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Cardioprotective Role of *Justicia Traquebareinsis* linn. Leaf Extract in Isoproterenol Induced Myocardial Infarction in Albino Rats

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ABSTRACT

Atherosclerosis is complex disease and the underlying cause of heart attack, stroke and peripheral vascular disease. It is the main cause of morbidity and mortality worldwide, characterized by an excessive inflammatory, fibro fatty, proliferative response to damage of the artery wall. The present study was designed to evaluate the cardioprotective role of *Justicia tranquebareinsis* Linn. leaf extract on isoproterenol induced myocardial infarction in Wistar albino rats. The rats were divided into five groups of six animals each. Group I served as a normal control, Group II rats were administered isoproterenol (20mg/kg, s.c) at the end of experimental period on the29th and 30th days. Group III and IV were pretreated with *Justicia tranquebareinsis* Linn. leaf extract (100 mg/kg, 200mg/kg ,respectively) for a period of 28 days and received a subcutaneous injection of isoproterenol (20mg/kg, b.w)at the end of experimental period for 2 consecutive days. Group V received aqueous extract of *Justicia tranquebariensis* Linn 200mg/kg b.w for 28 days. After the experimental period, blood was collected and serum was separated and used for the estimation of protein, cholesterol, triglycerides, phospholipids, and lipoproteins and the assay of marker enzymes. The heart homogenate was used for the assay lipid profile. Isoproterenol induced rats showed significant increase in the levels of triglycerides, total cholesterol and phospholipids in both serum and heart homogenate. A rise in the levels of LDL, VLDL with significant decrease in the level of HDL was also observed in the serum of isoproterenol-intoxicated rats. Significant increase in the level of myocardial marker enzymes (CK, LDH, ALT and AST) in serum was noted. The LDH & CK levels were low in the heart tissue. Oral administration of aqueous leaf extract of *Justicia tranquebariensis* Linn. (100 and 200mg/kg) to isoproterenol-induced rats daily for a period of 28 days proved the protective role of the aqueous extract of *Justicia tranquebariensis* Linn. The levels of the biochemical parameters in the plant treated groups were nearly the same as that of the normal control.

INTRODUCTION

Cardiovascular diseases are a group of disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis and pulmonary embolism. Most heart attacks are caused by atherosclerosis (stiffening and narrowing of the arteries).Atherosclerosis results from the formation of plaques inside the artery which is composed of high blood fats (triglycerides) and LDL or "bad" cholesterol, narrowing the passage and reducing the amount of blood that can flow through (Abbott *et al*, 2003). It is the main cause of morbidity and mortality worldwide, characterized by an excessive inflammatory, fibro fatty, proliferative response to damage of the artery wall. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. By 2030, almost 25 million people will die from CVDs, mainly from heart disease and stroke. These are projected to remain the single leading cause of death (Mukherjee, 1995). The effect of isoproterenol on the cardiovascular system is non-selective and relates to its actions on cardiac β1 receptors and β2 receptors on skeletal muscle arterioles. It has positive inotropic and chronotropic effects on the heart. In skeletal muscle arterioles it produces vasodilatation. These effects elevate systolic blood pressure. Isoproterenol produces an elevated heart rate (tachycardia), which predisposes patients to cardiac dysrhythmias (Shen,2008).

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Justicia tranquebariensis is a small shrub, which is widely distributed in southern parts of India. In this genus about 20 species have been chemically ivestigated and major secondary metabolites isolated were ligans, flavonoids,steroids and tri terpenes. The juice of small and somewhat fleshy leaves of genus *Justicia* is considered by natives of India as cooling and aperients, and is prescribed for the children in the smallpox (Subbaraju & Pillai, 1996)

Some species of the genus *Justicia* have been used in the traditional system of medicines for the treatment of fever, pain, inflammation, diabetes, diarrhoea and liver diseases. They also possess anti-inflammatory, anti-alergic, anti-tumoral, anti-viral and analgesic activities. The leaf juice of *J.tranquebariensis* has been used to treat jaundice and leaf paste is applied over affected area to treat skin diseases (Poongodi et al, 2011).

The present study was designed to evaluate the cardio protective role of the aqueous extract of *J.tranquebariensis* in Wistar strains of albino rats.

MATERIALS AND METHODS

Collection of Plant Material

Aerial parts of *Justicia tranquebariensis* were collected from in and around Trichy, identified with the help of Flora of Presidency of Madras and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's College, Trichy.

Preparation of Plant Extract

The plant materials were shade dried and coarsely powdered with electrical blender. 200gm of *Justicia tranquebariensis* Linn. was mixed with 1200 ml of water. Then it was boiled until it was reduced to one third and filtered. The filterate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening.

Experimental models

Wistar strains of Albino rats of both sexes weighing 150-200g were used for the study. Animals were housed in well ventilated cages in the CPCSEA approved animal house. The protocol was approved by the Institutional Animal Ethics committee. They were fed with pelleted rat chow and water ad libitum. They were acclimatized to the laboratory conditions for a week before the experiment.

Experimental Design

The rats were divided into five groups of six animals each. Group I served as a normal control, Group II rats were administered isoproterenol (20mg/kg, s.c) at the end of experimental period on the 29th and 30th days. Group III and IV were pretreated with *Justicia tranquebareinsi*s Linn. leaf extract (100 mg/kg, 200mg/kg ,respectively) for a period of 28 days and received a subcutaneous injection of isoproterenol (20mg/kg, b.w)at the end of experimental period for 2 consecutive days. Group V received aqueous extract of *Justicia tranquebariensis* Linn 200mg/kg b.w for 28 days. After the experimental period, blood was collected and serum was separated and used for the estimation of protein, cholesterol, triglycerides, phospholipids, and lipoproteins and the assay of marker enzymes. The heart homogenate was used for the assay lipid profiles.

Estimation of protein (Lowry *et al*, 1951)

Aliquots of the suitably diluted serum (0.1ml to 10ml by two serial dilutions) was made up to 1.0ml with water and 4.5ml of alkaline copper reagent was added to all the tubes including blank, containing 1.0ml water and standards containing aliquots of standard BSA and made up to 1ml with water. The tubes were incubated for 10 min at room temperature. 0.5 ml was added to all the tubes and incubated for 20 min at room temperature. The blue colour developed was read at 640nm.

Estimation of Cholesterol (Zak *et al*, 1953)

To 0.1 ml of the lipid extract, 4.9 ml of ferric chloride precipitaion reagent was added.Centrifuged for few minutes and the supenatant was collected.Take 2.5 ml supernatant, 2.5ml of ferric chloride diluting reagent. Add 4 ml of concentrated sulfuric acid. Suitable aliquots of the standards were made upto 5 ml with ferric chloride diluting reagent with 4 ml of concentrated sulfuric acid.The optical density was measured at 560 nm. The cholesterol content was expressed as mg/dl of serum.

Estimation of Triglyceride (Foster & Dunn, 1973)

1.0 ml of isopropanol was added to 0.1 ml of sample and mixed well, followed by 0.4g of alumina and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2.0ml of the supernatant was transferred to appropriately labelled tubes. The tubes were placed in a water bath at 65oC for 15 min for saponification after adding 0.6 ml of the sapponification reagent. After cooling 1.0 ml, of sodium metaperiodate was added followed by 0.5 ml of acetyl acetone reagent. After mixing the tubes at 65^{o}C 1/2hr.The contents were cooled and read at 430nm.

Estimation of Phospholipids (Bartlette 1959, Fiske and Subbbarrow, 1925)

0.1 ml of the sample was (tissue lipid) was digested with 0.2 ml of perchloric acid over a sand bath. Digestion was continued till it was colourless. The liberated phosphorus was estimated. 4.3 ml of H2O was added to the digested sample followed by 0.5 ml of ammonium molybdate. After 10 min 0.2 ml of ANSA was added. The tubes were shaken well and kept aside for 20 min. The blue colour developed was read at 620 nm.

Estimation of HDL Cholesterol (Friedewald *et al*., 1972)

1 ml serum was mixed with 0.1 ml of phosphotungstate reagent and 50 µl of magnesium chloride reagent. The content was centrifuged at ambient temperature for 30 minutes at 1500 rpm. 0.1 ml supernatent was added to 4.9 ml of ferric chloride

precipitating reagent, then it was mixed well and centrifuged. From this, 2.5 ml of supernatant was taken. An ice bath was set. 2.5 ml of diluting reagent and 4 ml of concentrated sulphuric acid were added by thorough mixing. Various concentrations of working standard solution were taken to which 5ml diluting reagent, and 4 ml of sulphuric acid were added. A blank was also maintained. The colour developed was read at 560nm.

Aggregation of VLDL

To 1 ml of plasma added 0.15 ml of SDS solution. The contents were mixed well and incubated at 37ºC for 2 hrs. The contents were centrifuged in a refrigerated centrifuge at 10,000 g for 30 min. VLDL C for 2 hrs. VLDL aggregates as pellicle at the top. The supernatant was a mixture containing HDL and LDL fraction. The values were expressed as mg/ dl.

$$\text{LDL Cholesterol} = \frac{\text{Total Serum Cholesterol} - (\text{Total serum TGL} - \text{HSL Cholesterol})}{5}$$

$$\text{VLDL} = \frac{\text{Total serum TGL}}{5}$$

Assay of SGlutamate Oxaloacetate Transaminase (King, 1965)

The assay mixture containing 1ml of substrate and 0.2 ml of serum was incubated for 1 hr at 37°C. To the control tubes serum was added after the reaction was arrested by the addition of 1ml of DNPH. The tubes were kept at room temperature for 30 min. Added 0.5 ml of NaOH and the colour developed was read at 540 nm.

Assay of SGlutamate Pyruvate Transaminase (King, 1965)

The assay mixture containing 1ml of substrate and 0.2 ml of serum was incubated 1 hr at 37°C. Added 1 ml of DNPH and kept at room temperature for 20 min. Serum was added to the control tubes after the reaction was arrested by the addition of 1 ml of DNPH. Added 5 ml of NaOH and the colour developed was read at 540 nm.

Assay of S Lactate Dehydrogenase (King, 1965)

To a set of tubes 1.0 ml of the buffered substrate and 0.1 ml serum was added and the tubes were incubated at 37˚C for 15 minutes. After adding 0.2 ml of NAD+ solution, the incubation was continued for another 15 minutes. The reaction was then arrested by adding 1.0 ml of DNPH reagent and the tubes were incubated for a further period of 15 minutes at 37˚C. 0.1 ml of serum was added to blank tubes after arresting the reaction with DNPH. 10 ml of 0.4N sodium hydroxide solution was added and the color developed was measured at 440 nm. Suitable aliquots of the standards were also analyzed by the same procedure. The enzyme activity was expressed as IU/litre.

Assay of S Creatine Kinase (Okinaka *et al*., 1961)

The incubation mixture containing 1.0 ml of double distilled water, 0.1 ml of serum, 0.1 ml of ATP solution, 0.1 ml of magnesium – cysteine reagent and 0.1 ml of creatine was incubated at 37˚C for 20 minutes. The tubes were centrifuged and the supernatant was used for the estimation of phosphorous by Fiske and Subbarow method.

Statistical Analysis

The values were expressed as mean± SEM. The statistical analysis was carried out by one way analysis of variance followed by "t" test. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The results obtained are tabulated which clearly indicates the protective role of the selected plant drug source. Table 1 depicts the changes in the lipid profile of the experimental models. The Gp II animals showed a significant increase in the levels of cholesterol, triglycerides and phospholipids in both serum and tissue. The pretreatment with the plant extract maintained the lipid profile near normal. Table 2 clearly indicates a raise in the LDL and VLDL levels with a decrease in HDL. The plant extract treated groups showed near normal level of lipoproteins. Table 3 indicates the levels of marker enzymes in serum. The isoproterenol induced group showed a marked elevation in the levels of the marker enzymes in the serum while the pretreated groups showed no significant alteration in the levels. Table 4 clearly indicated the levels of serum and tissue protein. The disease control group showed a decrease in both serum and tissue protein levels while the pretreated groups maintained the protein levels near normal.

Lipids play a vital role in CVD by the way of hyperlipidaemia, development of atherosclerosis and also by modifying the cellular membrane composition, structure and stability (Rajadurai & Prince, 2005).

Isoproterenol induced elevation in cholesterol levels could be due to 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 cholesterol (Deepa & Varalakshmi, 2005).

The increased phospholipids content in isoproterenol induced rats may be due to the damage caused in the myocardial membrane. The membrane stablilizing activity of the plant extract induced myocytes to synthesise more phospholipids which was necessary to repair the damaged membrane (James and Hrabison, 1982)

Hypertriglyceridemia was observed in isoproterenol intoxicated rats may be due to decreased activity of lipoprotein lipase in the myocardium resulting in decreased uptake TG from the circulation. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage. Pretreatment with the *J.tranquebariensis* Linn plant extract alters the activities of LCAT(Lecithin: cholesterol acyl esterase), lipo protein lipase, and cholesterol ester synthetase(CES)

and increases HDL, decreasing TG and cholesterol levels, indicating the potential lipid lowering effect of *J.tranquebariensis* Linn (Upaganlawar,2009). Isoproterenol administration increased LDL and VLDL levels with asignificant decrease in HDL. Present study also showed the significant decrease in HDL in isoproterenol treated animals, which was prevented by pretreatment with *Justicia tranquebariensis* Linn. HDL is known to be involved in the transport of cholesterol from tissues to the liver for excretion into the bile and thus called "good cholesterol".

Thus the cholesterol lowering activity of *J.tranquebariensis* Linn could be mediated through increasing the activity of extrahepatic lipoprotein lipase which increased hydrolysis of triglycerides that result in the transfer of lipids and apolipoproteins to HDL and thereby facilitate their excretion (Mahendra A Gunjal *et al*, 2010). Myocardium contains an abundant concentration of many enzymes, viz. AST, CK and LDH and once metabolically damaged releases its content into extracellular fluid (Sharma *et al*, 2001). Hence, in isoproterenol induced rats, the increased activities of serum ALT, AST, LDH and CK accompanied by their concomitant reduction in heart homogenate confirm the onset of myocardial necrosis (Saxena *et al*, 1998). Pretreatment with *Justicia tranquebariensis* Linn showed the normalization of the activity of diagnostic marker enzymes when compared with isoproterenol treated rats.

During active necrosis changes in serum protein level were reported in isoproterenol induced myocardial infracted rats (Ponnusamy Saranya *et al*, 2012) .

A decrease in serum protein is usually as a result of a fall in albumin or sometimes γ - globulin (Rekha Rajendran and Saleem Bhasha, 2008) Isoproterenol induced myocardial infarction is a free radical mediated tissue damage and may lead to production of more oxygen and hydrogen peroxide ions which in turn could bind with albumin and thus destroy it.

Table. 1: Effect of the aqueous extract of *J.tranquebariensis* on the lipid profile of the models .

Group	Cholesterol		Triglyceride		Phospholipids	
	Serum mg/dl	Tissue mg/g	Serum mg/dl	Tissue mg/g	Serum mg/dl	Tissue mg/g
I	68.51 ± 1.68	4.56 ± 0.38	156.25±1.42	1.56±0.11	10.52± 0.91	22.45± 0.59
II	132.41± 1.34*	8.72 ± 0.16*	312.5±2.31*	6.25±1.07*	29.63± 0.83*	44.56± 1.09*
III	85.91± 0.91	6.2 ± 0.96	176.92±1.33	4.68±1.02	17.55 ± 0.93	31.82 ±1.01
IV	70.91 ± 2.1*	4.81± 1.03*	155.31±1.08*	1.61±0.96*	12.35 1.03*	24.71± 0.69*
V	67.31± 0.11	4.32± 1.09	160.16±0.93	1.52±0.87	9.65± 0.91	21.38± 0.91

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with normal control
**p<0.05 statistically significant when compared with disease controlgroup

Table. 2: Evaluation of the changes in the lipoprotein levels in treated and control groups of the experimental models.

Groups	I	II	III	IV	V
LDL (mg/dl)	21.64± 1.36	52.6±1.6*	27.58±1.24	23.92±0.09**	22.17±1.02
HDL (mg/dl)	37.77 ± 1.09	20.37± 0.79*	31.59 ±1.03	34.88 ± 0.92**	33.41±1.09
VLDL (mg/dl	31.25±0.09	62.5±0.73*	48.56±0.93	36.49±0.79**	29.04±0.88

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with control group
**p<0.05 statistically significant when compared with disease control group

Table. 3: Assay of marker enzymes.

Groups	I	II	III	IV	V
Serum AST (IU/L)	17.46±0.56	104.76±0.91*	42.37±0.81	27±31±0.19**	18.41±0.91
Serum ALT (IU/L	22.38±0.99	82.9±1.01*	45±0.71	28.51±0.75**	27.7±0.51
LDH (IU/L)	80.33±1.41	157.21±0.89*	97.34±3.32	82.63±2.09**	78.74±1.21
CK (IU/L)	273.22±10.31	681.9±3.89*	368.38±3.04	281.14±4.02**	269.43±1.03

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with control group
**p<0.05 statistically significant when compared with disease control group

Table. 4: Changes in the Serum and Tissue protein levels in the experimental models.

Groups	I	II	III	IV	V
Serum protein (g/dl)	6.95±0.05	2.38±0.05*	4.22±0.98	5.99±0.86**	7.01±0.99
Tissue protein (g/g of tissue)	3.26 ± 0.03	1.12 ± 0.69*	2.23 ± 0.72	3.12 ± 1.07**	3.84 ± 0.74

Values are mean ± SEM (n=6)
*p< 0.05 statistically significant when compared with control group
**p<0.05 statistically significant when compared with disease control group

CONCLUSION

From the present study it is clearly evident that *Justicia tranquebareinsi*s Linn. possess a cardioprotective effect and the results obtained indicates its potential in protecting the Wistar strains of albino rats in isoproterenol induced myocardial infarction in.

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