Effects of Allium Sativum on hematological parameters and blood lipid profile in albino rat model

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\textbf{ABSTRACT}
To investigate the effect of Garlic extract on hematological parameters and blood lipid profile in albino rat model. Experimental/Analytical study Place and Duration: Animal House, Isra University Hyderabad from May to November 2013. Sixty adult albino rats were divided into four groups; Group I. controls received 0.9% isotonic saline, Group II. Received garlic extract orally (100 mg/kg), Group III. Received garlic extract orally (200 mg/kg) and Group IV. Received the garlic extract (300 mg/kg) for 30 days. Blood samples were collected for hematological analysis and plasma lipids. The data was analyzed on Statistix 8.1 (USA) using one-way ANOVA, Bonferroni and Chi-square tests. A p-value of ≤0.05 was taken statistically significant. The hematological and plasma lipids fractions showed significant differences between the controls, and experimental garlic groups at different doses (p=0.001). The hemoglobin, packed cell volume, red and white cell counts, and platelets were raised in garlic extract groups particularly in high doses of 300mg/kg. The differential white blood cell counts also revealed significant differences. The plasma lipids showed a reduction in total cholesterol, LDL-c and a rise in HDL-c levels. (p=0.001). The garlic extract influences hematological parameters in albino rats and reduces plasma lipids fractions which may be protective against cardiovascular disease.

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\textbf{Introduction}
\textit{Allium sativum} is commonly known as Garlic. \textit{Allium sativum} is a well known spice and herbal medicine for the prevention and treatment of a variety of diseases ranging from infections to heart diseases.\textsuperscript{1,2} It is reported \textsuperscript{2} that garlic shows antimicrobial activity. Garlic is considered as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular protecting effects.\textsuperscript{4} It is also reported that garlic has the tendency of lowering serum and liver cholesterol.\textsuperscript{5} In previous studies the positive effects of garlic supplements have shown significant differences on plasma lipids as reported.\textsuperscript{5,7} Previous studies have demonstrated that lowering plasma total cholesterol (TC), low density lipoprotein cholesterol (LDLc) and increasing high density lipoprotein cholesterol (HDLc) are beneficial in preventing risk of cardiovascular disease (CVD).\textsuperscript{8,9} There are divergent views on the effect of garlic on plasma cholesterol concentration and coronary heart disease. Recent studies have reported the ability of garlic to reduce plasma cholesterol in humans.\textsuperscript{10-12} Evidence on the role of garlic in lowering plasma cholesterol in developed populations abounds.\textsuperscript{13,14} Several studies have also reported the antihypertensive effects of garlic.\textsuperscript{13,14} Garlic extracts have been used in the treatment of a wide range of disorders in the past.\textsuperscript{15-16} Sang et al\textsuperscript{17} demonstrated that garlic oil is active against fatty change of the liver. Allicin, diallyl disulfide-oxide, an active ingredient released from garlic (alliin) is a systemic vasodilator.\textsuperscript{18} Also reported was garlic containing preparation which showed significant decrease in diastolic blood pressure in severely hypertensive patients.\textsuperscript{19} Either extracts of garlic and partially purified distilled extracts of garlic have been reported to inhibit human platelet aggregation in vitro.\textsuperscript{20} Treatment with garlic extracts was found to improve the activation of natural killer cells, the function of T-lymphocytes and the level of interleukin-2.\textsuperscript{21} Also in vitro and in vivo studies showed that aged garlic extracts stimulate immune functions.\textsuperscript{22} Many studies have been reported on effects of garlic on various organs in rat model; however, scanty information is available regarding effects of garlic on hematological parameters. The present study aimed to investigate the possible effects of aqueous garlic extract (GE) on hematological parameters and blood lipid levels in experimental albino rat model.

\textbf{Materials and Methods}
An experimental study was conducted at the animal house of Isra University in albino rat model from January to November 2013. Sixty male albino rats weighing 150-250 grams were included in the study. Sick animals and animals weighing more or less than mentioned in inclusion criteria were excluded from study. Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw and clean water were provided ad-libitum.
Garlic extract preparation

The aqueous garlic extract was prepared by dissolving garlic in pure water at a quantity of 100mg, 200mg and 300mg. The aqueous garlic extract was administered orally at a dose of 100, 200 and 300 mg/5ml of distilled water per kilogram of body weight.

- **Animals and experimental design**

At the end of experimental period, 12-hour-fasted rats were anesthetized during the post absorptive period. Blood was drawn from the vena cava into heparin tubes centrifuged and stored at 4°C; the plasma was stored in a freezer for later assays. The rats were given garlic in different doses for 30 days. The rats were divided into four groups:

  - **Group I. Control Group (n=15)** Rats received 0.9% isotonic saline orally throughout the experimental period.
  - **Group II. Experimental Group (n=15)** Rats were given aqueous garlic extract, administered orally at a dose of 100 mg/5ml of distilled water per kilogram of body weight.
  - **Group III. Experimental Group (n=15)** Rats were given aqueous garlic extract, administered orally at a dose of 200 mg/5ml of distilled water per kilogram of body weight.
  - **Group IV. Experimental Group (n=15)** Rats were given aqueous garlic extract, administered orally at a dose of 300 mg/5ml of distilled water per kilogram of body weight.

- **Blood sample:** The blood samples were collected from vena cava into EDTA tubes centrifuged and stored at 4°C; the plasma was stored in a freezer for later assays. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes and plasma lipids.

- **Plasma lipid profile:** Plasma triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high-density lipoprotein-cholesterol (HDL-C) were enzymatically determined using immunoassay kits (Asia Pharmaceuticals, Seoul) and an enzyme-linked immunosorbent assay reader (Pharmacia Biotech, Cambridge, United Kingdom) according to the manufacturer’s protocol. Plasma low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald equation.

The data was analyzed on Statistix 8.1. The continuous variables were presented as mean±SD using analysis of variance (ANOVA) and Bonferroni test. Chi-square test was used for categorical variables. A p-value of ≤ 0.05 was taken statistically significant.

**Result**

The present study observes major differences in the hematological parameters and blood lipid profile among controls and experimental groups. Significant differences were observed in the packed cell volume (PCV), hemoglobin, RBC count, white blood cells and differential white cell counts as shown in table. I, with a highly significant p-value for multiple comparisons (p=0.001).

Similarly major differences were observed in the lipid profile of controls and experimental groups. The most significant differences in lipid profile of experimental group IV and controls was observed (p=0.0001) as shown in table II.

**Discussion**

The present experimental study in albino rat model showed significant differences in the hematological parameters and lipids profile in controls and garlic extract groups as shown in table I and II.

The hemoglobin, packed cell volume, red blood cell counts, white blood cells counts and platelets revealed statistically significant differences between controls and garlic extract groups. The study showed that rats given higher doses of garlic had a preponderance of neutrophil over lymphocyte (table II). Also, a higher dose of 300mg/day garlic extract was observed to cause a significant increase in hematological counts and indices, and reductions in blood lipid fractions. These data therefore support the earlier reports by Sumiyoshi and Oluweli et al that garlic extracts stimulate immune functions. This observation may partly explain the role of garlic in activating the natural killer cells, the function of T-lymphocytes and the level of interleukin-2 as reported previously.

In the present study, within group analysis showed a significant decrease in plasma total lipids in the experimental groups fed on high garlic extract. There were however no significant changes in the plasma TG, HDLC and LDLC when compared groups II and III with controls. Reduced plasma TC, and LDLC and increased mean plasma HDLC were obtained in rats fed on high garlic amounts (300mg/dl). Earlier studies showed that garlic when administered raw caused significant reduction in total cholesterol in subjects with raised plasma cholesterol.

### Table I. Hematological parameters in different animal groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group. I (Controls)</th>
<th>Group. II (Garlic extract 100mg)</th>
<th>Group. III (Garlic extract 200mg)</th>
<th>Group. IV (Garlic extract 300mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (x10^6µL^-1)</td>
<td>4.59</td>
<td>4.78</td>
<td>4.79</td>
<td>4.80</td>
</tr>
<tr>
<td>Hemoglobin (gdl^-1)</td>
<td>15.1</td>
<td>14.9</td>
<td>15.3</td>
<td>15.7</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>43.9</td>
<td>44.3</td>
<td>43.9</td>
<td>45.9</td>
</tr>
<tr>
<td>White blood cells (µL^-1)</td>
<td>7900</td>
<td>7870</td>
<td>8790</td>
<td>9789</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>62.2</td>
<td>67</td>
<td>65</td>
<td>86</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1.9</td>
<td>2.1</td>
<td>2.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>35</td>
<td>29.5</td>
<td>31.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Basophils (µL^-1)</td>
<td>0.1</td>
<td>0.03</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>Platelets (x10^6µL^-1)</td>
<td>4.5</td>
<td>4.3</td>
<td>4.9</td>
<td>5.85</td>
</tr>
</tbody>
</table>

### Table II. Blood Lipid profile in different animal groups

<table>
<thead>
<tr>
<th>Plasma Lipids (mgdl^-1)</th>
<th>Group. I (Controls)</th>
<th>Group. II (Garlic extract 100mg)</th>
<th>Group. III (Garlic extract 200mg)</th>
<th>Group. IV (Garlic extract 300mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>110</td>
<td>145</td>
<td>105</td>
<td>78</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>135</td>
<td>131</td>
<td>109</td>
<td>98</td>
</tr>
<tr>
<td>Low density lipoprotein</td>
<td>43</td>
<td>39</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>High density lipoprotein</td>
<td>41</td>
<td>43</td>
<td>45</td>
<td>47</td>
</tr>
</tbody>
</table>
The groups that had garlic incorporated into the diet at high doses of 300 mg/dl had the highest mean plasma HDLC level. One proposed mechanism of cholesterol reduction is through the inhibition of HMG-CoA reductase by garlic, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis. On the other hand the plasma LDLC was significantly decreased in the groups of animals whose diet had garlic incorporated. This perhaps supports the ability of garlic to reduce LDL cholesterol concentration. Evidence from available studies showed that garlic can affect vasculature by increasing aortic elasticity as well as retardation of atherosclerosis progression11,14 perhaps through increase excretion of LDL cholesterol. As evident from this study, garlic also decreases plasma triglyceride level in the rats fed on garlic containing diets, most likely through the stimulation of lipase. Available reports from a similar study indicated that garlic is a potential stimulant of lipase. The results of present study suggest that garlic has hypolipidemic effect. Available report shows that garlic consumption is beneficial in the prevention of cardiovascular disease. The garlic extract in high doses increases hematological parameters and reduces bad cholesterol of plasma lipids as observed in present study.

Conclusion
The garlic extract influences hematological parameters in albino rats and also reduces plasma lipids which may be protective against cardiovascular disease. However, further studies are warranted.

References