Antihyperlipidemic Activity of methanolic extract of Garlic (Allium sativum L.) in Triton X-100 induced hyperlipidemic rats

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ABSTRACT

The present study is designed to evaluate the effect of garlic extract on lipid profiles in Triton X-100 induced hyperlipidemia in male wistar rats. Three fractional extracts (AS1, AS2, and AS3) were obtained by column chromatography from methanolic extract of garlic. All three garlic extract significantly increased (p < 0.0001) plasma HDL-Cholesterol and decreased plasma TC, LDL-Cholesterol and TG levels as compared with hyperlipidemic control. Among three fractions, AS3 has more significantly reduced the plasma lipid levels than others AS1 and AS2.

Keywords: Garlic, lipid profile, Allium sativum

1. INTRODUCTION

Hyperlipidemia is defined as an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides and phospholipids (1-2). An elevation of plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs or diseases. Despite of differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic rat models are extensively used in lipid research (3). The solution of Triton X-100 has successfully been used to induce hyperlipidemia in rats in previous studies (4) and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability.

Garlic (Allium sativum L.) is used as a spice and medicinal herb. Most recent research on garlic has used garlic in the form of tablets, flesh, raw, boiled, cooked and dried (5). Commercially available garlic preparations in the form of garlic oil, garlic powder and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (6). Garlic and garlic extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (7-8). Several studies have also shown that garlic contains active hypocholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide (9, 10). The present study investigated the antihyperlipidemic activity of methanolic extract of Allium sativum in Triton X-100 induced hyperlipidemic rats.

2. MATERIALS AND METHODS

2.1. Extraction of plant material

The fresh fruits of Allium sativum (AS) were obtained from the local market in Hyderabad, India. Dried and ground bulbs (about 100g) were submitted to extraction with 300ml ethanol (80%) in a Soxhlet apparatus for 72 hours. After extraction, the solvent was filtered and made to evaporate by Rotavapor. The obtained garlic alcoholic extract was stored in distilled water to give 0.4g garlic extract per one ml of the suspension and administered orally through orogastric tube. The volume of administrated extract was 1ml for each animal.

2.2. Fractional extraction of parent extract

The parent dried juice extract was then fractionated by using the solvents according to polarity in ascending order i.e. by using chloroform: acetic acid, methanol, pyridine and water. Each fraction was dried in oven at 40-50°C.

Column Chromatographic Isolation and Purification of Methanolic Extract

1. Column: Glass
2. Dimension: Length 50 cm, diameter 3.5 cm
3. Stationary phase: Silica gel
4. Sample: Methanolic ether extract
5. Mobile phase: Gradient elution

The Methanolic fraction of parent extract was subjected for isolation over the silica gel (mesh size- 100-250) column. Previously, the slurry of silica gel was prepared with the mobile phase. The column was washed with the mobile phase for sufficient period of time. Then Methanolic fraction was loaded over the silica gel. The mobile phase was passed continuously with constant flow rate (10 ml/min.). The fractions were collected at regular intervals of time, evaporated at temperature <40-50°C and subjected for evaluation of anti-hyperlipidaemic activity.
Isolation of compounds by Thin Layer Chromatography (TLC)

Active fraction obtained by column chromatography was used for further isolation by TLC. The solvent system developed on trial and error basis was n-butanol: methanol: water (6:2:2). Four spots were obtained and were named as AS$_1$, AS$_2$, and AS$_3$.

Isolated spots were collected by using preparative TLC.

2.3. Chemicals

Atorvastatin pure drug was a kind gift from Dr. Reddy’s Pharmaceutical Ltd, Hyderabad. All other chemicals used were analytical grade from SD fine chem., India. Cholesterol kit (Enzymatic Method) and HDL-C kit were procured from Qualigens Diagnostics, Mumbai. Triglycerides kit was obtained from E-Merck Limited, Mumbai, India.

2.4. Animals

Wister albino adult male rats weighing 200-220g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (Hindustan Lever Ltd., Bangalore) and water ad libitum. Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline after overnight fasting for 18 hours.

2.5. Anti-hyperlipidemic studies

The rats were divided into five groups of eight rats in each group and were treated with single dose/day (p.o) of standard drug or extracts of AS.

**Table 1: Effect of methanolic extracts of garlic on serum lipid profile levels in Triton induced hyperlipidemic rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Normal</td>
<td>83.51 ± 1.87</td>
<td>69.07 ± 1.93</td>
<td>43.73 ± 1.47</td>
<td>75.51 ± 2.24</td>
</tr>
<tr>
<td>Group II: HL(Triton)</td>
<td>152.51 ± 3.85</td>
<td>156.20 ± 5.70</td>
<td>25.66 ± 0.19</td>
<td>192.73 ± 4.23</td>
</tr>
<tr>
<td>Group III: Triton + AS$_1$ (10 mg/kg)</td>
<td>139.74 ± 0.51$^a$</td>
<td>127.81 ± 1.09$^a$</td>
<td>41.74 ± 1.04$^a$</td>
<td>151.49 ± 3.69$^a$</td>
</tr>
<tr>
<td>Group IV: Triton + AS$_2$ (10 mg/kg)</td>
<td>121.19 ± 2.09$^a$</td>
<td>104.79 ± 2.91$^a$</td>
<td>48.51 ± 0.92$^a$</td>
<td>124.92 ± 3.81$^a$</td>
</tr>
<tr>
<td>Group V: Triton + AS$_3$ (10 mg/kg)</td>
<td>119.25 ± 0.21</td>
<td>101.52 ± 0.91</td>
<td>56.01 ± 0.19</td>
<td>121.92 ± 0.72</td>
</tr>
<tr>
<td>Group VI: Triton + AT</td>
<td>112.17 ± 1.23</td>
<td>89.54 ± 0.86$^a$</td>
<td>53.93 ± 1.11$^a$</td>
<td>116.46 ± 2.80$^a$</td>
</tr>
</tbody>
</table>

Values are in mean ± SD; n=8; a= p < 0.0001 Vs Group II

Figure 1: Atherogenic index values in hyperlipidemic and garlic extracts treated group of rats

Isolation of compounds by Thin Layer Chromatography (TLC)

Active fraction obtained by column chromatography was used for further isolation by TLC. The solvent system developed on trial and error basis was n-butanol: methanol: water (6:2:2). Four spots were obtained and were named as AS$_1$, AS$_2$, and AS$_3$. Isolated spots were collected by using preparative TLC.
It is widely accepted that reduction in plasma HDL is a risk factor for atherogenic uptake and metabolism by different tissues. By controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Oral administration of all garlic fractions, AS1, AS2, and AS3, of A. sativum at the doses of 10mg/kg significantly (P < 0.0001) reduced the serum TC, TG and LDL-C levels and increased the serum HDL-C levels when compared to the hyperlipidemic (HL) control group. The change in lipid levels in groups of II, III and IV were comparable with group of atorvastatin treated rats. Among three fractions, AS3 reduced the elevated levels more significantly than the others. Oral administration of all garlic extracts significantly reduced the elevated lipid levels in rats possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process (15). Increased levels of HDL (cardio protective lipid) after administration of garlic extracts concluded that the extract is a potent cardio protective agent and this effect may be due to the increase in the activity of Lechitin: cholesterol acyl transferase (LCAT), which play a key role in incorporating the free cholesterol into HDL and transferring back to Very Low Density Lipoproteins (VLDL) or Intermediate Density Lipoproteins (IDL), which is taken back by the liver cells. Several studies show that an increase in HDL-C is associated with a decrease in coronary risk (2). High levels of TC and LDL-C are major coronary risk factors (16, 17). Administration of GARLIC lowered both total and LDL cholesterol in hyperlipidemic rats. This lowering of TC and LDL-cholesterol would reduce the incidence of coronary events (18).

3. RESULTS AND DISCUSSION

Natural remedies have been investigated for centuries for a wide variety of ailments. Garlic has received special attention for its beneficial effects (13-14), but until recently there has been little scientific support for its therapeutic and pharmacological properties. In the present study, garlic (Allium Sativum) was selected to screen for its antihyperlipidemic activity in Triton X-100 (100 mg/kg) induced hyperlipidemic rats.

As reported earlier, Injection of Triton X-100 (100 mg/kg) has successfully induced hyperlipidemia in rats by increasing the serum TC, TG and LDL-C levels (4). The effect of methanolic extracts of garlic on serum lipid profile levels was shown in Table 1. Treatment with methanolic extracts (AS1, AS2, and AS3) of A. sativum at the doses of 10mg/kg significantly (P < 0.0001) reduced the serum TC, TG and LDL-C levels and increased the serum HDL-C levels when compared to the hyperlipidemic (HL) control group. The change in lipid levels in groups of II, III and IV were comparable with group of atorvastatin treated rats. Among three fractions, AS3 reduced the elevated levels more significantly than the others. Oral administration of all garlic extracts significantly reduced the elevated lipid levels in rats possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

REFERENCES


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