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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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 tranquebariensis 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 the 29th and 30th days. Group III and IV were pretreated with Justicia tranquebariensis Linn. leaf extract (100mg/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.

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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 investigated and major secondary metabolites isolated were ligans, flavonoids, steroids and 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, diarrhea and liver diseases. They also possess anti-inflammatory, anti-allergic, 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 filtrate 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 tranquebariensis 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 precipitation reagent was added.Centrifuged for few minutes and the supernatant 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 65°C for 15 min for saponification after adding 0.6 ml of the saponification 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°C 1/2hr. The contents were cooled and read at 430nm.

Estimation of Phospholipids (Bartlette 1959, Fiske and Subbarrow, 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 heated 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.
L and LDL rum while the pretreated groups showed
mation of phosphorous by
˚C for 15
os observed in isoproterenol
ester synthetase exceeds the
e containing 1.0 ml of double
reaction was then
e for another 15 minutes. Th
was continued for another 15 minutes. After adding 0.2 ml of NAD+ solution, the incubation
ml serum was added and the tubes were incubated at 37
Assay of S Lac

Lipids play a vital role in CVD by the way of
hyperlipidaemia, development of atherosclerosis and also by
modification of the cellular membrane composition, structure and
(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 stabilizing activity of the plant extract
induced myocytes to synthesise more phospholipids which was
necessary to repair the damaged membrane (James and Hrabison,
1982)

Hypertriglyceridermia 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)

Aggregation of VLDL

To 1 ml of plasma added 0.15 ml of SDS solution. The
contents were mixed well and incubated at 37°C for 2 hrs. The
contents were centrifuged in a refrigerrated 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
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 asisgnificant 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 infarcted 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Cholesterol (mg/dl)</th>
<th>Tissue Cholesterol (mg/g)</th>
<th>Serum Triglyceride (mg/dl)</th>
<th>Tissue Triglyceride (mg/g)</th>
<th>Serum Phospholipids (mg/dl)</th>
<th>Tissue Phospholipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>68.51±1.68</td>
<td>4.56±0.38</td>
<td>156.25±1.42</td>
<td>1.56±0.11</td>
<td>10.52±0.91</td>
<td>22.45±0.59</td>
</tr>
<tr>
<td>II</td>
<td>132.41±1.34*</td>
<td>8.72±0.16*</td>
<td>312.5±2.31*</td>
<td>6.25±1.07*</td>
<td>29.63±0.83*</td>
<td>44.56±1.09*</td>
</tr>
<tr>
<td>III</td>
<td>85.91±0.91</td>
<td>6.2±0.96*</td>
<td>176.92±1.33</td>
<td>4.68±0.02</td>
<td>17.55±0.93</td>
<td>31.82±1.01</td>
</tr>
<tr>
<td>IV</td>
<td>70.91±2.1*</td>
<td>4.81±1.03*</td>
<td>155.31±1.08*</td>
<td>1.61±0.96*</td>
<td>12.35±1.03*</td>
<td>24.71±0.69*</td>
</tr>
<tr>
<td>V</td>
<td>67.31±0.11</td>
<td>4.32±1.09</td>
<td>160.16±0.93</td>
<td>1.52±0.87</td>
<td>9.65±0.91</td>
<td>21.38±0.91</td>
</tr>
</tbody>
</table>

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 control group

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

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

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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum AST (IU/L)</th>
<th>Serum ALT (IU/L)</th>
<th>LDH (IU/L)</th>
<th>CK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>17.46±0.56</td>
<td>22.38±0.99</td>
<td>80.33±1.41</td>
<td>273.22±10.31</td>
</tr>
<tr>
<td>II</td>
<td>104.76±0.91*</td>
<td>82.9±1.01*</td>
<td>157.21±0.89*</td>
<td>681.9±3.89*</td>
</tr>
<tr>
<td>III</td>
<td>42.37±0.81</td>
<td>45.0±0.71</td>
<td>97.34±3.32</td>
<td>368.38±3.04</td>
</tr>
<tr>
<td>IV</td>
<td>27.93±1.09**</td>
<td>28.5±1.75**</td>
<td>82.63±2.09**</td>
<td>281.14±4.02**</td>
</tr>
<tr>
<td>V</td>
<td>27.41±0.91</td>
<td>27.7±0.51</td>
<td>78.74±1.21</td>
<td>269.43±1.03</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum Protein (g/dl)</th>
<th>Tissue Protein (g/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6.95±0.05</td>
<td>3.26±0.03</td>
</tr>
<tr>
<td>II</td>
<td>2.38±0.05*</td>
<td>1.12±0.69*</td>
</tr>
<tr>
<td>III</td>
<td>4.22±0.98</td>
<td>2.23±0.72</td>
</tr>
<tr>
<td>IV</td>
<td>5.99±0.86**</td>
<td>3.12±1.07**</td>
</tr>
<tr>
<td>V</td>
<td>7.01±0.99</td>
<td>3.84±0.74</td>
</tr>
</tbody>
</table>

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 tranquebareinsis 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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