The study was conducted to evaluate cardioprotective effect of Unani formulation. Unani formulation in the dose of 100 mg / 100 gm body weight was administered per oral for 60 days before isoproterenol to test cardioprotective effect. The levels of various cardiac enzymes, glycogen and adrenal ascorbic acid were significantly altered in isoproterenol treated group as compared with control and formulation pre-treated groups. The levels were statistically similar in the groups. Unani formulation has been found to exhibit cardioprotective activity.

Keywords: *Bombbyx mori* Linn., *Nepata hindostana* (Roth) Haines, *Terminalia arjuna* (Roxb.) Wight & Arn., Isoproterenol, Cardioprotective activity, Unani formulation

IPC Int. Cl.: A61K36/00, A61P9/00, A61P9/04, A61P9/10, A61P9/12

Coronary artery disease (CAD) is progressively increasing in Asia. The four major risk factors for CAD are hypertension, hyperlipidemia, diabetes mellitus and cigarette smoking. Hypertension and dyslipidemia co-exist more often than alone1. All Unani physicians paid much attention to the treatment of this vital organ (heart) of the human body2. The Unani drugs are used in compounded form to have additive or corrective effect of each other3. Many of the compound formulations are described in standard *Qarabadeen* (Unani pharmacopeia) for cardiovascular disorders with a large number of ingredients. Some compound formulations containing lesser number of ingredients are effectively being used in clinical practices by Unani physicians with good effects in cardiovascular disorders. A possibility of errors of interpretation exists around the research on such drugs mentioned in old treatises. Research problems pertaining to this drug can be solved by applying the modern scientific principles. The formulation containing *Nepeta hindostana* (Roth) Haines, *Bombbyx mori* Linn. and *Terminalia arjuna* (Roxb.) Wight & Arn. are used as *muquawai-qalb* (cardiotonic) by Unani physicians in their clinical practices4,6. Therefore, it was considered to be worthy to evaluate cardioprotective effect of the Unani formulation in isoproterenol induced myocardial ischemia in rats.

**Methodology**

The study was conducted in the Department of Ilmul Advia, AK Tibbiya College in collaboration with the Department of Pharmacology, JN Medical College, AMU, Aligarh. Adult albino rats of either sex weighting 150-200 gm were provided standard diet (Lipton India) and water ad libitum. Twelve hours dark and light cycles were maintained. They were divided into 4 groups containing 6 animals each. The temperature was maintained 26±2 ⁰C. Isoproterenol hydrochloride purchased from Sigma USA. *N. hindostana* (*Badranj boya*), bark of *T. arjuna* and *B. mori* (Abraisham) were procured from Dawakhana Tibiyya College, AMU, Aligarh and were identified by comparison for its macroscopic and microscopic characters with authentic specimens at the Botanical Survey of India, Dehradun and the Forest Research Institute, Dehradun.

The bark of *T. arjuna* and whole plant of *N. hindostana* were dried in shade, powdered in electric grinder to a coarse 20-40 mash size and extracted in soxhlet apparatus with distilled water for 6 hrs separately. The aqueous extract of *B. mori* (raw silk cocoon) was obtained by reflex method. All 3 extracts were separately filtered, concentrated at water bath and stored at room temperature for use. All three extracts of *Terminalia arjuna*, *Nepata hindostana* and *Bombbyx mori* were combined in ratio of 1:2:1 respectively and freshly dissolved in distilled water on...
each day before administration. The animals were divided into 4 groups. Group I was given distilled water in same volume for same duration and served as control. Group II was given isoproterenol 20 mg/100 gm body weight subcutaneously twice at interval of 24 hrs to induce myocardial infarction. Group III was administered Unani formulation in the dose of 100 mg/100 gm of body weight po daily for 61 days, and Group IV was administered Unani formulation in the dose of 100 mg/100 gm of body weight po daily for 60 days followed by isoproterenol subcutaneously twice at interval of 24 hrs. The dose was calculated in the usual manner by multiplying the Unani clinical dose by factor 7 for rat. Twelve hours after second dose of isoproterenol, the animals were sacrificed. Blood, cardiac muscle and adrenal gland were collected for estimation of cardiac enzymes i.e. creatinine phosphokinase (CPK), isoenzymes (CPK-MB) lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT), cardiac glycogen and adrenal gland ascorbic acid. Histopathological examination of cardiac muscle was performed. The results were analysed by one-way analysis of variance (ANOVA) by using graph pad, prism. P <0.05 was considered statistically significant.

Result

It was observed that in isoprotarenol treated group (II), the serum CPK, CPK-MB, LDH, AST and ALT were significantly high as compared to their respective controls (Fig. 1) but the serum levels of these enzymes were statistically similar in control group I, Unani formulation treated group (III) and pre-treated group (IV). Cardiac glycogen in group II was significantly low as compared with control. It was statistically same in group I, III and IV. Adrenal ascorbic acid was significantly low in group II as compared with groups I, III and IV (Table 1). Microscopic examination of rat myocardium of group I showed normal myofibril structures with striation, branched appearance and continuity with adjacent myofibrils (Fig. 2). The microscopic examination of group II showed intercellular edema, focal haemorrhage with macrophages infiltration. The muscle fibres showed vascular changes with area of necrosis (Figs. 3 & 4). Microscopic examinations of Group IV showed minimal intercellular exudation without edema and absence of haemorrhage. The architect of the cardiac muscles fibers was relatively well preserved (Fig. 5). There was significant protection in myocardial damage in group IV as compared with group II.

Discussion

The ingredients of test formulation possess cardioprotective activity. B. mori, N. hindostana and T. arjuna have been reported as cardioprotective and cardiotonic drugs. Isoproterenol has been reported to cause ischemia or anoxia resulting in anaerobic

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPK</td>
<td>32.85±4.38</td>
<td>114.25±11.00*</td>
<td>35.00±5.36</td>
<td>46.63±5.67</td>
</tr>
<tr>
<td>CPK-MB</td>
<td>31.70±2.56</td>
<td>94.98±6.63*</td>
<td>29.50±5.83</td>
<td>36.85±3.04</td>
</tr>
<tr>
<td>LDH</td>
<td>70.20±4.76</td>
<td>201.42±9.32*</td>
<td>68.75±5.85</td>
<td>75.86±5.18</td>
</tr>
<tr>
<td>AST/SGOT</td>
<td>43.90±8.47</td>
<td>136.67±3.53*</td>
<td>42.05±3.71</td>
<td>52.71±7.17</td>
</tr>
<tr>
<td>ALT/SGPT</td>
<td>22.91±3.11</td>
<td>104.30±5.01*</td>
<td>20.70±2.21</td>
<td>42.71±3.38</td>
</tr>
<tr>
<td>Ascorbic acid**</td>
<td>04.31±0.47</td>
<td>02.23±0.32*</td>
<td>04.93±0.22</td>
<td>03.84±0.56</td>
</tr>
<tr>
<td>Cardiac glycogen**</td>
<td>06.16±0.51</td>
<td>02.86±0.17*</td>
<td>06.87±0.36</td>
<td>05.72±0.52</td>
</tr>
</tbody>
</table>

P<0.001

* measured micro gram / gram of wet tissue
Data are expressed as Mean ±S.D.
metabolism. Myocardial infarction causes rise in the plasma concentration of enzymes that are normally concentrated in cardiac cells. The biochemical markers that are most widely used in the detection of the myocardial infarction: creatinine phosphokinase (CPK) and its isoforms (CPK-MB) which is more sensitive and cardio-specific enzyme. CPK-MB has greater than 95% sensitivity and specificity for myocardial injury when measured within 24-36 hrs. Estimation of elevated serum enzymes SGOT, LDH, CPK-MB is useful guide for death of heart muscles.

Increased CPK activity in the blood may be found in a number of clinical conditions including acute myocardial infarction. The total CPK activity alone is a sensitive but nonspecific test of myocardial injury, while the combined determination of total CPK and CPK-MB activity of blood will greatly increase the specificity of the diagnosis of myocardial infarction. The serum CPK and CPK MB level was found to be significantly high in isoproterenol treated group and was nearer to control group, in Unani formulation treated and pretreated group.
LDH catalyses the oxidation of lactate to pyruvate and reduction of pyruvate to lactate. The single most important clinical significance of serum LDH determination is the detection of cellular hypoxia or necrosis in some tissues of human organism\(^2\). The level of LDH in isoproterenol treated group was significantly increased as compared with control and Unani formulation pretreated group\(^21,22\). Glutamic-oxaloacetic transaminase (SGOT) and Glutamic-pyruvic transaminase (SGPT) enzymes are widely distributed in the various tissues of human being. They are supportive in clinical diagnosis. The most frequent cause for the elevation of SGOT activity in the blood is liver and heart diseases. The levels of serum SGOT and SGPT are correlated with the size of infarction\(^23,24\).

Normal myocardium utilizes free fatty acids as the substrate for energy production. But in anoxic condition, free fatty acids utilization decreases and glucose consumption increases, as a result the glycolysis becomes the main source of energy. Catecholamines increase the glycogen breakdown making more carbohydrates available for glycolytic energy\(^25,26\). Thus, in myocardial necrosis the glycogen level is depleted. In the group treated with isoproterenol, the cardiac glycogen level was lower as compared to control and formulation treated and pretreated group. Acute myocardial infarction produces severe stress which causes enhanced adrenocortical activity and depletion of adrenal ascorbic acid\(^27\). There was significant depletion of adrenal ascorbic acid in group having myocardial infarction as compared to control. The typical microscopic changes of coagulative necrosis become detectable in the first 4 to 12 hrs; wavy fibers may be present at the periphery of infarct. An additional but sublethal ischaemic changes may be seen in the margins of infarct, so called vacuolar degeneration or myocystosis. The morphologic changes in myocardial infarction after 12-24 hrs showed on going coagulative necrosis\(^28\). Fibrillar collagens are generally damaged after the onset of myocardial infarction\(^29\).

References


