PROTECTIVE EFFECT OF A POLYHERBAL FORMULATION ON SERUM LIPIDS AND MARKER ENZYMES IN ISOPRENALINE HYDROCHLORIDE INDUCED CARDIOTOXICITY IN WISTAR RATS

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ABSTRACT
Cardioprotective potential of 70% ethanolic extract of a polyherbal formulation (PHF) containing the plants Allium sativum, Trigonella foenum-graecum and Linum usitatissimum on serum lipid profiles, myocardial marker enzymes in serum and heart homogenate in Isoprenaline hydrochloride (ISO) induced cardiotoxicity in rats were evaluated. Subcutaneous injection of ISO (25 mg/kg) to wistar rats showed a significant (p<0.05) increase in the levels of cholesterol, triglycerides, VLDL and LDL with significant decrease in the level of HDL. Increase in the levels of myocardial marker enzymes creatine kinase (CK), creatine kinase-MB, lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) was seen in serum and the same concomitantly decreased in heart. Oral administration of polyherbal formulation -PHF (250 and 500 mg/kg) to Isoprenaline hydrochloride induced rats daily for a period of 28 days showed a significant improvement in lipid profile along with marker enzymes in serum and heart homogenate. From the study the effect of 500 mg/kg dose was more pronounced.

KEYWORDS: Cardiovascular diseases, polyherbal formulation, herbal preparations, hypolipidemic activity, cardio protection.

INTRODUCTION
Cardiovascular diseases such as hypertension and myocardial infarction (MI) are the most important cause of mortality in developing countries due to changing lifestyles. MI is the...
acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demands.\cite{2} Isoprenaline hydrochloride (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction.\cite{3} MI induced by ISO in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia and increase in serum creatine phosphokinase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities.\cite{4,5} The endothelial damage which ultimately generate atheroma and plaque formation, are characterized by high cholesterol and lipid concentrations along with free radical oxidative stress. The involvement of hydroxyl radicals (OH) is a major causative factor for the peroxidative modification in circulatory LDL i.e. responsible for initiation and progression of atherosclerosis.\cite{6} There is increasing trend towards the application of herbal medicines to treat the cardiovascular diseases.\cite{7} Herbal medicines are increasingly gaining greater importance from the public and medical professionals due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life.\cite{8} From the literature survey and through folk medicine we found that the plants *Allium sativum*, *Trigonella foenum-graecum* and *Linum usitatissimum* are found to posses cardioprotective effect. Hence based on their anti-oxidant activity a polyherbal formulation (PHF) containing the three plants in 1:2:1 ratio was used for the study. The present study was designed to explore the cardioprotective activity of polyherbal formulation in ISO induced MI in rats.

**MATERIALS AND METHODS**

**Plant authentication**

The plant parts *Allium sativum* (bulbs), *Trigonella foenum-graecum* (seeds), *Linum usitatissimum* (seeds) were collected from the local market, Coimbatore and shade dried. All the plants were identified and authenticated from the authority of Botanical Survey of India, Coimbatore.

**Extraction**

The polyherbal formulation containing three plant constituents *Allium sativum*, *Trigonella foenum-graecum* and *Linum usitatissimum*. All the plants parts were ground and powdered. The dried and coarsely powdered plant parts were packed separately in soxhlet apparatus and subjected to extraction with 70% ethanol. The extracted materials were evaporated to dryness.
under reduced pressure at 45°C. The PHF comprising of plant parts mixed with (1:2:1) ratio is used for the study.

Phytochemical screening of the extracts
The ethanolic extracts of
(i) *Allium sativum* contains alkaloids, flavanoids, glycosides, saponins and phenolic compounds. (ii) *Trigonella foenum-graecum* contains tannins, saponins, alkaloids, flavanoids and terpenoids. (iii) *Linum usitatissimum* contains carbohydrates, proteins, steroids, alkaloids and flavanoids.

All the three plants were found to contain alkaloids, flavanoids and polyphenols by the phytochemical estimation due to which the plants are showing antioxidant activity.

Drugs and Chemicals
ISO was procured from Sigma Chemical Co., St. Louis, MO, USA, while all other chemicals used were of analytical grade.

Animals
Male wistar rats (150-200g) were obtained from KMCH College of Pharmacy, Coimbatore. Animals were kept in the department animal house under controlled conditions of temperature at 25±1°C, normal relative humidity, light and dark cycle of 12 hours ten days prior to the start of the experimental conditions. The animals were housed in polypropylene cages with sterile paddy husk bedding, food and tap water were made available throughout the experimental period. All the experiments were carried out with prior approval of Institutional Animal Ethical Committee (Ref. no: 300/2015/IAEC).

Experimental design
The experimental rats were divided into five groups, each group consisted of six animals.

Group I: Served as control.
Group II: Rats were administered subcutaneously with ISO (25 mg/kg/day) on 29th and 30th day for 2 days.
Group III: Rats were treated with standard drug Atorvastatin (10 mg/kg/day) for a period of 28 days and then ISO was administered subcutaneously on 29th and 30th day.
Group IV: Rats were treated with PHF (250 mg/kg/day) for a period of 28 days and then ISO was administered subcutaneously on 29th and 30th day.
Group V: Rats were treated with PHF (500 mg/kg/day) for a period of 28 days and then ISO was administered subcutaneously on 29th and 30th day.

At the end of the experimental period i.e., 12 h after the second dose of ISO injection, all the rats were killed. Blood was collected and serum separated after centrifugation. Serum was used for various estimations. The heart was dissected out, washed immediately in ice-chilled saline, blotted and weighed. A known weight (200 mg) of the heart tissue was homogenized in 5 ml of 0.1 M tris-HCl (pH 7.4) buffer solution. The homogenate was centrifuged at 3000 rpm for 5 min. The supernatant was used for the estimation of various parameters.

BIOCHEMICAL ESTIMATION

Processing of heart tissue
The hearts were taken out from formalin, weighed and a 10% homogenate was prepared in ice-chilled phosphate buffer (50mM, pH 7.4). Aliquots of homogenate were centrifuged at 5000 rpm for 20 min at 41°C and the supernatant was used for the estimation of LDH, AST, ALT, cholesterol, triglycerides, free fatty acids and phospholipids.

Biochemical assay in the serum and heart tissue
Estimation of serum enzymes
Lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) by the method of King, creatine phosphokinase (CK) by the method of Okinaka et al., creatine kinase-MB, serum triglycerides, total cholesterol and high density cholesterol (HDL) levels were determined enzymatically by colorimetric specific kits (Merck India Ltd.). LDL and VLDL were calculated by the method of Varley et al., free fatty acids and phospholipids.

RESULTS
The effects of PHF on the serum marker enzymes (CK, CK-MB, LDH, AST and ALT) and lipid profiles in serum and heart homogenate of ISO treated animals are shown in the table 1, 2 and 3 respectively. ISO treated rats showed a significant (p<0.05) increase in serum marker enzymes when compared with control group. Oral pretreatment of PHF (both 250 mg/kg and 500 mg/kg) to ISO treated rats significantly (p<0.05) decreased the levels of serum marker enzymes in serum. In heart homogenate, ISO treated group showed a significant (p<0.05) increase in total cholesterol, triglycerides, LDL and VLDL, when compared with control.
Oral pretreatment of PHF (both 250 mg/kg and 500 mg/kg) to ISO treated rats significantly (p<0.05) decreased the levels of total cholesterol, triglycerides, LDL and VLDL near to normal levels.

**Heart weight**

**Table 1: Effect of PHF on body weight and heart weight in control and experimental rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final body weight (g)</th>
<th>Heart weight (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>184.00 ±2.0</td>
<td>0.64±0.01^a</td>
</tr>
<tr>
<td>Group II</td>
<td>158.67 ± 7.02^b</td>
<td>0.85± 0.02^b</td>
</tr>
<tr>
<td>Group III</td>
<td>175.00 ± 7.02^c</td>
<td>0.66 ± 0.03^a</td>
</tr>
<tr>
<td>Group IV</td>
<td>162.33 ± 2.52^d</td>
<td>0.76 ± 0.01^a</td>
</tr>
<tr>
<td>Group V</td>
<td>176.00 ± 7.21^c</td>
<td>0.69 ± 0.01^a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

**Statistical comparisons**

a. represents comparison between group II and I.
b. represents comparison between group III and II.
c. represents comparison between group IV and II.
d. represents comparison between group V and II.

The symbols represent statistical significance *p<0.05

The heart weights of control and experimental rats were recorded and the results are depicted in the table 1. From the table, it was evident that Isoprenaline hydrochloride induction significantly (p<0.05) increased the heart weight when compared to normal control rats (group I). Pretreatment with low and high dose of PHF significantly (p<0.05) decreased the heart weight in group IV and V rats similar to that of standard control rats (group III) respectively, when compared to group II rats. This may be due to the reduction of the degree of the damage in the myocardium by polyherbal formulation.
Effect of PHF on LDH activities

Table 2: Effect of PHF on cardiac marker enzymes in serum of control and experimental rats.

<table>
<thead>
<tr>
<th>Parameters (IU/L)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK MB</td>
<td>135.33±6.48a</td>
<td>498.97± 7.03b</td>
<td>154.17± 11.75c</td>
<td>288.00± 6.06d</td>
<td>190.70 ± 8.98e</td>
</tr>
<tr>
<td>CK</td>
<td>84.67±2.32b</td>
<td>307.93±8.89o</td>
<td>92.87± 4.85</td>
<td>183.93± 3.27a</td>
<td>190.70 ± 8.98e</td>
</tr>
<tr>
<td>LDH</td>
<td>718.50±190.62a</td>
<td>1262.67±52.52b</td>
<td>844.33± 45.21a</td>
<td>912.00± 74.91a</td>
<td>755.80± 396.51a</td>
</tr>
<tr>
<td>AST</td>
<td>128.80±19.03a</td>
<td>192.33± 20.22b</td>
<td>148.20± 33.92a</td>
<td>141.70± 2.46a</td>
<td>124.00± 8.70a</td>
</tr>
<tr>
<td>ALT</td>
<td>76.43±14.40a</td>
<td>167.30±36.23b</td>
<td>82.33± 5.73a</td>
<td>103.97± 8.13a</td>
<td>92.30 ±3.06a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

Experimental design and statistical comparison are as in table 1.

LDH activities were significantly increased in groups treated with ISO, compared with normal control. Pre-administration of PHF (250 mg/kg and 500 mg/kg) showed significant fall in LDH activity in serum compared to ISO control (group II).

Effect of PHF on AST and ALT activities

AST is a biochemical marker for diagnosis of acute myocardial infarction. ISO induced rats, increased the activities of serum AST and ALT accompanied by their concomitant reduction in heart homogenate confirm the onset of myocardial necrosis. Pretreatment with PHF showed the normalization in the activity of these enzymes when compared with only ISO (group II) treated rats.

Effect of PHF on CK and CK-MB activities

Isoprenaline hydrochloride (ISO) significantly increased the levels of CK and CK-MB, compared to normal control group indicating myocardial infarction. Prior administration of PHF significantly decreased the ISO induced elevated levels of cardiac marker enzymes.

Effect of PHF on serum lipid profiles

The levels of serum triglycerides and cholesterol of control and treated animals are given in the table 3. Rats induced with ISO, showed a significant (p<0.05) increase in serum levels of triglycerides, total cholesterol, LDL, VLDL with significant (p<0.05) decrease in HDL when compared to control rats. Oral pretreatment with PHF (250 mg and 500 mg/kg) to ISO treated rats significantly (p<0.05) decreased the levels of cholesterol, triglycerides, VLDL, LDL and significantly (p<0.05) increased the HDL level at 500 mg/kg when compared to group II rats (ISO alone).
Table 3: Effect of PHF on lipid profiles in serum of control and experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>74.73 ±5.03a</td>
<td>44.27±2.91a</td>
<td>28.53±5.55a</td>
<td>37.35±1.07a</td>
<td>8.85±0.58a</td>
</tr>
<tr>
<td>Group II</td>
<td>103.77± 5.97b</td>
<td>73.73± 3.79b</td>
<td>19.67± 1.82b</td>
<td>69.35±9.19b</td>
<td>14.75± 0.76b</td>
</tr>
<tr>
<td>Group III</td>
<td>71.87± 3.30a</td>
<td>48.33± 4.50a</td>
<td>36.03± 2.96c</td>
<td>26.17± 5.92c</td>
<td>9.67± 0.90a</td>
</tr>
<tr>
<td>Group IV</td>
<td>87.00 ± 5.38d</td>
<td>57.17± 4.51c</td>
<td>25.33 ± 3.21a</td>
<td>40.58± 19.38d</td>
<td>11.43± 0.90c</td>
</tr>
<tr>
<td>Group V</td>
<td>75.83± 4.91a</td>
<td>47.30 ± 2.80a</td>
<td>29.67 ± 3.51a</td>
<td>36.71± 8.00a</td>
<td>9.46 ± 0.56a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

Experimental design and statistical comparison are as in table 1.

**Effect of PHF on total cholesterol in heart lipid profile**

![Fig. 1: Effect of PHF on total cholesterol in heart of control and experimental rats.](image1)

**Fig. 1: Effect of PHF on total cholesterol in heart of control and experimental rats.**

![Fig. 2: Effect of PHF on triglyceride in heart of control and experimental rats.](image2)

**Fig. 2: Effect of PHF on triglyceride in heart of control and experimental rats.**
Though the heart can utilize free fatty acids for its energy requirements, the excess free fatty acid may be used for the synthesis of triglycerides, resulting in hypertriglyceridemia. The level of total cholesterol, triglycerides and free fatty acids were found to be significantly (p<0.05) increased in ISO induced groups compared to control rats. Prior administration of PHF to group IV and V (treatment group) and group III (standard group) showed a significant decrease (p<0.05) in these lipid profiles. The phospholipids were also found to increase significantly (p<0.05) in pretreated groups (group III, IV and V) when compared to toxic control (group II).
DISCUSSION

The aim of the present study was to evaluate the role of polyherbal formulation (PHF) for its cardioprotective activity in ISO induced myocardial necrosis in rats. The results revealed the beneficial effects of PHF in Isoprenaline hydrochloride (ISO) induced myocardial necrosis in rats. ISO, a synthetic catecholamine is a β-adrenergic receptor agonist. In high dose, it has ability to destruct the myocardium and cause cardiotoxicity due to cytosolic Ca\(^{2+}\) overload. As a result of this myocardial destruction, cytosolic enzymes (CK, CK-MB, LDH, AST and ALT) are secreted in to the blood and serve as diagnostic markers of cardiotoxicity. Pathophysiological changes such as cell necrosis, contractile failure, ventricular arrhythmias and subcellular changes after the administration of ISO are comparable to those taking place in human myocardial ischemia/ infarction.[17] The pretreatment with PHF significantly lowered the ISO induced elevation in the activities of diagnostic marker enzymes. This may be because of the action of PHF in maintaining membrane integrity, there by restricting the leakage of these enzymes.

Lipids play a vital role in cardiovascular diseases by the way of hyperlipidemia, development of atherosclerosis and also by modifying the cellular membrane composition, structure and stability.[18] ISO induced elevation in the cholesterol levels could be due to increase in biosynthesis and decrease in its utilization. Hypertriglyceridermia was observed in ISO intoxicated rats may be due to decreased activities of lipoprotein lipase in the myocardium resulting in less uptake of triglyceride from the circulation. Pretreatment with PHF altered the activities of LCAT (Lecithin Cholesterol Acyl Esterase), lipoprotein lipase and cholesterol ester synthetase (CES) and increases HDL, decreasing triglycerides and cholesterol levels indicating the potential lipid lowering activities of PHF.[19] ISO increased the levels of LDL and VLDL and significantly decreased the level of HDL. In the present study the pretreated animals (group III, IV & V) were prevented from the toxic effects of ISO. HDL is known to be involved in the transport of cholesterol from tissues to the liver, for excretion in to bile and thus called “Good cholesterol”.

CONCLUSION

From the results of the study, it can be concluded that the PHF (bulbs of Allium sativum, seeds of Trigonella foenum graecum and Linum usitatissimum) exhibits potent effect in maintaining the cardiac marker enzymes as well as serum and tissue lipid levels in
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World Journal of Pharmacy and Pharmaceutical Sciences

isoprenaline hydrochloride (ISO) induced myocardial infarction in rats similar to standard control (Group III) rats.

**Abbreviations:** PHF, Polyherbal formulation; ISO, Isoprenaline hydrochloride; CK, Creatine kinase; LDH, Lactate dehydrogenase; ALT, Alanine transaminase; AST, Aspartate transaminase; LDL, Low density lipoproteins; HDL, High density lipoproteins; VLDL, Very low density lipoproteins; MI, Myocardial infarction.

**REFERENCE**


