Chapter 1

INTRODUCTION
1.1: Ayurveda and Heavy Metal Toxicity

People all over the world have an unbreakable trust in Ayurveda, because Ayurveda is a healing system considered being the oldest in the world, and it traces its roots to the Vedic period in ancient India. The name comes from the Sanskrit ‘ayur’ (life) and ‘veda’ (knowledge). It is a holistic system that includes the harmonious balance of mind and body, environment and behavior, spiritual healing, and herbalism. This system of medicine flourished through religious beliefs, spirituality and culture, rather than logical thoughts and experiments.¹ The use of plants is as old as human kind and it has been steadily increasing over the past 10 years. Plant-based remedies are now one of the most popular complementary treatments. Herbal supplements are receiving increasing exposure through media, including the internet, in lay journals and more recently in the scientific press. Interest in herbal medicine has been facilitated by multiple factors, including the perception that pharmaceutical medications are expensive, over prescribed and may often be dangerous. Alternatively, herbal medicine is often perceived as being "natural" and therefore is considered to be safe. However, the scientific literature supporting the efficacy of herbal therapies is incomplete.²

According to the principles of Ayurveda, the ancient science, Ayurvedic preparations are absolutely safe, free from side effects and residual toxicity. The Ayurvedic tradition considers all substances, whether they come from animals, vegetables, or the earth (minerals) as medicines, provided they are applied in a proper way and for specific purposes. Moreover Ayurveda claims that, ‘through Ayurvedic processing even a potent toxic substance can be converted into healing nectar’. So Ayurveda prepares medicines from herbs, animal products, minerals, and heavy metals as per the recipe and protocols mentioned in different classical Ayurvedic texts. What ever may be the claims and clarifications regarding Ayurveda, it is true that some health
professionals worldwide are worried about the presence of heavy metals in certain Ayurvedic preparations.³

In the present time heavy metal toxicity through Ayurvedic, Siddha or patented herbal drugs is an unnoticed but a prevailing problem in India and other countries. Ayurvedic medicine originated in India more than 2000 years ago and it relies heavily on herbal medicinal products (HMPs). These herbal preparations are made from bio-derived substances (plants and animals) and non-biologically derived substances like metals and non-metals.⁴ Ayurveda experts estimated that 35% to 40% of the approximately 6000 medicines in the Ayurvedic formulary intentionally contain at least 1 metal. The important elements used in Ayurveda and patented herbal drug industry are mercury, lead, tin, cadmium, zinc, copper, silver, iron, gold, sulphur, antimony and arsenic. Approximately 80% of India’s one billion populations use at least one or the other product of Ayurveda through more than one-half million Ayurvedic practitioners working in 2860 Ayurvedic hospitals and 22100 clinics.⁵

Mercury and lead in Ayurvedic medicines are, deliberately included for gaining therapeutic effect based on Ayurvedic Formulary of India. The most used heavy metals in Ayurveda are mercury and lead.⁶ Reports from the U. S., Canada, U.K., Australia, Germany, Holland, Italy, Poland and Croatia indicate that some Ayurvedic preparations contain excess amounts of mercury, lead, arsenic and cadmium, resulting in patients with severe toxicity symptoms. During the first decades of twentieth century extensive research studies have been done in Europe in experimental physiology and pharmacology with logical scientific approach.⁷ Based on these studies, many spurious metal and non-metal based drugs have been removed from modern medicine.
Nowadays the Ayurvedic medicines are very popular in the western world and many heavy metal toxicity cases have been reported. In a study conducted by Saper et al.,\textsuperscript{8} using 70 Herbal Medicinal Products (HMPs) reported that 20% of them contained either mercury or lead. The users of these HMPs later manifested the typical symptoms of lead and mercury toxicity.\textsuperscript{9} The 2003 annual report of the American Association of Poison Control Centers’ Toxic Exposure Surveillance System documented 3362 exposures to mercury or compounds containing mercury.\textsuperscript{10} Many of these victims consumed imported herbal preparations from different Asian countries. Similar study reports on heavy metal toxicity through Ayurvedic/patented herbal drugs are not available in India.

Ayurvedic remedies are available in general pharmacies, Ayurvedic shops, internets, supermarkets and even in village grocery shops as ‘Over The Counter’(OTC) drugs. Because many of the Ayurvedic preparations are marketed as dietary supplements, as aphrodisiacal drugs or as antioxidants, they are not regulated under Drug Controlling Act. Even in United States of America, the marketing of Herbal Medicine Products are regulated under the Dietary Supplement Health and Education Association (DSHEA), which does not require proof of safety or efficacy.\textsuperscript{11}

Mercury and lead toxicity by consuming Ayurvedic or patented herbal drugs have been noticed at different places of the world. Mercury poisoning generally manifest with stomatitis, colitis, progressive renal damage, anemia, and peripheral neuritis. The central nervous system is also affected by behavioral changes, mental depression, insomnia, and occasional hallucinations. In the case of lead toxicity the following symptoms are very common; status epilepticus, fatal infant encephalopathy, congenital paralysis and sensory neural deafness, and developmental delay.\textsuperscript{12} Since 1978 at least
55 cases of heavy metal intoxication associated with Ayurvedic preparations in adults and children have been reported in the United States.\textsuperscript{13}

1.1.1: Mercury Toxicity

Mercury was probably first used by man in the form of the sulphide ore, cinnabar, as a source of red pigment for his early artistic efforts. The extended use of mercury compounds in medicaments and amalgams is recorded by the Romans prior to and following the time of Christ. The early Hindu wise men thought that mercury had aphrodisiacal properties. The sages of ancient China thought it to have immortal attributes. The Arabian and European alchemists considered it to be one of the two “contraries”, the other “contrary” being sulphur, from which all other elements were believed to emerge. The alchemists named the liquid metal after the Fleet- Footed Greek God; Mercury.\textsuperscript{14}

Mercury was used similarly in the Greco-Roman world, with both Hippocrates and Galen recording its toxic effects. Since then, its toxicity has become well known among metal workers, miners, felt-hat manufacturers, dyers and paint manufacturers. Despite this, mercury has been incorporated into the treatment of man’s maladies from ancient times. Its main use has been to treat syphilis, from its first appearance in the West in the 15th century up to World War-II. Mercury and its salts have at various times been used as antiseptics, skin ointments, laxatives, diuretics, bowel washouts, for the treatment of colorectal cancer, and scabicides. It is still used today as a solvent for the silver-tin amalgams used in dental fillings.\textsuperscript{15}

Mercury is a major toxic metal ranked eighth in the ‘Toxic Substances List’ prepared under the Canadian Environmental Protection Act.\textsuperscript{16} The normal level of mercury in human blood is less than 1mg/dl. When it is increased to 2-5 mg/dl symptoms of toxicity appear and a mercury level
above 15mg/dl is fatal. The classical triad of chronic elemental mercury poisoning is (1) oral lesions (gingivitis, salivation and stomatitis), (2) tremor and (3) psychological changes (insomnia, shyness, emotional instability, memory loss). This triad of symptoms is called erethism.\(^1\) A second danger from metallic mercury is that it is bio-transformed into organic mercury, by bacteria at the bottom of lakes and in the intestine. The organic mercury thus formed in the bottom of the sea can be passed along the food chain and eventually to man. It was this process that led to the Japanese tragedy at Minamata bay in the late 1950s.\(^1\)

As time and science progressed, the various compounds of mercury, both inorganic and organic, were developed. Their usages in industry, agriculture, medicine and veterinary medicine gradually increased and resulted in serious environmental pollution and mercury toxicity in human beings and animals. So the usage of mercury and its compounds in medicine, veterinary science and agriculture are gradually being replaced by other compounds, except in Ayurveda and other traditional systems of medicines in India and other Asian countries. The United States Environmental Protection Agency (USEPA) banned the use of mercurials even in pesticides and fungicides in 1972.\(^1\)

The persistent presence of mercury in the environment naturally leads to the bio-accumulation and transport in the aquatic chain, and levels in variety of food make mercury among the most dangerous of all metals in the human food chain. The toxicological effects and relevant human exposures of mercury have been illustrated over the past few centuries. On the basis of toxicological characteristics, there are three forms of mercury: elemental, inorganic and organic compounds. Metallic mercury may be oxidized to inorganic divalent mercury, particularly in the presence of organic material formed in the aquatic environment. Methyl mercury, an important form of
organic mercury, can be taken up by fishes and eventually consumed by humans.\textsuperscript{20}

In the recorded history of mercury poisoning Minamata incident stands out as a milestone. The first heavy metal epidemic, Minamata disease, was caused by the consumption of shell fish from water that was heavily contaminated by waste water containing methyl mercury chloride and metallic mercury from a chemical plant. This disease was first observed in the communities near Minamata bay in south-western Japan; hence the disease is called Minamata disease. One hundred and twenty-one persons were poisoned, forty six fatally affected, from eating the contaminated fish.\textsuperscript{21} Dogs, cats, pigs, rats, and birds living around the bay developed classical clinical signs and many of them died.\textsuperscript{22,23} Methyl mercury has been extracted in crystalline form from shell fish, which had caused Minamata disease. The levels of methyl mercury chloride reached 50 ppm in fish and 85 ppm in shell fish obtained from the contaminated areas.

Irukayama\textsuperscript{24} detected methyl mercury directly from the sledge of Minamata bay. In Minamata patients, the mercury content in their hair was noted as 96.8-705 ppm.\textsuperscript{25} High concentrations of mercury were found in the internal organs of those died of Minamata disease; higher amount was detected in kidney and liver.\textsuperscript{26} Although the amount in the brain was less than that in the liver and kidneys, it is characteristic of methyl mercury poisoning that the amount deposited in the brain is higher than that seen in the case of inorganic mercury poisoning.\textsuperscript{27} Fujiki et al.,\textsuperscript{28} have estimated the mercury content in the hair of inhabitants in the Minamata area averaged 2.1 ppm to 14.82 ppm. This is slightly higher than the level found in other places of Japan.

The symptoms of Minamata disease began with numbness of the limbs (Figure-1) and peri-oral area, sensory disturbances and difficulty with hand
movements such as grasping things, fastening buttons, holding things, writing etc.... The lack of mental co-ordination, weakness and tremor, dysarthria (speech slow and slurred) and ataxic gait followed by disturbances of vision and hearing were also reported.\textsuperscript{29} These symptoms became aggravated and led to general paralysis, deformity of limbs, difficulty in deglutition, convulsions and death, the most typical cases presented the so called Hunter-Russel Syndrome.\textsuperscript{30}

**Figure-1.1.2: Deformity of hand in Minamata patient**

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{hand_deformity.png}
\caption{Deformity of hand in Minamata patient}
\end{figure}

1.1.3: Acute and Chronic Poisoning by Organic Mercurials

Methyl mercury compounds appear to be the most toxic of the mercurials, tending to be retained in the body, especially in the brain.\textsuperscript{31} The efficient absorption of methyl mercuric compounds from food may be due to the lipid solubility of their chloride complexes.\textsuperscript{32} When methyl mercury enters the body in large quantities there are symptoms of acute brain damage such as aberrations of consciousness, convulsions and paralysis, followed by death. If the intake mercury is lower, chronic symptoms are manifested, which include mental retardation, cerebral palsy, seizures, chorea, tremors, cataracts, small
body size, anorexia, and renal dysfunction.\textsuperscript{33,34} Pyramidal dysfunction symptoms like muscular atrophy and mental disturbances are prominent. Still lower dozes of methyl mercury cause mild, atypical or incomplete cases as seen in chronic Minamata diseases or may appear as a nonspecific disease.\textsuperscript{35} The other effects seen from poisoning organic mercury include cardiac arrhythmias, hepatic dysfunctions like elevation of bilirubin and liver enzymes, respiratory tract irritation, and blistering of the skin.\textsuperscript{36}

Berlin et al.,\textsuperscript{37} have reported the severity of methyl mercury as it can pass through blood brain barrier and accumulate in the human brain, thus being neurotoxic in both adult and fetus. The neurotoxic effect of methyl mercury may be due to the decreased glutamate uptake by astrocytes in brain and spinal cord, as demonstrated in cell culture experiments.\textsuperscript{38} Methyl mercury can affect the neurons by catalyzed hydrolysis of phospholipids composing the cell membrane. Chronic ethyl mercury poisoning leads to the manifestation of the following clinical symptoms like frequent polydypsia, polyuria, weight loss, emaciation, severe proteinuria, alopecia, pruritis of the palms, soles, anus and genitals.\textsuperscript{39} The combined nephrotoxic effects of methyl mercury and ethanol in acute and chronic conditions have been reported in rats as increased urea, creatinine and uric acid levels in the serum.\textsuperscript{40}

The clinical characteristics of congenital Minamata diseased patients were serious mental retardation, primitive reflex such as grasping reflex, cerebellar symptoms such as instability of neck, ataxia adiadochokinesis, dysmetria, intension tremor, dysarthria and nystagmus. They also manifested disturbance of gait like akinesia, hypokinesia and hyperkinesias (chorea, athetosis, etc...), hyper-salivation, character disorder, paroxysmal symptoms (such as generalized tonic convulsions, loss of consciousness, myoclonic jerk, etc...), and also strabismus, deformity of limbs, and pyramidal symptoms.\textsuperscript{41} The minimum lethal doze for methyl
Mercury is 3.92 mg per kg and the half-life period of methyl mercury was found to be calculated as seventy days.\textsuperscript{42} The biochemical studies on Minamata diseased patients revealed the disturbances in liver function and abnormalities in renal function. Diabetes and hypertension are abnormally prevalent in chronic mercury poisoning cases.\textsuperscript{43}

Toxicological studies on certain animals revealed the effect of mercury poisoning. Prolonged ingestion of a low level of inorganic mercury can result in a sub-acute or chronic syndrome of mercury poisoning. The animal that is thus exposed accumulates mercury due to the slow excretion rate. Sub-lethal exposure can result in excessive salivation, anorexia, oliguria, and a foul breath. The animal becomes emaciated and depressed, has a stiff-legged walk, and is weak. If the animal survived for a week or more, it developed generalized alopecia with scabby lesions around the mouth, anus and vulva. The animal develops a pruritis, and the gingival tissues become tender and inflamed, which can result in loosening and shedding of teeth. The animal has a chronic diarrhea, which does not respond to treatment, renal insufficiency start with oliguria and finally leads to anuria.\textsuperscript{43} Mercury poisoning also inhibits the activity of liver tyrosine aminotransferase enzyme in rats.\textsuperscript{44} In this way mercury poisoning affects liver enzyme production and activity.

There are four liver enzymes that are commonly used in the diagnosis of liver diseases. They are aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), alkaline phosphatase (ALP) and \(\gamma\)-glutamyltransferase (GGT). ALT and GGT are present in several tissues, but plasma activities primarily reflect liver injury. AST is found in liver, muscle and to a limited extent in red blood cells. Bone and liver are good sources of ALP in normal individuals, though it is seen in a number of other tissues. Based on tissue distribution, ALT and GGT would seem to be the most specific markers of liver injury.\textsuperscript{45}
Injury to liver, whether acute or chronic by toxic substances, eventually results in an increase in serum concentrations of aminotransferases. AST and ALT are enzymes that catalyze the transfer of \( \alpha \)-amino groups from aspartate and alanine to the \( \alpha \)-keto group of ketoglutaric acid to generate oxaloacetic and pyruvic acids respectively, which are important contributors to the citric acid cycle.\(^{46}\) The elevation of AST and ALT has clinical relevance mostly in the case of alcoholic liver disease rather than toxic liver disease.\(^{47}\)

The abnormal elevation of liver enzymes such as ALT (Alanine amino transferase), AST (Aspartate aminotransferase) and ALP (Alkaline phosphatase) can be taken as an index for liver injury or diseases. ALT is an enzyme normally present in the serum and tissues of the body, especially the tissues of the liver. The enzyme is released into the serum because of tissue injury and may increase in persons with acute liver damage. This enzyme usually rises higher than aspartate aminotransferase in liver disease with moderate increases (up to 10 times normal) in cirrhosis, infections, or tumors and increases up to 100 times normal in viral or toxic hepatitis. AST is an enzyme normally present in muscles, serum and in certain body tissues, especially those of heart and liver. The enzyme is released into serum because of tissue injury and thus may increase as a result of myocardial infarction and liver damage. ALP is an enzyme widely distributed in the body, especially in bone and liver ducts. Serum ALP levels may greatly increase with liver tumors and lesions, and may show a moderate increase with diseases such as hepatitis. The serum ALP level is normally 20-30 IU/L.\(^{48}\)

1.1.4: Pathological Findings of Minamata Disease

1. Changes in the cerebellum: Granular-cell-type cerebellar atrophy was seen. The disintegration following diffuse loss of granular cells was most severe, while the Purkinje’s cells were spared.
2. Focal changes in the cerebral cortex: In all cases the cortex of the calcarine region (area striata) was most grossly damaged in both the hemispheres; changes essentially similar to those in the calcarine regions of the pre-central cortex, post-central cortex, temporal transverse cortex, temporal cortex, and insular cortex were often found.

3. Slight changes in the cerebral nuclei: In the cerebral nuclei, brain stem and cord, relatively less severe changes were observed (in acute or sub acute cases).

4. Changes in the peripheral nerve: The selective destruction of sensory peripheral nerve fibers with de-myelination was observed.

5. Changes in other organs: In acute and sub acute cases, hypoplasia and aplasia of the bone marrow and hypoplasia of lymph nodes. Round cell infiltrations in the interstitial spaces with fatty degeneration of parenchymatous cells of liver and kidney were found.

Patho-physiological findings in Minamata diseased patients by autopsy have shown damage by methyl mercury to the liver, kidney, pancreas, and bone marrow. During chronic organic mercury poisoning, marked elevation of mercury levels were noted in urine but blood electrolytes, calcium, magnesium, glucose etc. remained normal for the first six weeks. This phenomenon has been demonstrated in experimentation in animals.

The reproductive system is sensitive to many toxic substances. Directly or indirectly, they affect on reproductive system: indirectly by affecting hormones; directly by affecting the egg, sperm and supporting structures or tissues. The main clinical manifestation of reproductive toxicity is infertility or sterility. The metals and trace elements which cause sterility are arsenic, lead, lithium, mercury, nickel and selenium. Harada has also reported the teratogenic effect of methyl mercury. When a female’s intake of poison is
high and she becomes ill with Minamata disease, pregnancy does not occur. When the dose is smaller, pregnancy will occur but the fetus is aborted spontaneously or fetus becomes a still born. An even smaller doze permits conception and birth, but the baby will suffer from congenital Minamata disease with severe neurological symptoms. Methyl mercury has been listed as one of the six most dangerous chemicals in the environment. The absence of blood brain barrier system against the transportation of methyl mercury to brain tissues may be the reason for consequent nervous malfunctions during methyl mercury poisoning.\textsuperscript{53} The neuropsychological disorders in children due to methyl mercury poisoning by consuming whale flesh were studied in Faeroe Islands.\textsuperscript{54} The teratogenic effects of methyl mercury in young women, pregnant women, infants and young children were also studied in the ocean fish eating community in Seychelles.\textsuperscript{55} Samhitha\textsuperscript{56} had studied about the susceptibility of human infants to heavy metal toxins. As a result of rapid growth, immaturity of kidneys, liver and vulnerability of the myelinising in central nervous system, infants are found more prone to heavy metals in their first year of life.

Animal exposure to mercury is a well studied topic in Japan. In the coastal area of Minamata, cats and dogs and some pigs went mad and died. The inhabitants called this striking malady the “dancing–cat disease”.\textsuperscript{57} Cats in the area that were spontaneously affected with Minamata disease (or dancing disease), and others in which the conditions had been induced experimentally by a diet of shellfish from Minamata bay, showed the following localized mercury levels: liver, 37.0-145.5ppm (as against 0.9-3.66ppm in controls) ; brain, 21.5-70.0 ppm (0.09-0.82ppm) ; and hair, 21.5-70.0 ppm (0.51-2.12 ppm).\textsuperscript{25}
1.1.5: Acute and Chronic Poisoning by Inorganic Mercurials

Acute mercury poisoning is commonly caused by inorganic mercury compounds (mercuric chloride, mercuric sulphide etc...) taken either with suicidal intent or accidentally. The characteristic clinical sign observed during acute inorganic mercury poisoning is violent abdominal pains with blood stained diarrhea. The inorganic mercurials have a coagulative necrotizing effect on the alimentary mucosa and the blood vascular system that causes an extensive hemorrhagic gastroenteritis and blood loss, which can lead to shock collapse and ultimately to death.\(^{58}\)

Among higher vertebrates including humans, inorganic and alkoxyalkyl compounds cause kidney damage, which usually leads to death. The uptake of inorganic mercury by kidney cells suggests that active transport is involved, but mostly by diffusion.\(^{59}\) Mercury reaches its highest concentration at this site, and the kidney may show evidence of disturbed function within a few minutes after the poison reaches the circulation. The affinity of mercury ions for thiol groups accounts for the accumulation of large amounts of inorganic mercury in the kidneys. There has been evidence that some damage may occur to the extent that both renin-angiotensin-1-converting enzyme activities were reduced and may modify systemic hemodynamics.\(^{60}\)

Mercury ion is known to promote oxidation of kidney cells and to disrupt renal mitochondrial function. The increased \(\text{H}_2\text{O}_2\) production by rat renal mitochondria is an indirect effect of inorganic mercury. The first response of the kidney may be a diuresis due to suppression of tubular reabsorptive function; soon the renal damage becomes so extensive and that results in oliguria and finally anuria. Renal lesions produced by mercury are confined largely to the tubular epithelium but the glomeruli are also injured.\(^{38}\) Excessive exposure to inorganic mercury compounds either through
inhalation of elemental mercury vapour, ingestion of divalent mercury salts, or the use of skin-lightening cosmetics containing inorganic mercury may entail a nephrotic syndrome (severe albuminuria) or an acute tubular necrosis which result elevation of blood urea nitrogen, creatinine and uric acid in the serum.\textsuperscript{61,62}

Chronic mercury poisoning means the exposure of small amount of mercury over an extended period of time. It is encountered in industries which utilize mercury or its salts.\textsuperscript{63} Chronic mercury toxicity also may result from mercury medication. The sign and symptoms of chronic inorganic poisoning are characterized as stomatitis, colitis, progressive renal damage, anemia, and peripheral neuritis. The commonest sign of progressive renal damage is proteinuria, reflecting glomerular damage. Hypercellular glomerulitis in the cortical region is a general characteristic, in which proliferation of mesengial cells or endothelial cells or polymorphonuclear neutrophils may occur in this condition.\textsuperscript{64} With high doses of inorganic mercury, a ‘Frank nephritic syndrome’ can develop, that has been seen both in industry and medicine.\textsuperscript{65}

El-Demerdash\textsuperscript{66} has reported the toxic effect of inorganic mercury in rats; mercuric chloride has been administered for five consecutive days at a doze of 0.5 μmol Hg/ml. The following biochemical alterations had occurred:
1) Decreased protein contents in brain, serum and liver. 2) Decreased acetylcholine-esterase (AChE) in brain and serum. 3) Decreased glutathione S-transferase (GST) activities in plasma and liver. 4) Increased acid and alkaline phosphatases (ACP and ALP), aspartate and alanine aminotransferase (AST, ALT) in serum and liver. 5) Increased lactate dehydrogenase (LDH) in plasma, brain and liver.

In acute inorganic mercury poisoning, Cassidy et al.\textsuperscript{43} reported that, the pathological symptoms are consistent with severe hemorrhagic gastro enteritis, edema, hyperemia and petechial hemorrhages. In addition to these
symptoms, the internal organs like liver and kidney are found swollen with congested lungs. Histologically, the alimentary tract will show extensive areas of coagulative necrosis, and kidney will contain advanced degenerative lesions of coagulative necrosis. The laboratory analysis of kidney and liver tissues revealed the presence of mercury. The highest level is noticed in kidney especially in the cortical region. The level of mercury in kidney tissue will generally be above 10 ppm on a wet weight basis. Actually any level of mercury above 3 ppm should be viewed with suspicion if the clinical signs and lesions are compatible with mercury poisoning. Mercury level above 100 ppm/L in the urine are indicative of an undue exposure. The analysis of urine provides diagnostic criteria to evaluate a low level exposure to aid in diagnosis.

In another avian study it is reported that, birds fed with inorganic mercury (mercuric chloride) showed a great reduction in food intake (anorexia) and consequent poor growth and weight loss. Increased liver enzyme production, decreased cardiovascular function, blood parameter changes like low blood cell count (RBC and WBC), hyper immune response, abnormal kidney function and structure, and behavioral changes were also reported in the same study.67

1.1.6: Mercury Vapour Poisoning

Acute toxicity of elemental mercury vapor causes symptoms within several hours such as weakness, chills, metallic taste, nausea, vomiting, diarrhea, dyspnoea, cough and feeling of tightness in the chest. Pulmonary toxicity may progress to interstitial pneumonitis with severe symptoms of respiratory dysfunction.68 Mercury vapour is readily absorbed from the lungs. Common feature of intoxication from mercury vapour is severe salivation and gingivitis. Renal dysfunction is a major problem in chronic exposure. It also manifests with goiter and increased uptake of radioiodine by thyroid,
tachycardia, labile pulse, gingivitis, dermographia and increased measure of mercury in urine.\textsuperscript{69}

Chronic mercury vapour poisoning strikingly affects the central nervous system and kidney. Tremor, initially involving facial muscles and eyelids, are present at rest, but aggravated by intention. It gradually becomes more pronounced and also starts to affect the limbs. Handwriting becomes illegible, with omission of letters and eventually whole words; erethism is manifested as excessive shyness, loss of confidence, vague fears, irritability, insecurity, and suicidal melancholia. Patient becomes unable to perform simple tasks such as dressing.\textsuperscript{70} If the exposure continues, tremor becomes severe and psychological changes manifest like depression, irritability, excessive shyness, insomnia, emotional instability, loss of memory, confusion and vasomotor disturbances such as excessive perspiration and uncontrolled blushing.\textsuperscript{71} Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic mercury and organo-selenium compounds have also been reported.\textsuperscript{72}

1.1.7: Biological Conversion of Inorganic Mercury to Organic Mercury

Mercury compounds are chemically classified as inorganic either in the form of the elemental metal or as salts in the mercurous or mercuric state. The organic compounds are those associated with carbon in their molecular structure.\textsuperscript{73} Mercury in elemental form, in liquid state is nontoxic, and a human being might ingest up to a pound without significant adverse effects.\textsuperscript{74} But, Lin et al.,\textsuperscript{75} have reported the toxic effect of elemental mercury in several case studies; 1) An individual with 30 years of experience in repairing mercury based sphygmomanometers was manifested with hand tremors. 2) In another case, one person intentionally ingested 220ml, approximately 3 kg of metallic mercury. For expelling mercury through feces took two weeks and noted 1000 ppm urine mercury levels. After six months he was admitted in
the hospital for glycemic control with jaundice and with impaired liver function.

The organic mercury can be originated from elemental mercury and inorganic mercury compounds. That happened in Minamata bay; the factory affluent reached in the bay contained both methyl mercury and elemental mercury. The elemental mercury was, subsequently, methylated by microorganisms in the mud on the bottom of the bay. Jensen et al., and Jonnalagadda have reported that the microorganisms were capable of methylating inorganic mercury. Wood et al., demonstrated that biological methylation of inorganic mercury was an enzymatic process involving vitamin B-12. He proved it by an experiment in which extracts from methane forming bacterium isolated from a culture of \textit{Methanobacterium emelenskii} and \textit{invitro} solutions of methyl cobalamine were able to methylate inorganic mercury to organic mercury. All the form of mercury appears to be directly or indirectly capable of conversion to organic mercury. In Minamata the level of methyl mercury chloride reached 50 ppm in fish and 85 ppm in shellfish obtained from inorganic mercury contaminated area, which indicates the bio-conversion of inorganic mercury to organic mercury.

The process by which the inorganic mercury changes into organic mercury was still unknown at that time. The microbial conversions of inorganic mercury to organic forms were experimentally proved by using \textit{Neurospora crassa} and \textit{Clostridium cochlearis} respectively. As far as the ingestion of inorganic mercury in human beings are concerned, any inorganic compound of mercury can be changed into an organic form during digestive process and also by the action of intestinal commensals. Under environmental condition, \textit{methanogens} in the soil and in the bottom of the river or water source with ample organic matter can change inorganic mercury to organic mercury, which might have happened in Minamata although the pollutant was
partially inorganic mercury. So it can be concluded that even inorganic mercury compounds intake may lead to organic mercury poisoning.

1.1.8: Presence of Mercury in food

Normal human diets generally contain less than 50μg mercury/kg food. The possibility of ingesting inorganic mercury through daily food is very rare; the daily intake is estimated to be below 1μg/day. In India sufficient studies on the mercury concentrations in food items are not available, but in USA, UK and Japan such studies are plenty. In the absence of gross contamination of soil or irrigation water, some of the commonly found mercury values for various food and food products are summarized below.

Table-1.1.9: Levels of mercury residues in food in several countries

<table>
<thead>
<tr>
<th>Foods</th>
<th>USA, μg/kg</th>
<th>UK, μg/kg</th>
<th>Japan, μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereal</td>
<td>2-2.5</td>
<td>5</td>
<td>12-48</td>
</tr>
<tr>
<td>Bread&amp;flour</td>
<td>20</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Meats(^a)</td>
<td>1-50</td>
<td>10-40</td>
<td>310-360</td>
</tr>
<tr>
<td>Fish(^b)</td>
<td>0-60</td>
<td>70-80</td>
<td>35-540</td>
</tr>
<tr>
<td>Milk</td>
<td>8</td>
<td>10</td>
<td>3-7</td>
</tr>
<tr>
<td>Cheese</td>
<td>80</td>
<td>170</td>
<td>----</td>
</tr>
<tr>
<td>Butter</td>
<td>140</td>
<td>10</td>
<td>---</td>
</tr>
<tr>
<td>Fruits</td>
<td>4-30</td>
<td>10-40</td>
<td>18</td>
</tr>
<tr>
<td>Fresh vegetables</td>
<td>0-20</td>
<td>10-25</td>
<td>30-60</td>
</tr>
<tr>
<td>Canned vegetables</td>
<td>2-7</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Egg white</td>
<td>10</td>
<td>ND</td>
<td>80-125</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>62</td>
<td>-----</td>
<td>330-670</td>
</tr>
<tr>
<td>Beer</td>
<td>4</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\)Includes beef, pork, beef liver, canned meats, and sausages.
\(^b\)Includes canned salmon, shellfish, and white fish.

Source compiled from Janssen\(^86\) and Concon.\(^87\)
1.1.10: Fate of Ingested Mercury in the Biological System

In a biological system, mercury forms a covalent bond with sulphur and accounts for its potent biological activity. The mercuric ion readily reacts with sulphydryl groups to form mercaptides. Therefore, mercurials even in very low concentration are capable of inactivating sulphydryl enzymes and in this way interfere with cellular metabolism and function.\textsuperscript{88} The affinity of mercury for thiol groups provides with the basis of treatment of mercury poisoning by dimercaprol and pencillamine. Mercury also combines with ligands of physiological importance like phosphoryl, carbonyl and amine groups.\textsuperscript{89} Absorption of mercury vapours by inhalation readily occurs, as mercury vapour freely crosses the alveolar membrane.\textsuperscript{90} 100% bio-availability is reported for absorbed mercury vapour. The absorbed mercury vapours are oxidized to divalent mercuric cations by catalase in the erythrocytes. Neurotoxicity is more common with vapor of mercury (elemental mercury).\textsuperscript{91}

Studies on mice revealed that, after mercury administration, the metal is found in the tissues in the following order of decreasing concentration: kidney, liver, spleen, intestinal wall, heart, skeletal muscle, lung and brain.\textsuperscript{92} Kidney is the major site of mercury deposition. As the mercury content of other tissues decreases after the first day of administration, that of the kidney increases for about 2 weeks to about 85 to 90\% of the total body burden. The mercury in the kidney is excreted via the urine at a rate of about 1\% of the body burden per day. Particularly large amounts of mercury do not appear in the liver. By the end of 2 weeks, as in the intravenous studies, most of the mercury is localized in the kidney.\textsuperscript{93}

The ingested soluble inorganic mercury (Hg\textsuperscript{2+}) gains access to the blood circulation and which can be quantitatively assessed as blood mercury level (BML). During mercury poisoning, BML is considered as an index of mercury absorption.\textsuperscript{92} GI tract absorption for inorganic mercury is 10\%. The
inorganic mercury does not cross blood brain barrier. Intestinal absorption ranges from 90% for methyl mercury in man; in rat it is about 50% for mercuric acetate, and 50-80% for phenyl mercury acetate. The inorganic mercury poisoning through local application of mercuric chloride in cattle is almost similar to organic mercury poisoning. For practical purposes, ingestion of oral elemental mercury as a single dose poses a negligible risk of severe toxicity. The oral LD$_{10}$ is reported to be 1429 mg/kg (in man), or approximately 100 g for a 70 kg adult. Percutaneous absorption is also low (approximately 2% of the rate of uptake by the lung).

The ingested elemental mercury is having low absorption rate from G I tract. It forms metal droplets, and is unable to react with bio-molecules. But inhaled mercury vapor is completely absorbed by the lung and is then oxidized to divalent mercuric cation by the catalase enzymes in the erythrocyte. A significant amount of mercury vapour crosses blood brain barrier and enters brain and causes CNS toxicity. Mercury is a cumulative poison and is stored mainly in the liver and kidney. The level of accumulation depends on the type of organism and the chemical form of mercury. The LD$_{50}$ for mercuric chloride in man is 37 mg/kg. When the mercurials enter the blood stream, the organic forms are bound mainly to the RBC, while inorganic mercurials are bound chiefly to thiol molecules of serum proteins.

Less soluble inorganic salt like calomel (mercuric chloride) undergoes oxidation to highly soluble mercurial compounds. The highest concentration of inorganic mercury is found in the kidney and retained longer than in other tissues. Concentration of inorganic mercurial is similar in whole blood and plasma. Mercury excretion from the body starts almost immediately after absorption, following a variety of routes, though principally by the kidneys. The renal excretion is around 15% of the mercury load. Inorganic mercury has a half-life period of 30-60 days in the kidney. Mercury deposited within
the brain has an elimination half-life that may exceed several years. In rats, the highest concentrations of mercury were found in the Purkinje cells of the cerebellum and in certain neurons of the spinal cord and midbrain.

1.1.1: Physical and Chemical Properties of Mercury

The metallic form has the characteristics of being the only metal that is a liquid at ordinary room temperature and high density of 13.59. It is silvery white mobile very heavy liquid, which at -38.87°C solidifies to a thin white malleable mass and which can be cut with a knife. Mercury is slightly volatile at room temperature. The volatility increases appreciably at increased temperature and it is emitted as an invisible mercury vapour. The vapours of mercury are surely hazardous to personnel working in laboratory and industry if careful handling procedures and proper ventilation are not employed.

The elemental metallic form of mercury, often referred to as quick silver, has an atomic weight of 200.59 and an atomic number 80 on the periodic table. Mercury has a valence of either 1 (mercurous) or 2 (mercuric). The chemical symbol Hg is derived from its Latin name Hdrargyram. The metal does not form its oxide during room temperature but at 356.58°C it slowly oxidizes to red oxide of mercury. Mercury readily forms amalgams with most metals with exemption of iron. It has great affinity towards sulfur and hence the term mercapto (sulphur capturing). This property leads mercury to a most biologically acting metal because it forms compound with sulfhydryl groups of all enzymes and impairs enzyme function. Therefore, mercurials even in very low concentration are capable of inactivating sulfhydryl enzymes and in this way interferes with cellular metabolism and function.

Mercury compounds are chemically classified as inorganic, organic or in the form of metallic mercury. Mercuric salts are available in the form of
mercurous or mercuric state. The organic form of mercury means those are associated with carbon in their molecular structure such as phenyl mercuric salts and alkyl mercuric compounds. Organic mercury compounds are easily absorbed and after ingestion have half life period varying from 60 to 120 days in humans, but up to 20 years in fish.\textsuperscript{105} Methyl mercury can easily pass through bio-membranes and are lipophilic in property. Orally ingested methyl mercuric chloride is absorbed 100\% in mice. Female mice retained 2:1 over males and retention 65-75\% in carcass, 8-10\% in kidneys but, only 1-1.6\% in brain. Hair is a major deposit for mercury after exposure to methyl mercury chloride. The metabolism of methyl mercuric chloride is found similar in mice and humans.\textsuperscript{106} Methyl mercury has been listed as one of the six most dangerous chemicals in the environment. This is why in USA, the Environmental Protection Agency (EPA) reduced the allowable intake of methyl mercury from 0.5 mcg to 0.1 mcg of mercury per kilogram per day.\textsuperscript{107}

1.1.12: Spiritual Basis of Mercurial Drugs

Mercurial drugs have a long history of use in India and by second century they were developed into a system of medicine known as \textit{Rasasashtra}.\textsuperscript{108} The text of Indian Alchemy (\textit{Raasavidya}) reveals that, a wide variety of inorganic and bio-derived substances were used to prepare mineral drugs (plants and animal products) but more the former. The important minerals are referred to as \textit{rasaas} and they are classified as \textit{maharasaas} (superior) and \textit{upa-rasas} (subsidiary). Mercury, though a metal, is extolled as the king of \textit{rasaas}, the \textit{maharasaas}, and has several names in the \textit{rasa sashthra} texts as \textit{paarada}, \textit{sita}, \textit{rasendra}, \textit{swarnakaraka}, \textit{sarvadathu pushti} and more significantly in a mythological setting \textit{Sivaja} (born of Siva) Siva \textit{veerya} (semen of Siva) and \textit{hara beeja} (seed of Siva).\textsuperscript{109}

The metal’s heavy weight, silvery appearance, fluidity and its property of readily combining with other substances might have reasoned to consider
mercury as the most potent of all substances, possessing divine and aphrodisiacal properties in *vedic* period. That is regarded as having spiritual and mythical dimensions as well as ‘scientific basis’. On spiritual basis the Sanskrit name of mercury is *paarada*; the sperm of Siva. To be safer for consumption, it must be mixed with a substance of equal power, the sulphur. The mercury and sulphur have natural attraction.\(^3\) According to the Indian Alchemists, when mercury melted with sulphur, molecular union occurs and their poisonous nature is transmitted into healing nectar called *Kajjali*. *Kajjali* is the alchemical child of Siva and Parvathy and the basic constituent for majority of mercurial drugs.\(^{109}\)

On spiritual reasons, the first emperor of China, *Qin-Shi-Huang*, was driven insane and killed by mercury pills which were intended to give him eternal life and he was buried in a tomb filled with mercury. Metallic mercury is also used in some religious practices, and is sold under the name "azogue" in botanicals stores. Botanicas are common in Hispanic and Haitian communities where azogue may be used as an herbal remedy or for spiritual practices. In China, forty cinnabar-containing traditional medicines are still used today.\(^{110}\)

The Ayurvedic teachings prescribe specific methods for the purification of heavy metals. The metal is heated and treated with oils, cow’s urine, milk, ghee, buttermilk or sour and gruel of grains. These ancient methods achieve subtler purification than mere chemical treatment and permit the human tissues to receive the metal’s influence without any toxic effect. *Rasasastra* again reveals the medicinal properties of mercury; as it is a very heavy and potent metal, helps to enkindle the enzyme transform and to regenerate the tissues. Mercury is considered the semen of God Siva in Indian mythology. It stimulates intelligence and awakens awareness. It should never be used alone, but always in conjunction with sulphur. The potency of certain
herbs is increased many thousand fold when used in conjunction with mercury and sulphur.

1.1.13: Mercury in Ayurveda

Herbs, minerals, and metals are used in Ayurvedic herbal medicine products. Ayurvedic theory attributes important therapeutic roles to metals such as mercury and lead. In Ayurveda mercury was an important constituent of drug for centuries as an ingredient in many diuretics, antibacterial, antiseptic skin ointments and laxatives. In modern medicine mercurial drugs are almost replaced in recent decades except thimerosal in vaccines as preservatives.

Venkataraman has reported that mercury and its salts are the largest used heavy metals in Ayurvedic chemical formulations. It occurs in nature as organic or elemental form. Salts of mercury occur in two oxidative states (1) mono-valent mercurous and (2) divalent mercuric salts. In the Ayurvedic Formulary of India, the authentic text for ayurvedic drug preparation and practice, there are about 55 formulations for mercury for various ailments. The daily dozes of mercurial drugs vary from 4 mg to 1g and the average doze administered by Ayurvedic practitioners is 125 to 250 mg per day. Usually mineral drugs are administered with adjuvants (anupaana drava) like cold water, honey, milk, ghee, juices of various herbs like ocimum, ginger and cumin etc… These adjuvants are acidic, basic or neutral in nature that determines the pharmacokinetics of the drugs after ingestion.

The time tested principles of Ayurveda believes in the purification of mercury compounds with different purification methods like marana, svedana and basmeekaran (calcinations). They transform mercury into complex chemical form and convert the heavy metal to non-toxic medicines. But according to the principles of modern chemistry, mercury can exist only in
three forms (1) elemental mercury (2) inorganic salts and (3) organic salts. As far as a biological system is concerned, the metabolites should be polar and water-soluble so that it is eliminated from body through urine and feces. Mercury mostly accumulates in the kidney and produce nephrotoxicity.

Mercury combines with one or more reactive ligands like –OH, –SH, –COOH, –S–S– that are essential for physiological functions. Mercury forms metal complex or coordinated compounds like Hg–O–Hg, Hg–S–Hg, and Hg–S–S–Hg; usually these legends are co factors or functional groups of many enzymes and biological molecule in the body will be affected. Since 1978, at least 55 cases of heavy metal (mercury and lead) intoxication associated with Ayurvedic herbal medicine products in adults and children have been reported in the US and other foreign countries. In England, 30% of Ayurvedic preparations sampled contained lead, mercury, or arsenic.111

1.1.14: Cinnabar (Hingula or Chayilyam)

Figure 1.1.15: Cinnabar

In Sanskrit cinnabar is known to be Hingula or Chayilyam. Cinnabar is an important ore of mercury. Its chemical composition is mercuric sulphide (HgS). According to modern science cinnabar is known to be highly toxic.112 It is widely used in Traditional Chinese medicine (TCM) and Ayurvedic drug preparation in India.
Although cinnabar is not used in Western medicine, TCM practitioners sometimes prescribe it as part of a medicinal mixture, often on the basis of the concept of “using poison to cure poison”. Used internally, cinnabar is believed to clear away “heat" and tranquilize the mind. It is also used as a tonic to reduce the incidence of heart palpitations, restlessness, and insomnia, and to treat some sore throats and cold sores that occur in the mouth and tongue. In addition, cinnabar is applied externally to treat certain skin disorders and infections.\textsuperscript{113}

In \textit{Rasasasthra} cinnabar is considered as a drug with rejuvenation (\textit{Rasa}yana) property and will improve the mental faculties. This mineral is used to prepare a wide range of mercurial drugs in Ayurveda. These drugs are prescribed for treating the disorders of eyes, skin, urogenital system and spleen. The same author further warns that, cinnabar may cause adverse effects. If used internally without proper purification it may cause delirium, diabetes mellitus, psychosis, blindness and tiredness.\textsuperscript{114}

1.1.16: Rasakarpura (Calomel or Horn Quick Silver)

Rasakarpura appears as a white sublimate with chemical composition of mercuric chloride. The artificially prepared rasakarpura is commonly available in the market, prepared from metallic mercury, sulphuric acid and rock salt. This compound is widely used for many ayurvedic preparations. It is also known as Mercury bi-chloride or Mercury per-chloride. It appears as crystals or white granules or as powder; in the toxicology field it has a nickname “\textit{violet poison}”. This inorganic form of mercury is a cumulative poison, which is mainly stored in kidney and liver. LD\textsubscript{50} in rat is approximately 37mg/kg.\textsuperscript{115}

Mercury salts, especially mercuric chloride primarily affect the gastrointestinal tract and the kidneys, and can cause severe kidney damage;
however, as they can not cross the blood-brain barrier easily, mercury salts inflict little neurological damage without continuous or heavy exposure.\textsuperscript{116} Mercuric chloride is highly toxic to human beings especially corrosive to mucous membranes. Ingestion may cause severe nausea, vomiting, hematemsis, abdominal pain, diarrhea, melena, renal damage and prostration. 1 or 2 gram of mercuric chloride is frequently fatal. In mice experiments it is reported that, 20\% of mercuric chloride was absorbed by the gastrointestinal tract after oral ingestion. Male mice retained 2:1 over females and the retention mainly in kidneys, liver, and carcass. The average brain accumulation is approximately 1\% of the total mercury retained for 14 days.\textsuperscript{117}
1.2: Lead Toxicity

Lead has been mined and used since ancient times and is present in most living creatures and plants.\textsuperscript{118} The ease of refining lead from galena, its malleable properties, and non-corrosive nature contributed much to the early wide spread use of this metal. At that time the use of metal was considered as aristocratic for wealthy people. During the golden age of Roman Empire, lead was used for plumbing, making of wine vessels and cooking utensils by the wealthy. This wide spread use of lead might be the major cause for plumbism, which led to speculation that chronic lead poisoning might have contributed to the fall of the Roman Empire.\textsuperscript{119}

Lead poisoning can be considered as an environmental disease, but nowadays it is a disease of life style or it is caused by the consumption of Ayurvedic drugs or ‘traditional herbal drugs’.\textsuperscript{120} Of all heavy metals, lead has probably the longest history of environmental contamination and toxicity to humans. Lead is one of the best-studied toxic heavy metal and it is not surprising that the toxic properties of lead were known as early as the second century BC. Lead is the most common environmental poison in India, about 30\% of population is already affected by lead poisoning.\textsuperscript{17}

Lead is a major toxic metal, ranked seventh in the Toxic Substances List prepared under the Canadian Environmental Protection Act. Lead is not biodegradable due to its non-corrosive nature. It never disappears but only accumulates. Lead provides no known biological benefit to human or any lower mammal. Lead has no known essential function in any system but can accumulate in many biological systems until it reaches toxic levels.\textsuperscript{121} Lead is a divalent metal and often competes with other divalent ions such as iron, calcium, and zinc with regard to absorption and biochemical processes. In calcium deficient persons lead absorption is in high rate. Iron deficiency has also shown enhancing intestinal absorption of lead.\textsuperscript{122} The absorption of lead
from food is estimated to be 10% in adults and 40% in children. Overall lead appears clinically exerting its toxic effects more in some tissues as opposed to others. The nervous, renal and circulatory systems appear to be sites where lead appears to have its greatest toxic impact.

1.2.1: Environmental source of lead

The content of lead in rain water ranged from 3-300 micro grams per liter in one study with an average of 40 microgram per liter. Another study reported an average concentration of 34 microgram per liter for 32 sampling areas throughout the United States. The average concentration of lead in soils is reported to be 16 mg per kg. These few examples point out the general presence of lead in the environment and evidence that the levels have increased over a period of years. Sukla et al., have viewed the history of lead use, the changing pattern of lead sources, atmospheric contamination and presence of lead in rain water and surface water. The wide spread use of lead in piping and soldering of water tanks and use of white lead in some countries contribute to incidents of lead poisoning. There is evidence that lead in the environment has increased during the past 200 years.

According to W H O, the Provisional Tolerable Weekly Intake (PTWI) of lead from all sources for a healthy adult is 50μg/kg body weight. Because of increased sensitivity of infants and children the PTWI suggested for this group is 25μg/kg body weight. Specific toxicities of lead vary with age and circumstances of the host, but major risk is toxicity to the nervous system. The most susceptible populations are children, particularly toddlers, infants in the neonatal period, and fetuses. Drinking water consumed directly and used in food processing also contributes lead to dietary intake. No organic forms of lead have been reported to occur in food. Lead in food stuffs exist exclusively as salts, oxides or sulfhydryl complexes. Most salts and oxides are insoluble in water, and hence, lead absorption is low.
The absorbed lead may accumulate in the body over decades and it is stored in the bones and teeth. More than 95% of retained lead is in bone, acting as a reservoir, where it is in continuous exchange with the soft tissue pools. The half-life of circulating lead in blood is about one month. The absorbed lead in plants are distributed differently, the lead levels in green leafy vegetables are somewhat higher than in fruits. Lead translocation from soil to the edible portion of vegetables is more easily, compared with that of fruits grown on trees and bushes. Another factor affecting lead content of vegetation is the growing location with regard to major highways. Perhaps not surprisingly, there is good correlation between average traffic counts and average soil and plant lead content at sites close to roadside. Sufficient study reports on lead concentrations in food materials are not available in India but lead contents of certain representative food items selected from United States of America is shown in table-2

**Table-1.2.2: Lead contents in some selected food items in U. S. A**

<table>
<thead>
<tr>
<th>No.</th>
<th>Food item</th>
<th>Range μg/100gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cereal grains</td>
<td>0-62</td>
</tr>
<tr>
<td>2</td>
<td>Cereal grain products</td>
<td>0-749</td>
</tr>
<tr>
<td>3</td>
<td>Sea food raw</td>
<td>17-250</td>
</tr>
<tr>
<td>4</td>
<td>Canned sea food</td>
<td>6-30</td>
</tr>
<tr>
<td>5</td>
<td>Meats</td>
<td>7-37</td>
</tr>
<tr>
<td>6</td>
<td>Gelatin</td>
<td>0-114</td>
</tr>
<tr>
<td>7</td>
<td>Egg whole</td>
<td>0-15</td>
</tr>
<tr>
<td>8</td>
<td>Vegetables, leafy</td>
<td>0-126</td>
</tr>
<tr>
<td>9</td>
<td>Legumes raw, dried or frozen</td>
<td>0-6</td>
</tr>
<tr>
<td>10</td>
<td>Canned legumes</td>
<td>3-11</td>
</tr>
<tr>
<td>11</td>
<td>Apples</td>
<td>38 (mean)</td>
</tr>
<tr>
<td>12</td>
<td>Pear</td>
<td>3 (mean)</td>
</tr>
<tr>
<td>No.</td>
<td>Food item</td>
<td>Range μg/100gm</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>13</td>
<td>Milk, whole and fresh</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>Milk, skim dried and packaged</td>
<td>2 (mean)</td>
</tr>
<tr>
<td>15</td>
<td>Milk, evaporated</td>
<td>4 -5</td>
</tr>
<tr>
<td>16</td>
<td>Tea-leaves</td>
<td>1.37 (mean)</td>
</tr>
<tr>
<td>17</td>
<td>Cocoa, dry</td>
<td>0.1 (mean)</td>
</tr>
<tr>
<td>18</td>
<td>Sugar white</td>
<td>0-7</td>
</tr>
<tr>
<td>19</td>
<td>Molasses</td>
<td>53 (mean)</td>
</tr>
<tr>
<td>20</td>
<td>Baking powder</td>
<td>150 (mean)</td>
</tr>
<tr>
<td>21</td>
<td>Yeast dry</td>
<td>117 (mean)</td>
</tr>
<tr>
<td>22</td>
<td>Black pepper</td>
<td>40 (mean)</td>
</tr>
<tr>
<td>23</td>
<td>Cinnamon</td>
<td>11 (mean)</td>
</tr>
<tr>
<td>24</td>
<td>Nutmeg</td>
<td>41 (mean)</td>
</tr>
<tr>
<td>25</td>
<td>Allspice</td>
<td>64 (mean)</td>
</tr>
<tr>
<td>26</td>
<td>Chili powder</td>
<td>18 (mean)</td>
</tr>
<tr>
<td>27</td>
<td>Bay leaves</td>
<td>55 (mean)</td>
</tr>
<tr>
<td>28</td>
<td>Cider, apple</td>
<td>90μg/L (mean)</td>
</tr>
<tr>
<td>29</td>
<td>Vinegar</td>
<td>100μg/L(mean)</td>
</tr>
<tr>
<td>30</td>
<td>Cola, two samples</td>
<td>16-85μg/L</td>
</tr>
<tr>
<td>31</td>
<td>Ginger ale</td>
<td>10μg/L (mean)</td>
</tr>
<tr>
<td>32</td>
<td>Beer, canned</td>
<td>40μg/L (mean)</td>
</tr>
<tr>
<td>33</td>
<td>Wine, red</td>
<td>50μg/L (mean)</td>
</tr>
<tr>
<td>34</td>
<td>Drinking water</td>
<td>1-5μg/L</td>
</tr>
<tr>
<td>35</td>
<td>Alcoholic beverages</td>
<td>50-100μg/L</td>
</tr>
</tbody>
</table>

Source compiled from Reilly,\textsuperscript{123} WHO,\textsuperscript{134} Deshpande et al.,\textsuperscript{135} Janssen.\textsuperscript{136}
Figure 1.2.3: Blood lead levels (BLL) in children and adults and its adverse effects
1.2.4: Acute and Chronic Lead Toxicity in Children

Lead is a pervasive environmental or induced poison that affects virtually every system in the body. Lead is a cumulative poison and is accumulated in tissues over the years; 90% of lead is seen in bones, 9% in blood and 1% in brain and kidneys. Lin-Fu\textsuperscript{137} reviewed the detrimental effect of lead poisoning in children. The absorbed lead can damage the kidneys, the nervous system, the reproductive system and can cause high blood pressure.\textsuperscript{138} Lead exposure in young children is of particular concern because children absorb lead more readily than adults due to the less developed myelin sheathes in the nervous system. Barltop\textsuperscript{139} found out a strong positive correlation between maternal and fetal blood lead values. It is especially harmful to the developing brains of fetuses and young children. The absorbed lead can easily cross the placental barrier and is present in cord blood at birth, the average value being reported as 13µg/100 ml.\textsuperscript{140}

According to Norton\textsuperscript{141} lead is a potent neurotoxicant, which causes damage to myelin, affecting oligodendrocytes or Schwann cells, resulting in encephalopathy if central white matter is involved or polyneuritis if peripheral cells are damaged. Lead can also affect synaptic junctions of the neuromuscular system. The synaptic clefts and the terminals of myelinated axons are uniquely vulnerable. Further the author reported that lead could cause lesions restricted in distribution, which primarily affect localized anatomical areas in the central nervous system. Growing children are highly sensitive to lead and manifest behavioral and learning difficulties. Memory loss and learning difficulties have been the subject of numerous reports.\textsuperscript{142} Lead poisoning targets both hippocampus and cortical brain regions. The degenerative changes in the cerebral cortex, further progress to late changes of nuclear fragmentation in neurons and cell death, the same are
experimentally proved by animal studies. Rodents and children may also exhibit impaired visual function during lead toxicity.

Lead poisoning on nervous system causes morphological alterations in the glial cells. Swelling of astrocytes and the presence of cytoplasmic electron-dense bodies and intranuclear inclusions are cell responses to lead toxicity. The astrocytes cover the vascular walls of the brain vessels, and lead can injure these structures. Neuromuscular disorders due to chronic lead poisoning can be called the lead palsy, which includes skeletal muscle weakness and fatigue which occur long before actual paralysis. Degenerative changes in the motor neurons and their axons have also been reported. The most serious manifestations of lead poisoning are lead encephalopathy. It is very common in lead poisoned children than adults. The early signs of syndromes are laziness, vertigo, ataxia, falling, headache, insomnia, restlessness and irritability. As the encephalopathy progresses, the patient may first become excited and confused, delirium with repeated clonic and tonic convulsions or lethargy and coma follows later.

The encephalopathy induced by lead toxicity is most likely due to a compromise in the blood-brain barrier. Brain edema occurs in the interstitial area and appears due to compromised blood vessel integrity. The brain capillaries and blood vessels have endothelial cells that contain tight junctions and act as a seal or barrier that excludes many plasma proteins and organic molecules and impaired sodium and potassium exchange. Elevated lead levels disrupt these vessels, and plasma proteins such as albumin enter the interstitial spaces, as do some ions. This increases osmotic pressure, and water accumulates in response. The lack of lymphatic structures within the central nervous system means that the fluid flows in to the cerebro-spinal fluid. This edema causes an increase in intracranial pressure and restricts blood flow to the brain, resulting in ischemia. The blood-lead level as low as 10μg/L is
associated with harmful effects on children’s learning and behavior (even though the normal average intake is 0.3 microgram/L). All children aged 6 months to 6 years are considered to be at high risk because it is at this time the development of brain completes. 

Elevated blood lead level can result in (1) Learning disabilities (2) Behavioral problems and (3) Mental retardation. Acute exposure of lead (70mg/L or more) can result in convulsive seizure, severe CNS depression and death. It is noticed that, the amount of lead that do not appear to harm an adult, can even slow down the normal mental and physical development of children. Lead levels once thought to be safe (25 micro/L) are associated with lower cognitive function, learning disabilities and shorter stature, hearing loss and neurobehavioral problems in children. In chronic lead poisoning, mild anemia, mental deterioration, hyperkinetic or aggressive behavior, peripheral neuropathy, lead palsy, and kidney damage are some of the clinical symptoms of chronic lead poisoning in children. Several dietary factors influence the level of absorption. A low body-calcium status, iron deficiency, and diet rich in carbohydrates but lacking protein and those containing high levels of vitamin-D results in increased absorption of lead.

1.2.5: Acute and Chronic Lead Toxicity in Adults

Lead poisoning continues to be one of the most prevalent occupational, environmental or life style illness affecting adult. In adults, lead poisoning also occurs by consuming certain Ayurvedic, Siddha, or traditional herbal drugs prepared in India and other Asian countries. Adults do not absorb lead as easily as children but the intensity of exposure is at high rate in adults than in children. It affects smooth muscles of gastrointestinal tract resulting in anorexia and muscle discomfort. Constipation is an early sign but occasionally diarrhea occurs. As intoxication advances, anorexia, and constipation become more marked. Intestinal spasm leading to colic is the most distressing feature of chronic lead poisoning. In addition to the
occupational exposure of lead in adults, lead poisoning due to the consumption of drug is more prevalent in adult, than in children. In the normal adult, about 90% of the ingested lead is generally excreted in the urine and feces. The levels of lead in bones, teeth and hair continue to increase with age, suggesting a gradual accumulation of lead in the body. Therefore, lead ingestion through food or medicine may possibly lead to chronic lead poisoning. Acute renal toxicity due to lead exposure includes reversible loss of renal function such as oliguria finally leads to anuria. Damage to the proximal tubules of nephrons, produces Fanconi syndrome, manifested by aminoaciduria, glycosuria and phosphaturia.\textsuperscript{151}

The effect of lead on gastro intestinal (GI) tract is manifested as colic, which is a consistent early symptom in occupationally exposed or drug consumed cases or in cases of acute intoxication. Initial non specific symptom appears at blood lead levels of approximately 80µg/L and manifest dyspepsia, anorexia, post prandial epigastritis, constipation, cramp and nausea. Gastro intestinal symptoms are aggravated when blood lead level reaches 100 µg/dL or higher and includes lead colic (severe abdominal spasm that resembles acute abdominal pain requiring surgery) and liver damage. Needleman et al.,\textsuperscript{151} reported that the clinical diagnosis of lead poisoning in the adult is often complicated by the lack of any clear symptom and sign.

Acute lead exposure causes drowsiness, loss of muscular co-ordination, kidney damage, fatigue, apathy and susceptibility to infection, gouty arthritic condition and extreme anemia.\textsuperscript{152} Chronic exposure with elevated blood lead level is associated with hypertension, head ache, confusion, irritability focal motor dysfunction and insomnia. The continuous prolonged high level exposure results in chronic and non-reversible effects associated with progressive interstitial fibrosis which may lead to renal damage characterized by the sclerosis of vessels, glomerular atrophy, reduced glomerular filtration and azotemia and finally death due to complete renal
shutdown. Chronic and massive exposure to lead may cause progressive tubulo-interstitial nephropathy that develops insidiously and often leads to kidney failure.\textsuperscript{153-156}

1.2.6: Hematotoxic Effect of Lead

The hematotoxic effect of lead is a well studied topic in heavy metal toxicology. During the synthesis of hemoglobin, lead can adversely affect the enzymatic synthesis of heme by inhibiting aminolaevulinic acid dehydratase (ALAD). This enzyme is zinc dependent and, thus, susceptible to the toxic effects of other heavy metals like lead, cadmium, copper, iron, cobalt and manganese.\textsuperscript{157,158} Lead inhibits nearly all enzymatic steps involved in heme synthesis. Hammond\textsuperscript{159} had well studied the hematotoxic effect of lead. During the heme synthesis lead inhibits ALAD by binding with the sulfhydryl groups of the enzymes. So it prevents the conversion of delta-aminolaevulinic acid to porphobilinogen. The inhibition of ALAD is manifested as elevated $\delta$-aminolaevulinic acid ($\delta$-ALA) excretion in the urine. In man elevated $\delta$-ALA excretion is a more sensitive and specific index of lead exposure. Measurement of $\delta$-ALA either in urine or serum is a helpful diagnostic aid in lead toxicity.

The intracellular bio-availability of lead in the target organs like kidney and brain appears to be largely determined by complexation with a group of low molecular weight proteins. These proteins are rich in aspartic and glutamic dicarboxyl amino acids. In rats, these proteins attenuate the lead inhibition of the heme pathway enzyme $\delta$-aminolaevulinic acid dehydratase by a mechanism involving both lead chelation and zinc donation to this highly lead-sensitive zinc-dependent enzyme.\textsuperscript{160,161} Lead also inhibits hemesynthetase, which is responsible for the introduction of iron into the tetra pyrrole porphyrine ring.\textsuperscript{162} The inhibition heme synthesizing enzymes lead to anemia. Anemia is a classic indication of lead toxicity.\textsuperscript{163} Basophilic stippling
(aggregation of RNA) occurs in erythrocytes. The common hematological result is hypochromic microcytic anemia. The effect of inhibition of hemesynthetase is manifested in the animal as protoporphyrinemia and as elevated coproporphyrine excretion in the urine. But the estimation of coproporphyrine is not commonly used to detect lead poisoning in human beings. The effect of lead on heme synthesis is diagrammatically represented below.

Figure-1.2.7: The effect of lead on heme synthesis

![Diagram of heme synthesis](image-url)
The absorbed lead may be distributed into three compartments (a) the freely diffusible lead, which probably includes blood lead and free exchangeable lead of soft tissues; (b) the more firmly bound but exchangeable soft tissues lead; (c) the hard tissue lead such as in bones, teeth, hair, and nails. After absorption, inorganic lead is distributed initially in soft tissues particularly the tubular epithelium of kidney and in the liver. In due course lead is redistributed and deposited in bone, teeth and hair. Small quantities of organic lead are accumulated in brain, with most of that in gray matter and basal ganglia. The half-life of lead in the hard tissues has been estimated to be greater than 20 years; that of blood lead is 27 days.\textsuperscript{166} Lead is practically present in every organ tissues of human body, with amount ranging from 1 to 1.7μg/g tissue.\textsuperscript{167} Over 90% of the lead in the human body stays in the bone. The retention of lead in soft tissues is greatest in the liver, followed by kidneys, aorta, muscle and brain in decreasing order. Urinary excretion is a more important route and the concentration of the lead in urine is directly proportional to that in blood plasma.\textsuperscript{168}

Acute lead poisoning adversely affects hematopoietic, nervous, gastrointestinal and renal functions. Local action of lead in mouth produce marked astringency, thirst and metallic taste. It causes nausea, abdominal pain and vomiting. Stool may be black, anorexia; dyspepsia and constipation are followed by an attack of colic with intense paroxysmal abdominal pain. Lead encephalopathy is also observed in young children. A shocking syndrome may be developed if large amount of lead is absorbed rapidly. Secondary to this, lose of fluid occurs. Acute CNS symptoms include parasthesia, pain and muscle weakness. Acute hemolytic disorders and hemoglobinuria may develop. The kidneys are damaged and oliguria is evident. Death may occur in one or two days. If patient survives, chronic lead poisoning is likely to occur.\textsuperscript{169}
For the detection of hepatotoxicity due to drugs and chemicals, the hepatic lesions can be mainly classified into two; the Type-I lesions and Type-II lesions.\textsuperscript{170}

In morphological classification, the liver injury can be broadly classified into five, which is the widely used.\textsuperscript{171} It describes five groups of reactions;

1. Zonal hepatocellular alterations without inflammatory reaction.
2. Intrahepatic cholestasis.
3. Hepatic necrosis with inflammatory reaction.
4. Unclassified group.
5. Hepatic cancer.

Unless the liver injury occurs on a massive scale, the necrotic lesions due to cell death are not necessarily critical because of the regeneration capability of the liver.\textsuperscript{172} The cell necrosis is preceded by a number of morphological changes such as cytoplasmic edema, dilatation of endoplasmic reticulum, disintegration of polysomes, accumulation of triglycerides, swelling of mitochondria with disruption of cristae, and dissolution of organelles and nucleus.\textsuperscript{173} Biochemical events that may lead to these changes include binding of reactive metabolites to proteins and unsaturated lipids (including lipid peroxidation and subsequent membrane destruction), disturbance of cellular $\text{Ca}^{2+}$ homeostasis, interference with metabolic pathways, shifts in sodium ions and potassium ions balance, and inhibition of protein synthesis.\textsuperscript{173} Mercury and lead are not generally causing hepatotoxicity and liver injury. That is why they are not included in the list of hepatotoxic agents, which cause liver necrosis, fatty liver, cholestasis (drug induced), hepatitis and carcinogenesis.\textsuperscript{174, 175}
Kehoe\textsuperscript{176} reported that, a dose of 0.62mg lead per day was sufficient to bring about a slight accumulation of lead in human body. An oral intake of 10 to 15 mg of lead per day results in gastro-enteric plumbism in about 30 days. The toxic effects of lead and the minimal blood lead levels at which the effects are most likely observed are summarized below.

**Table-1.2.8: The Lowest Lead Levels Cause Observable Adverse Health Effects in Children and Adults.**

<table>
<thead>
<tr>
<th>Effects (Symptoms or manifestations)</th>
<th>Blood lead concentration, μg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children</td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>80-100</td>
</tr>
<tr>
<td>Hearing deficit</td>
<td>20</td>
</tr>
<tr>
<td>Intelligence deficit</td>
<td>10-15</td>
</tr>
<tr>
<td>In utero effects</td>
<td>10-15</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>40</td>
</tr>
<tr>
<td>Hemotological</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>80-100</td>
</tr>
<tr>
<td>U-ALA\textsuperscript{a}</td>
<td>40</td>
</tr>
<tr>
<td>B-Epp\textsuperscript{b}</td>
<td>15</td>
</tr>
<tr>
<td>ALA inhibition</td>
<td>10</td>
</tr>
<tr>
<td>Py-5-N\textsuperscript{c} inhibition</td>
<td>10</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
</tr>
<tr>
<td>Neuropathy?</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin D metabolism</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Blood pressure (males)</td>
<td>30</td>
</tr>
<tr>
<td>Reproduction</td>
<td>40</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Aminolevulinic acid in urine.  
\textsuperscript{b}Concentration of erythrocyte protoporphyrin.  
\textsuperscript{c}Enzyme pyrimidine-5-nucleosidase inhibition results in accumulation of nucleotides in red blood cells, altering their energy metabolism and affecting their membrane stability and survival.\textsuperscript{177}
The chronic lead poisoning can be classified into six types:

1. Gastro Intestinal
2. Neuromuscular
3. CNS
4. Haemotological
5. Renal
6. Other types which have no organ specificity

Nephrotoxic compounds, mercury, lead, uranium, cadmium, platinum, chromium, arsenic, gold, antimony and thallium may lead to chronic or acute renal failure, which is manifested by the uremic syndrome. Uremia is characterized by oliguria and increase in blood nitrogen, mostly in the form of urea and other substances normally excreted in the urine. Renal damage by toxicants may be assessed by an evaluation of kidney function. Basically, the concentration of normal urine constituents is measured. For example, the concentration of sodium chloride in the urine may indicate a failure to concentrate urine or reabsorb water and sodium. Abnormal excretion of glucose in the absence of hyperglycemia, high urinary aminoacid creatinine ratio (amino-aciduria), proteins sediments, and urinary enzymes, such as glutamate oxaloacetate transaminase and alkaline and acid phosphatases are also indicative of renal failure.

Higher blood lead levels can lead to protein-lead complexes in the tubules which appear as dense accumulations. Gout may be a symptom of such toxicity due to increased reabsorption of uric acid. Continued accumulation of lead by the kidneys often leads to an increased accumulation of fibrotic connective tissue. Elevated blood urea nitrogen and creatinine are typical measures of lead induced renal failure. The mitochondria appear to be altered histologically in the proximal tubules as a result of lead
accumulation. Vascular lesions and atrophy of various portions of the nephron may appear.\textsuperscript{183} Inclusion bodies are commonly reported upon histological examination of glomeruli.\textsuperscript{184}

Prikle et al.,\textsuperscript{185} have studied the association of lead toxicity with hypertension; even low levels are associated with elevated blood pressure. Increased lead absorption leading to increased hypertension was reported in another study.\textsuperscript{186} Low levels of lead exposure have been reported to result in hypertension when other toxicity signs are absent.\textsuperscript{187} The same findings have been reported also in animal studies.\textsuperscript{188, 189}

\textbf{1.2.9: Chronic Lead Poisoning by Indian Herbal/Ayurvedic Drugs}

Dunbabin et al.,\textsuperscript{190} have pointed out the toxic effect of Indian Herbal (Ayurveda) drugs, Pushapdhanvaras and Shakthi tablets (lead drug preparations) on adults. Lead drugs adversely affect liver function than renal function. The liver function test showed a predominant hepatic picture, with alkaline phosphatase of 150µ/L, normal range being, 30-120µ/L. Alanine aminotransferase-270µ/L (<40µ/L), Aspartate aminotransferase-200µ/L (<30µ/L), and bilirubin- 45µ mol/L (<20µmol/L). Further the authors reported that the major clues for the diagnosis of lead poisoning was, the anemia with accompanying basophilic stippling of RBC. The preliminary symptoms of lead toxicity had been started with abdominal pain, anorexia, constipation arthralgia and hepatitis. The quantitative analysis of Pushapdhanvaras tablet and Shakthi tablet revealed the amount of lead as 7.93 percentages and 5.59 percentages respectively. Pushapdhanvaras tablets were deep red in colour, which would be consistent with lead salts, probably lead tetroxide.\textsuperscript{190} In India, Thatte et al.,\textsuperscript{191} have reported a case history of lead poisoning from Mahayograj Guggul (an Ayurvedic drug prescribed for arthritis). The patient manifested the typical symptoms of lead poisoning including severe anemia with classic basophilic stippling of the RBCs.
1.2.10: Lead Toxicity in Rats

Bogden et al.,\textsuperscript{192} have reported the effects of lead and calcium in female rats following delivery, the dams excreted 4.6 mg percentage of lead in the milk. Lead caused growth retardation and paraplegia in pups. The cerebellum is damaged and pathology involves capillary endothelial proliferation leads to encephalopathy. The effect of lead on lactating rats has been studied, the suckling rats manifested the symptoms of lead-encephalopathy, about 85-90% of young rats died in two weeks.\textsuperscript{193} Schlaepfer et al.,\textsuperscript{194} reported axonal and neuronal cell-body degeneration in lead poisoned rats. Snowdon\textsuperscript{195} demonstrated that the behavioral and neurophysiologic effects of lead in rats are greatest during the earliest stages of development. He also reported the effect of lead on hematopoietic systems by assessing the amount of ALA excretion through urine during lead acetate exposure at a dose of 0.52-0.8 mg/100 gm for 21 days. The same author in his second series of tests proved 100% reproductive failure in pregnant rats for 21 days of lead acetate injected intra-peritonially at 0.85-mg/100 gm dose. In renal function tests the blood urea-nitrogen and serum creatinine did not increase in the rats receiving lead in drinking water.\textsuperscript{196}

Krigman et al.,\textsuperscript{197} have reported urinary incontinence and caudal paraplegia in pups after 25 days of weaning with 4% lead carbonated feeding. Histological studies revealed the reduction of grey matter and thinner cortical mantle. Reduced or delayed subdivisions of dendrites and axons were also noted.\textsuperscript{197,198} The biochemical parameters like phospholipids, galactolipids, plasmalogens and cholesterol in the brain showed significant reduction.\textsuperscript{198} Moore et al.,\textsuperscript{199} have studied the composition of lead induced inclusion bodies in renal tubular cells of rats. The inclusion bodies can be separated by differential centrifugation and were found to be insoluble in physiological media. Teruo et al.,\textsuperscript{200} have reported the presence of lead induced intra nuclear
inclusion bodies in the neurons and astrocytes. Fowler\textsuperscript{160} confirmed the protein-binding property of lead in the brain and the deterioration of astrocytes. In another study, the hepatotoxic effects of lead acetate in Wistar albino rats were manifested with oxidative damage in the liver with increase in the liver enzyme production and intense catalase activity.\textsuperscript{201} Mahaffe\textsuperscript{202} has reported that low calcium diets dramatically increases the soft tissue storage of lead in rats.

In rats, if lead is given in combination with ethanol, produced more pronounced inhibition in the activities of hepatic glutamic oxalacetic transaminase (GOT/AST) and glutamic pyruvic transaminase (GPT/ALT) as compared to lead alone treatment. Simultaneous exposure to lead and ethanol produced a greater depression of dopamine (DA) and 5-hydroxytryptamine (5-HT) levels in the whole brain of rats, compared to rats treated with lead alone. The concentrations of lead in blood, liver and brain were significantly higher in rats exposed simultaneously to lead and ethanol.\textsuperscript{203} The results suggested that animals exposed to ethanol and lead were more vulnerable to the neurologic and hepatotoxic effects and the systemic toxicity of lead.

1.2.11: Lead Toxicity in Dogs

In chronic lead poisoned dogs, the histopathological studies revealed the presence of eosinophilic acid fast intra-nuclear inclusion bodies in renal and hepatic epithelium.\textsuperscript{204,205} Bone marrow hyperplasia especially of erythroid elements, necrosis of random striated muscle fibers, peripheral neuropathy, paucity of developing follicles in ovaries and sperm in testes and hemosiderosis in liver and spleen were also reported by the same authors. In another study, Pentshew\textsuperscript{206} reported that the lesions found in the brain of dog are quite similar to those found in lead encephalopathy of children. But in children, cerebral edema and lesions in the cerebellum are more common than in dogs. The lesions found in the brain are the signs of peripheral neuropathy,
similar to those occurring in plumbism of adult humans. In another study on lead poisoned dogs, high levels of lead have been detected in blood, urine, liver and hair. The animals exhibited paralysed oesophagi, thought to be due to vagal neuropathy.\textsuperscript{207}

Zook\textsuperscript{208} studied the effect of accidental lead exposure in dogs. The histology revealed lesions in the brain which involved vascular damage consisting of dilatation of blood vessels, swelling and laminar necrosis of endothelial cells, hyalinization and necrosis of certain arterioles and occasional thrombosis of capillaries. The damaged vessels are often surrounded by edema, fibrin and hemorrhage associated with the vascular changes of laminar vacuolation, gliosis and necrosis of neurons in the cerebral and cerebellar cortex. He also reported necrosis of renal proximal tubular epithelium in lead exposed dogs. The post mortem examinations of lead poisoned dogs were found as the bone marrows showing abnormal red colour. Proliferation of endothelial cells and new capillaries are seen in the cortical grey matter. Blackman et al.,\textsuperscript{209} have reported the similarity of lesions of lead encephalopathy in children, cattle, monkeys and dogs. All are characterized by vascular damage.

1.2.12: Physical and Chemical Properties of Lead

Lead is a metal of antiquity and has been used for many purposes for thousand of years. Lead appears as a bluish-white, silvery-grey metal. Highly lustrous when freshly cut, tarnishes upon exposure to air. The metal is very soft and malleable, easily melted, cast rolled and extruded. It has a cubic crystal structure, melting point-327.4\textdegree{}C, boiling point-1740\textdegree{}C, density-11.34 and heat of vapourization-1740\textdegree{}C.\textsuperscript{210} The atomic number and weight of lead are 82 and 207.2 respectively. The metal has a variable valence of 2 and 4. Four naturally occurring isotopes are available as Pb-204 (1.40 \%), Pb-206 (25.2\%), Pb-207 (21.7 \%) and Pb-208 (51.7\%). Lead is one of the metals
known to the ancient world and occurrence in the earth crest is about 15-
g/tone (0.002%). It occurs chiefly as sulfide in galena, anglesite (Pb SO₄),
cerussite (PbCO₃), minitite (PbCl₂.3Pb₃-(ASO₄)₂) and pyromorphite
(PbCl₂.3Pb₃(PO₄)₂).²¹⁰

Lead is highly resistant to corrosion but reacts with hot con. HNO₃,
with boiling con. HCl or H₂SO₄. Pure water and weak organic acids in the
presence of oxygen attack lead. The metal is resistant to tap water and
hydrofluoric acid. Usual valence state in inorganic lead compound is +2.
Solubility in water varies. Lead sulfide and lead oxide are poorly soluble and
the nitric, chlorate and chloride salts are reasonably soluble in cold water.
Lead also forms salt with organic acids such as lactic acid, acetic acid, and
stable organic compounds, for example tetraethyl lead and tetra methyl lead.

1.2.13: Lead Drugs in Ayurveda

Among the heavy metal drugs used in Ayurveda, lead stands in the
second position. There are hundreds of lead drug combinations in Ayurveda
and in patented herbal drug industry. Lad⁵ has reported that lead is a very
effective medicine for skin disease; it is used to treat leukhoria, vaginal
discharge, swelling, gonorrhea and syphilis. In the ‘Ayurvedic Formulary of
India’ there are about 20 authentic formulations for lead drug preparations.
Ang et al.,²¹¹ Baer et al.,²¹² Leukouch et al.,²¹³ and Alkhayat et al.,²¹⁴ have
conducted research work on heavy metal toxicity by consuming
Ayurvedic/herbal drugs and found out the presence of lead and mercury in
Ayurvedic preparations. They have also reported the toxicity symptoms in the
human subjects.

Ayurvedic traditional systems of medicine in India unfolded its
theories, and followed the earliest scripts and records till date without any
change and review. Heavy metal minerals are inorganic species of chemical
compounds, widely used in the preparation of Ayurvedic drugs. In the case of all Ayurvedic medicines, including mineral drugs, biotransformation studies are not conducted. Hence scientific remarks on toxic minerals are not possible. Ayurveda galena is called as *Neelanjana*, chemically it is Lead sulphide. Galena is the most important and available ore of Lead. Galena and other varieties of *anjanas* are widely used in the disorders of eye. In Ayurveda it is believed that, galena is having rejuvenating property. Lead being the major fraction of galena, the toxic reactions from lead salt is expected.114

1.3: Ayurvedic Drugs

Since antiquity Ayurvedic medicines have been used in India. It relies heavily on herbal medicine products. Herbs, minerals and metals are used in Ayurvedic medicinal products.4 It encompasses a wide range of technique to treat illness and encourages general well being. Translated from Sanskrit, Ayurveda means ‘the science of life’ and the central philosophy is that the mind and body are one and the same, and physical health can’t be achieved without emotional, mental and spiritual health. Though Ayurveda is gaining some popularity in the west, the numbers of scientific studies have been very little. From the evidence so far, it seems that the Ayurvedic approach can be effective in treating a number of disorders including digestive problems and allergies. Ayurveda can be used to treat a wide range of disorders including anxiety, digestive problems, eczema, hypertension, high cholesterol levels, rheumatoid arthritis and mental disorders.3 The scientific evidence on the pharmaco-therapeutic and physiological effects of Ayurvedic drugs has not been reported. So the claims and clarifications of Ayurveda or patented herbal drugs have to be researched with animal model and human experimentation.
1.3.1: Range of Therapies in Ayurveda

An Ayurvedic practitioner uses a range of healing therapies to balance the doshaas and bolster praana, including (1) acupuncture (2) aroma therapy (3) diet (4) herbal medicines or bio-derived medicines (5) massage (6) meditation (7) panchakarma (8) sound therapy or use of manthraas (9) yoga. Among these, special emphasis has been given to herbal medicines or drug synthesized from plants and animals. In this contest it has not been mentioned even the use of mineral based Ayurvedic drugs. But in the official book for Ayurvedic drug preparation and practice, there are a number of formulations for mercury, lead, tin, cobalt, copper, nickel, arsenic, sulphur, iron, silver and gold etc… Among these, mercury, lead, arsenic and sulphur constitute the majority and most of them are available in the form of Bhasmas (powdered ashes).

1.3.2: Bhasma Preparations in Ayurveda

According to ‘The Ayurvedic Formulary of India, powder of substances obtained by calcinations are called bhasmas. In this section it is applied to the metals and minerals and animal products, which are by special process, calcined in closed crucible or pits with dried cow dung cake (puta).

1.3.2.1: Methods of Preparation

Stage-1 (sodhana)

Bhasma are prepared from purified minerals, metals, marine and animal products. In Ayurveda, the process of purification is called sodhana. Chemical purification is different from medical purification; in chemical purification elimination of foreign matter occurs. Ayurvedic purification aims at (a) elimination of harmful matters from the drug (b) modification of undesirable physical properties in the drug (c) conversion of some of the
characteristics of the drug (d) the enhancement of the therapeutic action, there
by potentiating the drug. Sodhana is of two kinds (1) Saamanya sodhana
which is applicable to a large number of metals and minerals, as heating the
thin sheets of the metal and immersing them in taila, takra, gomutra etc. (2)
visesha sodhana which is applicable only to certain drugs and certain
preparations. Visesha sodhana consists of (1) bhaavana (2) svedana(3)
nirvaapana (4) mardana

Stage –2 (Marana)

The second stage is the preparation of bhasma. The purified drug is put
into a khalva (stone mortar and pestle) and ground with the juices of specified
herbs or kashaayas of drug mentioned for a particular mineral or metal. It is
ground for a specified period of time. Then small cakes or chakrikas are
made. The size and thickness of the cakes depend on the heaviness of the
drug. If the drug is heavy, the cakes must be made into thinner. These cakes
are dried under sunlight and placed in one single layer in a shallow earthen
plate (saraava). The edge is sealed with clay-smeared cloth in seven
consecutive layers and dried. A pit is dug in an open space. The diameter and
depth of the pit depends on the metal or mineral that is to be calcined. Half of
the pit is filled with cow dung cakes. The sealed earthen container is placed
in it and the remaining space is filled with more cow dung cakes. Fire is put
on all four sides and middle of the pit; when the burning is over, it is allowed
to cool completely. Then the earthen container is removed from the pit and
the seal is opened and contents are taken out, the medicine is ground into a
fine powder in a khalva. This process of triturating with juice, making
chakrikas and giving putas is repeated as many times as prescribed in texts or
till the proper fineness and quality are obtained.

The putas are described under different names to indicate the size of
the pit and the number of cow dung cakes to be used; they also indicate the
amount of heat required and period of burning. The following putas are commonly used in the preparation of bhasmas (1) Mahaa puta (2) Gaja puta (3) Varaaha puta (4) Kukkuda puta (5) Kapota puta (6) Bhaanda puta. The test for the properly prepared bhasmas are (1) there should be no chandrika (metallic luster) (2) when taken between index finger and thump and spread, it should be so fine as to get easily into the finger line (rekha puritha). (3) When a small quantity spread on cold and still water, it should float on the surface (vaaritham) and (4) the bhasma should not revert to the original state (apunarbhava). As it is mentioned above, the bhasma preparations are made by using the laborious protocols of Ayurveda and the proponents of Ayurveda argue that a properly prepared bhasma never contains heavy metals in elemental form. In contradictory to this statement, Naresh et al.,\textsuperscript{216} have reported the presence of heavy metals in elemental forms in many bhasma preparations.
Aim of the Study

1. To determine the amount of mercury and lead in some common Ayurvedic or patented herbal drugs.

2. To find out the source of mercury and lead in Ayurvedic or patented herbal drugs.

3. To observe the clinical (post-drug administration) symptoms manifested by mercurial and lead drug treated animals.

4. To determine the tissue (blood, kidney, liver and brain) levels of mercury and lead in experimental animals fed with Ayurvedic drugs.

5. To find out the physiological and biochemical effects of mercury and lead in experimental animals.

6. To find out the hematotoxic effect of lead in experimental rats by estimating delta-aminolevulinic acid levels in urine.

7. To find out the hepatotoxic, nephrotoxic and neurotoxic effects of mercury and lead in experimental rats.

8. To find out the histopathological manifestations of mercury and lead toxicity in experimental rats.