Effect of Abana an Ayurvedic Formulation, on Lipid Peroxidation in Experimental Myocardial Infarction in Rats

Sheela Sasikumar, C. and Shyam Devi, C.S.*, Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai, India.

ABSTRACT
The present study was conducted to elucidate the antioxidant role of an Ayurvedic formulation Abana in isoproterenol-induced myocardial infarction in rats. In myocardial necrosis induced by isoproterenol, a significant increase in serum iron content with a significant decrease in plasma iron binding capacity, ceruloplasmin activity and glutathione level were observed. There was also a significant increase in lipid peroxides levels on isoproterenol administration. Activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase, glutathione reductase were decreased significantly in heart with isoproterenol-induced myocardial necrosis. Abana, produced a marked reversal of these metabolic changes related to myocardial infarction induced by isoproterenol. In conclusion Ayurvedic formulation Abana exerts its effect by modulating lipid peroxidation and enhancing antioxidant and detoxifying enzyme systems.

Cardiovascular diseases including atherosclerosis and cardiac tissue injury after myocardial infarction is due to free radicals generated at the site of damage. Free radicals may be formed by infiltration of white cells into ischemic myocardium or may be formed in the endothelial cell by the action of xanthine oxidase during the period of ischemia. It is generally accepted that oxygen-centered free radicals are key mediators associated with ischemia-reperfusion injury of heart. Isoproterenol, is a beta-adrenergic agonist and has been reported to increase lipid peroxidation through enhanced free radical formation. Isoproterenol induced myocardial infarction has been used as model for the evaluation of cardioprotective agents.

Abana, is a cardiotonic formulation of selected ingredients, which provides significant protection against ischemia and hypertension. It is a cardioprotective drug with antithrombotic and antihypercholesterolaemic effects.

The present study was undertaken to evaluate the mechanism of myocardial damage in relation to lipid peroxides and antioxidant enzymes.

Male albino rats of wistar strain, weighing 100-150 g were used for the study. They were fed with commercial pelleted rat chow and given water ad libitum. The rats were divided into 4 groups of 6 animals each. Group I rats served as control. Group II rats were administered isoproterenol (20 mg/100 g body weight, subcutaneously twice at an interval of 24 hr) in saline. Group III rats received Abana (75 mg/100 g body wt.) orally for a period of 60 days. Group IV rats were orally administered with Abana at the above mentioned dosage for 60 days and isoproterenol (20 mg/100 g) was administered subcutaneously twice at an interval of 24 hr) at the end of experimental period.
After the experimental period the animals were sacrificed by cervical decapitation. Blood was collected in 2 different test tubes with and without anticoagulant from which plasma and serum was collected respectively. Heart was dissected out immediately, washed in ice-cold saline and homogenised in Tris-HCl buffer (0.1 M) pH 7.4. The homogenate was then centrifuged and supernatant obtained was used for the assay of various enzymes. Glutathione (GSH) was assayed by the method of Moron et al.\(^9\), superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich\(^10\) based on the inhibition of epinephrine auto-oxidation by the enzyme, catalase (CAT) activity was measured by following decomposition of H\(_2\)O\(_2\) according to the method of Beers and Sizer\(^11\), glutathione peroxidase (GPX) was assayed by the method of Rotruck et al.\(^12\) using H\(_2\)O\(_2\) as substrate, glutathione-s-transferase (GST) activity was measured using 1, Chloro 2,4 dinitro benzene as substrate according to Habig et al.\(^13\) and glutathione reductase (GRD) activity was assayed by the method of Pinto and Barley\(^14\). Iron content in serum was estimated by the method of Ramsay\(^15\), plasma iron binding capacity was determined by Ramsay’s dipyridyl method\(^16\) and ceruloplasmin activity was measured according to the method of Ravin\(^17\). Lipid peroxides in serum and heart were estimated using thiobarbituric acid reaction as described by Okhawa et al.\(^18\) Protein was estimated by the method of Lowry et al.\(^19\) using Bovine serum albumin as standard.

Drug: Abana an Ayurvedic herbal preparation containing several important herbs\(^20\) was obtained from The Himalaya Drug Company, Bangalore as gift sample.

Data were analysed statistically using student’s ‘t’ test. The value of \(p\) less than 5\% (\(p<0.05\)) was considered as significant.

The effect of Abana administration on serum iron, content, plasma iron binding capacity, ceruloplasmin activity and glutathione content are shown in Table 1. Isoproterenol administered rats showed a significant increase in serum iron content (\(p<0.001\)) with a significant decrease in GSH level, ceruloplasmin activity and iron binding capacity (\(p<0.001\)) when compared to control rats. All the alterations except glutathione were prevented in rats pre-treated with Abana (Group IV).

| Table 1: Levels of serum iron, plasma iron binding capacity, ceruloplasmin activity and glutathione content in control and experimental rats (Values are mean +/- SD of six rats per group) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Control         | Isoproterenol   | Abana           | Abana + Isoproterenol |
| Serum iron (µg/ml)              | 41.8 ± 1.8      | 63.3 ± 2.6*     | 42.4 ± 1.6      | 46.5 ± 2.2*       |
| Plasma iron binding capacity (µg/dl) | 44.3 ± 1.6      | 31.3 ± 1.2*     | 44.1 ± 1.06     | 40.5 ± 1.4*       |
| Ceruloplasmin (units/ml)        | 0.960 ± 0.05    | 0.615 ± 0.04*   | 0.906 ± 0.04    | 0.893 ± 0.02*     |
| Glutathione (mg/dl)             | 55.3 ± 2.5      | 34.6 ± 1.9*     | 56.3 ± 2.7      | 53.6 ± 2.2*       |

*Significantly different from control group \(p<0.001\)

Levels of GSH, lipid peroxides, SOD, CAT, GPX, GST and GRD are shown in Table 2. The antioxidant enzyme activities were decreased significantly (\(p<0.001\)) with a significant decrease in glutathione content in Group II rats when their values are compared to those of control rats. The activities of antioxidant enzymes, glutathione and lipidperoxide level were maintained at near normal (Group IV) when compared with Group II.
Table 2: Levels of lipid peroxides, glutathione and activities glutathione reductase, glutathione-s-transferase, superoxide dismutase and catalase in heart of control and experimental animals  
(Values are mean ± SD for six rats per group)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Isoproterenol</th>
<th>Abana</th>
<th>Abana + Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxides (nm of TBA reactants/mg protein)</td>
<td>3.6 ± 0.23</td>
<td>5.4 ± 0.42*</td>
<td>3.2 ± 0.20</td>
<td>4.2 ± 0.29*</td>
</tr>
<tr>
<td>Glutathione (nm of GSH/g tissue)</td>
<td>4.8 ± 0.24</td>
<td>2.5 ± 0.15*</td>
<td>5.1 ± 0.28</td>
<td>4.2 ± 0.20*</td>
</tr>
<tr>
<td>Glutathione reductase (µg of GSBG utilized/min/mg protein)</td>
<td>5.2 ± 0.43</td>
<td>3.6 ± 0.29*</td>
<td>5.5 ± 0.45</td>
<td>4.6 ± 0.41*</td>
</tr>
<tr>
<td>Glutathione peroxide (µg GSH utilised/min/mg protein)</td>
<td>3.2 ± 0.29</td>
<td>1.5 ± 0.12*</td>
<td>3.4 ± 0.3</td>
<td>2.9 ± 0.21*</td>
</tr>
<tr>
<td>Glutathione-s-transferase (nm of CDNB conjugated/min/mg protein)</td>
<td>921 ± 15.1</td>
<td>720 ± 21.5*</td>
<td>908 ± 18.3</td>
<td>889 ± 25.7*</td>
</tr>
<tr>
<td>Superoxide dismutase (units/mg protein)</td>
<td>3.3 ± 0.28</td>
<td>1.8 ± 0.19*</td>
<td>3.2 ± 0.31</td>
<td>2.7 ± 0.23*</td>
</tr>
<tr>
<td>Catalase (nm of H2O2 decomposed/min/mg protein)</td>
<td>4.5 ± 0.15</td>
<td>2.1 ± 0.12*</td>
<td>4.6 ± 0.18</td>
<td>4.0 ± 0.23*</td>
</tr>
</tbody>
</table>

*Significantly different from control group p<0.001  
#Significantly different from isoproterenol control group p<0.001

During myocardial infarction, reactive oxygen species like superoxide, hydrogen peroxide, and hydroxyl radicals are produced in enormous amount\textsuperscript{21}, which contribute to myocardial tissue injury. Cardiovascular actions of isoproterenol may also lead to cardiac necrosis\textsuperscript{22}. The results of the present investigation support this study.

The increased levels of free iron are usually associated with decreased plasma iron binding capacity in isoproterenol treated rats. During ischemia, free iron is released from heme dependent proteins like hemoglobin and myoglobin and a decreased iron binding capacity increases prostaglandin metabolism and in vivo lipid peroxidation\textsuperscript{23}.

There is increased mobilisation of iron from ferritin in the heart by the enzyme xanthine oxidase and overproduction of free radicals results in myocardial change\textsuperscript{24}. Abana pre-treatment decreases the level of serum iron by increasing iron binding capacity and prevents hemolysis, thereby preventing iron-catalysed lipid peroxidation.

Ceruloplasmin, activity is decreased significantly during isoproterenol administration\textsuperscript{25}. Abana pre-treatment increases cerulo-plasmin activity resulting in decreased production of free iron. Lipid peroxidation is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflects the various stages of the disease and its complications. Sushmakumari \textit{et al.}\textsuperscript{4} reported enhanced lipid peroxidation in serum and heart of isoproterenol treated rats when compared to control. Increased level of lipid peroxides injures blood vessels, causing increasing adherence and aggregation of platelets to the injured sites\textsuperscript{27}.

Abana pre-treated isoproterenol administered rats maintained the level of lipid peroxides to near normal in heart when compared to control. Abana, is a herbomineral formulation rich in vitamin C and beta-carotene, which enable the drug to perform as an antioxidant. \textit{Phyllanthus emblica} present
in the drug is a source of vitamin C, which can directly react with superoxide, hydroxy radicals and singlet oxygen\textsuperscript{28,29}. Abana is processed with Daccus carota which contains beta-carotene, a major carotenoid precursor of vitamin A. Beta-carotene a pigment found in the drug can efficiently quench singlet oxygen and function as an antioxidant\textsuperscript{30}. Abana inhibits platelet adhesion and platelet aggregation, which are the important events in myocardial infarction\textsuperscript{31}.

Isoproterenol treated rats showed a significant decrease in glutathione level, activities of glutathione-s-transferase, glutathione peroxidase and glutathione reductase in heart. Glutathione level and activities of glutathione dependent enzymes were restored at near normal in rats pre-treated with Abana (Group IV).

Glutathione reductase and glutathione peroxidase are essential for maintaining constant ratio reduced glutathione to oxidised glutathione in the cell. Decreased glutathione levels on isoproterenol administration may be due to its increased utilisation in protecting SH containing proteins from lipid peroxides. Reduced availability of glutathione also reduces the activity of glutathione peroxidase and glutathione-s-transferase, on isoproterenol administration\textsuperscript{32}. Inactivation of glutathione reductase in the heart, leads to accumulation of oxidised glutathione (GSSG) the oxidised product of GSH\textsuperscript{33}. GSSG inactivates enzymes containing SH groups and inhibits protein synthesis\textsuperscript{34}. Abana pre-treatment restores glutathione level and increases the activities of glutathione peroxidase and glutathione-s-transferase.

SOD and CAT activities were decreased on isoproterenol administration is in accordance with the observation of Majunla \textit{et al.}\textsuperscript{35}. During myocardial infarction, superoxide radicals generated at the site of damage modulates SOD and CAT, resulting in the loss of activity and accumulation of superoxide anion, which damages myocardium. Abana pre-treatment increases the activity of SOD and CAT and it scavenges superoxide radicals and reduces myocardial damage caused by free radicals.

The results obtained from this study indicate that Abana pre-treatment offers significant protection to the myocardium by inhibiting lipidperoxidation and activating antioxidant defence enzymes in the system.

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REFERENCES


