A Study on Hypolipidemic effect of Garlic in Dyslipidemic Patients

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ABSTRACT
Dietary components play an important role in the development of various human diseases. Certain phytochemicals are present in the food that have anticancer and anti-inflammatory properties, which confer many health benefits. One source of such phytochemicals is garlic, whose role in the prevention and treatment of cardiovascular diseases is best known throughout the world. Garlic (Allium sativum) and garlic extracts have been reported to be variably effective in managing hypertension and hypercholesterolemia, although remains to be proven conclusively. The aim of this investigation was to study the efficacy of an aqueous extract of raw garlic in controlling levels of cholesterol, and triglycerides in dyslipidemic patients. The present study was undertaken to assess the hypolipidemic role of garlic in dyslipidemic patients. 300 cases of dyslipidemia between the ages of 40-60 years were included in the study. Serum lipid profile was determined by enzymatic methods by kits obtained from Sigma-Aldrich. Total cholesterol, low density lipoprotein cholesterol, triglycerides were significantly dropped after the intake of raw garlic extract while High density lipoprotein cholesterol was significantly increased. Our findings suggest that raw garlic extract intake for significant duration causes significant drop in the level of Total Cholesterol, LDL Cholesterol, Triglyceride and significant increase in the level of HDL Cholesterol.

Key words: TC-Total cholesterol, LDLc-Low density lipoprotein cholesterol, VLDLc-Very low density lipoprotein cholesterol, HDLc-High density lipoprotein-cholesterol, TG-Triglyceride.

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INTRODUCTION
Dietary components play an important role in the development of various human diseases. Epidemiologic studies indicate that diets rich in fruits, vegetables, and spices are associated with lower risk of all-cause, cancer, and cardiovascular-disease death [1,2]. Certain phytochemicals are present in the food that have anticancer and anti-inflammatory properties, which confer many health benefits. One source of such phytochemicals is garlic, whose role in the prevention and treatment of cardiovascular diseases is best known throughout the world.
Garlic (Allium sativum) belonging to Alliaceae family is used universally as a flavoring agent, traditional medicine, and functional food to enhance physical and mental health. The therapeutic effects of garlic in treating a wide variety of human diseases have been known for centuries; thus, garlic has acquired a special position as a prophylactic and therapeutic medicinal agent. Many animal and human studies have shown that garlic extract is very appropriate for many diseases, because this medicine herb has a lot of useful effects, including; hypolipidemic and hypocholesterolemic effects, antimicrobial and antifungal activity, anticarcinogenic activities, antioxidant activity, anti-hypertensive activity, anti-diabetic, antihyperhomocysteinemia and antithrombotic effects [3].
It is even cited in the Egyptian Codex Ebers, a 3,500-year-old document, as useful in the treatment of heart disorders, tumors, worms, bites, and other ailments [1]. Garlic is also reported to inhibit the pathogenesis of cardiovascular disease and to prevent cancer and other chronic diseases associated with aging [4].

Garlic (Allium sativum) and garlic extracts have been reported to be variably effective in managing hypertension and hypercholesterolemia [5-7], although remains to be proven conclusively [8-9]. The aim of this investigation was to study the efficacy of an aqueous extract of raw garlic in controlling levels of cholesterol, and triglycerides in dyslipidemic patients treated orally daily for 24 weeks. Because there have been many inconsistent reports about the use of different preparations of garlic, we decided to use the aqueous extract of garlic.

MATERIAL AND METHODS
This prospective study was conducted on three hundred cases of primary dyslipidemia recruited from the Medicine O.P.D. of Major S.D. Singh Medical College Farrukhabad, India, from January 2012 to October 2014. Inclusion criteria were patient age (40-60 year old), a positive family history of hypercholesterolemia, a minimum fasting total cholesterol level at enrolment higher than > 185 mg/dl, participation in a dietary counselling program, and compliance with a National Cholesterol Education Program Step II diet for at least 6 months. Exclusion criteria include the presence of secondary cause of hyperlipidemia or a history of serious illness 3 months or less prior to the enrolment. All patients were entered in the study after written informed consent was obtained from the parent and the study was approved by the ethical committee of the Major S.D. Singh Medical College Farrukhabad.

After an overnight fast of 14-16 hours, 5 ml blood samples of patient and control were collected in vacuum tubes and allowed to clot at room temperature for 60-120 minute followed by centrifugation at 3000 rpm for 10 min. at 40C. Serum was stored at -20C, for estimation of lipid profile. The garlic (Allium sativum) was purchased from local market. Garlic (100gm) was washed first by distilled water and then by 95% ethanol. Garlic was homogenized using sterile mortar and pestle. And then sieved through double layer of sterile fine mesh cloth to make 100% extract. This extracts was stored at 4°C in refrigerator.

They were given garlic extract (equal to 400 mg garlic, 1 mg allicin) twice daily. All patients were put on NCEP type II diet and twenty four weeks later, lipid profiles were checked.

Estimation of triglyceride
Estimation of triglyceride was performed by method described by Kaplan [10] by using commercially available kit from Sigma- Aldrich. In brief, 0.02 ml of serum was mixed with 2 ml of reaction solution. The absorbance of sample was measured against the reaction solution at 540 nm, due to the formation of Quinonimine dye, which is directly proportional to the total triglyceride concentration in the sample.

Estimation of total cholesterol
Estimation of total cholesterol was performed by Pelkonen et al [11]. CHOD-PAP method by using commercially available kit from Sigma-Aldrich. In brief, 0.02 ml of serum was mixed with 2 ml of reaction solution (Enzyme solution with colour reagent). The absorbance of samples was measured at 540nm against the reagent blank value.

Estimation of serum HDLc
Estimation of serum HDLc was performed as described by Nikkila et al [12]. CHOD-PAP method by using commercially available kit from Sigma-Aldrich. In brief, 0.2 ml of serum was mixed with 0.5 ml of precipitating reagent solution and centrifuged at 4000 rpm for 10 minute. 0.1 ml of clear supernatant was mixed with 1 ml of reaction solution. The intensity of colour produced was directly proportional to the concentration of HDL cholesterol in the sample. The absorbance of samples was measured at 540 nm against the reagent blank value.

Estimation of serum LDLc
Estimation of LDLc and VLDLc was calculated by Friedewald equation suggested method of Sattyanaryanan [13].

\[ \text{LDLc} = \text{[TC]} - \text{[HDLc]} - \text{[TG/5]} \]

Statistical analysis
Data were analyzed by paired sample t test and a difference was considered statistically significant when the probability value (P-value) was < 0.001.
RESULTS
The study was conducted on 300 dyslipidemic cases.
Table No. - 1 shows the distribution of cases according to age group. The result shows maximum cases 48.0% were in the age group of 51 - 60 years followed by 36% were in age group of 46-50 years, while the least 16% were in age group of 40-45 years.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-45</td>
<td>48</td>
<td>16.0%</td>
</tr>
<tr>
<td>46-50</td>
<td>108</td>
<td>36.0%</td>
</tr>
<tr>
<td>51-60</td>
<td>144</td>
<td>48.0%</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>100%</td>
</tr>
</tbody>
</table>

DISCUSSION
In order to elucidate the hypolipidemic role of garlic, a large number of studies have investigated its effects on serum cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TG). Although there is no standardization of garlic preparations, dosages or periods of treatment, most findings suggest that administration of garlic lowers cholesterol and TG levels in patients with increased levels of these lipids. Garlic juice reduced serum cholesterol and TG in human subjects [14] and helped in preventing the rise of serum cholesterol, thus indicating a cholesterol-lowering property [15, 16]. In this study levels to TC, LDL, TG were measured in 300 dyslipidemic cases. It is found that TC, LDL, TG levels dropped significantly after the intake of raw garlic extract for 24 weeks. Similarly level of HDL increases significantly. These results are in favour of previous study carried out by Alder and Holub's study (11.5% decrease of total cholesterol, and 14.2% decrease of LDL-cholesterol) [17], Tohidi and Rahbani's trial (9.0% decrease of total cholesterol, 15.0% decrease of LDL-cholesterol) [18], study of Steiner et al (6.1% decrease of total cholesterol, 4.0% decrease of LDL-cholesterol) [19]. Also, other studies explained these benefits [20, 21].
Our results are also in agreement with results of Tohidi and Rahbani's study [22], and Mader's trial [23] in which Triglyceride dropped by prescription of garlic tablet by 6.3% (13.72 mg/dl). HDL-cholesterol should be investigated in more clinical trials.
The most probable mechanism behind this effect may be garlic extract antagonized LXRx expression in the intestine. Reverse expression of LXRx in these tissues may have important role in reduction of triglyceride and cholesterol by garlic. The livers X receptors (LXRα (NR1H3) and LXRβ (NR1H2)) are important regulators of cholesterol, triglyceride and glucose homeostasis that belong to the nuclear receptor superfamily. LXRs with regulation of ATP-binding cassette transporter (ABC) genes have a critical role in the reverse cholesterol transport. It has been demonstrated that LXRx agonists effectively block intestinal cholesterol absorption. LXRx also plays a role in cholesterol storage and steroidogenesis pathways [24, 25]. Wang Y, et al. have been shown that LXRx plays vital role in the controlling of cholesterol biosynthesis via directly silencing the expression of two main enzymes (squalene synthase and lanosterol14α-demethylase (CYP51A1)) [26]. Reverse expression of LXRx in these tissues may have important role in reduction of triglyceride and cholesterol by garlic.

CONCLUSION
From above study we reach to the conclusion that raw garlic extract lowers the total cholesterol, LDL Cholesterol, triglyceride level and increases the HDL Cholesterol level. But the effect of garlic extract on lipid profile still needs further trials as the form of garlic product, its dose needs special attention along with the effect of it on different of dyslipidemias.
REFERENCES