of enzymes into the serum.⁽¹⁷⁾ The significant rise observed in the levels of diagnostic marker enzymes in the serum of Group II isoproterenol-administered rats as compared to that of Group I control rats (Table I) is an indication of the severity of the necrotic damage to the myocardial membrane.⁽¹⁸⁾ Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue.⁽¹⁹⁾ The release of cellular enzymes reflects non-specific alterations in the membrane integrity and permeability as a response to β -adrenergic stimulation.

In the present study, pretreatment with the *M. calabura* leaf extract significantly prevented isoproterenol-induced elevation in the levels of the diagnostic marker enzymes of Groups IV, V and VI animals compared to Group II rats, probably due to the protective effect of the *M. calabura* leaf extract on the myocardium; this reduced the extent of myocardial damage and thereby restricted the leakage of these enzymes from the myocardium.⁽²⁰⁾ 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.⁽²¹⁾ In conclusion, the results of the present study indicate that the prior administration of the *M. calabura* leaf extract attenuates isoproterenol-induced MI. The cardioprotective effect of the *M. calabura* leaf extract is probably related to its ability to strengthen the myocardial membrane by its membrane-stabilising action. *M. calabura* leaf extract at the dose level of 300 mg/kg was found to be more effective than the other dose levels. In this study, the cardioprotective potential of *M. calabura* leaf extract is evident.

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