Review of Literature
Atherosclerosis is the leading cause of death and disability. It affects the various regions of the circulation and yields distinct clinical manifestations depending on the circulatory bed affected. Atherosclerosis is responsible for coronary artery disease, cerebrovascular disease, peripheral occlusive diseases and aortic aneurysm.

Atherosclerosis is a disease primarily of the elastic arteries preferentially large and medium sized muscular arteries. The basic lesion is the atheroma or fibrofatty plaque which consists of a raised focal plaque within the intima, having a core of lipid (mainly cholesterol and cholesterol esters) and a covering fibrous cap. (Robbins & Cotran, 1994). Various risk factors for atherosclerosis are present of them four are most significant i.e.

- Diet and hyperlipidemia (hypercholesterolemia, hypertriglyceridemia).

- Hypertension

- Cigarette smoking

- Diabetes
Others are obesity, physical inactivity, stress (type A personality), male gender, hyperhomocysteinemia etc.

Atherosclerotic plaques are rich in cholesterol and cholesterol esters which are mainly derived from the lipoproteins present in the blood.

Genetic disorders causing severe hypercholesterolemia manifests as premature atherosclerosis despite the absence of other risk factors eg. (congenital absence of LDL receptros).

Acquired diseases that cause hypercholesterolemia such as nephrotic syndrome and hypothyroidism increase the risk of IHD.

The Framingham heart study has elucidated the relation between total cholesterol, LDL-C, HDL-C, triglyceride levels and the risk for coronary atherosclerosis. During the 12 years follow up evaluation a total of 383 men and 227 women developed symptomatic CHD and showed a significant positive correlation with the categories of blood pressure, total cholesterol, LDL-C and HDL-C.

Framingham heart study also evaluated the association between the elevated plasma Lp(a) and CHD in a prospective manner\textsuperscript{13}. Lp(a) was determined to be an independent risk factors comparable
to the attributable risk of total serum cholesterol in excess of 240mg/dl or an HDL-C level of less than 35mg/dl.

**Cholesterol:**

Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. Lipoprotein transport free cholesterol in the circulation where it readily equilibrates with cholesterol in other lipoproteins and in membranes.

It is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as cholesterol or bile salts. Cholesterol is the precursor of corticosteroids, sex hormones, bile acids and vitamin D.

It occurs in foods of animal origin such as egg yolk meat, liver and brain and a major risk factor for atherosclerosis (Harper 1996).

**Triglyceride:**

Triglyceride is synthesized from phosphatidate which in turn is synthesized from acylation of glycerol 3 phosphate by enzyme glycerol 3 phosphate acyl transferase. These are the major energy storing lipids (Harper 1996). Some studies have shown that plasma triglycerides levels >130-150mg/dl are associated with low HDL cholesterol and small dense LDL particles. Meta analysis of several
prospective population studies confirms that triglyceride concentrations are independent risk predictor of coronary heart disease.

*Lipoproteins*:

Lipoproteins are spherical particles made up of hundreds of lipid and protein molecules. The major lipids of the lipoproteins are cholesterol, triglycerides and phospholipids. Triglycerides and cholesterol esters (esterified form of cholesterol) are hydrophobic and forms the core of the lipoprotein.

Phospholipids and a small quantity free (unesterified) cholesterol are amphipathic and cover the surface of the particle. According to the density lipoproteins of plasma have been grouped into four groups. As the proportion of lipid to protein in a lipoprotein increases, the density decreases. According to increase of density they are classified as follows:

**Table No.1**

Multiple studies have revealed that there is an inverse relationship between the HDL level and the risk of coronary events. HDL helps in reverse cholesterol transport from the tissue back to liver.
**Table No: 1**

Compositions of the lipoproteins in plasma of humans:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Source</th>
<th>Density</th>
<th>Protein (%)</th>
<th>Total lipid (%)</th>
<th>Composition</th>
<th>Percentages of total lipid</th>
<th>Free Fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Triacylglyceride</td>
<td>Phospholipid</td>
<td>Cholesteryl ester</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>Intestine</td>
<td>&lt;0.95</td>
<td>1-2</td>
<td>98-99</td>
<td>88</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>VLDL</td>
<td>Liver (Intestine)</td>
<td>0.95-1.006</td>
<td>7-10</td>
<td>90-93</td>
<td>56</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>IDL</td>
<td>VLDL</td>
<td>1.006-1.019</td>
<td>11</td>
<td>89</td>
<td>29</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>LDL</td>
<td>VLDL</td>
<td>1.019-1.063</td>
<td>21</td>
<td>79</td>
<td>13</td>
<td>28</td>
<td>48</td>
</tr>
<tr>
<td>HDL2</td>
<td>Liver &amp; Intestine</td>
<td>1.063-1.125</td>
<td>33</td>
<td>67</td>
<td>16</td>
<td>43</td>
<td>31</td>
</tr>
<tr>
<td>HDL3</td>
<td>VLDL, Chylomicrons</td>
<td>1.125-1.210</td>
<td>57</td>
<td>43</td>
<td>13</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>Albumin FFA</td>
<td>Adipose tissue</td>
<td>&gt;1.281</td>
<td>99</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

VLDL – Very low density lipoprotein, LDL – Low density lipoprotein
IDL – Intermediate density lipoprotein, HDL – High density lipoprotein
**Apolipoproteins:**

The protein moiety of a lipoprotein is known as an apolipoprotein. The apolipoproteins (apos) provide structural stability to the lipoproteins and determine the metabolic fate of the particles upon which they reside.

**Apolipoproteins of human plasma lipoproteins (Table No – 2)**

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Lipoprotein</th>
<th>Metabolic Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo AI</td>
<td>HDL, Chylomicrons</td>
<td>Structural component of HDL, LCAT activator</td>
</tr>
<tr>
<td>Apo AII</td>
<td>HDL, Chylomicrons</td>
<td>Unknown</td>
</tr>
<tr>
<td>Apo IV</td>
<td>HDL, Chylomicrons</td>
<td>Unknown, possibly facilitates transfer of other apos between HDL and Chylomicrons</td>
</tr>
<tr>
<td>Apo B48</td>
<td>Chylomicrons</td>
<td>Necessary for assembly and secretion of chylomicron from the small intestine</td>
</tr>
<tr>
<td>Apo B100</td>
<td>Chylomicrons, VLDL, IDL, LDL</td>
<td>Necessary for secretion of VLDL from liver, ligand for LDL receptor</td>
</tr>
<tr>
<td>Apo CI</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>May inhibit hepatic uptake of chylomicron and VLDL remnants</td>
</tr>
<tr>
<td>Apo CII</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>Activator –lipoprotein Lipase</td>
</tr>
<tr>
<td>Apo CIII</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>Inhibitor of lipoprotein Lipase, may inhibit hepatic uptake of chylomicron and VLDL remnants</td>
</tr>
<tr>
<td>Apo E</td>
<td>Chylomicrons, VLDL, IDL, HDL</td>
<td>Ligand for finding lipoprotein to LDL receptor.</td>
</tr>
</tbody>
</table>
LCAT: Lecithin cholesterol acyl transferase.

According to the National Cholesterol Education Programme (NCEP) expert panel on detection, evaluation and treatment of high Blood Cholesterol in adults (Adult treatment Panel III). Major risk factors (exclusive of LDL cholesterol) for CHD are as follows:

- Cigarette smoking
- Hypertension (blood pressure $\geq 140/90$ mm of Hg or on antihypertensive medication).
- Family history of premature CHD (CHD in male first degree relative $<55$ yrs; CHD in female first degree relative $<65$ years).
- Age (men $\geq 45$ years; women $\geq 55$ years).

Diabetes is regarded as a coronary heart disease risk equivalent i.e. a condition that carries an absolute risk for developing new CHD equal to the risk for having recurrent CHD events in persons with established CHD.

Hence forth, hypercholesterolemia and hypertriglyceridemia are considered as directly and indirectly predisposing factors for ischemic heart disease and it is presumed that garlic may be beneficial in the primary or secondary prevention.
Garlic (*Allium Sativum*):

Garlic (*Allium Sativum* family Liliaceae) is widely distributed and used in all parts of the world as spice and food. Garlic is a dietary supplement regarded simultaneously as a food and a medicinal herb and has been used as such from the times of Egyptians Pharoahs and the earliest of Chinese dynasties.

More than 1000 papers have been published in the past 20 years on garlic and related alliums. Garlic was called “Russian penicillin” during World War II because garlic was used to prevent wound infections.

Garlic exhibits potentially beneficial clinical activity as an antihyperlipidemic, antimicrobial, antiplatelet, antioxidant, antidiabetic and as a vasoprotective agent. Extensive clinical and scientific studies partially support the use of garlic for the treatment of hypercholesterolemia, infection and the prevention of atherosclerosis. Garlic also appears to slightly improve hypertension, protect against free radicals and slow blood coagulation.

Regular use of garlic may prevent cancer although it has been stated that garlic raises immunity but no real evidence is present to support the view. It has also been proposed as a treatment for asthma, candida and colds.
Mechanism of action

Garlic is composed of many natural sulfur compounds including a sulfur containing amino acid alliin (S-allyl-L-cysteine sulfoxide). Alliin is pharmacologically inactive. When garlic is crushed, alliin mixes with the enzyme alliinase it is converted to allicin (diallyl thiosulfinate). Allicin is unstable and upon steam distillation or maceration yields various diallyl and dimethyl sulphides plus E-ajoene and Z-ajoene.

The total activity of garlic is in its ability to produce allicin, which then produces other active principles which is referred to as the allicin yield.

Hypocholesterolemic action:

Garlic and wild garlic reduces serum cholesterol levels primarily by inhibiting cholesterol synthesis. Allicin sulphydryl binding ability explains its cholesterol lowering effect as sulphydryl containing compounds are involved in the synthesis of cholesterol. Sulfide bridges are formed by the disulfides found in garlic with 3-hydroxy-3methyl glutaryl CoA (HMG-CoA) reductase or the molecules found in lipids. HMG-CoA reductase is the rate limiting step in the synthesis of cholesterol as it catalyzes the intermediate step i.e. the formation of mevalonate from acetyl –CoA. Therefore,
allicin is commonly accepted as the pharmacologically active component in garlic.

**ANTIPLATELET ACTION**

Makheja et al has shown that garlic extract inhibits platelet aggregation, by suppressing thromboxane β2 synthesis. Garlic inhibits platelet aggregation by alteration in both the platelet cyclooxygenase and lipooxygenase pathway.

Ariga et al have isolated the component as methyl allyl trisulphite (MATS).

Apitz Castro et al suggested that garlic’s inhibitory effect might be mediated through modification of the physiochemical properties of the plasma membrane, rather than affecting the arachidonic or calcium metabolism of platelets.

**VASOPROTECTIVE EFFECT**

Garlic’s vasoprotective effect was demonstrated due to increased nitric oxide synthetase activity, which may facilitate endothelium dependent smooth muscle relaxation. Thus, garlic may improve aortic elasticity through restoration of impaired endothelium. Although other studies suggest that garlic also increases fibrinolytic activity.
In contrary to the common belief study conducted by Arora RC et al 1981 at Dept. of Medicine, M.L.B. Medical College, Jhansi showed that after 12 wks of intake of garlic, patients did not show any appreciable change in plasma fibrinogen levels or coagulation time.

**Hypoglycemic Action**

Garlic decreases blood glucose levels by increasing serum insulin and glycogen storage in liver.

The health benefits of garlic supplements remain a controversial topic. On one hand there appear to be quite a large number of studies indicating a beneficial cardiovascular effect of garlic supplements, on the other hand the most well controlled studies generally suggest a lack of any beneficial effect of garlic supplements.

(Bordia, et al 1974-75)\(^2-4\) have claimed that acute ingestion of garlic in healthy subjects will prevent fat induced changes of blood lipids, coagulation and fibrinolysis. Study was only of 10 cases and they were so impressed by the results that they proceeded to assess the effect of garlic on experimental atherosclerosis in cholesterol fed rabbits and found it superior to clofibrate.
Later (Sainani et al, 1979) study on 5 subjects confirmed the beneficial effects of garlic, as showed by Bordia et al. Moreover they concluded that garlic and onion would result in significantly lowered (p<0.0001) levels of serum cholesterol, serum triglyceride and β-lipoprotein (LDL).

(Arora RC, Arora S et al, 1981) evaluated the effect of essential oil extracts of garlic and onion in the doses recommended by these authors.

Twenty healthy males (age 26.4±5.12 yrs, weight 56.7±7.3Kg) and 13 proven cases of IHD (age 43.8±9.5 yrs, weight 64.9±8.2Kg) were randomly chosen from patients attending MLB Medical College & Hospital, Jhansi. and were given fat rich diet, fat rich diet + clofibrate, fat rich diet + garlic, fat rich diet + onion. The fasting & post prandial values of serum cholesterol and b-lipoprotein thus obtained in different test conditions did not show any appreciably change. Henceforth the so called beneficial effects of garlic and onion were not seen.

(Arora RC, Arora S, Gupta RK et al, 1981) undertook another study were garlic was given (a) for long period and (b) without fat induced hyperlimia.

This study comprised of 30 proven cases of IHD (Gr I, 26 males and 4 females, age 41.0±3.7yrs) and 20 healthy volunteers
(Gr II, 18 males and 2 females, age 24.0±4.1 yrs). Subjects were given essential oil of garlic i.e. six garlic capsules (each capsule containing 0.625mg of garlic oil) in 3 equally divided doses at meals for a period of 12 weeks. The STC, STG, β-lipoprotein and plasma fibrinogen and coagulation time values showed marginal fluctuations with insignificant ‘P’ values.

(Lau et al, 1987)\textsuperscript{14} conducted a study on 15 hyperlipidemic subjects they were treated with Kyolic aqueous garlic extract 1000 (4ml) daily for 24 weeks and 12 subjects were kept as control. Garlic significantly lowered cholesterol levels by about 9%.

(Vorberg & Schneider et al, 1990)\textsuperscript{15} conducted a trial in Germany over a 40 hypercholesterolemic subjects and they were counselled to take Kwai Tablets 900mg/day for 16 weeks. Garlic extract significantly lowered blood cholesterol >12%. Explanation of the large effect in this study was that there was less heterogenicity between the participants. Secondly this study used the highest daily and cumulative dose of garlic. It was estimated that the Kwai trials tested the equivalent of approximately one half to one clove fresh garlic per day.

(Mader et al, 1990)\textsuperscript{16} conducted a study in Germany. A total of 261 patients at 30 medical centers were given kwai tables (dried garlic extract) 800mg/day or placebo over the course of 16 weeks,
patients in the treated group experienced a 12% drop in total cholesterol and a 17% decrease in triglyceride levels. Patients who were having initial cholesterol levels of 250-300 mg/dl achieved maximum benefit. This study is considered to be one of the best study.

In the same period of 1990, (Auer et al)\textsuperscript{17} studied 47 subjects with hypertension average starting blood pressure of 171/100. Over a period of 12 weeks, half were treated with 600mg of garlic powder daily standardized to 1.3% alliin, the other half was given placebo. Results showed statistically significant drop of 11% drop in systolic blood pressure and 13% in diastolic pressure.

Review article- A \textbf{meta analysis effect of garlic on total serum cholesterol was published in Ann Intern. Med 1993 by Stephen Warschafsky, et al}\textsuperscript{23}, showed that garlic in an amount approximating one half to one clove per day (600-900mg) has been shown to decrease total serum cholesterol by about 9%.

\textbf{A meta analysis published in the Journal of the Royal College of Physician by (Silagy CS, Neil HAW, et al, 1994)}\textsuperscript{24} states that garlic supplements cause overall 12% reduction in total cholesterol over a placebo and it is evident after only 4 weeks, treatment and that this was likely to persist for as long as the study was continued.
(Leon A Simons, Balasubramaniam S et al, 1995)\textsuperscript{26} conducted a trial over 30 subjects with mild to moderate hypercholesterolemia after a dietary restriction for 28 day, subjects took kwai\textsuperscript{®} garlic powder tablets 300mg three times daily or matching placebo for 12 weeks, followed by 28 days washout, followed by a 12 weeks crossover on alternative preparation there was no significant differences in plama cholesterol, LDL-C, HDL-C, plasma triglycerides, Lp(a) concentration or blood pressure. This study clearly states against the findings of the previous meta-analysis.

Neil HAW et al 1996 undertook a trial of 115 subjects for six months by a kwai\textsuperscript{®} tablets 300mg three times daily and concluded that garlic is less effective for reducing serum cholesterol than suggested by his own meta analysis in 1994 and expected that previous reports may be a publication bias, overestimation of treatment effects in trials with inadequate concealment of treatment allocation.

Similarly (Berthold HK et al, 1998)\textsuperscript{35} and (Isaacsohn JL et al, 1998)\textsuperscript{36} showed that garlic oil preparation, garlic powder respectively had no influence on serum lipoprotein, cholesterol absorption or cholesterol synthesis.
(Koscielny J et al 1999) a double blind placebo controlled study that followed 152 individuals for 4 years found that garlic significantly reduced the development of atherosclerosis.

Lipid lowering drugs such as statins are an exciting advance, they decrease hepatic cholesterol synthesis by inhibiting HMG-CoA reductase. They are highly effective in reducing serum total cholesterol, LDL-C and usually increase HDL cholesterol. Long term efficacy and safety of the drug has been established.

With the publication of the Landmark Scandinavian Simvastatin survival study (4S) in 1994, where 4444 patients were treated for an average of 5.4 years. Simvastatin became the first lipid lowering agent proven to reduce morbidity and mortality in patients with CHD, as well as safe in long use. It showed that simvastatin improved patients survival by 30 percent and was well tolerated with a frequency of adverse events similar to those of placebo. Study was further reinforced by an eight year follow up of (4S) patients showing survival and safety were both continued.

Original 4S data have been reanalysed to demonstrate additional attributes of simvastatin in the secondary prevention of CHD.

- A reduction in the development of new or worsened angina pectoris by 26 percent.
In patients with clinical diabetes, a significant decrease (42 percent) in the risk of major coronary events\textsuperscript{53}.

In patients with impaired fasting glucose, a significant decrease (55 percent) in the risk of coronary mortality, total mortality (43 percent) and of major coronary events (38 percent)\textsuperscript{53}.

Simvastatin therapy resulted in a significant beneficial alteration of the lipid profile.

Patients were randomly assigned to receive either Placebo or 20mg of simvastatin per day. In the simvastatin group, total cholesterol was reduced by 28 percent, which was accompanied by a reduction in LDL-C levels of 38 percent. Simvastatin therapy resulted in 15 percent decrease in patients who had initial triglycerides levels were within the prescribed range of trial. Patients with significant hypertriglyceridemia before randomization had been excluded. HDL-C was increased by 8 percent.

Cerebrovascular events and new carotid bruits were also significantly reduced by simvastatin therapy.