Evaluation of Cardioprotective Effect of Tocotrienol Rich Fraction from Rice Bran Oil

Jayeeta Bardhan¹, Ananya Chatterjee², Smita Das², Sandip K. Bandyopadhyay²,³, Runu Chakraborty¹, Utpal Raychaudhuri¹⁴

¹Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, West Bengal, India.
²Central Research Laboratory, Department of Biochemistry, K.P.C. Medical College & Hospital, 1F, Raja S.C. Mullick Road, Kolkata, West Bengal, India.
³Department of Biochemistry, University College of Medicine, I.P.G.M.E & R, 244B A.J.C. Bose Road, Kolkata, West Bengal, India.

*Corresponding author’s E-mail: urcfoodtech@gmail.com

Accepted on: 28-10-2014; Finalized on: 31-12-2014.

ABSTRACT

This study was aimed to evaluate the protective effect of Tocotrienol Rich Fraction (TRF) prepared from crude rice bran oil on experimentally induced myocardial infarction (MI) in rats. To quantify the tocotrienol content of TRF, the HPLC analysis was performed. It has been found that in TRF, tocotrienol was present in the concentration of 0.252 mg ml⁻¹, δ tocotrienol 0.228 mg ml⁻¹ and γ tocotrienol 0.652 mg ml⁻¹ respectively. Rats were pretreated orally with TRF at the doses of 0.5, 1.0, 2.0, 4.0 mg tocotrienol kg⁻¹ body weight prior to induction of MI by injecting Isoproterenol bitartrate (ISO). The effects on cardiac marker enzymes and lipid peroxidation marker, thiobarbituric acid reactive substances (TBARS) were estimated. Heart tissue histology was done. Rats administered with ISO showed significant increase in serum levels of MI diagnostic marker enzymes, lactate dehydrogenase, creatine kinase MB (CK-MB), aspartate transaminase, alanine transaminase, lipid peroxidation marker TBARS, and triglyceride, cholesterol, LDL, VLDL levels in serum. The group of rats administered with ISO alone showed decrease in LDH, CK-MB, AST, ALT levels in heart tissue homogenate and also in serum HDL level. Pretreatment with TRF significantly inhibited the effects of ISO. The effect of TRF at the dose of 2 mg tocotrienol kg⁻¹ body weight was found to be most effective. Histopathological observations also support the biochemical data. The results of the study showed that TRF exhibited antioxidant, anti-lipidperoxidative, hypolipidemic and cardioprotective activities in experimentally induced myocardial infarcted rats.

Keywords: Cardioprotection, Lipid profile, Marker enzymes, Myocardial infarction, Rice bran oil, Tocotrienol

INTRODUCTION

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in the Western world, and according to the World Health Organization, it will be the major cause of death in the world by the year 2020.¹ In market, number of synthetic drugs are available but their long term use ultimately cause severe side effects. So the research in natural cardioprotective compounds is the need of the hour. A common view is that oil intake to be restricted for the cardiac impaired patients, but the present study revealed that TRF from crude rice bran oil protects the cardiac damage induced by isoproterenol [1-(3, 4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride] (ISO), a synthetic catecholamine and β-adrenergic agonist. However, rich in tocotrienols, rice bran oil is obtained from the rice mill byproduct, rice bran.² But these micronutrients are lost during the chemical refining steps.³ Thus, attempts were made to utilize crude rice bran oil for preparing TRF in which the micronutrients would be retained.⁴ Number of reports showed that tocotrienol has direct cardioprotective role,⁵ by lowering cholesterol synthesis and down regulates the expression of HMG Co A (a reductase protein, that is responsible for cholesterol synthesis) through post transcriptional process.⁶ Cardioprotection being the property of constituent components, it has been presumed that, TRF should exhibit protection against myocardial infarction. This has motivated to evaluate the potential use of TRF as cardioprotective agent. For the purpose, different clinical parameters determinant of cardiovascular diseases have been measured. Histopathological observations also have been studied in ISO induced myocardial infarcted rats after pretreatment with TRF.

MATERIALS AND METHODS

Chemicals and reagents

Isoproterenol bitartrate salt and bovine serum albumin (BSA) were purchased from Sigma Aldrich, USA. All other common chemicals used were of the highest analytical grade (BDH, India; SRL, India; Merck, India). Crude rice bran oil was obtained from a local oil mill.

Methods

Preparation of TRF

The crude rice bran oil was mixed with ethanol and kept in a shaker. The alcohol mixture was then evaporated under vacuum at low temperature (40°C) to obtain a fraction that contains many medicinally beneficial micronutrients of which tocotrienol is a major component.⁴

Tocotrienol Content Analysis of TRF

Each of α, β, γ and δ isomers of pure tocotrienol at a concentration of 0.5 mg ml⁻¹ has been used as standard. The HPLC system, Jasco LC Net II/ADC, Japan was used. The HPLC was equipped with Jasco PU-2080 Plus Intelligent HPLC pump, Jasco MD-2015 Plus Multi
wavelength Detector and Jasco Chrompass Chromatography Data System. The flow rate of the mobile phase was maintained at 1ml minute\(^{-1}\). The detection of the samples was carried out at 290nm by UV detector.

**Experimental Procedure**

The present study focused on whether TRF could exert protective effect on MI in ISO induced rats. For this purpose the rats were orally treated with different doses of TRF for 12 days and on 11\(^{th}\) and 12\(^{th}\) day, MI were experimentally induced to all the groups except the control group. Male Charles Foster rats were procured after obtaining clearance from the I.P.G.M. E. & R. Animal Ethics Committee Guidelines, Animal Ethical Committee, Sanction No. IAEC/SB-3/2008UCM-64 dated 15.5.2008-2011. The rats (100-120 gm weight) were fed on a balanced laboratory diet as per National Institute of Nutrition, Hyderabad, India, and given tap water ad libitum.

The animals were kept in a well ventilated animal house at 25 ± 2 \(^{o}\)C, 65-70% humidity under (12h/12h) light and dark cycle.

**Grouping of animals**

In this experiment, the rats were divided into six groups each consisting of six rats.

**Group I**

Normal control rats.

**Group II**

Rats daily fed orally with TRF in the dose of 0.5 mg tocotrienol kg\(^{-1}\) body weight for 12 days and received ISO 100mg kg\(^{-1}\) body weight on 11\(^{th}\) and 12\(^{th}\) day.

**Group III**

Rats daily fed orally with TRF in the dose of 1.0 mg tocotrienol kg\(^{-1}\) body weight for 12 days and received ISO 100mg kg\(^{-1}\) body weight on 11\(^{th}\) and 12\(^{th}\) day.

**Group IV**

Rats daily fed orally with TRF in the dose of 2.0 mg tocotrienol kg\(^{-1}\) body weight for 12 days and received ISO 100mg kg\(^{-1}\) body weight on 11\(^{th}\) and 12\(^{th}\) day.

**Group V**

Rats daily fed orally with TRF in the dose of 4.0 mg tocotrienol kg\(^{-1}\) body weight for 12 days and received ISO 100mg kg\(^{-1}\) body weight on 11\(^{th}\) and 12\(^{th}\) day.

**Group VI**

Rats received ISO 100mg kg\(^{-1}\) body weight on 11\(^{th}\) and 12\(^{th}\) day.

**Collection of serum and preparation of tissue**

At the end of the experimental period, 12 hours after the second injection, all the rats were sacrificed. Blood was collected without anticoagulant for serum. Heart tissues were excised immediately and rinsed in ice chilled normal saline. Known weights of the tissues were homogenized in 5.0 ml of 0.1 M Tris - HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

**Biochemical assays**

LDH activity and CK MB activity were determined by the method described by Vijayan and his coworkers.\(^9\) AST and ALT assays were determined according to Reitman and Frankel method.\(^9\) The sample was mixed with thiobarbituric acid - trichloroacetic acid reagent with thorough shaking, heated for 20 minutes and then cooled to room temperature. The absorbance of the pink chromagen present in the clear supernatant after centrifugation was measured spectrophotometrically.\(^10\) The method involves the hydrolysis of triglycerides coupled with enzymatic procedures for measuring the glycerol released. Glycerol measurement can be made by using glycerol kinase.\(^11\) Cholesterol in acetic acid forms a red color on treatment with ferric chloride and sulphuric acid which can be measured colorimetrically.\(^12\) Proteins in serum are precipitated with ferric chloride - acetic acid reagent. Equal volumes of protein free filtrate, containing cholestrol, and a blank containing ferric chloride acetic acid reagent are separately treated with sulphuric acid. LDL (low density lipoprotein), VLDL (very low density lipoprotein) and chylomicrons are precipitated by polyanions (phosphotungstate) in the presence of metal ions (magnesium ions) to leave HDL in solution. The cholesterol content of the supernatant fluid is then measured by the method of Lopez-Virella.\(^13\) The Friedewald’s equation is used to calculate VLDL and LDL cholesterol levels.\(^14\)

\[
VLDL = \frac{Triglycerides}{5}
\]

\[
LDL = Total cholesterol - (HDL + VLDL)
\]

Non HDL Cholesterol can be obtained as:

\[
Non\ HDL\ Cholesterol = Total\ cholesterol - HDL\ cholesterol.
\]

LDL : HDL Cholesterol was calculated.\(^15\)

**Histopathological Examination**

The heart tissues obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissues were processed embedding in paraffin. Then, the tissues were sectioned, stained with hematoxylin (H) and eosin (E), examined under high power microscope and microphotographs were also taken.\(^1\)

**Statistical Analysis**

The results are presented as mean ± S.D. The significance of difference among the groups was assessed using one way analysis of variance (ANOVA). Significance was set as p < 0.05.
RESULTS

HPLC Analysis of TRF

Figure 1a, 1b, 1c and 1d show the HPLC profiles of standard α, β, δ and γ isomers of tocotrienol at a concentration of 0.5 mg ml⁻¹ each. The HPLC profile of TRF has been shown in Figure 2. The comparative standard-TRF analysis demonstrates the presence of tocotrienol in the investigated TRF. The tocotrienol isomers in the TRF were identified by comparison of their retention times. The major compounds detected in TRF are α, δ, γ isomers of tocotrienol.

Table 1 records the amount of different isomers of tocotrienol present in the TRF. The TRF contains α tocotrienol in the concentration of 0.252 mg ml⁻¹, δ tocotrienol 0.228 mg ml⁻¹ and γ tocotrienol 0.652 mg ml⁻¹, the total concentration of tocotrienol in TRF being 1.132 mg ml⁻¹.

Table 1: Amount of Tocotrienol isomers present in TRF

<table>
<thead>
<tr>
<th>Sample</th>
<th>α Tocotrienol (mg ml⁻¹)</th>
<th>δ Tocotrienol (mg ml⁻¹)</th>
<th>γ Tocotrienol (mg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRF</td>
<td>0.252</td>
<td>0.228</td>
<td>0.652</td>
</tr>
</tbody>
</table>

Effect of TRF on the Cardiac Marker Enzymes of Serum

Table 2 shows the serum levels of LDH, CK-MB, AST and ALT in control and experimental rats. Rats, treated with ISO alone, showed significant increase in the levels of LDH (117%), CK-MB (508%), AST (95%) and ALT (44%) in the serum compared to control rats. Oral pretreatment with TRF in doses of 0.5, 1, 2, 4 mg tocotrienol kg⁻¹ body weight to ISO induced rats, daily for a period of 12 days, decreased the levels of these enzymes in the serum. TRF, at the dose of 2 mg tocotrienol kg⁻¹ body weight, showed better effect than the other 3 doses (0.5, 1, 4 mg tocotrienol kg⁻¹ body weight) in ISO induced rats. The LDH level is found to decrease in ISO induced rats, pretreated with TRF, in a dose-dependent manner compared to rats treated with ISO alone.

However, the rats, induced with ISO, pretreated with TRF in the dose of 4 mg tocotrienol kg⁻¹ body weight, showed the maximum effect (28%) on serum LDH level. The serum CK-MB level reduced with increasing amount of TRF orally administered to ISO induced rats. TRF in the dose of 4 mg tocotrienol kg⁻¹ body weight in ISO induced rats gave the maximum of 85% reduction of CK-MB value compared with ISO induced rats. However, in case of both AST and ALT, TRF in the dose of 2 mg tocotrienol kg⁻¹ body weight gave the maximum lowering effect on serum enzyme levels with 40% and 32% reduction respectively with ISO induced rats.

Effect of TRF on the Cardiac Marker Enzymes of Heart Tissue Homogenate (HTH)

The levels of LDH, CK-MB, AST and ALT in the HTH of control and experimental rats are represented in Table 3. The LDH, CK-MB, AST and ALT activities in HTH showed a significant decrease of 43%, 46%, 40% and 42% respectively in ISO induced myocardial infarcted rats compared to the control rats. Pretreatment with TRF in doses of 0.5, 1, 2, 4 mg tocotrienol kg⁻¹ body weight to ISO induced rats, minimized the alterations in the activities of these enzymes when compared to rats treated with ISO alone. The LDH activity has increased by 59% in the group of rats, with TRF in dose of 2 mg tocotrienol kg⁻¹ body weight, compared with the ISO alone treated group of rats. CK-MB and AST enzyme levels have gradually increased in a dose dependent manner. TRF in the dose of 4 mg tocotrienol kg⁻¹ body weight has been found to provide the maximal protection, by increasing the CK-MB and ALT values, closest to that of the control groups. AST values of the group of rats, with TRF in the dose of 2 mg tocotrienol kg⁻¹ body weight, showed the highest values, closest to the control group values.

Effect of TRF on Lipid Peroxidation Marker

Table 4 shows the levels of serum and heart tissue homogenate TBARS of control and ISO induced rats. ISO treated rats showed significant increase in the level of TBARS when compared to normal control rats. Pretreatment with TRF reduced the levels of TBARS in ISO treated rats when compared to ISO alone treated rats. Maximal reduction of 36% in serum TBARS and maximal reduction of 25% in heart TBARS both have been observed in the same group of ISO induced rats, pretreated with TRF in dose of 2 mg tocotrienol kg⁻¹ body weight.

![Figure 1(a): HPLC profile of standard tocotrienol α isomer](image1a)

![Figure 1(b): HPLC profile of standard tocotrienol β isomer](image1b)

![Figure 1(c): HPLC profile of standard tocotrienol δ isomer](image1c)

![Figure 1(d): HPLC profile of standard tocotrienol γ isomer](image1d)
Effect of TRF on Lipid Profile

Table 5 describes the lipid profile of the TRF pretreated ISO induced rats in comparison to the lipid profile of the isoproterenol induced myocardial infarcted rats. When compared to control rats, the ISO induced rats, showed a significant increase in serum levels of triglycerides, total cholesterol, LDL and VLDL with significant decrease in the level of HDL, called good cholesterol. Oral pretreatment with TRF in doses of 0.5, 1, 2, 4 mg tocotrienol kg\(^{-1}\) body weight to ISO treated rats significantly decreased the levels of triglycerides, total cholesterol, LDL, and VLDL with significant increase in the level of HDL when compared to ISO alone induced rats.

### Table 2: Effect of TRF on the levels of LDH, CK-MB, AST and ALT in serum in normal and isoproterenol induced myocardial infarcted rats

<table>
<thead>
<tr>
<th>Experimental Sets</th>
<th>LDH (IU/L)</th>
<th>CK-MB (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>350 ± 21*</td>
<td>12.9 ± 1.4*</td>
<td>110 ± 12*</td>
<td>21.2 ± 3*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>758 ± 51</td>
<td>78.5 ± 8.0</td>
<td>215 ± 17</td>
<td>30.5 ± 4</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 0.5 mg tocotrienol kg(^{-1}) body weight</td>
<td>756 ± 45 NS</td>
<td>16.5 ± 1.5*</td>
<td>211 ± 22 NS</td>
<td>30.6 ± 5 NS</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 1 mg tocotrienol kg(^{-1}) body weight</td>
<td>588 ± 42*</td>
<td>12.5 ± 1.4*</td>
<td>201 ± 21*</td>
<td>25.4 ± 4*</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 2 mg tocotrienol kg(^{-1}) body weight</td>
<td>576 ± 41*</td>
<td>12.3 ± 1.4*</td>
<td>128 ± 11*</td>
<td>20.8 ± 4*</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 4 mg tocotrienol kg(^{-1}) body weight</td>
<td>546 ± 41*</td>
<td>11.8 ± 1.4*</td>
<td>140 ± 12*</td>
<td>23.5 ± 2*</td>
</tr>
</tbody>
</table>

*\(p < 0.05\), significance compared to the isoproterenol induced myocardial infarction group of rats
NS: non significance compared to the isoproterenol induced myocardial infarction group of rats

### Table 3: Effect of TRF on the levels of LDH, CK-MB, AST and ALT in heart tissue homogenate in normal and isoproterenol induced myocardial infarcted rats

<table>
<thead>
<tr>
<th>Experimental Sets</th>
<th>LDH (IU/mg protein)</th>
<th>CK-MB (IU/mg protein)</th>
<th>AST (IU/mg protein)</th>
<th>ALT (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>205 ± 15*</td>
<td>15.2 ± 1.6*</td>
<td>215 ± 18*</td>
<td>19 ± 1*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>116 ± 11</td>
<td>8.2 ± 0.9</td>
<td>128 ± 11</td>
<td>11 ± 0.7</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 0.5 mg tocotrienol kg(^{-1}) body weight</td>
<td>120 ± 10 NS</td>
<td>8.9 ± 0.8 NS</td>
<td>130 ± 15 NS</td>
<td>12 ± 0.9 NS</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 1 mg tocotrienol kg(^{-1}) body weight</td>
<td>130 ± 11 NS</td>
<td>9.5 ± 1.0 NS</td>
<td>150 ± 16 NS</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 2 mg tocotrienol kg(^{-1}) body weight</td>
<td>185 ± 14*</td>
<td>13.5 ± 1.4*</td>
<td>209 ± 19*</td>
<td>14 ± 1.2*</td>
</tr>
<tr>
<td>Isoproterenol + TRF in dose of 4 mg tocotrienol kg(^{-1}) body weight</td>
<td>180 ± 16*</td>
<td>14.5 ± 1.6*</td>
<td>198 ± 20*</td>
<td>15 ± 1.1*</td>
</tr>
</tbody>
</table>

*\(p < 0.05\), significance compared to the isoproterenol induced myocardial infarction group of rats
NS: non significance compared to the isoproterenol induced myocardial infarction group of rats

**Figure 2:** HPLC profile of TRF where α, δ and γ isomers are present.
Table 4: Effect of TRF on the levels of TBARS in serum and heart tissue homogenate in normal and isoproterenol induced myocardial infarcted rats

<table>
<thead>
<tr>
<th>Experimental Sets</th>
<th>SERUM TBARS (nano mole/ml)</th>
<th>HEART TISSUE HOMOGENATE TBARS (nano mole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5 ± 0.7*</td>
<td>0.04 ± 0.003*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>11 ± 0.9</td>
<td>0.06 ± 0.005</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 0.5 mg tocotrienol kg⁻¹ of body weight</td>
<td>10 ± 0.8*</td>
<td>0.057 ± 0.005*</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 1.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>9 ± 1*</td>
<td>0.055 ± 0.005*</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 2.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>7 ± 0.6*</td>
<td>0.045 ± 0.003*</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 4.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>8 ± 0.9*</td>
<td>0.05 ± 0.004*</td>
</tr>
</tbody>
</table>

*p < 0.05, significance compared to the isoproterenol induced myocardial infarction group of rats
NS: non significance compared to the isoproterenol induced myocardial infarction group of rats

Table 5: Effect of TRF on the lipid profile values in serum in normal and isoproterenol induced myocardial infarcted rats

<table>
<thead>
<tr>
<th>Experimental Sets</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>LDL Cholesterol (mg/dl)</th>
<th>VLDL Cholesterol (mg/dl)</th>
<th>Non HDL Cholesterol (mg/dl)</th>
<th>LDL:HDLD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>62 ± 5*</td>
<td>109 ± 14*</td>
<td>35 ± 5*</td>
<td>62 ± 8*</td>
<td>12 ± 2*</td>
<td>74 ± 8*</td>
<td>1.77 : 1</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>119 ± 14</td>
<td>136 ± 12</td>
<td>24.5 ± 6</td>
<td>79 ± 9</td>
<td>18 ± 4</td>
<td>93 ± 10</td>
<td>3.22 : 1</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 0.5 mg tocotrienol kg⁻¹ of body weight</td>
<td>109 ± 7*</td>
<td>101 ± 11*</td>
<td>34 ± 4*</td>
<td>65 ± 7*</td>
<td>14 ± 2*</td>
<td>81 ± 6.9*</td>
<td>1.91 : 1</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 1.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>98 ± 8*</td>
<td>107 ± 14*</td>
<td>33 ± 5*</td>
<td>62 ± 9*</td>
<td>13 ± 3*</td>
<td>81 ± 9*</td>
<td>1.87 : 1</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 2.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>82 ± 10*</td>
<td>106 ± 9*</td>
<td>33 ± 4*</td>
<td>62 ± 7*</td>
<td>14 ± 2.2*</td>
<td>61 ± 8*</td>
<td>1.87 : 1</td>
</tr>
<tr>
<td>Isoproterenol+ TRF in dose of 4.0 mg tocotrienol kg⁻¹ of body weight</td>
<td>67 ± 9*</td>
<td>110 ± 11*</td>
<td>33 ± 3*</td>
<td>60 ± 6*</td>
<td>17 ± 2.8*</td>
<td>61 ± 8*</td>
<td>1.81 : 1</td>
</tr>
</tbody>
</table>

*p < 0.05, significance compared to the isoproterenol induced myocardial infarction group of rats
NS: non significance compared to the isoproterenol induced myocardial infarction group of rats

Figure 3a: Microphotograph showing normal cardiac cells with branching of normal untreated group (Magnification 400x)

Figure 3b: Microphotograph showing cardiac muscle with disappearance of nucleus of isoproterenol induced group (Magnification 400x)

Figure 3c: Microphotograph showing heart tissue with peripheral position of the nucleus of TRF treated isoproterenol induced group (Magnification 400x)
**Effect of TRF on Histopathology of Rat Myocardium**

The group pretreated with TRF in the dose 2mg tocotrienol kg⁻¹ body weight exhibits maximal protection against isoproterenol induced damage. Thus, the histological study was performed with the group of rats pretreated with TRF in dose of 2mg tocotrienol kg⁻¹ body weight. Figure 3a shows normal cardiac cells with the nuclei centrally located. Isoproterenol induced myocardial infarction is reflected in Figure 3b in which the nuclei disappear from the cardiac cells. Figure 3c shows the cardiac cells of TRF pretreated, isoproterenol induced, myocardial infarcted rats. In Figure 3c, it has been found that the nuclei are located in the periphery of the cardiac cells. This portrays the protective effect of the TRF pretreatment on the cardiac cells.

**DISCUSSION**

Myocardial infarction (MI) occurs when the blood supply to a region of myocardium is interrupted due to occlusion of coronary artery and the resulting hypoxia causes the death of heart tissue. In this study, ISO was administered at the dose of 100 mg kg⁻¹ body weight to induce MI in rats. ISO was subcutaneously injected, twice, at an interval of 24 hours, with no mortality of animals during the treatment period. Cardio protection of TRF, which is enriched form of different isomeric forms of tocotrienols as shown in Figure 2, has been assessed by estimating serum and heart LDH, CK MB, AST, ALT levels, lipid profile, lipid peroxidation marker (Table 2-5). The presence of the LDH, CK MB, AST and ALT, diagnostic markers of MI, in heart tissue homogenate are indicative of myocardial integrity and their release in serum signifies myocardial injury. These marker enzymes are released from the heart into the blood during myocardial damage might be due to ISO induced necrosis in the myocardium. When myocardial cells containing LDH and CK-MB are damaged or destroyed, the cell membrane becomes permeable or may rupture, which results in the leakage of these enzymes. Membrane rupture could purely result from a mechanical effect (excessive cardiomyocyte contraction) or from osmolarity problems resulting from energy deficiency that induce cellular swelling. This accounts for the increased activities of serum LDH and CK-MB in ISO treated rats.

Thus, upon ISO treatment, resulting myocardial infarction causes elevation of serum LDH and reduction of HTH LDH level. Damage to the membrane, induced by the ISO, causes release of enzymes in the serum and deficiency of these enzymes in HTH reflects the damage to the myocardium. Pretreatment with TRF decreased the ISO induced elevation of serum LDH and it might be by protecting the cell membrane from the destructive effects of free radicals. An increase in the activities of AST and ALT in serum, with subsequent decrease in the myocardium of ISO induced TRF pretreated rats, has been observed in ISO induced rats. The ISO treated group shows significant elevation in the level of these serum markers, which confirmed the cardiac damage. ISO induction produces free radicals via β adrenoreceptor mechanism, affecting the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis. Pretreatment with TRF decreased the activities of these enzymes in the serum and increased the activities of these enzymes in the HTH of isoproterenol induced rats. TRF restored the activities of these enzymes in the serum and HTH closest to that of the control. This might be due to the protective effect of TRF on the myocardium, reducing the cardiac damage, thereby, restricting the leakage of these enzymes (Table 2 & 3). ISO also causes the production of cytotoxic free radicals through its auto-oxidation. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane. This alters membrane permeability, thus leading to the loss of function and integrity of myocardial membranes. Lipid peroxidation is an indication of the severity of ISO induced necrotic damage of the heart, and has been linked with altered membrane structure and enzyme inactivation. ISO treated rats showed marked increase in lipid peroxidation in myocardium, measured as TBARS content. Alteration in the metabolism of lipid peroxides is closely associated with myocardial damage due to free radicals produced by ISO. Administration of TRF markedly reduced lipid peroxidation as evidenced by reduction in myocardial TBARS level in comparison to ISO treated groups (Table 4). Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzymes. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with mediated atherosclerosis. Activation of lipid peroxidation corresponded with changes in lipid composition. Alterations in lipid composition, observed in necrosis impaired myocardial tissue, appear to occur due to destruction of cardiomyocytes. The significant increase observed in the lipid profile in rats, treated with ISO alone, could be due to enhanced lipid biosynthesis by cardiac CAMP on ISO administration. In ISO administered rats, cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides increased significantly with a significant decrease in HDL cholesterol. In rats, treated with TRF and ISO, the levels of cholesterol and triglycerides in the lipoprotein fractions were retained near control values. The most significant risk indicators for cardiovascular alterations, which are considered to be parameters of oxidative stress, are increased serum cholesterol, triacylglycerol, LDL-cholesterol and decreased HDL cholesterol. Various theories have suggested that cardiovascular damage was the result of an oxidative stress process. The high level of VLDL and triglycerides on ISO administration could be due to the decreased activity of extra hepatic lipoprotein lipase. The results obtained in the present study indicate that TRF pretreatment offers protection in myocardial infarction, experimentally induced by isoproterenol (Table 5).
Histological changes, induced by excessive amounts of isoproterenol, include degeneration and necrosis of myocardial fibers, accumulation of inflammatory cells, interstitial edema, lipid droplets and endothelial hemorrhage. The results obtained in the present study indicate that TRF pretreatment offers protection in myocardial infarction experimentally induced by isoproterenol (Figure 3).

CONCLUSION

The present results clearly emphasize the beneficial actions of TRF as cardioprotective agents. This finding proves the beneficial cardioprotective effect of these combinations against cardiac stress, in which oxidative stress was long known to contribute to the pathogenesis. Future works are recommended to link the mechanistic pathways between the cardioprotective and antioxidant action of the constituent compounds. Further research is also required to identify whether there exists any synergistic effect among the constituents. The natural cheap agricultural byproduct, TRF needs to be explored further to evaluate its other medicinal properties.

REFERENCES

1. Rajadurai M, Prince PSM, Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in Wistar rats: Biochemical and histopathological evidences, Toxicology, 228, 2006, 259–268.

Source of Support: Nil, Conflict of Interest: None.