Protective Effect of Abana, a Poly-herbal Formulation, on Isoproterenol-induced Myocardial Infarction in Rats

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SUMMARY

Objective: To find out the possible role of lipid peroxidation and glutathione in the pathogenesis of myocardial infarction and the protective role of Abana, a polyherbal drug.

Methods: The effect of Abana pretreatment (75 mg/100 g) for a period of 60 days on isoproterenol (20 mg/100 g s.c. twice at an interval of 24 hrs) induced lipid peroxidation was studied in rats. Marker enzymes levels such as creatinine kinase, lactate dehydrogenase, alanine transaminase and aspartate transaminase were assessed in serum and heart homogenate. Glutathione content and lipid peroxide levels were also estimated.

Results: In isoproterenol administered rats, a significant decrease was observed in the levels of marker enzymes in the heart with a corresponding increase in their levels in serum. Lipid peroxide level measured in terms of “TBA reactants” increased significantly in serum and heart. In rats pretreated with Abana, the alterations observed in the marker enzymes and lipid peroxide level were minimum on isoproterenol administration, and the levels were retained at near normal values.

Conclusion: Abana pretreatment may offer protection in experimental myocardial infarction induced by isoproterenol.

Key words: Abana; cardioprotective; lipid peroxide; isoproterenol; marker enzymes.

INTRODUCTION

Abana, is an Indian Ayurvedic herbomineral preparation of selected ingredients, which provides significant protection against ischaemia and hypertension. It possesses anti-thrombotic, anti-hypercholesterolemic and anti-arrhythmic properties. Its most important plant ingredients are Terminalia arjuna, Withania somnifera, Terminalia chebula, Phyllanthus emblica, Nardostachys jatamansi, Tinospora cordifolia, Glycyrrhiza glabra, Zingiber officinale and Nepeta hindostana etc., known for the beneficial effects in the Indian system of medicine.

Isoproterenol, a β-adrenergic agonist has been found to cause severe stress in the myocardium resulting in the infarct like necrosis of heart muscle. The pathophysiological changes following isoproterenol administration are comparable to those taking place in human myocardial infarction. Since lipid peroxidation is the major deleterious effect produced by many toxic agents, the alleviating effect of Abana on myocardial infarction was assessed by studying the
level of lipid peroxide and changes in the activities of marker enzymes which are the diagnostic indicator of myocardial infarction.

MATERIALS AND METHODS
Ayurvedic formulation Abana, was gifted by The Himalaya Drug Company, Bangalore. Isoproterenol, α-ketoglutarate, bovine serum albumin was obtained from Sigma Chemical Company (St. Louis, Mo, USA). All other chemicals used were of analytical grade.

Adult male albino rats of Wistar strain weighing 120-150 g were used for the study. They were fed with commercial pelleted rat chow and given water *ad libitum* and were maintained in a clean polypropylene cages at 25°C. The rats were divided into 4 groups each consisting of 6 animals.

Group I served as control, Group II rats were administered with isoproterenol (20 mg/100 g subcutaneously twice at an interval of 24 hrs). Group III rats were given Abana (75 mg/100 g) for a period of 60 days orally. Group IV rats were pretreated with Abana at the above mentioned dosage for 60 days and were given isoproterenol (20 mg/100 g body weight subcutaneously twice at an interval of 24 hrs) at the end of treatment period.

After the experimental period the rats were sacrificed by cervical decapitation. Blood was collected and the serum separated was used for the assay of marker enzymes lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and creatine kinase (CK). The heart was dissected out, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). Homogenate was centrifuged and supernatant was used for the assay of marker enzymes and glutathione. Lipid peroxides in serum and heart homogenate was determined by the method of Yagi. Protein was estimated by the method of Lowry *et al.*

The values were subjected to ANOVA and the significance between the various groups arrived at by using Student’s *Numann Keul Test*. For statistical analysis Group I was compared with Group II and Group II was compared with Group IV by using Student’s ‘*t*’ test.

RESULTS
Table 1 represents the activities of enzymes CK, LDH, AST and ALT in serum of control and experimental animals. Isoproterenol administered rats showed a significant increase in the activities of these enzymes. Abana pretreatment maintained the activities of these enzymes to near normal as in Group IV rats.

The activities of cell lysis marker enzymes such as CK, LDH, AST, ALT in heart homogenate of control and experimental animals are shown in Table 2. There was significant decrease in the
activities of cell lysis marker enzymes in Group II rats when compared to control. Abana pretreated rats restored the activities of cell lysis marker enzymes at near normal values.

Table 1: Activities of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) in serum of control and experimental animals

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>LDH</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.8 ± 0.18</td>
<td>1.26 ± 0.10</td>
<td>0.45 ± 0.05</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>6.5 ± 0.21*</td>
<td>2.06 ± 0.18*</td>
<td>0.77 ± 0.04*</td>
<td>0.36 ± 0.05*</td>
</tr>
<tr>
<td>Abana</td>
<td>4.7 ± 0.16</td>
<td>1.21 ± 0.13</td>
<td>0.46 ± 0.05</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td>Abana + Isoproterenol</td>
<td>5.0 ± 0.16*</td>
<td>1.37 ± 0.05</td>
<td>0.51 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for 6 animals in each group. The level of CK, LDH, AST and ALT in serum are expressed as µ k at/litre. *p<0.001 significantly different from control group; †p<0.001 significantly different from isoproterenol group.

Table 2: Activities of CK, LDH, AST and ALT in heart of control and experimental animals

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>LDH</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>168.3 ± 2.60</td>
<td>1903.3 ± 20.20</td>
<td>730.0 ± 10.5</td>
<td>300.0 ± 3.9</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>105.0 ± 1.70*</td>
<td>1381.6 ± 15.70*</td>
<td>480.0 ± 5.7*</td>
<td>201.6 ± 2.8*</td>
</tr>
<tr>
<td>Abana</td>
<td>165.3 ± 2.40</td>
<td>1928.3 ± 19.60</td>
<td>716.0 ± 10.2</td>
<td>290.0 ± 3.7</td>
</tr>
<tr>
<td>Abana + Isoproterenol</td>
<td>153.3 ± 2.60*</td>
<td>1830.0 ± 19.70</td>
<td>675.0 ± 9.6</td>
<td>273.3 ± 3.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for 6 animals in each group. The level of LDH, AST and ALT are expressed as nanomoles of pyruvate liberated/sec/g protein. The level of CK is expressed as micromoles of phosphorous liberated/sec/g protein. *p<0.001 significantly different from control group; †p<0.001 significantly different from isoproterenol group.

Rats administered with isoproterenol alone showed a significant increase in the levels of TBA reactive substances in serum and heart depletion of glutathione content in blood and heart. The alterations were minimum in rats pretreated with Abana (Table 3).

Table 3: Levels of lipid peroxides and glutathione in serum and heart of control and experimental animals

<table>
<thead>
<tr>
<th></th>
<th>Lipid peroxides</th>
<th>Glutathione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Heart</td>
</tr>
<tr>
<td>Normal</td>
<td>2.4 ± 0.18</td>
<td>3.6 ± 0.23</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4.2 ± 0.22*</td>
<td>5.4 ± 0.42*</td>
</tr>
<tr>
<td>Abana</td>
<td>2.2 ± 0.13</td>
<td>3.2 ± 0.20</td>
</tr>
<tr>
<td>Abana + Isoproterenol</td>
<td>2.8 ± 0.15*</td>
<td>4.2 ± 0.29</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for 6 animals in each group. The level of lipid peroxides in serum is represented as nanomoles of TBA reactants/litre and in heart is expressed as nanomoles of TBA reactants/gm protein. Glutathione content in blood is expressed as micromoles/litre and in heart as nanomoles of GSH/gm tissue. *p<0.001 significantly different from control group; †p<0.001 significantly different from isoproterenol group.
DISCUSSION

The diagnostic marker enzymes of myocardial infarction are creatine kinase, lactate dehydrogenase, alanine transaminase and aspartate transaminase\textsuperscript{12}. Rats administered with isoproterenol showed a significant decrease in activities of enzymes such as CK, LDH, AST and ALT in the heart with subsequent increase in their activities in serum when compared to normal. Manjula\textsuperscript{13} et al., have also studied the effect of aspirin on isoproterenol-induced myocardial infarction in rats. In this study they have stated that isoproterenol treatment results in significant decrease in the activities of enzymes like CK, LDH, AST and ALT in heart with subsequent increase in the activities in serum when compared to normal and aspirin treatment minimised the changes. An increase in the activities of marker enzymes in serum could be due to the leakage of enzymes from heart as a result of isoproterenol induced necrosis\textsuperscript{14} and the amount of enzymes appear in serum in proportion to the number of necrotic cells\textsuperscript{15}. Abana pretreatment maintained the activities of marker enzymes in serum and heart at near normal.

A significant increase in the levels of lipid peroxides in serum and heart on isoproterenol administration indicates enhanced lipid peroxidation by free radicals\textsuperscript{16}. Due to increased lipid peroxidation, glutathione levels were lowered significantly in blood and heart of Group II rats. Decreased glutathione level may be due to its increased utilisation in protecting SH groups containing proteins from the action of free radicals. Glutathione participates directly in the destruction of hydrogen peroxide\textsuperscript{17} and also promotes the formation of reduced form of ascorbate, which has high antioxidant activity\textsuperscript{18}. Abana pretreatment decreases lipid peroxide level and maintain glutathione content to near normal.

Abana is a complex herbal preparation and some of the ingredients like Terminalia arjuna, Nardostachys jatamansi and Glycyrrhiza glabra has been reported to prevent cardiovascular disorders\textsuperscript{19} in Indian system of medicine. Phyllanthus emblica present in the drug increases body resistance against disease and decay\textsuperscript{20}. Phyllanthus emblica and Daccus carota present in the formulation are rich source of ascorbic acid and β-carotene\textsuperscript{20} respectively. Ascorbic acid can directly react with singlet oxygen, superoxide and hydroxy radicals\textsuperscript{21}. β-carotene is the major carotenoid precursor of vitamin A, which can efficiently quench singlet oxygen and function as an antioxidant\textsuperscript{22}. These antioxidants present in the herbal formulation could have maintained the glutathione content, which react with free radicals and terminate lipid peroxidation thereby preventing tissue damage.

The results obtained from the above study indicates that Abana pretreatment offers significant protection to myocardium against the damage caused by isoproterenol induced lipid peroxidation.
REFERENCES


