Role of Arogh, a polyherbal formulation to mitigate oxidative stress in experimental myocardial infarction

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Antioxidant role of Arogh in isoproterenol induced myocardial infarction in rats has been studied. The activity of heart tissue antioxidants like glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase were significantly decreased in isoproterenol administered group. The activity of ceruloplasmin and levels of glutathione, vitamins E and C were also found to be substantially decreased in serum with a concomitant rise in lipid peroxide levels after isoproterenol exposure to rats. The synergistic effect of Arogh pretreatment, significantly suppressed the alterations induced by isoproterenol alone in rats.

Keywords: Arogh, Myocardial infarction, Oxidative stress, Polyherbal formulation, Rat

Myocardial infarction (MI) one of the major causes of mortality, is associated with ischemic necrosis of cardiac muscle due to compromised supply of blood to a portion of myocardium for proper physiological function. Recent studies suggest that increased free radical formation and subsequent oxidative stress associated with the occurrence of a relative deficit in the endogenous antioxidants, may be one of the mechanisms for the development of heart failure after myocardial infarction.

Isoproterenol, a β-adrenergic agonist and synthetic catecholamine has been reported to cause a severe oxidative stress in the myocardium through free radical formation. The pathogenesis and gross microscopic infarct like lesions in rats are reminiscent of the classical description of human myocardial infarction.

Arogh an ayurvedic formulation is a cocktail of nine herbs.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Plant</th>
<th>Parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nelumbo nucifera (Gaertn.)</td>
<td>Petals</td>
</tr>
<tr>
<td>2.</td>
<td>Rosa damascena (Mill.)</td>
<td>Petals</td>
</tr>
</tbody>
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3. *Terminalia chebula* (Retz.): Fruit pulp
4. *Zingiber officinale* (Rosc.): Rhizome
5. *Eclipta alba* (Hassk.): Leaves
6. *Hibiscus rosasinensis* (Linn.): Petals
7. *Hemidesmus indicus* (R.Br.): Roots
8. *Quercus infectoria* (Olivier): Gall
9. *Glycyrrhiza glabra* (Linn.): Roots

The present study was undertaken to find out the protective effect of Arogh pretreatment on the antioxidant levels after isoproterenol administration in experimental rats which leads to myocardial infarction (MI).

Animals — Adult male albino rats of Wistar strain weighing 120 g-150 g were fed commercial pellet of rat chow and water ad libitum. The rats were divided into four groups of six animals each and maintained under standard laboratory conditions with 12:12 hr light:dark cycle.

Group I - Normal rats
Group II - Administered isoproterenol (20 mg/100 g sc, twice at an interval of 24 hr) in 0.9% saline.
Group III - Rats pretreated rats with Arogh (150 mg/100g, po for a period of 60 days)
Group IV - Rats pretreated with Arogh (150 mg/100 g, po for a period of 60 days) + isoproterenol (20 mg/100 g, sc, twice at an interval of 24 hr) administered on the 59th and 60th day of arogh pretreatment.

At the end of the experimental period, the animals were anaesthetized with pentobarbital sodium (35 mg/kg, ip). Blood was drawn from the external jugular vein of the rat and serum was separated by centrifugation. The animals were consequently sacrificed and the heart dissected, washed in ice-cold saline and homogenised in Tris-HCl buffer (0.1 M) pH 7.4. The homogenate was centrifuged and supernatant obtained was used for the assay of various enzymes. Glutathione (GSH) was assayed by the method of Moron et al.\(^5\), superoxide dismutase (SOD) was assayed according to the method of Misra and Fridovich\(^6\) based on the inhibition of epinephrine autoxidation by the enzyme, catalase (CAT) activity was measured by following decomposition of H\(_2\)O\(_2\) according to the method of Cali borne\(^7\). Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al.\(^8\) using H\(_2\)O\(_2\) as substrate, glutathione-s-
transferease (GST) was assayed by the method of Habig et al.\textsuperscript{9}, vitamin E was estimated by the method of Quaife and Dju et al.\textsuperscript{10}, vitamin C was estimated by the method of Omaye et al.\textsuperscript{11}, ceruloplasmin was measured according to the method of Ravin\textsuperscript{12}, lipid peroxides in serum and heart were estimated using thiobarbituric acid reaction by the method of Ohkawa et al.\textsuperscript{13}

**Drug — Method of preparation of Arogh — Arogh**, an Ayurvedic formulation was obtained from Rumi Herbal Research Institute (Pvt) Limited, Chennai. Arogh 5 g was added to 150 ml of boiling water and boiling continued for 2 min. The decoction was cooled, filtered and the filtrate 35 ml is considered to represent 5 g of Arogh. This was orally administered to rats using an intra-gastric tube.

**Chemicals** — Isoproterenol, epinephrine, 1,1′,3,3′ tetra methoxy propane, NADPH, bovine serum albumin, glutathione (reduced) were purchased from Sigma Chemical Company (St. Louis, Mo; USA). All other chemicals were used of the analytical grade.

Student's *t* test was used for statistical analysis of data.

Isoproterenol administered rats showed a significant increase in serum and heart lipid peroxide levels when compared to control (Table 1). Increased lipid peroxidation, results in irreversible damage to the heart, of isoproterenol administered rats\textsuperscript{14}. The decreased level of lipid peroxides in group IV rats pretreated with Arogh in comparison with group II implies the inhibitory effect of Arogh on lipid peroxidation.

The activities of SOD and CAT in the heart tissue were decreased on isoproterenol administration as shown in Table 2. During MI, these enzymes are structurally and functionally impaired by free radicals, resulting in myocardial damage\textsuperscript{15}. In Arogh pretreated isoproterenol administered rats, the activities of SOD and CAT were found to be near normal. The combined effect of the plant extracts, may have protected the cells against the threat of superoxides and peroxides generated by isoproterenol.

The levels of glutathione and the activities of the glutathione dependent systems GPx and GST were found to be significantly decreased in the heart tissue of isoproterenol treated rats. The decreased level of glutathione on isoproterenol administration reduces the activities of GPx and GST and is the condition seen in isoproterenol treated rats\textsuperscript{11}. Arogh pretreatment enhanced the activity of the antioxidant enzymes to near normal status.

The levels of the vitamin E, vitamin C, ceruloplasmin and glutathione were found to be significantly decreased on isoproterenol administration and is consistent with earlier reports\textsuperscript{16}. Vitamin E\textsuperscript{17}, vitamin C\textsuperscript{18} and ceruloplasmin\textsuperscript{19} scavenge the superoxide radicals and thereby prevent free radical formation and lipid peroxidation. Arogh pretreatment (group IV) increases the levels of these non-enzymic antioxidants to near normal levels.

| Table 1 — Serum levels of peroxide and antioxidants in control and experimental groups |
|------------------------------------------|----------------|----------------|----------------|
|                                        | Group I  | Group II  | Group III  | Group IV  |
| LPO (nmoles of TBARS/mg protein)       | 2.18 ± 0.09 & 0.13 | 4.36 ± 0.13 * | 1.98 ± 0.07 | 2.30 ± 0.08 * |
| Vitamin C (mg/dl)                      | 2.36 ± 0.13 | 1.93 ± 0.25 * | 2.51 ± 0.21 | 2.12 ± 0.18 * |
| Vitamin E (mg/dl)                      | 12.38 ± 0.42 | 9.22 ± 0.28 * | 11.69 ± 0.43 | 11.08 ± 0.39 * |
| Ceruloplasmin (units/ml)              | 0.960 ± 0.04 | 0.630 ± 0.03 * | 0.910 ± 0.04 | 0.88 ± 0.03 * |
| Glutathione (mg/dl)                    | 70.11 ± 2.94 | 52.21 ± 1.94 * | 69.38 ± 2.22 | 63.07 ± 2.34 * |

*P<0.001; *Compared with group I and \( ^* \)Compared with group II

| Table 2 — Peroxide and antioxidants in the heart tissue of the experimental and control groups |
|------------------------------------------|----------------|----------------|----------------|
|                                        | Group I  | Group II  | Group III  | Group IV  |
| LPO (nmoles TBARS/mg protein)       | 3.45 ± 0.12 | 5.12 ± 0.15 * | 3.39 ± 0.18 | 3.98 ± 0.25 * |
| GSH (nmoles/mg protein)              | 4.50 ± 0.14 | 2.52 ± 0.25 * | 4.40 ± 0.2 | 4.01 ± 0.37 * |
| SOD (unit/min/mg protein)            | 3.48 ± 0.06 | 2.35 ± 0.04 | 3.29 ± 0.06 | 3.10 ± 0.05 * |
| CAT (nmoles of H\(_2\)O\(_2\) released/min/mg protein) | 4.32 ± 0.07 | 2.28 ± 0.03 * | 4.17 ± 0.07 | 3.93 ± 0.05 * |
| GPx (nmoles of GSH oxidized/mg protein) | 58.08 ± 0.79 | 33.94 ± 0.47 * | 55.02 ± 0.69 | 52.09 ± 0.72 * |
| GST (nmoles of CDNB conjugated/min/mg protein) | 930.0 ± 4.5 | 672.73 ± 4.24 * | 918.03 ± 9.50 | 900.08 ± 3.04 * |

\( ^* P<0.001; ^* \) Compared with group I and \( ^* \) Compared with group II
The protection offered by Aragh may be due to the combined action of the various plant constituents as well as of the isolated active principles rather than by any single component and may be useful as a drug for MI patients.

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References