SESSION 1: INTRODUCTION

The increase in the number of agents that cause environmental pollution is major global health problem. This is because of the frequent uses of xenobiotic substances or toxic chemicals or synthetic compounds such as heavy metallic compounds. Of these compounds, heavy metals and their salts are the important group of environmental pollutants. The use of heavy metals is intimately connected to human history. Men discovered metals as important materials for making tools and implements as early as in pre-historical times. These heavy metals are persistently accumulated but not metabolized into other intermediate harmless compounds. They do not easily break down in the environment and induced potential harmful effects at low doses. There are over 50 elements that can be classified as metals, 17 of which are considered to be very toxic. Toxicity level depends on the type of metal; its biological role and the type of organisms that are exposed to it. The important heavy metals included are lead, iron, nickel, copper, zinc, chromium, gold, mercury etc. The high concentration intake of cadmium causes itai itai disease and mercury intake leads to minamata disease and other heavy metals cause various types of poisoning.

Heavy metal pollution can originate from natural and anthropogenic sources. The circulation and migration of heavy metals in the natural environment are mainly due to the processes as rock decay, volcanic eruptions and evaporation of oceans, forest fires and soil formation processes. The origin of heavy metal pollution in the environment by anthropogenic sources include different branches of industry, transport, municipal waste management, waste dumping sites, fertilizers and waste used to fertilize soil. All these sources contaminate soil, water and air directly or indirectly and allow these heavy metals to enter the human or animal body and they accumulate mainly in kidney, liver, lungs, hair, adrenal gland, skin and brain. The human body cannot break down a heavy metal and it readily forms stable covalent complexes and interfere normal cell and tissue function through
multiple pathways including interactions with macromolecules such as proteins, enzymes, hormones etc. These metals have high affinity for thiol group containing enzymes and proteins, which are responsible for normal cellular defense mechanism.

The resultant accumulation of heavy metals in the human body poses a significant health risk with wide array of symptomatology including anemia, learning deficits, reduced intelligence, behavioral and cognitive changes, tremor, gingivitis, hypertension, irritability, cancer, depression, memory loss, fatigue, headache, hyper uricemia, gout, chronic renal failure, male infertility, osteodystrophies and possibly multiple sclerosis and Alzheimer’s disease. The toxic manifestations of these metals are primarily caused due to imbalance between prooxidant and antioxidant homeostasis known as oxidative stress. Heavy metals possess the ability to generate reactive radicals, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNAs. Change in gene expression of some hepatic enzymes like HMG CO.A reductase, which in turn depresses LDL receptor gene expression and leads to the rise in serum lipid profile through increased lipolysis and induced rise in serum LDL, VLDL and TAG and fall in serum HDL. Generation of ROS by heavy metals initiates lipid peroxidation and leads to oxidative stress resulting in metabolic dysfunction.

Heavy metal pollution is a global public health challenge due to its stable and persistent environment contaminants causing various alterations in target tissues of exposed animals. Of these lead is considered to be one of the most common ubiquitous and industrial pollutants that has been detected in almost all phases of biological system and to be toxic even at low concentration and exerts extensive damage to the brain causing severe learning and memory disabilities in children. Lead is a cumulative tissue poison and get stored in different parts of the body especially in bones, liver, kidney and brain and causes neurological, hematological, gastrointestinal, reproductive, circulatory and immunological diseases.
Recent studies suggest that oxidative stress is a potential contributor to lead toxicity, disrupt directly or indirectly the delicate pro oxidant/ anti oxidant balance in the cells. The causes of lead induced oxidative stress may be due to the direct binding of lead to the cell membrane thereby increasing the susceptibility of membranes to lipid per oxidation (LPO) or may be due to the alteration in biological antioxidant defense system or by the auto oxidation of $d$-ALA may lead to the formation and accumulation of ROS. The current therapeutic approach to lead toxicity is to increase the excretion of lead by chelation but their side effects are numerous. The most commonly used chelators such as calcium disodium ethylene diamine tetra acetic acid (CaNa$_2$EDTA; versenate), meso-2,3-dimercaptosuccinic acid (DMSA; succimer),sodium2,3-dimercapto-1-propanesulfonate(DMPS), and penicillamine may deplete essential metals and redistribute lead from bones to the kidney and brain. Thus there has been increased interest in the treatment of lead toxicity by using various products of medicinal plants with antioxidant properties for reducing reactive oxygen species (ROS) induced tissue injury. Garlic extract is one such product used by a Bulgarian scientist.

Antioxidant is a substance that can prevent oxidation in the biological system in different ways such as promoting catalytic removal of free radicals by enzymes or by donating electrons. Certain products that form can act in the form of proteins complexes with metals and minimize the prooxidant such as metal ions availability. Antioxidants can act at different stages for the prevention, interception or repair. Natural compounds involve a wide array of biological processes that include activation of antioxidant defenses and such other processes, because of these properties natural compounds play an important role in the protection from various diseases that are caused by reactive oxygen species. Nutraceuticals are chemically identified compounds with antioxidant or related immune specific, antiviral or antibacterial property and they are naturally present in our diet. These nutraceuticals have
dual role to play as food and as therapeutic agents. They are as essential as the vitamins to prevent or cure many diseases such as cancer of all types, cardiovascular diseases, hepatotoxicity, arthritis, radiation damages, hypercholesterolemia and oxidative damages to various tissues, cataract, allergies, inflammation, hypertension, platelet aggregation and for detoxification of heavy metals\textsuperscript{14}. Vitamins are the compounds included under nutraceuticals which can prevent or cure diseases. Example Vitamin E is a nature’s important lipid soluble chain breaking antioxidant that is protecting biological membrane and lipoproteins from oxidative stress\textsuperscript{15}. The important biological role of vitamin E is its direct influencing of cellular response to oxidative stress through the modulation of signal transduction pathways. Traditionally each vitamin has got its own identity status and nutritional importance. Many of the researchers particularly doctors shall continue to speak and write on vitamins as the major or only members of the nutraceuticals as at present what they are knowing and doing. This will enable them to ignore a dozen of other nutrients which are true nutraceuticals. The major beneficial nutritional medicines (nutraceuticals) include plant products like terpenes, phytosterols, polyphenoles like catachins, resveratrol, flavanoids and poly sulfides in alliums etc. Numerous studies support that sulfur containing antioxidants protect the body against oxidative damage related with the disease development and progression and has suggested a protective role through multiple antioxidant mechanisms such as ROS scavenging and metal binding\textsuperscript{16}. Of these sulfur containing nutraceuticals such as allium plants importantly garlic and onion are good sources of ideal and multifunctional antioxidants which can prevent or cure various diseases such as diabetes mellitus, cardiovascular diseases, cancer, cataract, cirrhosis, inflammation etc produced due to oxidative stress. Swati etal\textsuperscript{17} reported that an allium plant such as garlic has some beneficial effect in preventing the heavy metals such as nickel and chromium induced alteration in serum lipid profile. Further garlic oil as well as its non polar fraction showed protection against damages induced by CCl\textsubscript{4}\textsuperscript{18}. Our previous work showed protective effects of garlic and onion oils against the damages
induced by drinking water containing lead acetate and alcohol solutions separately or in combination as compared to the beneficial effects of Vitamin E."}

**1.1. AIM AND OBJECTIVES OF THE PRESENT STUDY**

Pollution with lead salt is a health hazard to modern man. Many data showed that antioxidants play an important role in abating some hazards of lead. Very little work is carried out by other people for the detoxification of health hazard causing heavy metal lead by supplementing the daily diet with nutraceuticals. Therefore we decided to focus our study on the counteracting effects of nutraceuticals such as active fractions of garlic and onion oils as compared to Vitamin E on such damages produced by feeding daily lead acetate solution to the rats. Our studied are focused on

- Oxidative damages produced by lead poisoning due to daily consumption of lead acetate solution.
- To evaluate the biochemical effects of active fractions of the allium oils – polar and non polar fractions of garlic and onion oils.
- To compare the curative and prophylactic effects of garlic and onion oil fractions in lead poisoning
- To compare the protective effects of polar fractions of garlic and onion oils with the non polar fractions of the same oils in lead poisoning.
- To compare the counteracting effects of nutraceuticals such as the more active fractions of garlic and onion oils with the standard vitamin E in lead poisoning.

- To evaluate in vitro protective effects of allium oils in lead poisoning.

For this we divided our studies into 4 broad phases
Phase I:

Studies on the biochemical effects of the polar and non polar fractions of garlic and onion oils and vitamin E in rats.

The following aspects were studied in detail

a. Hematological parameters
b. Antioxidant enzymes
c. Lipid profile
d. Lipid peroxidation
e. Histopathological changes of the tissues- liver, heart and kidney were studied

Phase II:

Studies on the curative effects of polar and non polar fractions of garlic and onion oils and vitamin E in lead acetate fed rats

The following aspects were studied in detail

a. Hematological parameters.
b. Blood lead level
c. Marker enzymes affected by toxicity were estimated.
d. Lipid profile.
e. Lipid peroxidation.
f. Histopathological changes in the various tissues such as liver, heart and kidney.

Phase III:

Studies on the prophylactic effects of two active polar fractions of allium oils in lead acetate fed rats for one month as compared to that of vitamin E

The following aspects were studied in detail
a. The changes in the hematological indices such as hemoglobin, heamatocrit, MCH, MCV, lymphocyte count, monocyte count, eosinophil count, neutrophil count were analysed.
b. Peripheral blood smear analysis
c. Markers affected by lead toxicity - ALAD and lead level were estimated.
d. Oxidative stress marker enzymes such as antioxidant enzyme activities were estimated.
e. Changes in the level of various lipid parameters in serum and tissues were estimated.
f. Level of lipid peroxidation product in blood and tissues were estimated.
g. Activities of toxicity marker enzymes ALT and AST in serum and tissues were estimated.
h. Levels of vitamin E and vitamin C in serum were estimated.
i. Histopathology of various organs such as liver, heart and kidney were studied.

**Phase IV:**

**In vitro study:** Studies on the protective action of allium oils against lead acetate induced hemolysis of RBC in normal saline were conducted.
SESSION 2: REVIEW OF LITERATURE

1.2.1. LEAD

Lead is a heavy metal with a bluish grey colour. It is having low melting point and is easily moulded and shaped and can be combined with other metals to form alloys. For these reasons humans used lead for millennia. Today lead is used widespread in products as diverse as; pipes, storage batteries, paints and pigments, glazes, vinyl products, ammunication, cable covers and radiation shielding. Depending on the exposure level, lead is said to have both mild and adverse effects like cognitive dysfunction, neurobehavioral disorders, neurological damage, hyper tension and renal impairment. Lead toxicity can directly interrupt enzyme activation, competitively inhibit trace minerals absorption, bind to sulphhydryl proteins and enzymes, alter calcium homeostasis and lower the level of sulphhydryl (SH) antioxidant reserves in the body. All these are the direct result of the oxidant effect of lead on tissues and cellular components.

1.2.2. Chemistry of Lead

The chemical symbol for lead is Pb which came from the Latin name of Lead i.e. Plumbum. Lead has an atomic number of 82 and atomic weight of 207.2. The density of lead is 11.34g/cm³ with low melting point such as 327.46°C or 621.43°F. Two forms of lead are seen in the nature-inorganic lead and organic lead.

Inorganic lead is found in the old paint, soil, dust and various consumer products. Depending upon the chemical form the colour varies. The most common forms are: white lead-lead carbonate compound, yellow lead-lead chromate/lead monoxide and red lead- lead tetra oxide. Organic leads are extremely dangerous and they are highly toxic to the brain and CNS than the inorganic lead. Tetra ethyl lead is the form of organic lead used in leaded gasoline. All forms of lead are toxic. Lead mimics biologically important
minerals such as calcium, iron and zinc and interfere the biological functions of these minerals.

**1.2.3. Naturally Occurring Ores of Lead**

0.002 % of the earth crust comprises of lead ores. The important ores of lead are:

<table>
<thead>
<tr>
<th>Ore</th>
<th>Chemical Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galena</td>
<td>lead sulfide</td>
</tr>
<tr>
<td>Anglesite</td>
<td>lead sulfate</td>
</tr>
<tr>
<td>Cerussite</td>
<td>lead carbonate</td>
</tr>
<tr>
<td>Mimetite</td>
<td>lead chloro arsenate</td>
</tr>
<tr>
<td>Pyromorphite</td>
<td>lead chloro phosphate</td>
</tr>
</tbody>
</table>

**1.2.4. Sources of Lead**

The most of the lead concentration in the environment is due to the result of human activities\(^{22}\). Several industries such as petroleum, mining, smelting, lead acid battery manufacturing waste increasing and mining industries release lead into the atmosphere such as air, soil and water. Lead paint is a primary source of lead exposure, drinking water, lead solder, lead pipes, lead glazed ceramics; glass, pottery items, toys, food cans, and cigarette smoke are the other sources of lead exposure. Workers are exposed to high levels of lead during in certain occupations such as the manufacturing of ammunition, sheet lead, solder, lead acid batteries, some brass and bronze, plumbing, fetal monitors, some surgical equipments, radiation shields, circuit board and military equipments like turbine engines, military tracking system etc\(^{23}\). Certain high levels of lead found in the eye cosmetics such as Kohl and Suruma commonly used as eyeliners. Herbal medicine used in India and South Asia may also be a potential source of lead exposure\(^{24}\).
1.2.5. *Metabolism of Lead*

1.2.5.1 *Absorption of Lead*

Absorption of lead mainly depends on the physical and chemical state of the metal and it is influenced by age, Physiological status and genetic factors. Exposure to lead occurs mainly through the skin, mouth, respiratory and gastro intestinal tract. Approximately 35-50% of inhaled lead of particle size less than 1µm is deposited in the alveolar tract and the remaining is absorbed into the blood stream\textsuperscript{25}. Gastro intestinal absorption of lead mainly depends on age and nutritional status. Diet having low levels of calcium, iron, copper, zinc, selenium and phosphate can increase the absorption of lead\textsuperscript{26}. In adults approximately 10-15% of ingested lead is absorbed from food, water, contaminated dust or soil in the gut where as in children absorption is up to 50% of ingested lead\textsuperscript{27}. Dermal absorption of inorganic lead from food, water, paint, toys and vinyl products is minimal, but the organic lead such as tetra ethyl lead or alkyl lead is well absorbed through the skin\textsuperscript{28}.

1.2.5.2 *Transport, Distribution and Storage of Lead*

99% of absorbed lead is transported primarily in the erythrocytes bound to plasma proteins\textsuperscript{26}. Absorbed lead is distributed by blood to mineralizing systems - bones and teeth and soft tissues such as liver, renal cortex, aorta, brain, lungs, spleen. The half life of lead in blood is 36 days, the half life of lead in soft tissues is 40 days and the half life of lead in bone is 27 years\textsuperscript{29}.

In adults approximately 90% of the total body burden of lead is seen in bones but in children 70 % of total body burden of lead is seen in the bones, while the concentration increases with age\textsuperscript{26}.In chronic exposure lead becomes deposited in the form of insoluble lead phosphate in the rapidly growing areas of skeleton such as radius, tibia and femur. Bone lead is readily mobilized to blood, mobilization of lead from bone to the more bio available blood compartment happens in such situations that increase bone turnover.
such as pregnancy, lactation, postmenopausal osteoporosis and hyperthyroidism\textsuperscript{30,31,32}. Lead is readily transferred to the fetal skeleton via placenta from the mother during pregnancy\textsuperscript{33}.

1.2.5.3 Excretion of Lead

75-80\% of absorbed lead is primarily excreted in urine and 15\% is excreted through feces and the rest includes secretion into bile, sweat, saliva, hair and nails and breast milk which are minor routes of excretion \textsuperscript{34}.

1.2.6 Health Effects of Lead

Lead toxicity is an insidious hazard with the potential of causing irreversible health effects.

1.2.6.1 Effects on Heme Biosynthesis

Heme is the iron containing constituent that combines with protein to form hemoglobin. It is also an essential component of the respiratory pigment cytochromes which plays a key role in energy metabolic reactions\textsuperscript{35}. Lead is a divalent cation, it can strongly bind with sulphydryl proteins and create interference with enzymes and structural proteins.

Lead directly affects the synthesis of heme by inhibiting the various key enzymes such as d'ALA synthase - a mitochondrial enzyme for the catalytic formation of amino levulinicacid, delta-ALAD- a cytosolic enzyme for the catalytic formation of porphobilinogen from ALA and ferrochelatase- a mitochondrial enzyme for the catalysis of the insertion of iron into proto porophyrin to form heme\textsuperscript{36}. That leads to the reduction in the life span of circulating erythrocytes by increasing the fragility of cell membrane when whole blood lead level (PbBs) exceeds 20\mu g/dl the activity of ALAD is inhibited by 50\%. Inhibition of ferrochelatase leads to increased excretion of coproporphyrin in urine and accumulation of protoporphyrin in erythrocytes. Thus the collective inhibition of these three key enzymes blocks the heme
synthetic pathway both for hemoglobin and for cellular respiration, resulting to fatigue and anemia. Anemia caused on account of lead poisoning is of 2 types—hemolytic anaemia that is associated with acute high level lead exposure and Frank anaemia that is associated with significant elevation of blood lead level for prolonged period. Lead can impair the activity of pyrimidine nucleotidase that leads to the increase of pyrimidine nucleotides in RBCs and prevent the maturation of erythroid elements, which leads to decreased RBC count and anaemia. One of the earliest potential biomarker for the detection of lead poisoning is basophilic stippling and premature erythrocyte hemolysis. Basophilic stippling and microcytic or normocytic, hypochromic anaemia occur only after significant levels of lead exposure such as the PbBs over 50µg/dl in adults and 25-40 µg/dl in children.

**FIGURE 1- Effects of Lead on Heme Biosynthesis**

![Diagram of heme biosynthesis](image-url)
1.2.6.2 Renal Effects of Lead

Renal functional abnormality due to lead toxicity is of 2 types - acute nephropathy and chronic nephropathy\textsuperscript{39}. Acute nephropathy is characterized by functionally impaired tubular transport mechanism and degenerative changes in the tubular epithelium along with the occurrence of nuclear inclusion bodies, which contain lead protein complexes. Signs include abnormal excretion of glucose and organic anions, proteinuria and lowered GFR. Classically acute poisoning is accompanied by abdominal pain, cognitive defects, Peripheral neuropathy, arthralgias and anaemia with basophilic stippling. Chronic nephropathy is much more severe and leads to irreversible functional and morphological changes such as glomerular and tubulo intestinal changes leads to renal breakdown, hyperuricemia and hypertension\textsuperscript{40}. Renal damage has been occurring at lower levels such as approximately 10 µg/dl has been reported in Grant\textsuperscript{41} but the renal dysfunction mostly occurs at high levels of lead exposure such as > 60 µg/dl.

1.2.6.3 Cardiovascular Effects of Lead

The major disorder of lead toxicity leads to ischemic coronary heart disease, cerebro vascular accidents and peripheral vascular disease. Both chronic and acute lead poisoning cause cardiac and vascular damages with potentially lethal consequences that include hypertension and cardio vascular disease\textsuperscript{42}. Elevated blood lead level with a range such as 20-29µg/dl significantly increases circulatory and cardiovascular mortality\textsuperscript{43}.

1.2.6.4 Reproductive Effects of Lead

Lead causes a number of adverse effects on the reproductive system in both men and women such as reduced libido reduced sperm motility and sperm count, abnormal prostatic function, changes in serum testosterone. Women are more susceptible to infertility, miscarriage, premature membrane
rupture, pregnancy hypertension and premature delivery\textsuperscript{44}. A blood lead level of 40µg/dl showed effects on sperm.

1.2.6.5 Effects of Lead in Bone

The primary site of lead storage in the human body is bone\textsuperscript{45}. In the bone, lead is stored in two compartments - the exchangeable pool at the bone surface and the non exchangeable pool deeper in cortical bone. Lead can enter into plasma at ease from the exchangeable pool but can leave the non exchangeable pool and move to the surface of the bone when bone is actively being resorbed. In adults 85-95 % of the lead is stored in bones but in children only 70% is stored in bone, so in children higher concentration of lead is in soft tissue. The storage and mobilization of lead depends on the factors such as dose of lead exposure, age, pregnancy, gestation and race. Lead appears to have an osteoporotic effect in bone\textsuperscript{46}.

1.2.6.6 Effect of Lead on Nervous System

Nervous system is the most sensitive and chief target for induced toxicity. Both the central nervous system and the peripheral nervous system become affected on lead exposure. Early symptoms of lead neurotoxicity in both children and adults are irritability, headache, decreased attention, memory loss and cognitive impairment. Peripheral nervous system is exposed to lead toxicity in adults while in children central nervous system is more prone to be affected by lead toxicity\textsuperscript{47,48}. Children may appear inattentive, hyperactive and irritable even at low level lead exposure. Children with greater lead level may be affected with delayed growth, decreased intelligence, short-term memory and hearing loss. Higher levels of lead can cause permanent brain damage and even death\textsuperscript{49}. Blood level such as 10-35 µg/dl lowered IQs along with behavior, concentration ability and attentiveness of the children\textsuperscript{28,38}.

One of the major reasons for lead’s neurotoxic effects is that it competes with or mimics the action of calcium. At extremely very small
concentrations, lead competes for the binding sites in the cerebellum for phosphokinase C. This process leads to the calcium entry into cells and neuronal function and alters mitochondrial structure, leading to inhibited cellular respiration and altered calcium based reactions and neuronal signaling with a result of an increase in spontaneous neurotransmitter release and an inhibition of controlled stimulated release. This toxicity is particularly damaging to the developing nervous system of the fetus. Lead is highly toxic to the immature astrocytes and interferes with myelin formation leading to disruption in maturation of the blood brain barrier and leads to the entering of molecular proteins like albumin into the tissues of the CNS, resulting edema and encephalopathy\textsuperscript{34}.

1.2.7 Mechanism of Lead Toxicity

Lead toxicity causes alterations in various cellular, intracellular and molecular mechanisms of actins in the body.

1.2.7.1 Oxidative Stress

Oxidative stress means the imbalance between the production of free radicals and the body’s ability to detoxify the ROS or to repair the resulting damages produced by the free radicals\textsuperscript{44}. Oxidative stress is the major mechanism of lead induced toxicity and it leads to two different but related pathways, operative simultaneously; 1) the generation of reactive oxygen species like hydro peroxides, singlet oxygen and hydrogen peroxide and 2) the direct depletion of antioxidant reserves in the biological system\textsuperscript{21}.
The antioxidant defenses of the body come into play to nullify the generated ROS. The important antioxidant found in the cell is reduced glutathione (GSH). Glutathione is a cysteine based molecule produced in the interior compartment of the lymphocyte. It is a tri peptide having a sulphydryl group and is found in mammalian tissues in milli molar concentration. Glutathione is an important antioxidant for quenching free radicals. Glutathione exists in both reduced and oxidized form. The reduced state of glutathione molecules donate reducing equivalents (H⁺ + e⁻) from their thiol contains cysteine residues to ROS and make them stable. After donating a hydrogen with an electron, one glutathione combines with another glutathione and form glutathione disulfide (GSSG) in the presence of the enzyme glutathione peroxidase. Then with the presence of glutathione reductase reduced glutathione is generated from GSSG. In normal condition 90% of total glutathione in the body exists in the form of reduced glutathione. Under a condition of oxidative stress, the concentration of oxidized glutathione (GSSG) is higher than that of reduced glutathione (GSH). A toxic metal such as lead...
binds with the Sulfhydryl complex of glutathione and inactivates the glutathione molecule, and then it becomes unavailable as an antioxidant or as a substrate in the pathways of metabolism\textsuperscript{50}. Lead also inactivates enzymes having functional sulfhydryl groups and makes them nonfunctional. For e.g: lead inactivates enzymes like $\delta$-amino levulinic acid dehydratase, glutathione reductase, glutathione peroxidase and glutathione-S-transferase, which further leads to depress the glutathione level\textsuperscript{51}.

**FIGURE 3- Effect of Lead on GSH Metabolism**

Note: $2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2^\cdot$ or $\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^\cdot + \text{Fe}^{3+}$

If catalase action is not sufficient or inactivated ROS are produced from $\text{H}_2\text{O}_2$ as smoking, alcoholism or over eating etc, Produce excessive number of electrons and they are trapped by respiratory oxygen and form superoxide. ETC also plays a role here. $\text{H}_2\text{O}_2$ is produced from super oxide by the action of SOD.

$$\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^\cdot$$ \hspace{1cm} \text{(1)}

$$2\text{O}_2^\cdot + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2$$ \hspace{1cm} \text{(2)}

Lead inactivates antioxidant enzymes such as SOD and catalase. Decrease in SOD concentration reduces the disposal of free radicals such as superoxide radical; catalase impairs scavenging of superoxide radical. Lead
replaces zinc ions, an important cofactor for antioxidant enzymes and inactivates them\textsuperscript{52}. The generated free radicals capture electrons from the lipids present inside the cell membranes and damage the cell. Apart from lipid peroxidation, lead also causes oxidation of hemoglobin that directly causes RBC hemolysis. This occurs due to inhibition of \( \delta \) ALAD and leads to the increase of ALA in blood and urine. The elevated ALA levels generate hydrogen peroxide and superoxide radical and also interact with oxy hemoglobin resulting in the generation of hydroxyl radical\textsuperscript{53}. All these mechanisms make the cell extremely vulnerable to oxidative stress and may lead to cell death.

**FIGURE 4- Lead Induced Oxidative Stress**

1.2.7.2 Ionic Mechanism of Lead Toxicity

Ionic mechanism of action for lead mainly arises due to its ability to substitute other cations like \( \text{Ca}^{2+} \), \( \text{Mg}^{2+} \), \( \text{Fe}^{2+} \) and \( \text{Na}^{+} \) affecting various fundamental biological processes of the body. Lead even in picomolar
concentration replaces calcium and thereby affecting key neurotransmitters such as protein kinase C that regulates long-term neural excitation and memory storage. Lead also affects Na\(^+\) ion concentration, this interaction between lead and sodium impairs the normal sodium dependent functioning of the cell. Toxic effects of lead are more pronounced in developing nervous system comprising immature astroglial cells that lack lead binding proteins and obstruct the formation of myelin sheath.

### 1.2.8 Toxicology

#### 1.2.8.1 Acute Toxicity

Acute toxicity is defined as the toxic effects produced by a single exposure of a substance or drug by any route for a short period of time. Acute toxicity of lead causes GI disturbances, dullness, restlessness, irritability, poor attention span, headaches, hepatic and renal damage, hypertension, hallucination and encephalopathy.

#### 1.2.8.2 Chronic Toxicity:

Chronic toxicity is defined as the toxic effect occurring due to the repeated daily exposure of a substance or drug at different doses for an expected life span. Chronic lead exposure causes hematological effects such as anaemia, basophilic stippling or neurological disturbances including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis. Chronic lead exposure also causes cardiovascular and renal toxicity. In children, lead exposure may lead to cognitive deficits such as decrease in IQ.

#### 1.2.8.3 Toxic Dose of Lead

- Lowest toxic oral dose for rats: 79mg/kg
- Lowest toxic dose of inhalation for rats: 10mg/m\(^3\)/24h
- Lowest lethal dose intra peritoneal for rats: 1000mg/kg
1.2.8.4 Lethal dose of lead acetate\textsuperscript{54,55}

\begin{itemize}
  \item LD\textsubscript{50} for rats orally : 4665mg/kg
  \item LD\textsubscript{50} for rat’s intra peritoneally : 174mg/kg
  \item LD\textsubscript{50} of lead acetate in humans : 714mg/kg
\end{itemize}

1.2.9 ALLIUM  PLANTS

\begin{itemize}
  \item Kingdom : Plantae
  \item Division : Angiosperms
  \item Class : Monocots
  \item Subclass : Liliidae
  \item Super Order : Liliiianae
  \item Order : Amarylidales/Asparagales
  \item Family : Alliaceae / Amaryllidaceae
  \item Subfamily : Allioideae
  \item Tribe : Allieae
  \item Genes : Allium
\end{itemize}

The alliums are a large genus widely and one of the world’s oldest cultivated vegetables, distributed over the holarctic region from the dry subtropics to the boreal zone. The genus is of great economic significance because it includes several economically important vegetables and flowering ornamentals as well as wild species from Europe, Asia and the Americans\textsuperscript{56}. The genus allium containing about 750 species include Allium cepa, Allium sativum, Allium fistulosum and Allium roylei etc\textsuperscript{57}, distributed all over Europe, North America, Northern Africa and Asia. Each differing in taste, colour and form but closely related in biochemical, phytochemical and nutraceutical contents\textsuperscript{58} including flavanoids, sulfur and selenium containing
compound\textsuperscript{59,60}. The botanical name allium is derived from the celtic word all which means pungent and it betrays the presence of a host of remarkable flavorants and odorants, all having in common one element Sulphur. Sulphur-Sulphur bonds and their oxides impart all medicinal properties to allium principles.

All these allium plants especially allium vegetables are rich with sulphur containing chemicals such as thiosulfinates and the other organo sulfur compound, the well known lachrymatory factor. The thiosulfinates or alkane (ene) thial-s-oxide are formed by the action of the enzyme alliinase (E.C.4.4.1.4) from their respective s-alk(en)yl cysteine sulfoxides, which are majorly responsible of onion flavor and produce the eye irritating compound that induce lachrimation\textsuperscript{58}. From all Allium species under differing conditions, thiosulfinates can decompose to form additional sulfur constituents including diallyl, methyl allyl and diethyl mono-, di-, tri-, tetra-, penta- and hexa sulfides, Vinyl dithiins and ajoene\textsuperscript{58}. Because of the presence of all these compounds alliums may become a unique source for health promoting compounds. Allium plants are generally consumed for their flavours, while their nutritive values have been appreciated only recently. Garlic (Allium sativum L) and onion (Allium cepa L) are the two important allium species with numerous health benefits, including prevention of cancer and cardiovascular disease \textsuperscript{61}.

Onion and Garlic are two important allium species which have been studied for their therapeutic uses such as antibiotic, antidiabetic, antioxidant, antiatherogenic, anticancer, hypo cholesterolemic and fibrinolytic activity\textsuperscript{56}. Of these two allium species garlic is more effective than onion in its biological action. Biological action of allium products are due to the presence of organo sulfur compounds having allyl (CH\textsubscript{2}=CH-CH\textsubscript{2}-) or its isomer propenyl (CH\textsubscript{3}-CH=CH-) group. Garlic has the more active allyl group (CH2=CH-CH2-), while onion has a less active propenyl or saturated methyl or propyl group.
However the disulfide/poly sulfide groups in these allium products confer them their biological effect against numerous diseases.

1.2.9.1 Garlic (Allium Sativum L)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>Class</td>
<td>Monocots</td>
</tr>
<tr>
<td>Subclass</td>
<td>Liliidae</td>
</tr>
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<td>Super Order</td>
<td>Liliianae</td>
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<td>Order</td>
<td>Asparagales</td>
</tr>
<tr>
<td>Family</td>
<td>Amaryllidaceae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Allioideae</td>
</tr>
<tr>
<td>Genes</td>
<td>Allium</td>
</tr>
<tr>
<td>Species</td>
<td>Sativum</td>
</tr>
<tr>
<td>Botanical name</td>
<td>Allium sativum</td>
</tr>
</tbody>
</table>

Common name:

- Hindi: Lasun
- English: Garlic
- Sanskrit: Rasonams and Lahsuna
- Malayalam: Veluthulli
- Tamil: Vellapoode
1.2.9.1.1 History of Garlic:

Garlic is a most popular perennial bulb used throughout the world, appears to have originated in Central Asia and then spread to China, the near East and the Mediterranean region\textsuperscript{62}. Garlic is a member of the alliaceae family with the history of human use of over 7000 years. In the bible, garlic is mentioned as a traditional treatment in many countries particularly in the near East, China and India. It is a valuable spice and a popular remedy for various ailments and physiological disorders as preventive and treatment alternatives because of the presence of many components inside garlic (Allium sativum). Jews learnt its culinary use from Egyptians when they stayed there as slaves to pharach. When they escaped from Egypt and wandered through the deserts to reach Canaan they asked Moses to return to Egypt to get garlic which they missed in the desert. Jews used garlic as an ingredient for bread and food supplements. Christians followed the same later.

1.2.9.1.2 Chemistry of garlic (Allium sativum L)

Garlic (Allium sativum L) has been used in world cuisines as well as remarkable remedy for multiple diseases, claimed to help prevent everything from high cholesterol to cancer\textsuperscript{63}. The chemistry of garlic is extremely complex but research has shown that it is organo sulphur compounds that promote its broad range of lipid lowering hypoglycemic, antithrombotic, antiblood coagulation, antihypertension, anticancer, antioxidant and antimicrobial effects. Intact garlic cloves contain only a few medicinally active compounds. The main chemical constituents of intact garlic are γ-glutamyl-s-allyl-L cysteines and S-allyl- K- cysteine sulfoxide which is known as alliin. Alliin is the primary odorless sulfur containing amino acid i.e. an alkyl derivative of cysteine sulfoxide\textsuperscript{64,65}. γ-glutamyl-S-allyl-L-cysteines are converted into S-allyl cysteins (SACS) through an enzymatic transformation with γ-glutamyl peptidase when garlic is extracted with an aqueous solution\textsuperscript{66}. When fresh garlic cloves are crushed or chopped or garlic powder that has been carefully
dried is added to water, they release the vascular enzyme allinase that rapidly
lyses the cytosolic cysteine sulfoxide to form sulfenic acid (R-SOH), that
immediately condenses to form allicin (thiosulfinates). Thus, no thiosulfinates
are found in intact garlic.

**FIGURE 5 – Alliin metabolism**

At least 100 sulfur compounds, 17 amino acids, steroids, terpenoids, flavanoids, phenols and minerals such as selenium were isolated from garlic\(^{66,67}\). Of these the thiosulfinates released from crushed garlic are reactive biological molecules and undergo a number of transformations depending on the temperature, solvent and pH on incubation of thiosulfinate in water the principal products formed are diallyl trisulphide, diallyl disulphide and allyl methyl trisulphide. Incubation of allicin or allyl methane thiosulfinate in low polarity solvents or without solvent, primarily produces 1,3-vinyl dithiin, 1,2-vinyl dithiin and lesser amount of ajoene and sulfides. Incubation of allicin in methanol yields diallyl trisulfide, ajoene and vinyl dithiins. Steam distillation of crushed garlic bulbs yield garlic oil that consists of diallyl, allyl methyl and dimethyl (mono to hexa) sulfides.
Figure 6 – Enzymatic reaction on crushed garlic and conversion of alliin into various sulfur containing compounds

Garlic oil, aged garlic and steam distilled garlic do not contain significant amounts of alliin or allicin, but instead contain various products of allicin transformation. If an aqueous extract of crushed garlic is distilled under vacuum the oil separated contains some allicin and if this sample is kept in 4°C allicin shall be converted to ajoene⁶⁴.
1.2.9.2 Onion (Allium cepa L.)

Kingdom : Plantae
Division : Angiosperms
Class : Monocots
Order : Asparagales
Family : Amaryllidaceae
Subfamily : Allioideae
Genes : Allium
Species : Cepa
Botanical name : Allium cepa
Common name :

Hindi : Pyaj
English : Onion
Sanskrit : Polanduh
Malayalam : Cylvannulli
Tamil : Venkayam, Ulligadda

1.2.9.2.1 History of Onion (Allium cepa L)

Onion (Allium cepa L.) is one of the oldest cultivated perennial herbs for over 4000 year. It is varying in size and shape from cultivar to cultivar with different colour (white/coloured)\textsuperscript{68}. Central Asia is considered to be the region
of origin but it was introduced to the Mediterranean and is cultivated worldwide. The earliest records come from Egypt, where it was cultivated at the time of the old kingdom. The onion (Allium Cepa L.) was among the first cultivated plants taken to the Americas from Europe, beginning with Columbus in the Caribbean. Later it was reported several times and established in the early 17th century in what is known the northern USA. Europeans took the species to East Asia during the 19th century.

In India the onion (Allium cepa L.) was mentioned as early as the 6th century B.C, from that time it was used as diuretics and was believed to promote a healthy heart, eyes, and joints. Onion (Allium cepa L.) was a popular folk remedy with rich flavanoids such as quercetin and sulphur compounds. Onion (Allium Cepa L.) was traditionally used as carminative, emmenagogue, contraceptive, and expectorant, antihelminthic, aphrodisiac and tonic. Onion (Allium cepa L.) was used for the treatment of cholera, bronchitis, bruises, ear ache, colic, insect bites, tuberculosis, diabetes, dropsy, catarrh, scurvy, epileptic fits, hysterical fits, fevers, hypertension, jaundice, pimples and sores. Onion (Allium cepa L.) have a unique combination of three families of compounds such as fructans, flavanoids and organo sulfur compounds that are believed to have salutary effects on human health. Fructans are small carbohydrate molecules that help to maintain the gastrointestinal health by sustaining beneficial bacteria. All these biologically active compounds produce biological responses such as reduction of risk factors for cardiovascular diseases, cancer, stimulation of immune function, enhanced detoxification of foreign compounds, hepato protection and antioxidant effect.

1.2.9.2.2 Chemistry of Onion (Allium cepa L)

Onion (Allium cepa L.) has an important status among vegetables because of their particular flavor and their ability to enhance the flavor of other foods and to promote beneficial health effects. Flavanoids and organo sulphur compounds are the two major classes of secondary metabolites in onion (Allium
cepa L.) that give health effects. According to Block et al\textsuperscript{73} the organo sulfur compounds give the anti inflammatory, anti allergic, antimicrobial, and antithrombotic activity by inhibition of cyclooxygenase and lipoxygenase enzyme.

The organo sulfur compounds of Allium cepa include thiosulfinates, thiosulfonates, cepaenes, S-oxides, S, S\textsuperscript{1}-dioxides, mono sulfides, disulfides, trisulfides and zwiebelanes. When the onion bulb is crushed, minced or processed L-cysteine sulfoxides (S-methyl, S-propyl or Z prop-1-enyl) are released from compartment. These L-cysteine sulfoxides contact with the enzyme alliinase seen in adjacent vacuoles leads to hydrolysis to produce Sulfenic acid (R-SOH) within second, condensation of the reactive intermediate such as sulfenic acid form the compounds\textsuperscript{71} such as thiosulfinates, cepaenes and propanethial-S-oxide. The characteristic odour of the oil is due to the presence of several unsaturated sulfur and other organic compounds. The compounds identified in the onion oil included are the following \textsuperscript{74}.

**Mono Sulphides:** Dimethyl sulphide, allyl methyl sulphide, methyl propenyl sulphide, allyl propyl sulphide, propenyl propyl sulphide and di propenyl sulphides.

**Oxygen compounds:** Propanol, dimethyl furan, 2-methyl pentanal 2-methylpent-2-enal, tridecan-2-one and 5-methyl-2-n-hexyl-2,3- dihydrofuran 3-one.

**Di sulphides:** Di methyl disulphide, methyl propyl disulphide, allyl methyl disulphide, methyl cis-propenyl disulphide, methyl trans-propenyl disulphide, isopropyl propyl disulphide, dipropyl disulphide, allyl propyl disulphide, trans-propenyl propyl disulphide, diallyl disulphide, allyl propenyl disulphide and di propenyl disulphide.

**Thiophene derivatives:** 2,5-dimethylthiophene, 2,4-dimethylthiophene, 3,4-dimethylthiophene and 3,4- dimethyl 2,5 dihydrothiophen-2-one.
**Trisulphides:** Dimethyl trisulphide, methyl propyl trisulphide, allyl methyl trisulphide, methyl cis propenyl trisulphide, methyl trans propenyl trisulphide, diisopropyl propyl trisulphide, iso propyl trisulphide, di propyl trisulphide, allyl propyl trisulphide, diallyl trisulphide, cis-propenyl propyl trisulphide and trans-propenyl propyl trisulphide.

**Thiols:** Hydrogen sulphide, methanethiol, propanethiol, allylthiol.

**Tetra Sulphide:** Dimethyl tetra sulfate

Quercetin and kaempferol are the major flavanoids in the onion (Allium cepa L). These flavanoids functions as an antioxidant that can deactivate molecules that are injurious to cells in the body.

**Figure 7 – Formation of Sulfur Compounds from the Precursor Forms of Onion**

Onion thiosulfinates are allicin type compounds with some antiviral and antibacterial activity. Binding crushed onion or garlic pastes on wounds helps to heal them quickly. This is a home remedy.
1.2.9.3 Biological Roles of Allium Principles

Flavanoids and organo sulfur compounds are majorly responsible for the biological actions of allium products. Presence of different organo sulfur compound is the main characteristic of this genus allium. These organo sulfur compounds possess protective effects such as antiinflammatory, antiallergic, antimicrobial and antithrombotic activity\(^73\). Most likely these organo sulfur compounds react with other system through their Sulfur- sulfur or sulfur -oxygen linkages and alkenyl side chains (unsaturated propenyl or allyl groups)\(^75\).

1.2.9.3.1 Anti oxidant activity

Oxidizing agents or free radicals are attacking our bodies constantly and tend to produce potential damages to our cells which lead to various diseases. Allium extracts have variable but interesting antioxidant properties due to its total phenolic and sulfoxide contents which are high in red, purple onions and garlic. More than the phenolic compounds alliums have various sulfur compounds which are excellent antioxidants\(^76\). Substance which have been found to protect us from free radical induced cell damages include bio flavanoids, polyphenols like catachins, vitamins E, A and C, β- carotene, selenium etc. However these could not give any protective effect to DNA. The reason may be due to their antioxidant actions might have been nullified by the action of their metabolites on reduced thiols like GSH which are endogenous antioxidants for vitamin regeneration and accompanied inhibition of gene induction due to sweeping away of ROS and RNS\(^77,78\). This type of GSH exhaustion can be avoided with such antioxidants which trap electrons, regenerate themselves and enhance GSH production. Allium principles containing alkyl and alkenyl disulfides and their sulfoxides are able to do these feat with very little quantities of each according to the findings of Klans-Dieter\(^79\) and other researchers work\(^80,81\).
The mechanism of action of organic disulfides/sulfoxides in which R=CH₃, C₃H₅, C₃H₇ etc is

\[
2\text{RSS (O)R}_1 + e^- \text{aq} \rightarrow \text{RSSR}_1^- \cdot + \text{RSS (O}_2 \text{) R}_1 \quad (1)
\]
(Disulfide oxide) (Disulfide radical ion and Thiosulfonate)

\[
\text{RSS- R}_1^- \cdot \rightarrow \text{RS}^- \cdot + \text{R}_1\text{S}^- \quad (2)
\]
(Disulfide radical ion) (Free radical and Sulfide anion)

\[
\text{R}_1\text{S}^- + \text{OH}^- \rightarrow \text{R}_1\text{S}^- \cdot + \text{OH}^- \quad (3)
\]
(Free radical repair)

\[
\text{R}_1\text{S}^- \cdot + \text{RS}^- \rightarrow \text{R}_1\text{SSR (Termination)} \quad (4)
\]

RSSR1 can also trap an electron and repeat the reactions

Disulfide and thiols can also scavenge free radicals

\[
\text{R}_1\text{SSR} + \text{OH}^- \rightarrow \text{R}_1\text{SOH} + \text{RS}^- \quad (5)
\]

\[
\text{RSH} + \text{OH} \rightarrow \text{RS}^- \cdot + \text{H}_2\text{O} \quad (6)
\]

In due course 4RS\cdot \rightarrow 2\text{RSSR O}_2 \rightarrow 2\text{RS(O)-S-R and the 1}\text{st step is repeated}

The thyl radical RS\cdot helps in the repair of free radicals and it does not react with critical molecules, hence they serve as very good scavengers of free radicals and as antioxidants. These disulfides, their poly sulfides and sulfoxides are formed in the crushed or ground pulps of onions and garlic. A small amount of disulfides can scavenge many numbers of free radicals by a cycle of actions by repair, regeneration and electron trapping as shown in the equation. These types of free radicals repair reactions prevent tissue damages and lipid peroxidation particularly in liver and heart \(^{82}\). The lipophilic organo sulfur compounds such as diallyl sulfide (DAS) and diallyl disulfide (DADS) and
hydrophilic organo sulfur compounds such as S-ethyl cysteine (SEC) and n-acetyl cysteine (NAC) will protect the cells against lipid related oxidations by the activation of associated antioxidant enzymes and modification of several enzymes such as 3-hydroxy 3-methyl glutaryl CO A reductase, glutathione-S-transferase and catalase. The precursors of allium oils viz; the sulfoxide aminoacids in alliums are palatable, mild and less smelling antioxidants. If the alliums are crushed the enzyme alliinase acts on the sulfoxide amino acids and yield oils. In a series of studies Lau and associates demonstrated that several garlic compounds can minimize oxidation of LDL cholesterol in vitro. Short term supplementation of garlic in human subjects has demonstrated an increased resistance of LDL to oxidation. This is due to Allium sativum L that activates lecithin: cholesterol acyl transferase that can convert cholesterol into HDL. In addition to that there is enhancement of the activity of plasma and hepatic lipoprotein lipase that cause reduction in levels of LDL and VDL.

Garlic extract exerts antioxidant action by scavenging reactive oxygen species (ROS) produced by the oxidative stress, enhancing the cellular antioxidant enzymes such as SOD, CAT and GPX and increasing reduced glutathione (GSH) in the cells. Garlic inhibits lipid per oxidation and oxidative modification of LDL. Garlic protects DNA against free radical damage and mutation.

Onion (Allium cepa L) contains Sulfur compounds such as diallyl disulfides and their oxidized thiols can trap electrons from other systems and act as antioxidants. Mc Anlis et al reported that direct protective effect showed by quercetin against oxidative LDL modification, the flavanoid showed its protective effect in vivo at the cellular level by preventing cell damage from already oxidized LDL. Reactive nitrogen species (RNS) cause oxidative damage to cellular proteins, tissues and DNA. Quercetin that reduces the level of peroxy nitrates an extremely powerful oxidant in the brain by scavenging the super oxide anion. Thus cellular modification, lipid peroxidation and risk of neuro degenerative disease in the CNS may be diminished. The inhibitory effects of specific sulf hydryl compounds and flavanoids in dietary onion may
decrease the risk of nitrosamine formation and reduce the cellular damage. In onion oil disulphides only methyl, propenyl and propyl groups are present. The unsaturated bond in propenyl group is in position one i.e., close to the sulfur bond and this makes it a weaker antioxidant. Alkyl groups like CH$_3$ and C$_3$H$_7$ are still less helpful for antioxidant action. Saturated groups weaken the antioxidant actions of onion oil. In this respect alkyl group in garlic compounds shows a better antioxidant action. Moderate hypoglycemic, hypolipidemic and antioxidant effects were observed with the onion oil and their precursors like SMCS and SP(en)CS and a far better action was shown by garlic oil and its precursor SACS, in diabetic rats and alcohol fed rats $^{90,91,92,93}$. As the above reported claims by various research workers consumption of extracts of garlic or onion or their oils and their precursor amino acids significantly improved the condition of diabetic rats and humans according to the measurement of their blood sugar and lipids, lipid peroxidation, enzymes etc. All these effects make allium principles as very important nutraceuticals that can correct the metabolic derangement through their antioxidant activity $^{94}$. According to some reports S-allyl cysteine is also an antioxidant with nutraceutical properties. See the following figure.

**FIGURE 8 – Antioxidant Mechanism Associated to Sulfur Compound of Allium S- allyl cysteine (SAC)$^{95}$**
1.2.9.3.2 Hepato protective effects

Free radicals and oxidative stress have been the reason for the liver injury in animals and humans. Allium principles protect the liver against deleterious agents and free radical mediated toxic damages to the liver cells. Garlic (Allium sativum L) helps the liver to maintain their normal function by accelerating the regenerative capacity of the cell. Obioha et al\textsuperscript{96} reported a decrease in hepatic activities of ALT, AST and ALP and concomitant increase in the serum ALT and AST activities in rats with Cd induced oxidative damages. Onion and garlic (Allium principles) extract feeding significantly attenuated these adverse effects. Prophylactic effects of garlic oil and its non polar fraction were observed satisfactory as compared to Vitamin E in CCl\textsubscript{4} administrated rats. A very encouraging data was observed in the form of correcting hepatic damages by CCl\textsubscript{4} in rats on simultaneous feeding of the above nutraceuticals\textsuperscript{18} to a similar group of rats subjected to CCl\textsubscript{4} administration. When CCl\textsubscript{4} was administrated to rats for a month it deranged enzyme activities of ALP, ALT, AST, GR, HMG CO.A reductase, G-6-PDH and malic enzymes to a significant extent in rats. Simultaneous feeding of both polar and non polar fractions of garlic to different groups of CCl\textsubscript{4} fed rats, the hepatic damages and the altered parameters such as serum, liver and heart lipids, lipid peroxidation, serum proteins, A/G ratio and the above sited enzymes were significantly corrected towards normal values. The modulatory effects of the oil have been attributed to ajoene and related polysulfides in garlic oil as they increase glutathione-S-transferase activity and lower N-nitroso dimethylamine demethylase activity\textsuperscript{93,97}.

1.2.9.3.3 Anti diabetic effect

Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and glycosuria which leads to affect the eyes, nerves, blood vessels, heart, skin and kidneys. Several animal and human studies showed allium principles contain valuable therapeutic agents that ameliorate the bad
effect of diabetes. Feeding of garlic extracts and oil reduced blood glucose in streptozotocin induced as well as alloxan induced diabetes in rats, mice and rabbits\textsuperscript{98,99,100,101}. Anwar M.M and Meki A R \textsuperscript{102} reported that garlic oil may effectively normalize the impaired antioxidant status in streptozotocin induced diabetes mellitus in rats. Treatment of diabetic rats with garlic oil significantly reduced cell phosphatase. Isolated product from garlic such as S-allyl cysteine sulfoxide having the antioxidant property may contribute its beneficial effects in diabetes\textsuperscript{103}. The hypoglycemic action of garlic compounds may be due to effective binding of them with compounds like cysteine which in turn might increase pancreatic secretion of insulin from β- cells or its release from bound insulin or enhancement of insulin sensitivity \textsuperscript{101}. \textit{In vivo} analysis of the effects of quercetin in onion on human diabetic lymphocytes showed a significant increase in the protection against DNA damage from hydrogen peroxide at the tissue level\textsuperscript{104}. Both allium oils and their precursors viz; SMCS and SACS are hypoglycemic agents because they are insulin secretagogues as well\textsuperscript{103}.

\textbf{1.2.9.3.4 Cardio protective effects}

Disorders of the heart and the circulatory system are due to the obstruction or clogging of the coronary arteries. Arteries that supply blood and oxygen to the heart become increasingly narrower as plaques buildup over time and blood supply becomes so restricted to that portion of the heart and heart attack occurs. High blood pressure and high serum cholesterol levels are the important predictors of heart disease. Both of these determinants are impacted by the therapeutic action of allium products such as garlic (Allium sativum L) and onion (Allium cepa L). Many researchers showed that long term feeding of both garlic and their preparations on experimental atherosclerosis induced by a high cholesterol diet in rabbits caused significant reductions in serum and atheromatous lesions\textsuperscript{105,106,107,108,109}. Garlic inhibited the biosynthesis of cholesterol. Garlic derived components combine with the sulfhydryl (–SH) groups of enzymes like HMGCO reductase. Both \textit{in vivo} and \textit{in vitro} studies observed reduced conversion of acetate into cholesterol\textsuperscript{110,111,112}. Garlic oil
reduced total cholesterol, caused decrease in LDL and VLDL level and concomitant increase in HDL-cholesterol\textsuperscript{113}. Patient treated with garlic powder at the dose of 900mg/day showed 9-18\% reduction in plaque volume, a 4\% decrease in LDL cholesterol, 8\% increase in HDL cholesterol and 7\% decrease in blood pressure\textsuperscript{114}.

The earliest manifestation of atherosclerosis is an accumulation of intracellular lipid such as cholesterol in the sub endothelial intimal cells and it is accompanied by stimulation of cells proliferation and lipidosis, enhanced proliferation and accumulation of extracellular connective tissue matrix. Garlic directly suppresses lipid synthesis by inhibiting biosynthesis of cholesteryl esters and TAGS in atherosclerotic cells. In addition to that garlic extract inhibits the activity of the enzyme such as acyl COA: cholesterol acyl transferase (ACAT) involved in the formation of cholesteryl esters that is the main component of the excessive fat accumulated by cells. In atherosclerotic cells ACAT activity is three fold higher than the normal cells and they are loaded with cholesteryl esters. Aqueous extract of garlic powder decreases this enzyme activity to normal level. Further garlic extract degrades cholesteryl esters in atherosclerotic cells by the stimulation of cholesteryl ester hydrolase\textsuperscript{66}.

Allicin of garlic (Allium sativum L) and similar compounds of onion (Allium cepa L) are inhibitors of acetyl CO. A synthesis and disulfide (DADS) is an inhibitor of HMG CO.A reductase, the rate limiting enzyme of cholesterol synthesis\textsuperscript{115,116}. LDL oxidation and endothelial cell damage are believed to be the reason for the development of atherosclerosis. Many researchers found that presence of quercetin commonly found in onion and fruits reduced LDL oxidation in vitro\textsuperscript{117,118}.

\textbf{1.2.9.3.5 Anti hypertensive effect}

Hypertension is a systolic blood pressure higher of 140 mm Hg or a diastolic blood pressure of 90mmHg or both. Elevated blood pressure is one of
the significant risk factors for stroke and coronary heart disease. Prevention and proper management of hypertension decreases the incidence of related morbidity and mortality. Nutraceuticals such as allium products have shown potential beneficial effects in the treatment of hypertension. Single or multiple doses of aqueous garlic extract reduced thromboxane B$_2$ and prostaglandin E$_2$ level and thereby reduced hypertension in rats$^{119}$. Blood pressure reducing properties of garlic have been linked to hydrogen sulfide production and allicin content in allium products$^{66}$. Allicin inhibits certain enzymes involved in the conversion of some hormones responsible for the regulation of blood pressure and garlic juice shows a direct relaxant effect on smooth muscles. Antihypertensive action of garlic is due to the prostaglandin like effects of its principles which decreases peripheral vascular resistance. The γ-glutamyl cysteines are the peptides found in garlic which inhibit the enzyme angiotensin converting enzyme that changes angiotensin I into angiotensin II and vaso dilating effects in vitro$^{64}$. Garlic modulates the production and functions of endothelium derived relaxing and constricting factors and contribute the protective effect against hypoxic pulmonary vaso constriction. Benavoids et al$^{120}$ proved that the antihypertensive effect of dietary garlic is largely mediated in the generation of hydrogen sulfides. They demonstrated that garlic derived organic polysulfide is converted into hydrogen sulfide gas (H$_2$S) by erythrocytes. H$_2$S is a powerful gaseous signaling molecule that will help to relax vascular smooth muscle, induce vaso dilation of isolated blood vessels and reduce blood pressure.

Onion and garlic organic sulfides can combine with NO and enhance vaso dilation and reduce hypertension in mammals according to Ku et al$^{121}$ and Schwartz et al$^{122}$. A similar effect is proposed for the antihypertensive effect of onion in spontaneously hypertensive rats$^{123}$. 
1.2.9.3.6 Fibrinolytic activity

Fibrin is an insoluble protein that can be dissolved by thrombin and plasmin. Both are proteolytic enzymes activated by circulating plasma precursor plasminogen or prothrombin. They could be activated by tissue type plasminogen activator (tPA) or urokinase plasminogen activator (UPA) few days after a wound is repaired. Inhibition of fibrinolytic activity or deficiency of factors involved for this activity might upset the hemostatic balance and allow excessive deposition of fibrin. This unwanted accumulation of fibrin caused decreased fluidity of blood circulation and cause cardiovascular diseases such as stroke and myocardial infarction. Acute or chronic intake of garlic oil, raw garlic or the allicin, the component of allium plants increased fibrinolytic activity. Gupta et al were the first to report that a fat induced decrease in blood fibrinolytic activity (BFA) was reduced by 23% (p ≤ 0.01) four hour after ingestion of a high fat and onion containing diet in 20 healthy adult males. Bordia and Augusti K T et al did similar experiment with feeding juices and extracted oils of garlic and onions and observed encouraging results. Mechanism of action of fibrinolysis may be based on the action of various sulfides in allium oils, i.e.; exchange reactions between Sulfides and fibrin clot. Alternatively oils may stimulate liberation of plasminogen activator from blood vessels. Sulfur compounds of onion oils also inhibited the formation of thromboxanes and the platelet activating factors.

1.2.9.3.7 Platelet aggregation

Platelets can adhere to the exposed collagen, laminin and van willebrand factors in the injured vessel wall and that is called platelet activation. It is also produced by ADP and thrombin. The activated platelets change shape, put out pseudopodia, discharge their granules and stick to other platelets, initiating the process of platelet aggregation. Aqueous extract of garlic inhibited platelet aggregation induced by ADP, epinephrine, collagen and
arachidonate and inhibited biosynthesis of prostacyclin in rat aorta\textsuperscript{129}. Garlic reduced the formation of thromboxane, inhibited the phospho lipase activity and lipoxygenese products formed in platelets. These effects may be the reason for the inhibition of platelet aggregation by garlic extract. It may be also due to the inhibition of uptake of calcium into platelets, thereby lowering cystolic calcium concentration \textsuperscript{129}.

According to Lawson et al\textsuperscript{130} there is an interaction product of allicin with endogenous thiol viz; S-(allyl thio) cysteine may be responsible for much biological effects of garlic within the body. Many of the studies showed that components of freshly cut garlic or onions that contain allicin type compounds inhibit platelet aggregations and smooth muscle contraction through the inhibition of cyclo oxygenase and related enzymes\textsuperscript{131}. Makheja et al\textsuperscript{132} have demonstrated that garlic and onion oil fractions containing allicin or poly sulfides effectively inhibited ADP, arachidonic acid or collagen induced platelet aggregation. Allium oils show this property due to their blockage of thromboxane and prostaglandin biosynthesis through inhibition of fatty acid oxygenase. Diallyl trisulfide a breakdown product of allicin shows anti platelet activity and inhibits formation of thromboxane\textsuperscript{133}. In fresh garlic and onion juices allicin type compounds are initially formed. It takes time for them to get transformed into alkyl or alkenyl poly sulfides such as e.g. DATS, DMTS, AMTS etc which are the true inhibitors of platelet aggregation.

### 1.2.9.3.8 Wound healing potential of allium products

Successful wound healing depends upon angiogenesis. An impaired angiogenesis is a hall mark of the chronic wounds encountered with diabetes and venous or arterial insufficiency. In the study of Chitra Shenoy et al\textsuperscript{134} they showed that alcoholic extract of onion (Allium cepa L) has a better wound healing activity in excision, incision and dead space wound models as compared to chloroform and water extracts. Different concentrations of aged
garlic solutions on wound healing showed better wound closure, re-
epithelialization, dermatrix regeneration and angiogenesis\textsuperscript{135,136}.

1.2.9.3.9 Effect on sickle cell anaemia

Sickle cell anaemia is a life threatening hereditary disease. Oxidative
stress plays a prominent role in sickle cell anaemia. Antioxidant protection of
the RBCs lead to a significant reduction in the sickle cell anaemia.\textsuperscript{137} Aged
garlic extract showed significant improvement in erythrocyte deformobility
through stabilization of erythrocyte membrane in non – sickle RBC. AGE may
also improve erythrocyte membrane stability in sickle RBC with antioxidant
activities. It is suggested that there was reduction of Heinz bodies in sickle RBC
with daily administration of AGE\textsuperscript{138}.

1.2.9.3.10 Neuro protective effect

The nervous system is the major communication network in the
human body. Its normal functioning mainly depends upon the maintenance of
its structural integrity and many complex metabolic processes. According to
the processes that disrupt normal structure or metabolism or both are capable
of producing neurological disease. Aged garlic extract protects neurons from
abeta neurotoxicity and apoptosis. Aged garlic extract prevents ischemia or
reperfusion related neuronal death and thus improves learning and memory
retention\textsuperscript{139}.

\textbf{Kyolic - This is an odorless garlic product produced in Japan. This was
formed to be a convenient form of garlic for experiments on both man and
animals.}

1.2.9.3.11 Protection against radiation by allium products

Lau and researchers\textsuperscript{140} conducted an experiment to
determine whether garlic could provide protection against radiation by
incubating human lymphocytes in tissue culture for 2 hours both with and
without kyolic (2.5mg/ml) or fresh garlic extract (2.5mg/ml). They then irradiated all but one of each set of cultures with 2000 rads from a Therac 20 linear accelerator and each unirradiated culture served as control. They then tested viability of the cells with trypan blue dye 3, 24, 48 and 72 hours following irradiation. Irradiated control cultures not infused with protection agents steadily declined; within 72 hours only 25% of the cells were still viable. Cells incubated with kyolic extract enjoyed significant protection; a few cells died within the initial few minutes, but almost all others were viable at the end of the test period. Sulfur compounds such as poly sulfides of allium products gave protection to lymphocytes from radiation possibly through a repair mechanism applying free radical scavenging of the irradiation products in the culture viz; break down products of water i.e., OH• and H•. Free radicals oxidize cells and destroy them. This is prevented by scavenging them by poly sulfides/ thiols.

\[
\text{R-S-S-R} + 2 \text{H}^\bullet \rightarrow 2 \text{RSH} \quad \text{(1)}
\]

\[
2\text{RSH} + 2 \text{OH}^\bullet \rightarrow \text{R-S-S-R} + 2 \text{H}_2\text{O} \quad \text{(2)}
\]

\[
\text{R-S-S-R} + 2\text{OH}^\bullet \rightarrow 2\text{RSOH} \quad \text{(3)}
\]

1.2.9.3.12 **Antibiotic activity**

Allicin and related compounds are formed in crushing garlic and onions. Antimicrobial effects of allicin type compound from onions are weaker as the double bond in the alkyl group is at position one rather than at two as in allicin. Onion juice is a home remedy to cure eye infections such as conjunctivitis. In bruised parts of fingers a bandage with bruised onion peels are also useful to cure the injuries. If 2-3 cloves of garlic consumed per day for 2-3 days the injuries on the body get cured easily without pus formation. Garlic paste along with the little dry vinegar has been used in China to treat all viral infections and a garlic oil preparation that contains diallyl trisulfide has been used to treat crypto coccal meningitis. Few days garlic pulp consumption
prevents infections from jaundice. A food supplement composed of black pepper, dry ginger and garlic paste in a ratio of 1:2:4 prevented Chikunguniya and also its after effects, viz; joint pains and swelling\textsuperscript{142}.

1.2.9.3.13 Immuno modulatory effect

Some of the new and frightening viral diseases like AIDS and flesh eating bacteria have so far no effective treatment, but have strengthening the body’s ability to fight off infection become more important. Allium principles stimulate the body’s immune system, particularly by enhancing the macrophages and lymphocytes which destroys bacteria, virus and cancer cells. Certain diseases can be caused by immune dysfunction; garlic might contribute immuno modification to the prevention of diseases. The hypothesis that the immune stimulation and other beneficial effects of garlic can reduce the effect of cancer should be confirmed further as per the study of Lamm DL et al\textsuperscript{143}. When garlic extract (Japanese Kyolic) was injected subcutaneously and intra peritoneally that produced more leucocytes activity as assessed by chemiluminescence method in mice.

The immune cells such as B-lymphocytes respond to various stimuli by the production of antibodies that fight off many common infections\textsuperscript{144}. The other immune cells such as T- lymphocytes, phagocytes and the natural killer cells (NK cells) attack directly the foreign invaders such as cancer cells, bacteria or fungi. Some carry out their attack by secreting chemicals called cytokines such as interferon and interleukins. NK cells kill tumor cells or virally infected cells by direct membrane contact or through antibody dependant cell mediated cytotoxicity. These cells are activated by substances called interleukins and interferon. NK cells recognize infected cells especially the cells attacked by viral agencies and destroy them and also have the ability to attack aberrant cells that could cause tumors\textsuperscript{82}. 
Garlic principles attract immune cells to the site of injury. Some researchers\textsuperscript{145} showed that Kyolic garlic showed more tumor cell destruction than by raw garlic. The major ingredients of kyolic extracts are S-allyl cysteine and cycloalliin and these compounds have showed better immuno modulatory activity than garlic extracts. Garlic is full of nutrients with polysulfides which amino acids play a role in beefing up the body’s defenses against disease.

1.2.9.3.14 Anti Inflammatory effect

Inflammation is a protective mechanism of tissues against endogenous and exogenous antigens. Chronic inflammation is a pathological condition characterized by continued active inflammation response and tissue destruction. The immune cells such as macrophages, neutrophils and eosinophils are involved directly or by the production of inflammatory cytokine production in chronic inflammation\textsuperscript{146}. The allium principles such as thiosulfinates viz; allicins and cepaens have been shown to possess anti-inflammatory properties. This is due to the inhibition of inflammatory cell influx by thiosulfinates and cepaens. Inflammation is related to the action of cyclooxygenase that is promoting the synthesis of prostaglandins. Allicin and related sulfides inhibit cyclooxygenase action\textsuperscript{135}. Ajoene inhibits the pain receptors at dorsal root of spinal cord, which results in the inhibition of pain signal transduction\textsuperscript{147}.

1.2.9.3.15 Effect on Enzymes

Allicin inhibits the thiol group of enzymes such as succinate dehydrogenase, xanthine oxidase, hexokinase, cholinesterase, triose phosphate dehydrogenase, lactate dehydrogenase, tyrosinase, ALP etc. Garlic oil influences oxidative phosphorylation in liver mitochondria of mice. Garlic extract constituent most probably allicin acts as an uncoupler of oxidative phosphorylation, inhibits the oxidation of succinate and increases the activity
of mitochondrial ATPase by 100% and the membrane becomes clearly more permeable to certain substrates such as ATP and sensitive to hypotic solution\textsuperscript{94}. Liver enzymes lipase and phosphorylase are increased significantly while the activity of glucose-6-phosphatase was decreased by garlic principles. The lipid lowering activity and supply of glucose and ATP to the tissue by the action of garlic principles are due to the secretion of insulin\textsuperscript{75,94}. Allicin showed a short term effect and its transformed forms of ajoene and polysulfides showed a long term effects. Both onion and garlic contained sulfoxide amino acids SMCS and SACS which showed similar effects in diabetic rats\textsuperscript{148}. Garlic oil polysulfide oxides interact with SH group enzymes such as HMG CO. A reductase and fatty acid synthase, thus limiting the serum and tissue levels of cholesterol and FFA. Garlic and onion oils and their sulfoxide aminoacids increase the activities of SOD, catalase and GR and GSH content in the tissue\textsuperscript{94}. Methanol extracts of fresh onion (Allium Cepa L) bulb inhibited lipoxygenase activity in the rat platelets\textsuperscript{71}. Garlic oil and its sulfides such as DADS and DATS are potent inducers of the phase II enzymes for carcinogens viz; quinone reductase, glutathione reductase and S-transferase\textsuperscript{94}. Thus the activation or inductions of various enzymes are possible by garlic compounds.

1.2.9.3.16 Antimicrobial Effects

Garlic and onion have been used for centuries in several societies against microbial infections. Sulfur compounds are the main active antimicrobial agents. The antimicrobial activity was affected by alk (en)yl group found in garlic and onion. The order for antimicrobial activity was; allyl ≥ methyl ≥ propenyl\textsuperscript{58}.

1.2.9.3.17 Antibacterial Effects

Historically garlic has been used as an antimicrobial agent, which fights bacterial infections. Numerous reports showed garlic extract has a wide range of antimicrobial activity against gram negative gram positive bacteria. Bolton et al\textsuperscript{149} reported that garlic had a remarkable cure rate for tuberculosis.
2 mg/ml of garlic extract was required to inhibit one mycobacterium tuberculosis strain.

Recently investigators demonstrated an inhibitory effect by aqueous extracts on numerous bacterial species such as Helicobacter pylori, Bacillus subtilis, Escherichia coli, Flavo bacterium sp., Listeria monocytogens, pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus and Vibrio para haemolyticus. Onion extract is effective *in vitro* against many bacterial species including bacillus subtilis, salmonella and E coli. Onion is not as efficient as garlic due to saturated alkyl groups and that the sulfur compounds in onion is only about one quarter the level seen in garlic\textsuperscript{58}.

The antimicrobial effects have been attributed to the action of thio sulfinates found in the allium principles. The mechanism is due to the inhibition of certain thiol containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups\textsuperscript{74}. Allicin inhibited bacterial enzymes such as acetate kinase and phosphotransacetyl co.A synthetase. Allicin inhibits bacterial cell growth by delaying and partially inhibiting DNA and protein synthesis\textsuperscript{58}.

**1.2.9.3.18 Antifungal Effects**

Fungal infections have become an important aspect of modern infectious disease practice. High dilutions of extracts of allium sativum showed significant fungi static and fungicidal activity *in vitro* and *in vivo*. Raghunandana RR et al\textsuperscript{150} and Yamada Y et al\textsuperscript{151} were the first to report that allicin was effective *in vitro* against candida cryptococcus, Trichophyton, epidermophyton and Microsporum. Allicin induced morphological abnormalities in hyphae of Trichophyton and the percent germination of spores was greatly decreased. The aqueous garlic extracts inhibited protein and nucleic acid synthase enzymes and arrested lipid synthesis completely in Candida albicans\textsuperscript{152}.
1.2.9.3.19 Antiviral Effects

Allium principles shown to have *in vitro* and *in vivo* antiviral activity against the human cytomegalovirus, influenza B, herpes simplex virus type 1 and 2, para influenza virus type 3, vaccinia virus, vascular stomatitis virus and human rhinovirus type 2\textsuperscript{153}. Ajoene blocks the integrin-dependent processes in a human immunodeficiency virus infected cell\textsuperscript{154}. In addition to sulfur compounds, quercetin a component found in onion possesses antiviral activity and enhances the bioavailability of some antiviral drugs.

1.2.9.3.20 Anthelmentic and Anti protozoal Effects

Sulfur compounds of allium plants such as allicin, diallyl trisulfide (DAT) and ajoene can reduce developing different protozoan parasites such as *Giardia lamblia*, *Leishmania* and *Leptomonas colosome*\textsuperscript{155,156,157,158}. Allium sativum L and its components were active against amoeba, 30 µg/ml of allicin totally inhibited the growth of amoeba cultures. The methanolic extract of Allium sativum showed anti leishmanial activity against *Leishmania* strain and *Leishmania donovani* strain both *in vivo* and *in vitro*\textsuperscript{159}. Allium cepa also showed anti parasitic activity for many helminthes and protozoa such as *Trichinella spiralis* and *Leishmania*\textsuperscript{160, 161}.

1.2.9.3.21 Anti Aging Effect\textsuperscript{94}

Aging is related to the over production of free radicals in the body and a lack of capacity of the body to scavenge them and repair the cells. Moreover destruction of receptors in various cells and neurons may fasten the aging process in the body. Antioxidant property of allium principles slow down aging process in the body. Garlic treatment maintained a relatively youthful morphology of the skin fibroblasts at comparable cumulative population doubling levels (CPDLS) between treated and untreated groups. Garlic extract has a dual role to suppress the growth of potentially abnormal cells as well as to give beneficial effects to maintain normal cells in a very healthy pattern of
growth and long term span of life. The mechanism of action is dependent on free radical scavenging and enhancement of endogenous enzymes like SOD, catalase, Glutathione reductase, Glutathione-S-transferase etc.94.

1.2.9.3.22 Detoxification of Heavy Metal Poisoning and other toxins: (Alliums Prevent heavy metal toxicity)

One of the most difficult toxins to neutralize in the body is heavy metal poisoning. Treating heavy metal poisoning has involved a process called chelaton. According to Petkov11 the sulfides of garlic are useful as antidotes for ‘saturnism’ chronic lead poisoning. Garlic and onion juices considerably diminished the symptoms of lead poisoning162. Nickel and chromium induced alterations in lipid profile were normalized by the administration of garlic163. The toxic effects of arsenic in mice have also been shown to be significantly reduced by the feeding of aqueous garlic extract164. Garlic also showed good protective effects against experimental intoxication with cadmium, mercury and organo metallic compounds. In another study garlic inhibited the hepatotoxicity of CrCl3 and the concomitant use of garlic and CrCl3 decreased AST and ALT levels165.

The cell membrane is the main target of the oxidative damage produced by xenobiotics including heavy metals. A heavy metal such as lead produces oxidative damage by enhancing peroxidation of membrane lipids166. Direct peroxidative activity of lead leads to the generation of ROS. The deleterious effects of oxidative stress are counteracted by endogenous antioxidant enzymes such as SOD and catalase and the antioxidant GSH. Arti Sharma et al167 reported that, SOD, catalase and GSH antioxidant were reduced by lead nitrate, thus exposing the tissues to per oxidative damage. Lead toxicity directly interrupts enzyme activation, competitively inhibits trace mineral absorption and bind to -SH proteins. Garlic organo sulfurs such as alliin, allyl cystiene, allyl di sulfide and di allyl disulfide possess antioxidant properties and neutralize several types of ROS168. The biologically active
compounds might have chelated lead and enhanced the excretion from the body that leads to the decreased accumulation of lead in tissues. Garlic also contains biologically active lipophilic sulfur bearing compounds such as allicin, SAC, DADS and DAS which can easily permeate through phospholipids membranes\textsuperscript{169} and reduce intracellular lead.

The removal of Cu\textsuperscript{2+}, Pb \textsuperscript{2+}, Al\textsuperscript{3+} and Hg\textsuperscript{3+} from their solution was showed by Lau\textsuperscript{140} on addition of a liquid extract of garlic (kyolic). The above salt solutions lyse red blood cells which could be prevented if 0.05ml solution of 1:10 diluted kyolic is added in test tubes containing 15ml of a 5\% suspension of human RBC and different heavy metal salt solution.

1.2.9.3.23 Anticancer Effect

One in three people usually develops cancer at some time during their life and one in five will die out of them. The use of garlic as anticancer agent began in the 1950’s and that the thio sulfinates of alliums inhibited the tumor cell growth. Garlic and onion intake decreased the risk of carcinoma in various tissues and organs viz; stomach, colon, esophagus, prostate, bladder, liver, lungs, mammary glands, skin and brain. Mei et al\textsuperscript{170} reported from China that in Cangshan country areas where people regularly eat garlic daily (20g/day), suffered least from stomach cancer (3 in 100,000) as compared to a 13 fold increase in Qixia country areas where people used no garlic (40, in 100,000). It was shown that Cangshan areas people’s gastric juice had lower concentration of nitrites than those in Qixia. Nitrites are converted to amines i.e., powerful carcinogens- nitrosamines in our system. Garlic principles may be protecting the system from nitrite accumulation\textsuperscript{170,140}. Lau\textsuperscript{171} suggested that garlic principles, ajoene and di allyl disulfide, detoxify carcinogens and protect the body from the toxins aflatoxin B1 and benzo (a) pyrene. Di allyl disulfide inhibits \textit{in vivo} activation of nitrosamines. Unsaturated polysulfide in allium principles inhibits tumor promotion perhaps by enhancing glutathione dependent detoxification of enzymes. Garlic oil increased glutathione
peroxidase activity in isolated epidermal cells incubated in the presence or absence of the potent tumor promoter-12-tetra-decanoyl phorbol-13-acetate (TPA) and inhibited the decline in the intracellular ratio of reduced (GSH)/oxidized (GSSG) glutathione\textsuperscript{172}. Another mechanism is that garlic and its constituents inhibited the nuclear factor kappa B (NF-kB) activation induced by various receptor agonists including lipopolysaccharide\textsuperscript{173} and tumor necrosis factor α (TNFα)\textsuperscript{174}. In addition to that garlic extracts have the ability to inhibit the Toll-like receptor level. Same way many mechanisms are proposed for the anticancer activity of Allium cepa such as inhibition of cell proliferation, inhibition of protein tyrosine kinase, inhibition of carcinogen activation and modulation of phase II enzyme activity\textsuperscript{175}.

1.2.9.4 Toxicity of Allium Plants (Garlic and Onion)\textsuperscript{176}: LD\textsubscript{50} of the ethanolic extract of Garlic in lab mice was shown to be about 8000mg/kg body weight. The toxic signs during 24 hrs after the oral feeding of the extract were rapid breathing, dullness and then death.

**1.2.9.4.1 Acute toxicity**

Acute toxicity studies show that this drug is safe up to the dose of 3000mg/kg body weight. No mortality was observed on the 14\textsuperscript{th} day of the acute toxicity. In randomized controlled trials side effects in those taking garlic included heartburn, nausea, vomiting, diarrhea, flatulence, bloating, mild orthostatic hypotension, flushing, tachycardia, headache, insomnia, sweating and dizziness as well as offensive odor.

1.2.10 Vitamin E

Vitamin E is a fat soluble vitamin, including eight naturally occurring components essential for health such as to exhibit antioxidant activity and can be stored by the body. The vitamin E such as chroman-6-ols collectively known as toco chromanols include tocopherols and tocotrienols, both are ingested along with fat containing food\textsuperscript{177}. Vitamin E is exclusively synthesized
by photosynthetic eukaryotes and other oxygenic photosynthetic organisms such as cyano bacteria\textsuperscript{178}. Vitamin E is found naturally in some foods. The major food sources of vitamin E are vegetable oils, nut oil seeds, egg yolk, margarine, cheese, soya beans, wheat germ, oat meal, avocados, olives, green leafy vegetables etc\textsuperscript{179}. Vitamin E is an interesting group of compounds, have been able to exert many and different biological activities in plant, animal and human cells. Vitamin E deficiency is rare in humans. Although it may develop in premature infants and in persons with chronic malabsorption of fats, as well as mild anemia, ataxia and pigment changes in the retina\textsuperscript{178}.

\textbf{1.2.10.1 Absorption, Transport and Metabolism}

Vitamin E is a fat soluble vitamin, absorbed from the intestine along the same manner as fat. Nearly all of the vitamin E absorbed across the intestinal mucosa is free tocopherol. Specifically unique tiny spheres with a hydrophilic outer layer called micelles engulf the Vitamin E and help to carry it across the gut. Chylomicrons produced by the small intestine carry these micelles into the lymph. In the lymph the enzyme lipoprotein lipase breaks down the majority of chylomicrons to produce chylomicrons remnants, which is released into the blood stream. The majority of the chylomicron remnants reach the liver which release the vitamin E from the remnants and puts it into the freshly produced VLDL. VLDL is broken down by lipoprotein lipase to produce the LDL. The main carrier of vitamin E in our blood is LDL fraction (55-65 \%). LDL freely exchanges Vitamin E with HDL (24-27\%)\textsuperscript{179}. HDL and LDL have been delivering vitamin E to the tissues.

\textbf{1.2.10.2 Chemistry}

Vitamin E consists of eight different compounds having four tocopherols and four tocotrienols designated as alpha, beta, gamma and delta\textsuperscript{180}. Our dietary food contains all these eight compounds of Vitamin E. Tocopherols and tocotrienols have same basic chemical structure and some differences in their molecules. Both consist of identical head –chromane ring, having an active
antioxidant group viz; phenolic hydroxyl and a phytyl tail for tocopherol. Tocotrienol differ from tocopherol on their tail. Tocotrienol have three unsaturated sites while tocopherols have a saturated side chain. The compounds including α, β, γ and δ homologues differ in number and position of the methyl substituents in the chroman ring. α–tocopherol with three methyl groups in 5, 7, 8 positions is the most active of all homologues. β–tocopherol has 5, 8 dimethyl, γ tocopherol has 7, 8 dimethyl, and δ tocopherol has 8 methyl groups respectively.

### 1.2.10.3 Structure of

![Tocopherol (Vitamin E)](image)

All tocochromanols are amphipathic molecules: the lipophilic isoprenoic side chain is associated to the membrane lipids and the polar chromanol ring is exposed to the membrane surface. The presence of a phenolic hydroxyl group in the tocochromanols showed its antioxidant activity.

### 1.2.10.4 Biological Role of Vitamin E

#### 1.2.10.4.1 Antioxidant activity

Vitamin E is an important component of our antioxidant system. They are the chain breaking antioxidants i.e., they break the chain reaction of lipid per oxidation by neutralizing free radicals and they protect the cell membranes by lipid repair and lipid replacement. In this way by preventing the propagation
of free radicals, vitamin E reduces or prevent many of the diseases related to the cause by the production free radicals viz; heart disease, cataract etc\textsuperscript{181,182}.

1.2.10.4.1.1 Mechanism of prevention of lipid peroxidation

The auto oxidation of membrane lipids proceeds as a chain reaction. It occurs in 3 steps - Initiation, propagation and termination\textsuperscript{183}.

A) Initiation Phase: The primary event is the production of a carbon centered radical (R\textsuperscript{•}) by the interaction of a PUFA with a free radical such as OH\textsuperscript{*}, HO\textsubscript{2}\textsuperscript{*} or O\textsubscript{2}\textsuperscript{-} generated by other means.

\[
R + \text{OH}^* \rightarrow R^* + \text{H}_2\text{O}
\]

(PUFA) (PUFA Radical)

B) Propagation Phase:

The carbon centered radical (R\textsuperscript{*}) rapidly reacts with molecular oxygen forming a peroxyl radical (ROO\textsuperscript{*}), which can attack another polyunsaturated lipid molecule. (RH stands for unsaturated carboxylic acid)

\[
R^* + \text{O}_2 \rightarrow \text{ROO}^* \quad \text{(Peroxyl radical)}
\]

\[
\text{ROO}^* + \text{RH} \rightarrow \text{ROOH} + R^*
\]

\[
\text{ROOH} \rightarrow R_1^* + \text{aldehyde}
\]

R\textsubscript{1}\textsuperscript{*} is shorter than R\textsuperscript{*} in the number of carbons in the chain

There is a simultaneous conversion of a carbon centered radical to a peroxyl radical (ROO\textsuperscript{*}). This would lead to continuous production of hydroperoxide with the consumption of equi molar quantities of PUFA. One free radical generates another free radical in the neighboring molecule.

C) Termination Phase

The reaction would proceed unchecked till a peroxyl radical reacts with another peroxyl radical to form inactive products.
1.2.10.4.2 Reaction of Vitamin E for the Prevention of Free radicals

Vitamin E is a most effective naturally occurring chain breaking antioxidant in tissues; it would intercept the peroxyl free radical and inactivate it before a PUFA can be attacked.

\[
\text{T - OH} + \text{ROO}^\cdot \rightarrow \text{TO}^\cdot + \text{ROOH}
\]

\[
\text{TO}^\cdot \rightarrow \text{An inactive product that decomposes to harmless metabolites.}
\]

The phenolic hydrogen of the alpha tocopherol reacts with the peroxyl radical and converts it into hydro peroxide product. The tocoperoxyl radical thus formed is stable and cannot propagate the cycle further but the tocoperoxyl radical can react with another peroxyl radical to convert it into inactive product.

1.2.10.4.3 Prevention of Cardiovascular Disease

Oxidation of LDL cholesterol initiates the development of atherosclerotic plaque. Vitamin E might help to prevent the formation of blood clot that could lead to a heart attack or venous thrombo embolism\(^{184}\). Vitamin E inhibits the oxidation of LDL cholesterol, which is if accumulated is believed to be the first step in atherosclerosis.

1.2.10.4.4 Prevention of Cancer

Unchecked free radical production leads to the development of cancer. Vitamin E might block the formation of carcinogenic nitrosamines formed in
the stomach from nitrites in food. Vitamin E enhances immune function and protect against cancer. Tocotrienols showed anti proliferative and apoptotic activities on normal and cancer human cells\textsuperscript{185}. The mechanism was related to induction of apoptosis mainly via mitochondria – mediated pathway and to cell cycle arrest due to the suppression of cyclin D by vitamin E\textsuperscript{186}. Vitamin E also inhibits vascularization - reducing proliferation and malignant proliferation.

\textbf{1.2.10.4.5 Prevention of alzheimer’s Disease}

Oxidative stress leads to the development of alzheimer’s disease. Antioxidants such as vitamin E prevent oxidative stress in most of the studies. Allium principles retard aging and promote healthy growth of cells\textsuperscript{187}.

\textbf{1.2.10.4.6 Protection against xenobiotics}

Vitamin E is the most potent endogenous antioxidants against metal poisoning and other xenobiotics. In arsenic intoxicated animals\textsuperscript{188} the supplementation of vitamin C and α-tocopherol has been found to alter the extent of DNA damage by reducing TNF-α level and inhibiting the activation of caspase cascade. Supplementation of vitamin E to lead treated erythrocytes prevent the inhibition of δ-ALAD activity and lipid oxidation\textsuperscript{189} both \textit{in vivo} and \textit{in vitro}. Vitamin E protects membrane lipids and notably prevents protein oxidation produced by lead intoxication. Similar effects were observed in our studies also on supplementing the food of rats subjected to lead toxicity with garlic and onion oil polar and non polar fraction (paper sent for publication).

\textbf{1.2.10.4.7 Other roles of vitamin E}

Vitamin E protects skin directly from the UV-radiation. Vitamin E increased the power of disease fighting T - cells, improved delayed type hypersensitivity skin response. Vitamin E protect against developing macular degeneration\textsuperscript{190}. 
1.2.10.5 Safe and effective use levels\textsuperscript{190}

Vitamin E from our diet is less than 15mg/day. Considerably higher doses are believed to be necessary for prevention of disease and promotion of wellness. The most common supplemental doses are 100, 200, 400 and 800 IU.

1.2.10.6 Toxicology

1.2.10.6.1 Acute toxicity

Vitamin E has a very low acute oral toxicity. The LD\textsubscript{50} for α - tocopherol is >2000mg/kg body weight in adult and neonate rats\textsuperscript{191}.

1.2.10.6.2 Sub-chronic toxicity

α-tocopheryl acetate in rats at doses of 125-2000mg/kg/body weight/day, elevated TSH levels by 30 to 100%. At a dose of about 500mg/kg/body weight / day biochemical indices of hepato toxicity such as serum ALP, ALT and AST were elevated and liver weight was increased\textsuperscript{192}.

1.2.10.6.3 Chronic toxicity

Wheldon et al\textsuperscript{193} studied two long term studies up to 16 months and 2 years duration respectively in rats. In the second of these studies, the rats were received at the doses 500, 1000 or 2000mg/dl and α - tocopheryl acetate /kg/body weight/ day. All dose levels between 15 and 18 weeks the rats developed spontaneous hemorrhages in the gut, urinary tract, meninges, and orbit at sites of minor injury.