Screening of cardioprotective effect of *Terminalia arjuna* Linn. bark in isoproterenol - Induced myocardial infarction in experimental animals.

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ABSTRACT

Medicinal plants are sources of important therapeutic aids for alleviating human diseases. *Terminalia arjuna* Linn. (family- Combretaceae) is used in Indian Ayurvedic medicine for the treatment of various diseases. The present study was designed to investigate the cardioprotective effect of ethanol and aqueous extracts of *T. arjuna* bark against isoproterenol induced myocardial infarction. Myocardial infarction in rat was induced by the administration of isoproterenol at a dose of 85 mg/kg, i.p., the rats were pretreated with the ethanol and aqueous extracts of *T. arjuna* in the dose of 250 mg/kg through the oral route. Isoproterenol alone- treated rats showed serum totals cholesterol, triglyceride and LDL levels were significantly increased and HDL level was decreased and, decreased myocardial tissue levels and increased serum concentration of lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate amino transferase (AST) levels due to myocardial damage produced by isoproterenol. This is further conformed by histopathological changes of heart tissues. The oral administration of *T. arjuna* bark extracts significantly restored the level of total cholesterol, triglyceride, LDL, HDL and Myocardial and serum LDH, CK, AST. The extract effect was compared with standard drug verapamil which also offered similar protection in biochemical and histopathological changes. The overall conclusion, *Terminalia arjuna* bark possess significant cardioprotective activity.

Keywords: *Terminalia arjuna*, Isoproterenol, myocardial infarction, Histopathology, cardioprotection.

INTRODUCTION

Myocardial infarction (MI) is the interruption of blood supply to part of the heart, causing heart cells to die, commonly due to occlusion (blockage) of a coronary artery. It create a major important cause of morbidity and mortality in developing counters due to increased high prevalence of risk factors and also aging of their populations [1]. Risk factors for cardiovascular disease (CVD) are smoking, hypercholesterolemia, hyperlipoproteinemia, high low density lipoprotein and low high density lipoprotein, diabetes, high blood pressure, older age, obesity. Complications of myocardial infarction (MI) include arrhythmias, congestive heart failure, cardiogenic shock, ventricular Aneurysm, pericarditis, dressler syndrome and pulmonary embolism [2].

According to WHO 17.3 million peoples died from CVDs in 2008, over 80% of CVD death take place in low and middle income countries [3]. An estimated that by 2030 more than 23 million peoples in world 2.6 million peoples in India's will die annually from CVDs [4,5]. There are different way of preventing and treating cardiovascular disease. Besides drug therapy and life style changing, dietary modification and supplementation play an increasingly important role in the conservative treatment of CVDs. Current interest has focused on plant based natural drug treatments.

Terminalia arjuna Linn. (Combretaceae) is a large tropical woody tree distributed throughout subtropical regions of India. It is a traditional Indian medicinal herb which has many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic system of medicine [6]. Its barks are widely used for various therapeutic applications. The Bark of *T. arjuna* tree contains calcium salts, magnesium salts, and glucoside has been used in traditional Ayurvedic medicines. Its leaf juice is used to cure dysentery.

T. arjuna helps in maintaining the cholesterol level at the normal rate, as it contains the antioxidant properties similar to the Vitamin E. It strengths the heart muscles and maintains the heart functioning properly. It also improves functioning of cardiac muscle and treatment of coronary artery disease, heart failure, angina and hypercholesterolemia. Its bark power possesses asthma, diuretic, prostaglandin enhancing and coronary risk factor modulating properties [7]. *T. arjuna* promotes well-organized cardiac performance and regulates blood pressure to normal. So, the present research has been designed to evaluate the cardioprotective property of the aqueous and ethanol extract of *Terminalia arjuna* Linn. Bark in using isoproterenol induced myocardial injured *albino* rats to support the traditional claim.

Figure 1: *Terminalia arjuna* tree with fruits.

Figure 2: *Terminalia arjuna* bark.

MATERIALS AND METHODS

Drugs Used

Stem bark of *Terminalia arjuna* Linn. were collected from Adhiparasakthi Agricultural College, Medicinal garden, kalavai, Vellore district, Tamil nadu, India. And authenticated by Dr. P. Jayaraman, Professor, Institute of Herbal Botany Plant Anatomy Research Center, Chennai. A voucher specimen (No: PARC/2013/2026) was deposited in center. Isoproterenol hydrochloride was purchased from Sigma chemical, Bangalore. All other reagents and chemicals used in this study were of analytical grade with high purity.

Preparation of Plant Extract

Aqueous Extract

After the collection of stem bark they were placed in clean tray and allowed for shade drying. The bark was subjected to surface sterilization using ethanol and then dried in shade. The dried whole plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh). The powdered sample (250 g) was boiled in hot water for 30 min. after which it was filtered using a piece of white cotton guaze. The filtrate was evaporated to dry at 40^{o}C producing brown color solid residue [8]. The residue was weighed (yield; 35% w/w) and stored in air and water proof containers, kept in refrigerator at 4^{o}C. From this stock, fresh preparation was made whenever required.

Ethanol Extract

The powdered sample (350 g) was defatted by treating with petroleum- ether (60-80^{o}C) and then extracted to exhaustion (Soxhlet) with ethanol. After extraction the extracts were filtered through wattman filter paper No: 40. The filtrates were evaporated to dryness in vacuum at (35-40^{o}C) to get some solid mass (yield; 37.4% w/w). The dried extracts were stored separately in screw cap vial at 4^{o}C until further use.

Animals

Healthy adult *wistar albino* rats (weighing 150 - 200g) were used in the experiments. Animals were housed in polypropylene cages at 22±2^{o}C with relative humidity of 45- 55% under 12 hour's light and dark cycle. They were feed with standard laboratory animal feed (Hindustan Lever Ltd., India) and water *ad libitum*.

Approval of Protocol

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Adhiparasakthi College of Arts and Science, kalavai, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 282/ac/09/CPCSEA). Ethical guidelines were strictly followed during all the experiments.

Oral Acute Toxicity Studies

Acute toxicity study was performed according to Organisation for Economic Co-operative and Development guidelines (OECD) No. 423. *Albino* rats of either sex were divided into six groups with six animals each. *Terminalia arjuna* ethanol extract was administered orally as single doses to rats at different dose levels of 50, 250, 500, 1000, 1500, 2000 and 2500 mg/kg b.w. Animals were observed individually during the first 30 minutes and periodically during 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total 14 days. At the end of the study the animals were observed for general toxic signs, morphological behaviour and mortality [9].

Induction of Myocardial Infarction

At the end of treatment period, all the animals, except the normal untreated rats that served as the control group, were administered isoproterenol (ISO) 85 mg/kg, interaperitoneal injection for two consecutive days on the 31 and 32 day at an interval of 24 h. to induce myocardial injury [10] After 48 hours rats were anaesthetized with anaesthetic ether, then sacrificed and the hearts were harvested for biochemical and histological studies.

Allocation of groups and experimental Protocol

The rats were randomly divided into five groups with six rats in each group. Group I, normal animals received saline 10ml/kg b.w with standard feed and water to allow *ad libitum* throughout the experimental period. Group II, the rats were orally fed normal saline once daily for 30 days and in addition, received isoproterenol (85mg/kg body weight) on the 31 and 32 day at an interval of 24 h. Group III, rats were pre-treated with verapamil (5µmol/kg body weight, intera vein) for a period of 14^{th} day and 30^{th} days only and in addition, received isoproterenol 85 mg/kg body weight on the 31 and 32 day at an interval of 24h. Group IV-V, rats were pre-treated with *Terminalia arjuna* ethanol extract (TAEE) and *Terminalia arjuna* aqueous extract (TAAE) at 250mg/kg body weight respectively for a period of 32 days and in addition, received isoproterenol 85mg/kg bw. on the 31 and 32 day at an interval of 24 h.

Collection of Blood and Heart Tissues

At the end of 32^{th} day, after treatments, all the animals were sacrificed by decapitation by mild ether anaesthesia and the fasting blood sample of each group were collected separately into sterilized dry centrifuge tubes, and allowed to coagulate for 30 min. at 37^{o}C. The clear serum obtained after centrifugation was used for the estimation of biochemical enzymes like lactate dehydrogenase (LDH), creatine kinase (CK), serum aspartate amino transferase (AST), and the lipid profile of total cholesterol (TC), triglyceride (TG), HDL and LDL were using the respective kits. The heart was excised immediately and immersed in physiological saline. It was suspended in 10% (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) and cut into small pieces. The required amount was weighed and homogenized using a Teflon homogenizer (Inco, India). The clear supernatant was used for estimation of cardiac enzymes of LDH, CK and AST were using the respective kits.

Histological Examinations

The heart was excised immediately and washed immediately with ice-cold saline; then fixed in 10% buffered formalin; 10% stored buffered formalin were embedded in paraffin; 5µm thick sections were cut and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histological changes.

Statistical Analysis

The statistical analysis was performed by ANOVA under one way classification followed by Bonferroni multiple comparison test, changes were considered significant at the P-value of < 0.05 and < 0.01 level of significance. The values were expressed as mean ± SD.

RESULTS

Oral Acute toxicity study

In acute toxicity study, it was found that the animal were safe up to a maximum dose of 2500mg/kg b.w. There were no changes in the normal behavioural pattern and no signs and symptoms of toxicity and mortality were observed.

Lipid Profile Compounds

Table 1 show that the serum totals cholesterol, triglyceride and LDL levels were significantly (P<0.01) increased and HDL level was decreased in ISO induced myocardial infarction rats. Pretreatment with daily oral administration of ethanol and aqueous extracts of *Terminalia arjuna* bark (250mg/kg body weight) significantly (P<0.01) decreased in total cholesterol, triglyceride and LDL level and the HDL level was return back to normal when compared to ISO treated group (Table 1).

Heart Marker Enzymes

In Table 2 represent, the exposure to ISO (Group - II) significantly (P<0.01) decreased the myocardial LDH, CK levels when compared to normal group, but there was no significance changes in the level of myocardial AST in Group - III and Group - IV-V. The pretreatment with ethanol and aqueous extracts of *Terminalia arjuna* bark (250mg/kg body weight) significantly (P<0.01) increased LDH and CK when compared to ISO treated groups rats.

Serum Marker Enzymes

From the experimental reports in table 3 represent, the significant (P<0.01) increase in the level of serum LDH, CK and AST in ISO treated rats when compared with normal animals. The pretreatment with ethanol and aqueous extracts of *Terminalia arjuna* bark (250mg/kg body weight) significantly (P<0.01) decreased levels of cardiac damage marker enzymes compared to ISO-treated and standard drug verapamil groups.

Histopathological Examination

Histopathological examination of the myocardium of normal rat showed clear integrity of myocardial cell membrane (Figure 3). Endocardium and pericardium were within normal limits. No inflammatory cell infiltration was observed.

The group of ISO-treated rats showed moderate to marked myocytic necrosis with moderate infiltration of lymphocytes and macrophages (Figure 4). The changes were more prominent along the endocardium and in papillary muscles.

Minimal-to-mild focal myocytic necrosis and minimal diffuse lymphocytic infiltration along the endocardium was seen in the heart sections of the standard drug verapamil treated group (Figure 5).

The TAEE treatment (Figure 6) showed mild multifocal myocytic necrosis with removal of sarcoplasm and mild diffuse lymphocytic infiltration along the endocardium. Minimal-to-mild multifocal myocytic necrosis with removal of sarcoplasm and mild diffuse inflammatory cell infiltration along the endocardium was observed in the TAAE group (Figure 7).

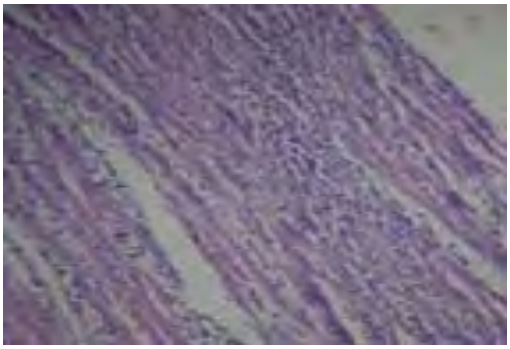

Fig – 3: Hematoxylin and Eosin Staining of Heart of Normal Rats: 10 X 10x = 100x

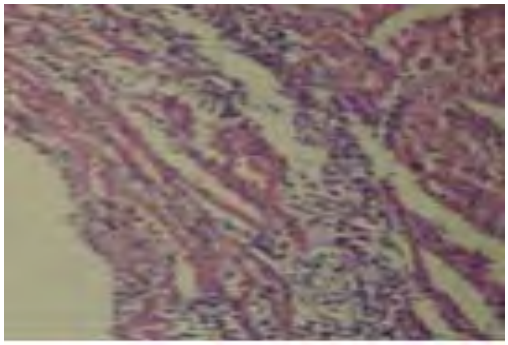

Fig - 4: Hematoxylin and Eosin Staining of Heart of ISO-treated Rats: 10 X 10x = 100x

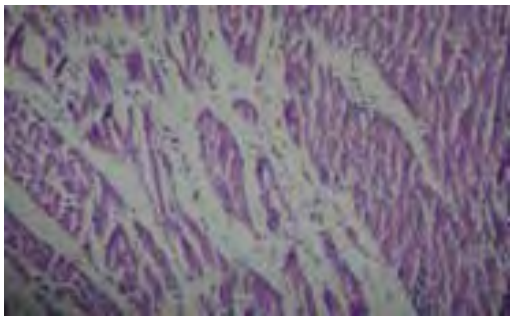

Fig – 5: Hematoxylin and Eosin Staining of Heart of Rats Treated with verapamil (5µmol/kg) and ISO:10 X 10x = 100x

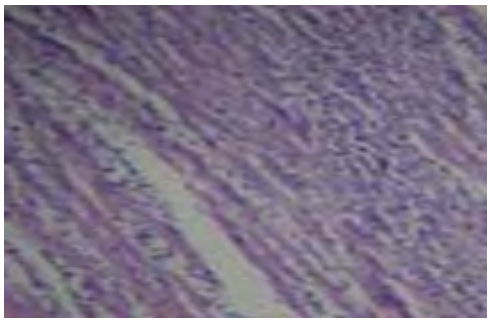

Fig - 6: Hematoxylin and Eosin Staining of Heart of Rats Treated with TAEE (250 mg/kg) and ISO:10 X 10x = 100x

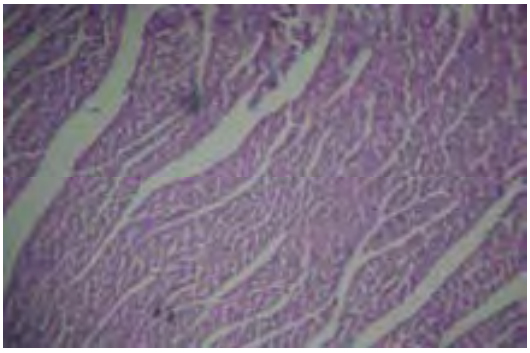

Fig – 7: Hematoxylin and Eosin Staining of Heart of Rats Treated with TAAE (250 mg/kg) and ISO: 10 X 10x = 100x

DISCUSSION

Isoproterenol induced myocardial infarction is widely used as a model of evaluating cardioprotective drugs [11]. Isoproterenol, a potent synthetic catecholamine, induces subendocardial myocardial ischemia, hypoxia, and finally fibroblastic hyperplasia with decreased myocardial compliance which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction [12].

The high dose of isoproterenol is ability to destroy myocardial cells. As a result of this, cytosolic enzymes such LDH, CK and AST were released into the blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in heart reflects the alteration in plasma membrane integrity and/of permeability [13]. Changes in the level of myocardial markers LDH and CK in both serum and heart homogenate in ISO-treated rats (Table 2) conforms the onset of myocardial necrosis. Chronic oral administration of *Terminalia arjuna* bark extracts (TAEE & TAAE 250mg/kg b.w) caused significant changes in the level of cardiac markers (LDH, CK & AST) in both serum and myocardium.

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 myocardium. High levels of circulating cholesterol along with TG and their accumulation in the heart tissue is usually accompanied by cardiovascular damage [14]. In the present study, ISO evidenced its hyperlipidemic effect by increasing serum TC, TG and LDL levels and decreased levels of HDL in comparisons with normal controls. High levels of LDL show positive correlation with MI, while increased levels of HDL have a negative correlation. Our earlier studies [15] reported hyperlipidemia in ISO induced myocardial necrosis. An increase in LDL and along with a decrease in HDL was observed in ISO induced rats. LDL is capable of carrying the highest concentration of cholesterol is evidence to increased serum TC [16].

Pretreatment with TAEE & TAAE (250 mg/kg b.w) significantly decreased the increased TC, TG, LDL and AST levels and increased the levels of HDL (Table 1). These alterations in lipid profile might be due to the presence of major active constituents of *Terminalia arjuna* bark.

CONCLUSION

In summary, it has been concluded from the biochemical and histopathological evidence that the *Terminalia arjuna* ethanol extract (TAEE) and *Terminalia arjuna* aqueous extract (TAAE) at 250 mg/kg body weight, both produced significant cardioprotection in isoproterenol induced myocardial infarction animals. When compared to aqueous extract methanol extract have highly significantly preventing the myocardial damages in rats.

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Table - 1. Effect of TAEE and TAAE treatment on lipid profile marker compounds in ISO- induced myocardial infarction.

Parameters	Normal (Saline 10ml/kg b.w)	Isoproterenol (85mg/kg.b.w)	Verapamil (5µmol/kg b.w)	TAEE- (250mg/kg b.w)	TAAE- (250mg/kg b.w)
Total Cholesterol (mg/dl)	87.31±1.0	138.10±0.1***	112.42±1.2***	119.61±1.1***	122.41±3.1***
Triglycerides (mg/dl)	77.11±1.3	123.21±1.2***	109.64±3.2***	112.31±1.3***	115.41±1.0***
HDL (mg/dl)	35.11±1.0	19.32±2.3***	33.41±2.1***	31.65±3.0***	31.31±1.3***
LDL (mg/dl)	21.02±1.2	43.46±3.2***	25.20±2.2***	22.52±1.3***	22.61±2.2***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test, *** P<0.01, ** P<0.05, TAEE: *Terminalia arjuna* ethanol extract, TAAE: *Terminalia arjuna* aqueous extract, HDL: High density lipoprotein, LDL; Low density lipoprotein.

Table - 2. Effect of TAEE and TAAE treatment on myocardial marker enzymes in ISO- induced myocardial infarction.

Parameters	Normal (Saline 10ml/kg b.w)	Isoproterenol (85mg/kg.b.w)	Verapamil (5µmol/kg b.w)	TAEE- (250mg/kg b.w)	TAAE- (250mg/kg b.w)
LDH(IU/l)	472.21±3.1	312.08±2.3***	441.03±1.2***	437.63±2.1***	432.21±1.3***
CK (IU/l)	163.11±4.1	60.12±1.0***	135.42±2.7***	124.4±1.1***	129.1±4.1***
AST (IU/l)	67.42±1.1	63.34±4.1**	62.41±61***	59.32±3.4***	57.38±1.1***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test, *** P<0.01, ** P<0.05, TAEE: *Terminalia arjuna* ethanol extract, TAAE: *Terminalia arjuna* aqueous extract, LDH: Lactate dehydrogenase, CK: Creatine kinase, AST: Aspartate transaminase.

Table - 3. Effect of TAEE and TAAE treatment on serum marker enzymes in ISO- induced myocardial infarction.

Parameters	**Normal (Saline 10ml/kg b.w)**	**Isoproterenol (85mg/kg.b.w)**	**Verapamil (5µmol/kg b.w)**	**TAEE- (250mg/kg b.w)**	**TAAE- (250mg/kg b.w)**
LDH(IU/l)	358.11±2.2	745.38±1.2***	452.32±4.2***	464.61±1.1***	472.01±1.2***
CK (IU/l)	150.12±3.2	473.02±1.0***	223.21±1.6***	241.1±1.0***	243.41±1.2***
AST (IU/l)	157.51±0.1	318.14±0.1***	161.22±2.0***	163.12±3.1***	172.12±0.2***

All value expressed as mean±SD; One way analysis of variance followed by Bonferroni multiple comparison test, *** P<0.01, ** P<0.05, TAEE: *Terminalia arjuna* ethanol extract, TAAE: *Terminalia arjuna* aqueous extract, LDH: Lactate dehydrogenase, CK: Creatine kinase, AST: Aspartate transaminase.