PROPHYLACTIC AND CURATIVE EFFECTS OF GARLIC OIL AS COMPARED TO ALPHA-TOCOPHEROL AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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Abstract

Myocardial infarction is the leading cause of death in the developed and developing countries like India. It is associated with elevated lipid levels in the blood. Treatment of hyperlipidemia is one of the major approaches towards decelerating the atherogenic process. The objective of the study was to evaluate prophylactic and curative effects of garlic oil as compared to Vitamin E in isoproterenol(ISP) induced myocardial infarction. Myocardial infarction is induced in rats by administration of Isoproterenol (100mg/kg, s.c. Once for one day). The degree of protection was determined by measuring the levels of serum and cardiac TG, TC, HDL-C, LDL-C, VLDL-C, cardiac CPK, LDH,AST,ALP,A/G ratio,Catalase,TBARS and FFA levels. Myocardial infarction was evidenced by elevated levels of serum and cardiac TG, TC, LDL-C and VLDL-C, TBRS and decreased levels of Catalase. Atherogenic model rats also have lowered levels of cardiac, CPK, LDH and elevated serum levels of LDH and CPK. Garlic oil and Vitamin E pretreatment (100mg/Kg,p.o. for 7 days) significantly reduced the hyperlipidemia, and increases the lowered levels of cardiac CPK, LDH,Catalase and decreases the elevated serum levels of LDH and CPK. In ISP administered group, the cardiac tissue TC, TG, and FFA were significantly increased. Rats pre-treated with garlic oil and Vitamin E showed significant decreases in TC, TG, FFA and TC as compared to ISP administered rats. Garlic oil can minimise the deleterious effects caused by isoproterenol induced damages in rats. This effect is due to the presence of different antioxidants in garlic oil. Garlic oil is a potential source of natural antioxidants and it is used in Ayurvedic preparations very commonly.
Keywords: Hyperlipidemia, Myocardial Infarction, Isoproterenol, Antioxidants

Introduction

Most of the biochemical studies associated with myocardial infarction (MI) have been based on the parameters of serum and tissues of animals in which MI was induced experimentally. A number of procedures have been evolved to produce experimental MI. They are mainly artificial coronary occlusion by surgical intervention¹ and use of sympathomimetic amines like epinephrine, norepinephrine or isoproterenol². Among this isoproterenol (3,4-dihydroxyphenyl)-2-isopropylamine ethanol hydrochloride) induced MI in experimental animals is most frequently used. Mechanism of action of isoproterenol in inducing MI in experimental animals involves specific receptors of it present in myocardium³. Isoproterenol is a non-selective adrenergic receptor's agonist. By interacting with receptors, adrenergic drugs directly alter rate and force of contraction of heart and tone of blood vessels. When the balance between the oxygen need and blood supply to the myocardium is altered acute coronary insufficiency results which leads to the hypoxia of the myocardia and severe necrosis. The cardiac effect of isoproterenol may lead to ECG variations, palpitation, sinus, tachycardia and more serious arrhythmias. These aspects of the deleterious action of isoproterenol and of the protective action of garlic oil on the blood vessel prompted us to investigate on the prophylactic and curative action of the latter as compared to vitamin E in isoproterenol injected rats.

Materials and Methods

Preparation of Garlic Oil

Locally purchased garlic (Allium Sativum Linn) was cleaned and sliced. It was then ground to a pulp in a mortar. Enough methanol was added to keep it fully immersed and left in a conical flask overnight. The next day the mixture was filtered through an ordinary filter paper. The residue left behind was further extracted with methanol by keeping it over night and the procedure was repeated. Both the extracts were combined. Methanol was then distilled off and the oil left behind was collected. This oil was extracted with diethyl ether to obtain the polar fraction. This process was repeated thrice to recover all the polar fraction of the oil. Ether was finally evaporated off on a water bath and the oil left behind was collected (Yield 4g/kg). A stock solution of the garlic oil (G.O.) was prepared in glycerol(50mg/ml).
Experimental animals.

Adult male albino rats (Sprague-Dawley Strain) weighing about 150g were divided into seven groups containing six rats in each so that rats of similar weights were distributed in all the groups. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) . A diet for the rats was prepared by mixing the following ingredients in terms of weights (g/100g diet) as followed by others.

Corn starch – 71, Casein – 16, Ground nut oil –8, Salt mixture – 4, Vitamin mixture – 1. The diet was mixed properly and made into small balls with water. All the rats were given drinking water ad libitum.

Experiment I:-

The following groups of rats with six in each were used to study the prophylactic effects of garlic oil and vitamin E against isoproterenol induced damages in rats.

Group I – Normal control. This group was given the normal diet and they were orally administered 2ml glycerol/day along with water ad libitum. After 10½ days they were sacrificed for various estimations in serum and tissues.

Group II – Isoproterenol injected control group. Feed is the same as above. After 12 hr fasting this group of rats were subcutaneously injected with a single dose of isoproterenol (100mg/kg) and they were orally administered 2ml glycerol/day. After 3½ days they were sacrificed for various estimations in serum and tissues.

Group III – One week prophylactic treatment with garlic oil (G.O). G.O. dissolved in glycerol was orally administered daily to each rat (100mg/Kg/day) for 7 days to study its prophylactic effect against isoproterenol induced damages. On the 7th day the same 12 hr fasting rats were given a subcutaneous injection of a single dose of isoproterenol (100mg/kg). These rats were sacrificed after 3½ days for various estimations in serum and tissues.

Group IV – One week prophylactic treatment with Vitamin E. Vitamin E dissolved in glycerol was orally given daily (100mg/Kg/day) to each rat of this group for 7 days to study its prophylactic effect against isoproterenol induced damages. On the 7th day the same 12 hr fasting rats of this group were given a subcutaneous injection of a single dose of isoproterenol (100mg/Kg/day). Later they were sacrificed after 3½ days for various estimations in serum and tissues.

Experiment II: Group V - A group of 12 hr fasting normal rats was subcutaneously injected with a single dose of isoproterenol (100mg/Kg). Feed was the same as in the first experiment. After 10½ days they were sacrificed for various estimations in serum and tissues.
**Group VI** – Curatively treated group with garlic oil. A group of 12 hr fasting rats was subcutaneously injected with a single dose of isoproterenol (100mg/Kg) and after 3½ days they were administered orally with garlic oil (100mg/Kg/day) for 7 days. Feed was the same as above. After 10½ days of IP injection they were sacrificed for various estimations in serum and tissues.

**Group VII**- Curatively treated with Vitamin E. A group of 12 hr fasting rats was subcutaneously injected with a single dose of isoproterenol (100mg/Kg) and after 3½ days they were administered orally with Vitamin E (100mg/Kg/day) for 7 days. Feed was the same as above. After 10½ days of IP injection they were sacrificed for various estimations in serum and tissues.

**Result**

Table-1: Comparative prophylactic and curative effects of garlic oil and vitamin E on serum lipids and atherogenic index of isoproterenol injected rats. Values are Mean of 6 rats ± S.D. Prophylactically treated groups are 3 & 4 and curatively treated groups are 6 & 7.

<table>
<thead>
<tr>
<th>Groups and type of treatment</th>
<th>Total chol. (mg/dl)</th>
<th>LDL chol. (mg/dl)</th>
<th>HDL chol.(mg/dl)</th>
<th>Atherogenic index</th>
<th>TAG (mg/dl)</th>
<th>FFA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal control</td>
<td>100.3±5.0</td>
<td>32.2±2.32</td>
<td>45.8±2.32</td>
<td>2.07±0.163</td>
<td>85.0±2.21a</td>
<td>92.3±3.01</td>
</tr>
<tr>
<td>2. Isoproterenol injected control</td>
<td>172.2±3.18</td>
<td>97.3±2.12</td>
<td>58.0±2.61</td>
<td>2.98±0.232</td>
<td>145.0±2.12</td>
<td>184.8±2.31</td>
</tr>
<tr>
<td>3. Do (after garlic oil treatment)</td>
<td>128.2±2.85a</td>
<td>44.3±2.70a</td>
<td>79.3±2.23b</td>
<td>1.58±0.147c</td>
<td>91.0±3.96ab</td>
<td>135.7±3.26a</td>
</tr>
<tr>
<td>4. Do (after vitamin E treatment)</td>
<td>132.2±2.86a</td>
<td>49.3±2.50a</td>
<td>74.8±3.19b</td>
<td>1.82±0.147a</td>
<td>97.3±1.86c</td>
<td>142.00±4.20a</td>
</tr>
<tr>
<td>5. Isoproterenol injected control</td>
<td>144.7±3.01</td>
<td>73.2±2.32</td>
<td>50.8±2.32a</td>
<td>2.95±0.187b</td>
<td>111.17±2.31</td>
<td>154.5±4.23</td>
</tr>
<tr>
<td>6. Do and garlic oil treatment</td>
<td>134.2±2.86a</td>
<td>49.3±3.27a</td>
<td>77.8±2.32b</td>
<td>1.75±0.105ac</td>
<td>93.3±3.78bc</td>
<td>139.7±3.26a</td>
</tr>
<tr>
<td>7. Do and vitamin E</td>
<td>135.0±3.7a</td>
<td>52.0±2.12a</td>
<td>73.3±3.27b</td>
<td>1.80±0.141a</td>
<td>99.2±3.71c</td>
<td>145.8±3.19a</td>
</tr>
</tbody>
</table>

ANOVA Level of significance is fixed at P<0.01. Values with similar small letters a to c in the same column are not significantly different from each other when any group is compared with another group. However when isoproterenol injected groups 2 and 5 are compared with normal group 1 or when treated groups 3 & 4 and 6 &7 are compared with
the isoproteranol injected groups 2 and 5 respectively, except one value noted as NS all others are significantly different (P<0.001).

Table 2 – Comparative prophylactic and curative effects of garlic oil and vitamin E on the activities of various marker enzymes in serum and A/G ratio in isoproteranol injected rats. Values are Mean of 6 rats ± S.D. Prophylactically treated groups are 3 & 4 and curatively treated groups are 6 & 7. Units of enzyme activities are noted as foot note.

<table>
<thead>
<tr>
<th>Groups and type of treatment</th>
<th>CPK(IU/L)</th>
<th>ALT(IU/L)</th>
<th>AST(IU/L)</th>
<th>LDH(IU/L)</th>
<th>ALP(IU/L)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal control</td>
<td>171.1±3.43</td>
<td>27.8±2.32</td>
<td>27.0±3.03</td>
<td>189.0±4.73</td>
<td>60.8±5.5\textsuperscript{a}</td>
<td>1.95±0.19\textsuperscript{a}</td>
</tr>
<tr>
<td>2. Isoproteranol injected control</td>
<td>470.5±7.21</td>
<td>49.5±1.87</td>
<td>55.3±2.18</td>
<td>330.2±5.98</td>
<td>115.0±10.6\textsuperscript{c}</td>
<td>2.00±0.14\textsuperscript{a}</td>
</tr>
<tr>
<td>3. IP injection after garlic oil treatment</td>
<td>209.7±4.13</td>
<td>35.4±2.48\textsuperscript{a}</td>
<td>33.3±2.17\textsuperscript{a}</td>
<td>250.8±3.63\textsuperscript{a}</td>
<td>66.8±6.5\textsuperscript{a,b}</td>
<td>1.80±0.13\textsuperscript{a}</td>
</tr>
<tr>
<td>4. IP injection after vitamin E treatment</td>
<td>220.3±4.13\textsuperscript{a}</td>
<td>39.5±1.87\textsuperscript{a,b}</td>
<td>34.2±2.32\textsuperscript{a}</td>
<td>256.2±2.86\textsuperscript{a}</td>
<td>65.6±7.2\textsuperscript{a,b}</td>
<td>1.78±0.15\textsuperscript{a}</td>
</tr>
<tr>
<td>Curatively treated groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Isoproteranol injected control</td>
<td>340.5±7.12</td>
<td>40.8±2.31\textsuperscript{b}</td>
<td>45.2±4.07</td>
<td>349.5±5.01</td>
<td>100.5±7.1\textsuperscript{c}</td>
<td>3.02±0.23\textsuperscript{b}</td>
</tr>
<tr>
<td>6. IP injection and garlic oil treatment</td>
<td>228.2±3.19\textsuperscript{a}</td>
<td>37.8±1.96\textsuperscript{a,b}</td>
<td>36.7±2.16\textsuperscript{a}</td>
<td>251.5±3.08\textsuperscript{a}</td>
<td>70.0±6.0\textsuperscript{b}</td>
<td>2.70±0.14\textsuperscript{b}</td>
</tr>
<tr>
<td>7. IP injection and vitamin E treatment</td>
<td>242.5±3.61</td>
<td>37.00±1.41\textsuperscript{ab}</td>
<td>33.2±2.48\textsuperscript{a}</td>
<td>270.6±4.51</td>
<td>64.0±4.5\textsuperscript{a,b}</td>
<td>2.80±0.14\textsuperscript{b}</td>
</tr>
</tbody>
</table>

ANOVA Level of significance is fixed at P<0.01. Values with similar small letters a to c in the same column are not significantly different from each other as followed in table 1. Comparison between test groups and their respective control are made and all those values without similar small letters are highly significant. (P<0.001).

Figure 1- Comparative prophylactic and curative effects of garlic oil and vitamin E on the activities of Catalase(IU/L) and TBARS(MOLES OF MDA/mg PROTEIN X10) enzymes in cardiac tissues in isoproteranol injected and control rats. Values are Mean of 6 rats ± S.D. Prophylactically treated groups are 3 & 4 and curatively treated groups are 6 & 7.
Discussion

Both Garlic oil and Vitamin E have significant effects in raising Catalase activity and lowering TBRS values in ISP injected rats. An increase in the lipid parameters and histopathology of cardiac tissues could be due to the damages caused by ISP on tissues. Similarly an increase in the activities of the marker enzymes in serum could be due to the leakage of them from heart tissue as a result of necrosis caused by the stress on ISP injection. The amount of enzyme released from the damaged myocardium is a measure of the size of infarction. Damage to the tissues could be due to free radical mediated lipid peroxidation by isoproterenol. Prophylactic treatment with G.O or vitamin E in rats showed a better but only at a nonsignificant level as compared to their effects in the curative treatment on a majority of parameters. The beneficial effects of treatment could be due to the efficient free radical quenching property of α-tocopherol and garlic oil. According to the antioxidant hypothesis, the primary function of α-tocopherol in vivo is the prevention of the destructive peroxidation of PUFA. Similar antioxidant action of G.O could also be responsible for an equal or in some cases for a better action of it in ISP injected rats. There are certain minor advantages of treatment with G.O. than with Vitamin E and at no time the latter proved to be a better drug than the former in our studies. This can be seen in the control of TAG and other lipid parameters and CPK in the serum of the prophylactically treated groups and in the maintenance of a better catalase activity for both prophylactically and curatively treated groups of rats with G.O.

There are reports that arginine and mineral rich tender coconut water and garlic containing indigenous drug and an extract of Terminalia Chebula could ameliorate the damages brought about by ISP injection. Garlic oil contains polysulfides and they can combine with the vasodilator nitric oxide (NO) forming nitroso derivatives. By increasing the concentration of NO on the sites of vasoconstrictions G.O may be very effective in preventing the damages by ISP.
Biological membranes contain double layers of TAGs and phospholipids which are also rich in aliphatic hydrocarbons. Arachidonyl residues of PL is one such group that helps to stabilize biological membranes by specific physiochemical interactions with aliphatic side chain of vitamin E as mentioned above and on the same account possibly with diallyl or dialkyl groups of garlic oil also. Similarly according to our study G.O which belongs to the thiol group of nutraceuticals is an effective drug for treatment both prophylactically and curatively on IP injected rats. Various reports reveal that there exists an inverse relationship between serum α -tocopherol concentration and lipid peroxidation levels. So garlic oil may also be added to the list as one of the most potent drug against the condition of MI and people may be advised to use garlic oil or homogenized garlic equivalent to 15g as it contains many sulfur compounds other than the oil with useful therapeutic effects as per WHO recommendations and reports by others.

**Conclusion**

In the present study, it is found that Garlic oil possess cardio protective, hypolipidemic and antioxidant activities as compared to Vitamin E against isoproterenol induced toxicity in experimental rats. The mechanism of these protective activities may be attributed to its various medicinal properties. The observations highlight that Garlic oil may be one of the promising drug for improving defence mechanisms in the physiological systems against oxidative stress caused during myocardial infarction. The histopathology of myocardium of treated groups shows the curative effects of garlic oil and Vitamin E in ISP injected rats.

**Acknowledgement**

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**Histopathology of myocardium**
(a): Normal control showing normal myocardium

(b): Isoproterenol (ISP:100mg/kg) alone showing fragmentation of myocardial fibers and greater focal interstitial inflammatory response.

(c) Garlic oil + ISP(100 mg/kg) showing reduced fragmentation of myocardial fibers and focal interstitial inflammatory response.

(d) Vitamin E + ISP (100 mg/ kg) showing reduced focal interstitial inflammatory response

References


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