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Cardioprotective Activity of Ethanolic Extract of Terminalia arjuna in Isoproterenol-Induced Rats

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ABSTRACT

One of the main reasons for the high mortality rate worldwide is myocardial infarction (MI). We describe how Partharishtam, an Ayurvedic polyherbal formulation, protects albino rats against myoproterenol-induced myocardial infarction. Significant alterations in important biomolecules measured in blood serum and cardiac tissues indicate that isoproterenol causes MI in normal albino rats. In terms of some of the known identifying markers of MI, including Troponin I and T, creatine phosphokinase serum (CPK-S), creatine phosphokinase myoglobulin isozyme fraction (CPK-MB), and oxidative enzymes like superoxide dismutase (SOD), reduced glutathione (GSH), and catalase, the cardioprotective effects of Partharishtam and the standard medication Propranolol were compared. Troponin 1 and T, CPK-S, and CPK-MB levels significantly decreased in isoproterenol-induced MI rats after partharishtam therapy. Studies using in vivo antioxidant enzymes also showed that when Partharishtam was administered to MI rats, the levels of SOD, GSH, and catalase increased to almost normal levels. This is quite similar to the widely used medication Propranolol, which is used to treat MI humans. In a rat model, histopathological research validated Partharistham's cardioprotective qualities. When Partharishtam was tested on healthy rats, we found no toxicity or adverse effects. Partharishtam, a polyherbal compound, may thus be regarded as a safe medication for MI. Key words: creatine phosphokinase, SOD, GSH, catalase, partharishtam, polyherbal, isoproterenol, propranolol, troponin I, troponin T, and myocardial infarction.

INTRODUCTION

A myocardial infarction occurs when the heart muscles malfunction as a result of coronary artery blockage, which causes a heart attack. The World Health Organization (WHO) projects that by 2020, MI will be the leading cause of mortality globally. One Every year, around 17.9 million individuals pass away from cardiovascular diseases (CVDs), accounting for 31% of all fatalities worldwide. Heart attacks, strokes, and other CVDs are caused by a poor diet, tobacco use, excessive alcohol use, and a lack of physical exercise. 2. The production of Reactive Oxygen Species (ROS), elevated inflammation, and cardiac muscle necrosis are the causes responsible for this dysfunction. 3–5 4-[1-hydroxy-2-(isopropylamino) ethyl] isoproterenol When given in supra-maximal dosages to rats, the synthetic catecholamine and β-adrenergic agonist benzene-1,2-diol hydrochloride promotes cardiac muscle necrosis and oxidative stress, which results in MI. 6, 7 Propranolol is the typical medication used to treat MI. As a beta adrenergic blocker, propranolol lowers blood pressure, tremors, and irregular heartbeats by inhibiting the action of the adrenaline hormone. 8, 9 However, a number of adverse effects, including nausea, stomachaches, dizziness, fatigue, and changes in eyesight, have been recorded with this medication. 10, 11 The usage of antioxidants as dietary supplements or in the eating of fruits and vegetables that naturally contain antioxidants is now on the rise. By using these substitutes, the heart is shielded against oxidative stress brought on by pollutants, diverse lifestyle variables, and even age. A variety of therapy procedures for cardiac conditions are offered in Ayurvedic and Sidhha medicine. Twelve Partharishtam, an Ayurvedic polyherbal formulation, was evaluated for its cardio-protective properties in an animal model in the current research. Because of its cardio-tonic qualities, which include boosting cardiac function by controlling blood pressure and cholesterol and strengthening and nourishing heart muscles, partharishtam is widely utilized. The Ayurvedic book Bhaishajya Ratnavali Hrudroga Adhikara (Section) 33/75-77 API, AFI, served as the basis for the creation of Partharishtam. The majority of the plant components of Partharishtam, including the bark of Terminalia arjuna, the flowers of Woodfordia fruticosa, the fruits of Vitis vinifera, and the flowers of Madhuca indica, have been shown in animal models to have cardioprotective and cardiotonic properties. 13–23 Nevertheless, there are no data on the cardiotonic effects of Partharishtam's polyherbal formulation on the control of the main myocardial infraction biomarkers. The following plant components are combined to make partharishtam: Madhuka – Madhuca indica (Family: Sapotaceae) – blossoms – 960 grams, Mrudvika – Vitis vinifera (Family: Vitaceae) – dried grapes – 2.4 kg, and Arjuna tvak – Terminalia arjuna (Family: Combrataceae) – stem bark – 4.8 kilogram Dhataki, or Woodfordia fruticosa (Lythraceae): 960 grams of flowers, 4.8 kg of guda, or jaggery, and 49.152 lits of water. Prominent Ayurvedic pharmaceutical businesses, including Zandu, Dabur, Baidyanath, Arya Vaidya Sala, and Kottakkal, make this formulation.

The myocardial cell membranes leak as a result of necrosis and/or inflammation, releasing vital proteins and enzymes that are then carried by the blood. Troponin I and Troponin T proteins, as well as enzymes such as lactate dehydrogenase (LDH), alkaline aminotransferase (ALT), aspartate aminotransferase (AST), CPK-S, CPK-MB, and others, are utilized as biomarkers to predict when MI may start. The oxidative stress on the heart muscles that causes MI is also indicated by decreased levels of antioxidant enzymes such as SOD, GSH, and Catalase. As a result, tracking these enzyme levels also provides a hint for MI prediction. The cardioprotective effectiveness of Partharishtam, an indigenous polyherbal medication, has been confirmed by testing its effects on a few clinical biomarkers of MI. In order to test for any potential negative effects, the acute and chronic toxicity levels of partharishtam were also investigated.

MATERIALS AND METHODS

Animal model

Wistar Rats, 150-200 g body weight were offered by KMCH College of Pharmacy, Coimbatore. All the rats were kept at room temperature and allowed to acclimate in standard conditions under 12 hours light/ 12 hours dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study. The experimental procedure was approved by IAEC (Institution of Animal Ethical Committee) of KMCH governed by CPCSEA, Government of India. Proposal number: 685/PO/02/a/CPCSEA/ 2015-2016.

Experimental design and drug treatments

Group I. Normal; Group II. Isoproterenol (85 mg/kg) control. Group III. Isoproterenol (85 mg/kg) + Propranolol (10mg/kg); Group IV. Isoproterenol (85 mg/kg) + Partharishtam (200mg/kg); Group V. Isoproterenol (85 mg/kg) + Partharishtam (400mg/kg).

The animals were divided into five groups of six animals each: Group I: animals served as control and received normal saline; Group II. Isoproterenol (85 mg/kg - treated twice only on day 20 and 21); group III received 10 mg/kg of propranolol for 21 consecutive days (which helps cure myocardial infarction). Group IV and group V received of Partharishtam (200mg/kg and 400 mg/kg) for 21 consecutive days. After the prolonged pretreatment of Propranolol and Parthatistham the animals were treated twice with isoproterenol (85mg/kg) subcutaneously at an interval of 24 hours (day 20 and 21) to induce MI. This test was conducted to see whether pre-treatment of Partharishtam has any curative role on myocardial infarction as compared to that of propranolol. Two consecutive injections of isoproterenol at an interval of 24 hrs. were observed to be sufficient to induce MI in animal model. All the animals were sacrificed on day 23 (i.e., after 24 hrs of the final injection of propranolol) to evaluate the cardio protective effect of Partharishtham and its side effects if any.

Acute and chronic toxicity study on rats

To examine the side effects of Partharistham, the animals were sacrificed on day 23 after pretreatment of Partharistham (alone) with two different doses (200 and 400mg/ kg body weight). To test the side effects we tested the urea, creatinine, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) in blood to test the kidney functions. In addition, we measure the organ weight, body weight, and water and food intake along with complete blood count.

Test of key biomarkers in MI rat tissues

We inspect the key biomarkers like creatine kinase in serum (CPK-S in blood) and creatine kinase in myoglobulin (CPK-MB in heart muscles) enzyme activities together with the levels of two marker proteins Troponin I and T (from blood serum) to test the medicinal function of Partharistham in MI induced rat.

Tissue collection

24 hrs after the administration of final dose of Isoproterenol (day 22), the rats were anaesthetized with diethyl ether and blood collected from the retro-orbital plexus. The blood was allowed to clot for half an hour and then centrifuged at 10,000 rpm for 10 min to separate the serum from the blood cells. The separated serum was then immediately stored at -20ºC for further biochemical analysis. Following the collection of blood, the animal was sacrificed by cervical dislocation and the heart was removed and washed immediately with ice cold saline to clean the blood and other cellular debris. One hundred mg of heart tissue was weighed and homogenized in 5ml of Tris-hydrochloride buffer (pH 7.4) in ice cold condition. The homogenate was then centrifuged at 2500g for 10min at 4°C. The supernatant obtained was then used for the (CPK-MB) assays.

In Vivo antioxidant study

In vivo antioxidant studies were conducted for catalase, super Oxide dismutase and reduced glutathione enzymes in heart muscle tissues using the methods of Samuel *et al*, 1950; McCord and Fridovich, 1969 and Moron *et al*, 1979, respectively.[24-26]

Histopathological studies

The cardio protective effective of Partharishtam was examined using hematoxylin and eosin staining in Isoproterenol induced MI heart tissues of rat. The histological architecture of cardiac muscles were analyzed and to find out the cardio protective role of Partharishtam.

RESULTS

Acute and chronic toxicity study on rats

Effect of two different doses of Partharishtam did not shows any changes of complete blood count parameters when compared to normal saline injected animals indicating its safe nature (Supplementary Table 1).

No perceptible changes on organ weight, body weight, water and food intake were observed at low and high doses of Partharishtam again reveal that Partharishtam does not have any side effects on normal rats (Supplementary table 2 and 3).

Biochemical parameters like blood urea, creatinine, SGOT and SGPT does not affect negatively indicating that Partharishtam is a safe medicine without any adverse effects on kidney functions (Supplementary Table 4).

Effect of Partharishtam on key biomarkers in blood and cardiac tissues of MI rats

Increase in serum CPK is one of the important biomarker of skeletal muscle injury including higher CPK-MB in cardiac tissues may point more directly to heart damage. We observe that two consecutive injections of isoproterenol drastically increase CPK (282%) in serum and CPK-MB (623%) in cardiac tissues which confirm MI in rat model. Treatment of propranolol significantly declines the isoproterenol induced elevated level of CPK in both the tissues as expected. Interestingly, Partharishtam also exhibited its recovery effect in MI rats by declining the CPK levels in blood serum and cardiac tissues (Figure 1) close to the level of propranolol. We used two different doses of

Partharishtam and the higher dose (400 mg/kg) perform better that the lower doses ((200 mg/kg).

Troponin is an integral protein in cardiac muscles required for muscle contraction. It is a complex of three regulatory proteins (Troponin I, Troponin T and Troponin C). High level of Troponin is one of the major indications of cardiac injury. We have specifically tested Troponin I and Troponin T in blood serum to examine the cardio protective effects of Partharishtam. Both the doses of Partharishtam (200 and 400mg/kg) significantly decrease the level of troponin proteins in blood serum in isoproterenol induced elevated level of this proteins in MI rat which is very much comparable to the standard drug propranolol used for myocardial infraction (Figure 2).

In vitro antioxidant studies of Partharishtam

Antioxidant activities of Partharishtam were observed in three different antioxidant enzymes (SOD, CAT and GSH) in heart muscles of Isoproterenol prompted MI rats. Isoproterenol injected rats showed sharp decline in SOD (55.22%), CAT (62.95%) and GSH (73.68%) activity reveals myocardial defects due to oxidative stress. It is interesting to note that both the doses of Partharishtam were able to rescue the isoproterenol induced decline in antioxidant enzymes. The results are very much comparable to that of the standard drug propranolol (Figure 3). The antioxidant study results clearly indicate that Partharishtam has a very good antioxidant potential which could be one of the reasons for its curative properties in terms of MI.

Effect of Partharishtam on histopathology of MI rats

Histopathological study reveals that there was no deformity in myocardial tissues in normal saline treated control rats (Figure 4a, a1). Treatment of Isoproterenol causes severe destruction of myocytes with inflammatory infiltrations of neutrophils (Figure 4b, b1). We observed that stroma is edematous with necrotic and dead myocytes surrounded by a mixture of neutrophils and macrophages. Blood vessels also severely congested. Proprenolol treated rats showed quite normal myocardium architecture in isoproterenol induced MI rats. However, we observed fewer infiltrations of neutrophils with normal blood vasculatures in the cardiac tissue (Figure 4c, c1). Surprisingly, Partharishtam treated (400mg/kg) rats showed very normal and regular myocardial architecture of with no dead myocytes and inflammatory infiltrations together with very normal blood vasculature in isoproterenol persuade MI rats (Figure 4d, d1).

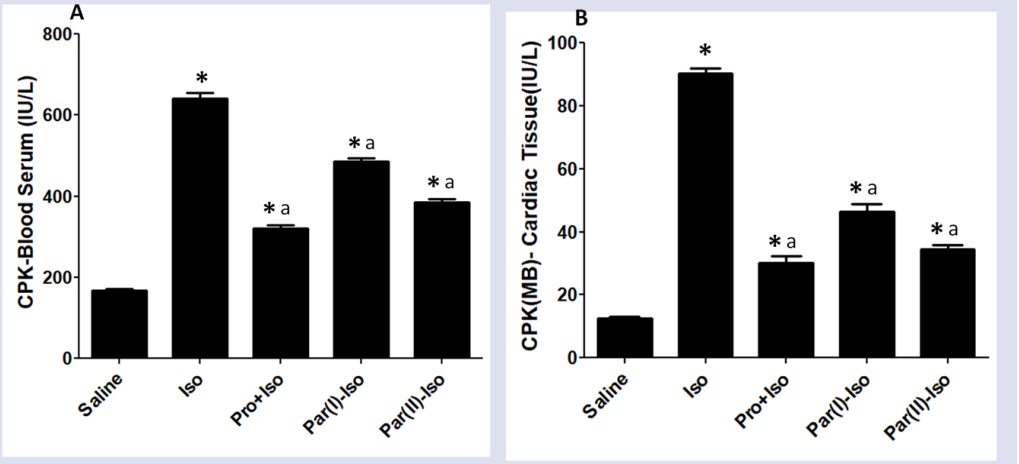

Figure 1: Effect of Partharishtam on Creatin Phosphate Kinase (CPK) in Blood serum (A) and Cardiac tissue (B) of Isoproterenol induced MI rats. * = P<0.001 (Saline Control *VS* all other treatments); a= P< 0.001 (Isoproterenol *VS* all other treatments); Iso (Isoproterenol); Pro (Propranolol); Par (Partharishtam).

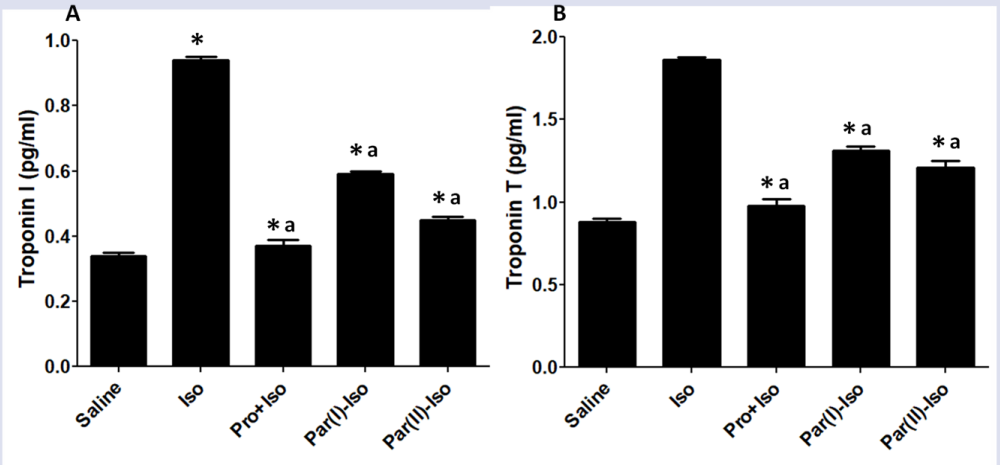

Figure 2: Effect of Partharishtam on Troponin I (A) and Troponin T (B) in blood serum of Isoproterenol induced MI rats. * = P<0.001 (Saline Control *VS* all other treatments); a= P< 0.001 (Isoproterenol *VS* all other treatments); Iso (Isoproterenol); Pro (Propranolol); Par (Partharishtam).

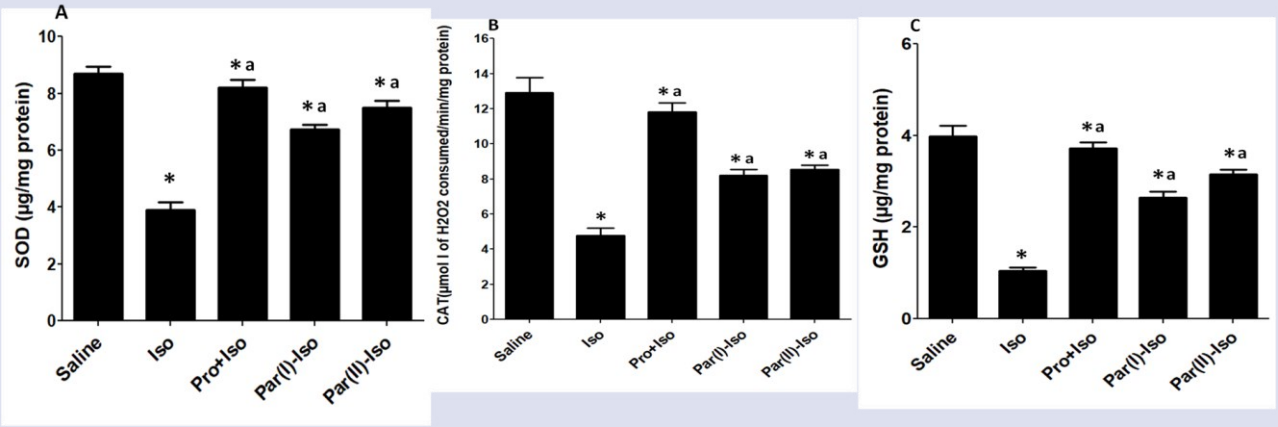

Figure 3: Effect of Partharishtam on antioxidant enzymes (A) SOD (B) CAT and (C) GSH in isoproterenol induced MI rats. * = P<0.001 (Saline Control *VS* all other treatments); a= P< 0.001 (Isoproterenol *VS* all other treatments); Iso (Isoproterenol); Pro (Propranolol); Par (Partharishtam).

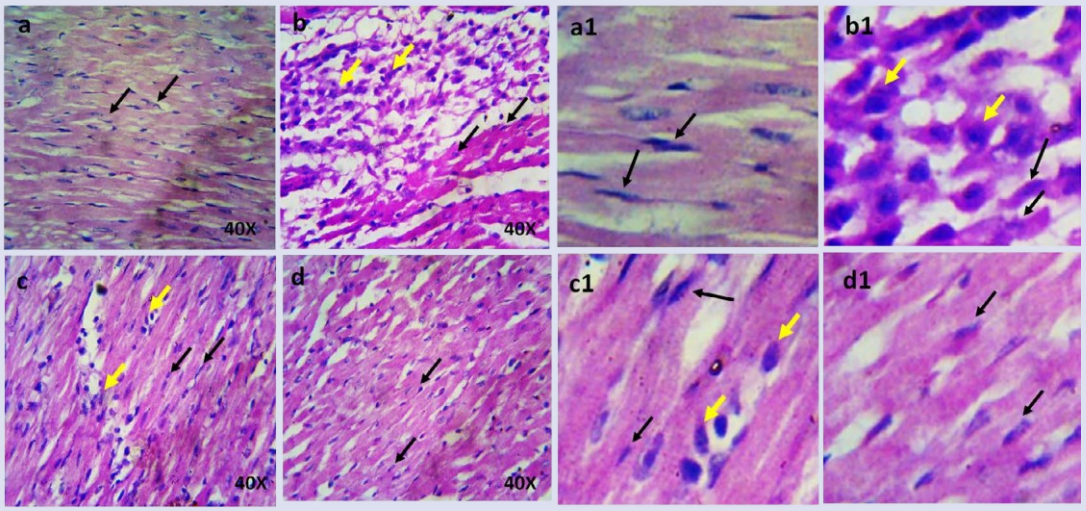

Figure 4: Effect of Partharishtam on histopathology of cardiac muscles in isoproterenol induced MI rats. A black arrow indicates the normal myocytes. Yellow arrow shows the neutrophelic infiltration in the damaged areas of cardiomyocites. (1a) Normal saline treated control cardiac muscles; (1b) Isoproterenol treated cardiac muscle showing damaged myocytes; (1c) Commonly used drug propernolol, rescued isoproterenol induced damage in cardiac muscles; (1d) Partharishtam with higher dose (400mg/kg) rescued isoproterenol induced damage in cardiac muscles; a1, b1, c1 and d1 are the enlarged views of the corresponding treatments of 1a,1b, 1c and 1d.

DISCUSSION

Propranolol, a beta blocker, is a common and effective treatment for ischemic heart disease (IHD), hypertrophic cardiomyopathy, essential tremor, rapid heartbeat caused by antipsychotic drugs, and myocardial infarction prevention (27–29). For both acute and chronic situations, propranolol combinations with fluoxetine, diltiazem, and other medications are now showing better results. However, it is still true that all of these medications have a number of negative side effects and disrupt the body's balance. As a general cardio tonic, partharishtam is a well-researched Ayurvedic polyherbal composition. Comparing Partharishtam to the commonly used standard medication Propranolol, the current trials and findings show a reasonable level of medical effectiveness. Partharishtam may be able to protect cardiac tissues produced by isoproterenol therapy from oxidative stress, as seen by the findings' lowered troponin I and T levels and increased levels of antioxidant enzymes. Here, it might be recommended that patients receiving care for Partharishtam is a supportive cardio tonic that helps speed up the recovery of individuals with myocardial infarction and ischemic heart disease when taken with regular medications. Although there are several findings in this area, the absence of conventional biochemical and pharmacological data sometimes raises doubts about the mechanism of action of Ayurvedic and other traditional forms of therapy. 30-43 The primary disadvantages of these conventional alternative herbal remedies are their lack of pharmacological validation regarding their safety, effectiveness, and mode of action, as well as their lack of standardization. We demonstrate that partharistham has no negative effects and may be used as a cardioprotective medication. We verified that this medication has no adverse effects, at least not on blood parameters or renal functions. In addition to additional substances like 2-(3-chlorophenoxy)- N'-(2,4- dicholorobenzylidine) acethydrazide, methyl 2-(4-chlorophenyl)- 6-metoxy-7-chlorocinchoninate, 1,3-dipentyl-heptabarbital, and biomolecules such glycerol tricaprylate, piperine, and pyrazole,

According to the GC MS study of Partharisthatam, 2-pyridone, 3,5-diiodo-n-methyl-1-dimethyl derivative, and stanzolol were identified. According to reports, all of the aforementioned chemicals exhibit biological properties including anti-inflammatory, antioxidant, and cardiovascular protection (Sadanandham et al, 2015). 44 The combination of these plant ingredients and their proportions for making partharishtam itself demonstrates how brilliant the proponents of Ayurveda were in creating a formulation for a cardiac tonic.

CONCLUSIONS

Since partharishtam had no negative effects on rats' normal behavioral patterns or other physiological characteristics, such as kidney function, it is a safe medication. Partharistham's ability to save lives shown that it is a highly safe medication with no negative side effects. Because of its natural origin and biocompatibility, this plant-derived medication may also be regarded as a safe herbal remedy. According to the findings of a study on myocardial infarction in a rat model, a higher dose of partharishtam (400 mg/kg body weight) demonstrated cardioprotective effects that were essentially equivalent to those of the standard medication propranolol (20 mg/kg) in terms of CPK (serum), CPK (myoglobulin), Troponin I, and Troponin T levels. Histological markers improved as a consequence of these outcomes, and they were on par with normal medication therapy for MI. Partharishtam is thought to protect the myocardial cell and membrane's structural and functional integrity, avoiding cardiac damage and the release of troponins and creatine kinase enzymes into the bloodstream. However, Partharishtam's exact mode of action for regulating the different parameters is yet unknown. In conclusion, partharishtam may be regarded as a safe and efficient medication for both general cardiac tonics and myocardial infarctions in particular.

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