# THE TOXICITY STUDIES OF MERCURY AND LEAD IN AYURVEDIC PREPARATIONS

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*By*

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# A word of gratitude….

*Dr. V. K. Sasidharan (late) former Professor and Head of the Department of Life Sciences, University of Calicut, was my motivation to select this topic and his guidance helped me to finish the majority of experiments. His sad demise in 2004 affected me a lot and on this occasion, I express my deep indebtedness to him. Again, with thanks, I remember his wholehearted support, constant encouragement, timely help and personal freedom rendered to me during my research. I realize that no word of thanks does justice to what he has done for me throughout this research project.*

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# DECLARATION

I do hereby declare that the thesis entitled, ***"The Toxicity Studies of Mercury and Lead in Ayurvedic Preparations"*** is an authentic record of the research work carried out by me under the supervisions of Dr. V. K. Sasidharan (late), Professor and former HOD of Life Sciences, University of Calicut. After his sad demise, the research work was handed over to Dr. Fathimathu Zuhara K. (Principal guide), Professor and former HOD of Life Sciences, University of Calicut, and Dr. D. M. Vasudevan, MD (co-guide), Distinguished Professor of Biochemistry, College of Medicine and former Principal of AIMS, Cochin. No part of this thesis has been presented for any other degree or diploma earlier.

CU Campus
August 2009.

**M. P. Poal**

.... to Prof. (Dr.) V. K. Sasidharan (late)

# CERTIFICATE

This is to certify that the thesis entitled ***"The Toxicity Studies of Mercury and Lead in Ayurvedic Preparations"*** is a bonafide research work of Mr. Poal M.P conducted in the Department of Life Sciences, University of Calicut. The research work was started under the guidance of Dr. V.K. Sasidharan (late), professor of Biochemistry and former HOD of Life Sciences, University of Calicut. After his sad demise, Prof. (Dr.) Fathimathu Zuhara K. took charge as the guide, while the co-guide was transferred to me. The research work was completed under our guidance and supervision.

This thesis has not previously formed the basis for award of any PhD. or other similar title of any other University.

Cochin
August 2009.

**Dr. D M Vasudevan, MD,**
Principal (retired) and
Distinguished Prof. of Biochemistry,
College of Medicine,
AIMS & Research Center,
Cochin.

# CERTIFICATE

This is to certify that the thesis entitled ***"The Toxicity Studies of Mercury and Lead in Ayurvedic Preparations"*** is an authentic record of research work carried out by Mr. Poal M.P conducted in the Department of Life Sciences, University of Calicut. The research work was started under the guidance of Dr. V.K. Sasidharan (late), professor of Biochemistry and former HOD of Life Sciences, University of Calicut. After his sad demise, the guideship was transferred to me and Dr. D.M Vasudevan, MD took charge as the co-guide. The research work was completed under our guidance and supervision.

This thesis has not previously formed the basis for award of any PhD. or any degree, diploma, associateship in any University or institution.

CU Campus
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**Dr. Fathimathu Zuhara, K**
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# ABBREVIATIONS

| | | |
|---|---|---|
| AAS | : | Atomic absorption spectrophotometry |
| AAT | : | Aspartate amino transferase |
| AChE | : | Acetylcholine esterase |
| ACP | : | Acid phosphatase |
| δ-ALA | : | Delta-aminolaevulinic acid |
| ALAD | : | Aminolaevulinic acid dehygratase |
| ALP | : | Alkaline phosphatase |
| ALT (SGPT) | : | Alanine amino transaminase (transferase) |
| AMP | : | Amino methyl propanol |
| ANOVA | : | Analysis of variance |
| AR | : | Analytical reagent |
| AST (SGOT) | : | Aspartate amino transaminase (transfrase) |
| BC | : | Before Christ |
| BCG | : | Bromo cresol green |
| BLL | : | Blood lead level |
| BML | : | Blood mercury level |
| CIFT | : | Central institute of fisheries technology |
| CNS | : | Central nervous system |
| CYM | : | Chayilyam |
| DA | : | Dopamine |
| DPX | : | Dextrene polysterene xylene |
| DSHEA | : | Dietary supplement health and education association |
| GGT | : | Gamma glutamyl transpeptidase (transferase) |
| GI | : | Gastro intestinal |
| GST | : | Glutatione s-transferase |
| H&E | : | Hematoxylene and eosine |
| HT | : | 5-Hydroxytryptamine |
| IISR | : | Indian institute of spices research |
| IU/L | : | International unit per liter |
| LD | : | Lethal dose |

| | | |
|---|---|---|
| LDH | : | Lactate dehydrogenase |
| Ma | : | Milli ampere |
| MDH | : | Malate dehydrogenase |
| NAD | : | Nicotinamide adenine dinucleotide |
| NADH | : | Nicotinamide adenine dehydrogenase |
| ND | : | Not detected |
| NGB | : | Nagabhasmam |
| NRC | : | National research council |
| OTC | : | Over the counter |
| PCT | : | Proximal convoluted tubule |
| PHD-1 | : | Patented herbal drug-1 |
| Ppm | : | Parts per million |
| PTWI | : | Provisional tolerable weekly intake |
| RBC | : | Red blood corpuscles |
| RNA | : | Ribonucleic acid |
| RS | : | Rasasindhuram |
| SG | : | Swasanandam |
| SPCA | : | Society for the prevention of cruelty against animals |
| SPSS | : | Statistical package for social studies |
| TCM | : | Traditional Chinese medicines |
| USEPA | : | United states environmental protection agency |
| WHO | : | World health organization |

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**SUMMARY AND CONCLUSION**

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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-bioderived 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.,[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.[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.[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.[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, colititis, 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.[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.[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.[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.[15]

Mercury is a major toxic metal ranked eighth in the 'Toxic Substances List' prepared under the Canadian Environmental Protection Act.[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.[17] 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.[18]

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.[19]

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.[20]

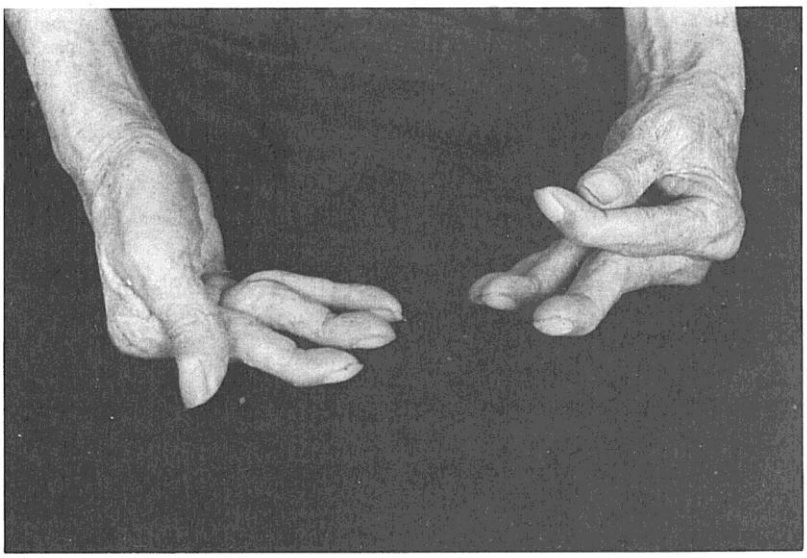
In the recorded history of mercury poisoning Minamata incident stands out as a milestone. The first heavy metal epidemic, Minamata disease, was caused by the consumption of shell fish from water that was heavily contaminated by waste water containing methyl mercury chloride and metallic mercury from 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.[21] Dogs, cats, pigs, rats, and birds living around the bay developed classical clinical signs and many of them died.[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 85ppm in shellfish obtained from the contaminated areas.

Irukayama[24] detected methyl mercury directly from the sledge of Minamata bay. In Minamata patients, the mercury content in their hair was noted as 96.8-705ppm.[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.[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.[27] Fujiki et al.,[28] have estimated the mercury content in the hair of inhabitants in the Minamata area averaged 2.1ppm 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.[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.[30]

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



### 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.[31] The efficient absorption of methyl mercuric compounds from food may be due to the lipid solubility of their chloride complexes.[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.[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.[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.[36]

Berlin et al., [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.[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.[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.[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, asynergy, 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.[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.[42] The biochemical studies on Minamata diseased patients revealed the disturbances in liver function and abnormalities in renal function. Diabetes and hyper tension are abnormally prevalent in chronic mercury poisoning cases.[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.[43] Mercury poisoning also inhibits the activity of liver tyrosine aminotransferase enzyme in rats.[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 γ-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.[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 α-amino groups from aspartate and alanine to the α-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.[49] This phenomenon has been demonstrated in experimentation in animals. [50]

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.[51] Harada[52] 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.[53] The neuropsychological disorders in children due to methyl mercury poisoning by consuming whale flesh were studied in Faeroe Islands.[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.[55] Samhitha[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".[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).[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 $H_2O_2$ 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.[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.[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.[64] With high doses of inorganic mercury, a 'Frank nephritic syndrome' can develop, that has been seen both in industry and medicine.[65]

El-Demerdash[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.,[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.[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.[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.[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.[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.[73] Mercury in elemental form, in liquid state is nontoxic, and a human being might ingest up to a pound without significant adverse effects.[74] But, Lin et al.,[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 micro organisms in the mud on the bottom of the bay.[76] Jensen et al.,[77] and Jonnalgadda[78] have reported that the microorganisms were capable of methylating inorganic mercury. Wood et al.,[79] 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 *Methanobacterium emelenskii* and *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.[80]

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 *Neurospora crassa* and *Clostridium cochlearis* respectively.[81, 82] 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, *methenogens* 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.[83] The possibility of ingesting inorganic mercury through daily food is very rare; the daily intake is estimated to be below 1µg/day.[84, 85] 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**

| Foods | USA, µg/kg | UK, µg/kg | Japan, µg/kg |
|---|---|---|---|
| Cereal | 2-2.5 | 5 | 12-48 |
| Bread&flour | 20 | --- | --- |
| Meats[a] | 1-50 | 10-40 | 310-360 |
| Fish[b] | 0-60 | 70-80 | 35-540 |
| Milk | 8 | 10 | 3-7 |
| Cheese | 80 | 170 | ---- |
| Butter | 140 | 10 | --- |
| Fruits | 4 -30 | 10-40 | 18 |
| Fresh vegetables | 0-20 | 10-25 | 30-60 |
| Canned vegetables | 2-7 | 20 | 0 |
| Egg white | 10 | ND | 80-125 |
| Egg yolk | 62 | ----- | 330-670 |
| Beer | 4 | --- | --- |

[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 sulfhydryl enzymes and in this way interfere with cellular metabolism and function.[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.[89] Absorption of mercury vapours by inhalation readily occurs, as mercury vapour freely crosses the alveolar membrane.[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).[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.[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.[93]

The ingested soluble inorganic mercury ($Hg^{++}$) 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.[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.[94] The inorganic mercury poisoning through local application of mercuric chloride in cattle is almost similar to organic mercury poisoning.[95] 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.[96] 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.[97] 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.[98]

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.[99] 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.[100] The renal excretion is around 15% of the mercury load.[101] 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.[102] 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.[103]

### 1.1.11: 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 $-38.87^0$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 $356.58^0$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 sulf-hydryl groups of all enzymes and impairs enzyme function.[104] 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.[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.[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.[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 *Rasasashtra*.[108] The text of Indian Alchemy (*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 *rasaas* and they are classified as *maharasaas* (superior) and *upa-rasas* (subsidiary). Mercury, though a metal, is extolled as the king of *rasaas*, the *maharasaas*, and has several names in the *rasa sashthra* texts as *paarada*, *sita*, *rasendra*, *swarnakaraka*, *sarvadathu pushti* and more significantly in a mythological setting *Sivaja* (born of Siva) Siva *veerya* (semen of Siva) and *hara beeja* (seed of Siva).[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. *Rasasaastra* 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[99] 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.[6] 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.[99] 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.[113]

In *Rasasasthra* cinnabar is considered as a drug with rejuvenation (*Rasayana*) 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.[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 "*violet poison".* This inorganic form of mercury is a cumulative poison, which is mainly stored in kidney and liver. $LD_{5O}$ in rat is approximately 37mg/kg.[115]

Mercury salts, especially mercuric chloride primarily affect the gastro-intestinal 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.[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.[117]

## 1.2: Lead Toxicity

Lead has been mined and used since ancient times and is present in most living creatures and plants.[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.[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'.[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.[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.[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.[122] The absorption of lead

from food is estimated to be 10% in adults and 40% in children.[123] 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.[124]

### 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.[125] 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.[126] 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.,[127] 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.[128] 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.[129] 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.[130]

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.[131] The absorbed lead in plants are distributed differently, the lead levels in green leafy vegetables are some what 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.[132,133] 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

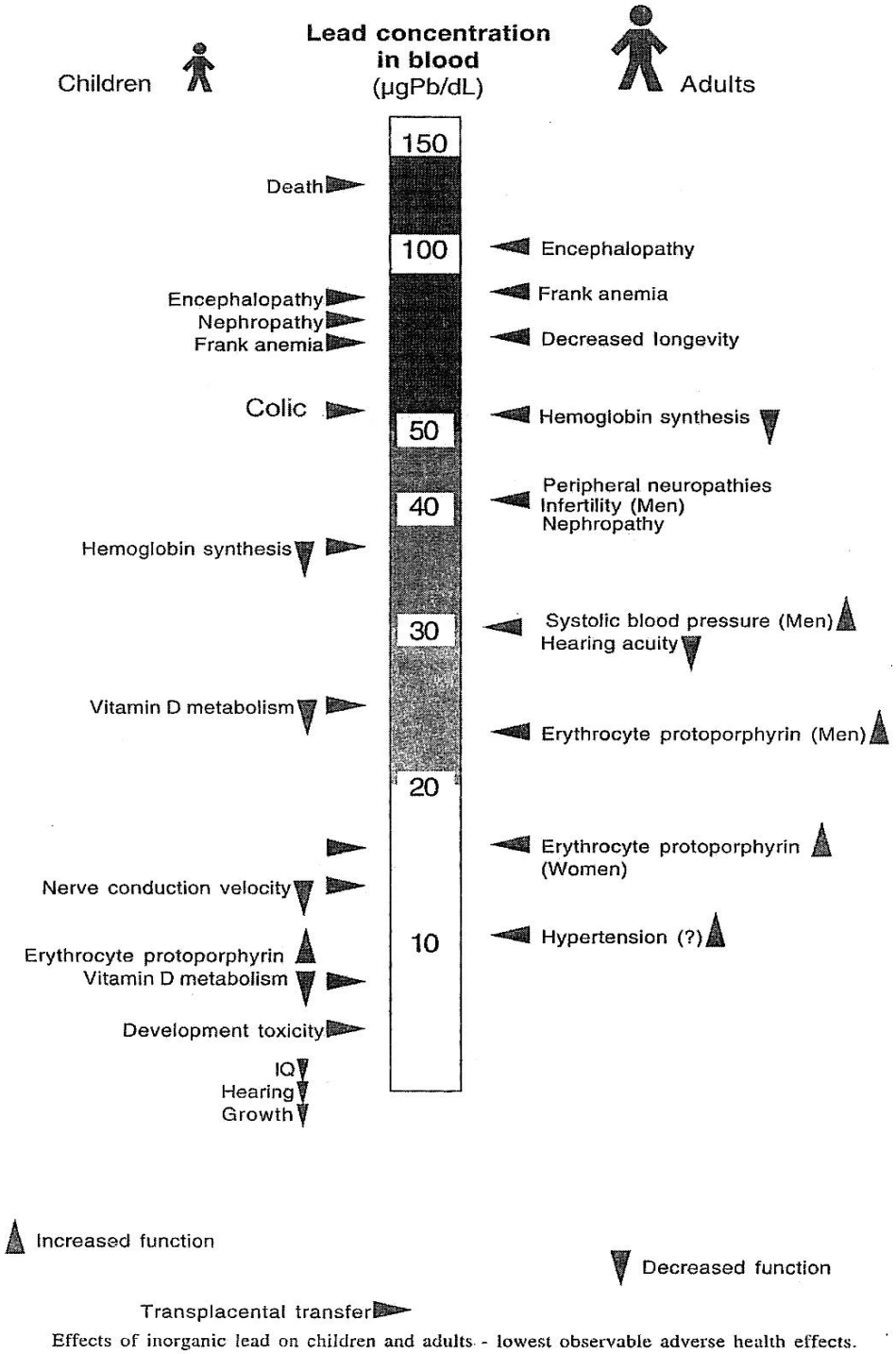
**Table-1.2.2: Lead contents in some selected food items in U. S. A**

| No. | Food item | Range µg/100gm |
|---|---|---|
| 1 | Cereal grains | 0-62 |
| 2 | Cereal grain products | 0-749 |
| 3 | Sea food raw | 17-250 |
| 4 | Canned sea food | 6-30 |
| 5 | Meats | 7-37 |
| 6 | Gelatin | 0-114 |
| 7 | Egg whole | 0-15 |
| 8 | Vegetables, leafy | 0-126 |
| 9 | Legumes raw, dried or frozen | 0-6 |
| 10 | Canned legumes | 3-11 |
| 11 | Apples | 38 (mean) |
| 12 | Pear | 3 (mean) |

| No. | Food item | Range µg/100gm |
|---|---|---|
| 13 | Milk, whole and fresh | 0 |
| 14 | Milk, skim dried and packaged | 2 (mean) |
| 15 | Milk, evaporated | 4 -5 |
| 16 | Tea- leaves | 1.37 (mean) |
| 17 | Cocoa, dry | 0.1 (mean) |
| 18 | Sugar white | 0-7 |
| 19 | Molasses | 53 (mean) |
| 20 | Baking powder | 150 (mean) |
| 21 | Yeast dry | 117 (mean) |
| 22 | Black pepper | 40 (mean) |
| 23 | Cinnamon | 11 (mean) |
| 24 | Nutmeg | 41 (mean) |
| 25 | Allspice | 64 (mean) |
| 26 | Chili powder | 18 (mean) |
| 27 | Bay leaves | 55 (mean) |
| 28 | Cider, apple | 90µg/L (mean) |
| 29 | Vinegar | 100µg/L(mean) |
| 30 | Cola, two samples | 16-85µg/L |
| 31 | Ginger ale | 10µg/L (mean) |
| 32 | Beer, canned | 40µg/L (mean) |
| 33 | Wine, red | 50µg/L (mean) |
| 34 | Drinking water | 1-5µg/L |
| 35 | Alcoholic beverages | 50-100µg/L |

Source compiled from Reilly,[123] WHO,[134] Deshpande et al.,[135] Janssen.[136]

**Figure-1.2.3: Blood lead levels (BLL) in children and adults and its adverse effects**




Effects of inorganic lead on children and adults - lowest observable adverse health 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[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.[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[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.[140]

According to Norton[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.[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.[143] Rodents and children may also exhibit impaired visual function during lead toxicity.[144]

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.[145]

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.[146] 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.[147,148] 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.[149]

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.[150] 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.[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.,[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.[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.[153-156]

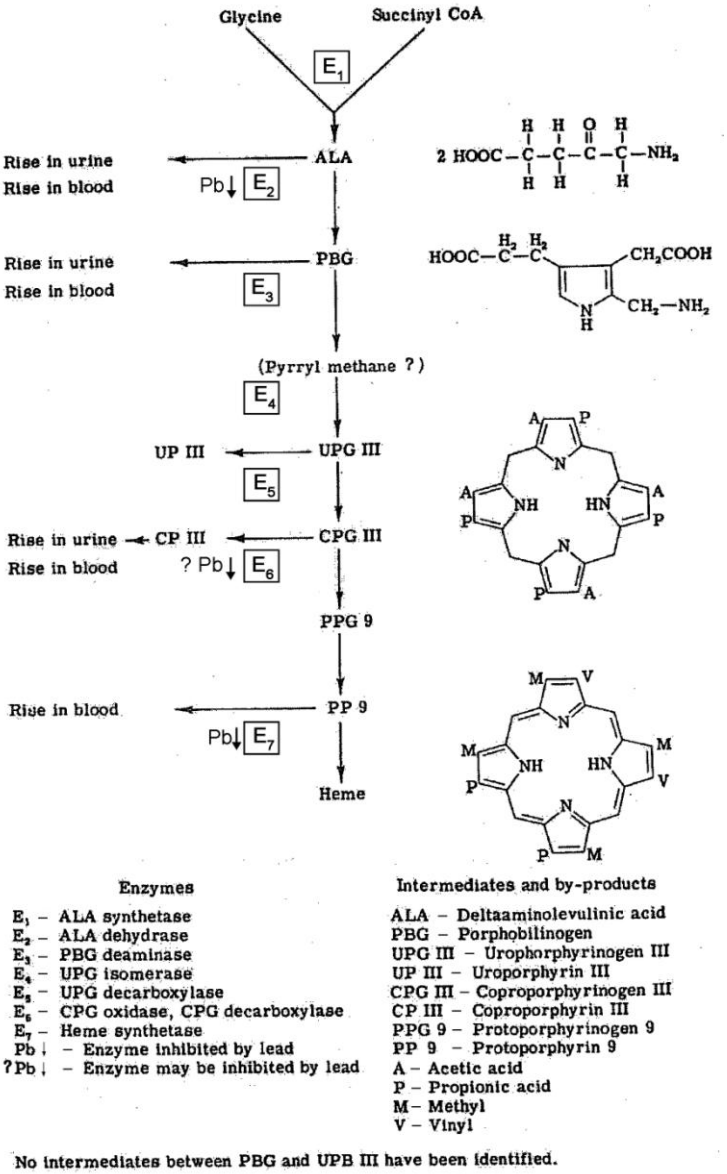
### 1.2.6: Hematotoxic Effect of Lead

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.[157,158] Lead inhibits nearly all enzymatic steps involved in heme synthesis. Hammond[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 δ-aminolaevulinic acid (δ-ALA) excretion in the urine. In man elevated δ-ALA excretion is a more sensitive and specific index of lead exposure. Measurement of δ-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 δ-aminolaevulinic acid dehydratase by a mechanism involving both lead chelation and zinc donation to this highly lead-sensitive zinc-dependent enzyme.[160,161] Lead also inhibits hemesynthetase, which is responsible for the introduction of iron into the tetra pyrrole porphyrine ring.[162] The inhibition heme synthesizing enzymes lead to anemia. Anemia is a classic indication of lead toxicity.[163] Basophilic stippling

(aggregation of RNA) occurs in erythrocytes. The common hematological result is hypochromic microcytic anemia.[164] 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[165] is diagrammatically represented below.

**Figure-1.2.7: The effect of lead on heme synthesis**



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.[166] Lead is practically present in every organ tissues of human body, with amount ranging from 1 to 1.7μg/g tissue.[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.[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.[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.[170]

In morphological classification, the liver injury can be broadly classified into five, which is the widely used.[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.[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 organells and nucleus.[173] Biochemical events that may lead to these changes include binding of reactive metabolites to proteins and unsaturated lipids (including lipid peroxydation and subsequent membrane destruction), disturbance of cellular $Ca^{2+}$ homeostasis, interference with metabolic pathways, shifts in sodium ions and potassium ions balance, and inhibition of protein synthesis.[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.[174, 175]

Kehoe[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.**

| Effects (Symptoms or manifestations) | Blood lead concentration, µg/dL | |
|---|---|---|
| | Children | Adults |
| **Neurological** | | |
| Encephalopathy | 80-100 | 100-112 |
| Hearing deficit | 20 | |
| Intelligence deficit | 10-15 | |
| In utero effects | 10-15 | |
| Peripheral neuropathy | 40 | |
| **Hemotological** | | |
| Anemia | 80-100 | 80-100 |
| U-ALA[a] | 40 | 40 |
| B-Epp[b] | 15 | 15 |
| ALA inhibition | 10 | 10 |
| Py-5-N[c] inhibition | 10 | |
| **Renal** | | |
| Neuropathy? | 40 | |
| Vitamin D metabolism | <30 | |
| Blood pressure (males) | | 30 |
| Reproduction | | 40 |

[a]Aminolevulinic acid in urine.
[b]Concentration of erythrocyte protoporphyrin.
[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.[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.[178] Uremia is characterized by oliguria and increase in blood nitrogen, mostly in the form of urea and other substances normally excreted in the urine.[179] 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.[180]

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.[181] 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.[182] 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.[183] Inclusion bodies are commonly reported upon histological examination of glomeruli.[184]

Prikle et al.,[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.[186] Low levels of lead exposure have been reported to result in hypertension when other toxicity signs are absent.[187] The same findings have been reported also in animal studies.[188, 189]

### 1.2.9: Chronic Lead Poisoning by Indian Herbal/Ayurvedic Drugs

Dunbabin et al.,[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.[190] In India, Thatte et al.,[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.,[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.[193] Schlaepfer et al.,[194] reported axonal and neuronal cell-body degeneration in lead poisoned rats. Snowdon[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.[196]

Krigman et al.,[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.[197,198] The biochemical parameters like phospholipids, galactolipids, plasmalogens and cholesterol in the brain showed significant reduction.[198] Moore et al.,[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.,[200] have reported the presence of lead induced intra nuclear

inclusion bodies in the neurons and astrocytes. Fowler[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.[201] Mahaffe[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.[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.[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 hemosedorosis in liver and spleen were also reported by the same authors. In another study, Pentshew[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.[207]

Zook[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.,[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$^0$C, boiling point-1740$^0$C, density-11.34 and heat of vapourization-1740$^0$C. [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 $SO_4$), cerussite ($PbCO_3$), minitite ($PbCl_2.3Pb_3$-$(ASO_4)_2$) and pyromorphite ($PbCl_2.3Pb_3(PO_4)_2$).[210]

Lead is highly resistant to corrosion but reacts with hot con. $HNO_3$, with boiling con. HCl or $H_2SO_4$. 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. Lad[5] 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.[5] 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).[215]

### 1.3.2: Bhasma Preparations in Ayurveda

According to 'The Ayurvedic Formulary of India, powder of substances obtained by calcinations are called *bhasmas*.[215] 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[152, 215]

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. V*isesha 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 earthern 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 earthern 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 *chakrika*s 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.,[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.

Chapter 2

# MATERIALS AND METHODS

## 2.1: Collection of Ayurvedic and Patented Herbal Drugs.

This study was conducted in the Physiology Division of Department of Life Sciences, University of Calicut, Kerala, during the period of August 2001 to June 2006. Animal experiments have been conducted on Wistar Albino rats bred in the Animal House (Reg. No. 426/01/C/C-SPCA) of the Department.

Ayurvedic or patented herbal drugs used for common ailments, and those used as general health supplements and aphrodisiacal were selected from Ayurveda and patented herbal drugs' catalogue. From this group about forty Ayurvedic drugs, including patented herbal drugs (table 3.1.1), were purchased from Pharmacies and general supermarkets around Kozhikkode and Calicut University areas. The collected drugs were subjected to Atomic Absorption Spectrophotometric analysis for mercury and lead.

## 2.2: Atomic Absorption Spectrophotometric Analysis (AAS).

Principle: -

Alan Walsh suggested the principle of AAS in 1950. It states that, the electrons of the atoms in the atomizer can be promoted to higher orbitals for a short amount of time by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy (the power) put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer Lambert-law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured.[217]

Quantitative analysis of mercury and lead have done by AAS. The di-acid digested samples were aspirated through a nebulizer. The metal to be detected was used as a cathode lamp. The absorbance at 254 nm for mercury and 283nm for lead has been used.

### 2.2.1: Details of the AAS instrument Used.

| | | |
|---|---|---|
| Name (brand) of the instrument | : | VARIAN SB-1.18, CAN 004559540, Mangrove-Victoria, Australia-1989 |
| Wave length | : | 405.8 nm |
| Slit width | : | 0.1 nm |
| Fuel | : | Acetylene |
| Support | : | Nitrous oxide |
| Lamp current | : | 5 Ma |
| Cathode lamps | : | Lead/Mercury |
| Absorbance for lead | : | 283 nm. |
| Absorbance for mercury | : | 254nm. |

### 2.2.2: Preparation of Test Samples by Di-acid digestion Technique (Wet-Ashing) for AAS Analysis.[218]

Requirements:-

I. Instruments

(1) Heating mantle with thermostat.

(2) Kjeldhal flask of 25-100 ml. capacity (Borosil).

(3) Glass funnels.

(4) Whatmann number 42- filter paper.

II. Reagents

(1) Con. $HNO_3$ (AR grade Product No. 25,812-1, Zigma-Aldrich, India)

(2) 60 % $HClO_4$ (AR grade 68% w/w, Product No. 17,674-5, Zigma-Aldrich, India)

(3) 2N HCl (AR grade Product No. 33,925-3, Zigma-Aldrich, India )

Protocols: -

(1) Weighed 0.25 gm to 1gm of dried drug samples are taken in 25-100 ml Kjeldhal flask.

(2) Added 5 to 10 ml of Con. $HNO_3$, placed a funnel on the mouth of the flask, and kept intact for overnight at a covered place or acid digestion chamber with sufficient exhaust for pre-digestion (cold digestion).

(3) After pre-digestion, when samples are no more visible, added 5-10 ml of con. $HNO_3$ and 2 to 3 ml of $HClO_4$.

(4) Kept the volumetric flask with samples in a heating mantle in acid proof digestion chamber having fume exhaust system and heated at about $100^0$C for the first hour and then increased the temperature to about $200^0$C. The temperature must not be increased above $250^0$C in which mercury may be vaporized.

(5) Continued digestion until the contents become colorless and only white fumes appeared.

(6) Reduced acid contents to about strictly 2-3 ml by continuing heating at the same temperature. The sample was not allowed to dry up.

(7) Removed from the heating mantle, allowed to cool and added 10 ml of dilute HCl (2N) (perfectly colourless).

(8) Warmed slightly and filtered through Whatmann No.42 filter paper into 25 ml or 50 ml volumetric flask.

(9) Given 3-4 washing of 10-20 ml of quartz (double) distilled water and made the volume to 25 or 50 ml.

(10) Serial dilutions were used to test highly concentrated drug samples by AAS analysis.

**2.2.3: Preparation of Blank Solution.**

The blank solution has been prepared by following the same procedure, omitting the active ingredients that have been added for the test sample preparation.

**2.2.4: Preparation of Standards.**

1. Mercury-standard solution contains 1mg/ml – 250mg/ml of Hg in 1 wt. percentage $HNO_3$, (Product No. 20729-2, Zigma-Aldrich).

   Lead-standard solution contains 1mg/ml – 250mg/ml of Pb in 1 wt. percentage $HNO_3$, (Product No. 201723-3, Zigma-Aldrich).

**2.2.5: AAS Analysis of drug samples for mercury and lead.**

The mercury and lead contents in the drug samples were estimated by atomic absorption spectrophotometry. For this purpose the di-acid digested drug samples were sent to Indian Institute of Spices Research (IISR), Chelavoor, Calicut and Central Institute of Fisheries Technology (CIFT), Matsyapuri, Cochin, where the AAS was carried out. The results were collected and recorded as per laboratory norms.

## 2.3: Animal Model Experiments.

### 2.3.1: Selection of Drugs and Anupana Dravas for Animal Studies.

Atomic absorption spectrophotometric analysis of forty Ayurvedic drugs, including patented herbal drugs had been done. Among these drugs, two each from mercury and lead group and a native mineral of mercury have been selected for animal study. The details of drugs used for animal experiments are given below.

### 2.3.2: Mercurial Drugs

(1) Rasasindhuram powder

| | | |
|---|---|---|
| Code used in drug test | : | RS |
| Reference for drug preparation | : | Rasaratnasamuchayam |
| Drug manufactured by | : | Kerala based company |
| Batch number | : | 39 |
| Date of manufacture | : | 28-11-2001 |
| Expiry date | : | Not mentioned |
| Category of drugs | : | Ayurvedic |
| Raw materials used | : | Balarasam (Pure Mercury) and Suddha Gandhakam (pure sulphur) |
| Anupaana dravaas/ adjuvants | : | Juice of ocimum/juice of ginger or honey |
| Therapeutic use | : | Fever (Jvara), Rheumatic fever, Respiratory diseases, Hemorrhagic cancer<br>Gonorrhea, syphilis, leprosy, arthritis, leukhoria etc… |
| Dosage | : | 50 mg to 100 mg twice a day for 7to 14 days |
| Diet control | : | Compulsory and according to the type of disease |

Dose of Drug for Test Animals and Concentration of Mercury

| | | |
|---|---|---|
| Dose for a 200 gm rat | : | 0.66mg/day (3.3 mg/kg/day) |
| Percentage of Hg in the drug | : | 3.827% (38.270mg/gm) |

**(**2) Swasanandam tablet

| | | |
|---|---|---|
| Code used in the drug test | : | SG |
| Reference for drug preparation | : | Arogyakalpadrumam |
| Drug manufactured by | : | Kerala based company |
| Batch Number | : | 858 |
| Date of manufacture | : | Sep.2002 |
| Expiry date | : | Not mentioned |
| Category of drug | : | Ayurvedic |
| Raw materials used | : | Pure chayilyam (cinnabar; red sulphide of mercury) and pure camphor |
| Dosage of drug | : | One tablet twice or thrice a day for14-28 days or continuous use for chronic conditions. |
| Anupaana dravaas (adjuvants) | : | Juice of ginger/ocimum/lemon |
| Therapeutic use | : | Bronchiospasm, asthma, fever due to respiratory dysfunctions |
| Diet control | : | Necessary with avoiding cold food items fatty and meaty items and avoid cold climate and dusty surroundings. |

Dose of Drug for Test Animals and Concentration of Mercury

| | | |
|---|---|---|
| Doze of drug for 200 gm Rat | : | 1.07mg/day (5.38mg/Kg/day) |
| Percentage of Hg in the drug | : | 2.8% (28.07 mg/gm) |

(3) Chayilyam or Hingulam

| | | |
|---|---|---|
| Code used in the drug test | : | CYM |
| Reference for mineral selection | : | Ayurvedic Formulary of India-1978 |
| Mineral manufactured by | : | Available in any Ayurveda raw material shop |
| Batch Number | : | Nil |
| Date of manufacture | : | Nil |
| Expiry date | : | Nil |
| Category of mineral | : | Mineral of mercury |
| Major ingredients | : | Mercuric sulphide (cinnabar) |
| Dosage of drug | : | Same dose as that of Swasanandam |
| Anupaana dravaas (adjuvants) | : | Juice of ginger/ocimum/lemon |
| Therapeutic use | : | Not mentioned |
| Diet control | : | Same as that of swasanandam |

Dose for Test Animals and Concentration of Mercury

| | | |
|---|---|---|
| Dose of drug for 200 gm Rat | : | 1.07mg/day (5.38mg/Kg/day) |
| Percentage of Hg in the drug | : | 65.58% (655.848mg/gm) |

### 2.3.3: Lead-containing drugs

(1) Nagabhasmam powder

| | | |
|---|---|---|
| Code used in the drug test | : | NGB |
| Reference for drug preparation | : | Raasatharangini |
| Drug manufactured by | : | Kerala based company |
| Batch Number | : | 13 |
| Date of manufacture | : | 1-7-2002 |
| Expiry date | : | Not mentioned |
| Category of drug | : | Ayurvedic |
| Ingredients used | : | Naaga (Lead) |
| Dosage of drug | : | 100 mg to 200 mg twice a day for 7-14 days |
| Anupaana dravaas (adjuvants) | : | Honey, lime juice or sugar and cold water |
| Therapeutic use | : | *Atisaara* (diarrhea with vomiting), *grahini*, *gulma*, piles, hemorrhoids and diabetes mellitus. |
| Diet control | : | Not necessary |

Dose of Drug for Test Animals and Concentration of Lead

| | | |
|---|---|---|
| Dose of drug for 200 gm Rat | : | 0.66mg/day (3.33mg/kg/day) |
| Percentage of Pb in the drug | : | 84.49% (844.977 mg/gm) |

(2) Patented Herbal Drug-1

| | | |
|---|---|---|
| Code used in the drug test | : | PHD-1 |
| Reference for drug preparation | : | Traditional patented drug |
| Drug manufactured by | : | Delhi based company |
| Batch Number | : | 4743 |
| Date of manufacture | : | 12-8-2002 |
| Expiry date | : | Not mentioned |
| Category of drug | : | Traditional Ayurvedic |
| Raw materials used | : | Not mentioned |
| Dosage of drug | : | One or two tablet twice a day for 2-6 weeks |
| Anupaana dravaas (adjuvants) | : | Cow milk or cold water |
| Therapeutic use | : | To increase body vigor and sexual stamina |
| Diet control | : | Not mentioned |

Dose of Drug for Test Animals and Concentration of Lead

| | | |
|---|---|---|
| Dose of drug for 200 gm Rat | : | 2.6mg/day (13.02mg/kg/day) |
| Percentage of Pb in the drug | : | 7.9% (79.327 mg/gm) |

## 2.4: Selection of Test and Control Animal Groups

### 2.4.1: Test Animal Groups

The test animal models consisted of 40 Wistar Albino rats, with males and females almost in equal numbers. The test animals, which were aged 4 to 6 months, were categorized into five groups. All the test animals have had an average weight of 200±10grams. Rats were kept in labeled cages as per the standard protocols of animal house keeping. All the rats were fed with normal rat feed (Godrej Agrovet Rat feed, supplied by Kamadhenu Enterprises, Banglore-1) and water *adlibitum***.** The test animals have been divided into two different drug models; mercury and lead.

### 2.4.2: Control Animal Groups

Control groups consisted of 16 Wistar Albino rats, with males and females almost in equal numbers. This group has been divided into two groups (eight animals each); one for the study on mercury and the other for the study on lead. The control animals were appeared healthy and have had an average weight of 200±10grams. Each control group was treated with the corresponding anupaana dravas (adjuvants) of drugs at same dose and duration, and followed all the tests and observation procedures that have been done for the drug treated group.

## 2.5: Mercury Drug Models

Mercury drug models consisted of three groups with eight animals each: rasasindhuram (RS), swasanandam (SG) and chayilyam (CYM) groups.

### 2.5.1: Rasasindhuram Drug Group

The animals of this drug model were treated with rasasindhuram powder (RS) at a dose of 2.5 mg/kg/day for 14 days. The powder was mixed with juice of ginger as adjuvant (anupaana drava) and made into a paste by using a mortar and pestle. This paste was diluted with ginger juice, according to the dose calculated for the animal group.

### 2.5.2: Swasanandam Drug Group

The animals of the second drug model were ingested with swasanandam tablet (SG) at a dose of 5.38 mg/kg/day for 14 days. The tablets were made into pastes with anupaana drava (juice of ginger) by using a mortar and pestle. The paste was diluted again with anupaana drava according to the dose calculated for the animal group.

### 2.5.3: Chayilyam Drug Group

The animals of the third group were treated with a mineral of mercury; the chayilyam (CYM) at a dose of 5.38 mg/kg/day for 14 days. The solid mineral was made into a paste with anupaana drava (juice of ginger) by using a mortar and pestle. The paste was diluted again with anupaana drava according to the dose calculated for the animal group.

## 2.6: Lead Drug Models

This drug model consisted of two groups (eight animals each); nagabhasmam (NGB) and patented herbal drug-1 (PHD-1) groups.

### 2.6.1: Nagabhasmam Drug Group

The animals of this drug model were treated with nagabhasmam powder (NGB) at a doze of 3.33 mg/kg/day with cold water (adjuvant) for 14 days. The drug was made into a paste with anupaana drava (cold water). The

paste was diluted again with anupaana drava according to the dose calculated for the animal group.

### 2.6.2: Patented Herbal Drug-1 Group

The animals of this group were treated with patented herbal drug-1 (PHD-1) at dose of 13.02 mg/kg/day for 14 days. The tablets were made into a paste with cold water by using a mortar and pestle. The paste was diluted again with cold water according to the dose calculated for the animal group.

## 2.7: Method of Drug Administration (ingestion)

Tablets and powders were made into paste with specific anupaana dravaas (adjuvant) and diluted with the same to prepare the dose prescribed for each drug. Forced (compulsory) ingestion has been done by using syringe and feeding tube. Drug administration has been done between 7 PM and 8 PM because of the nocturnal behavior of the animal. During drug administration period (14 days), the prescribed doses and durations for each drug were strictly followed.

## 2.8: Observation of Post-Drug Administration Symptoms

The drug treated groups were fed with normal feed and water *adlibitum*. Recurrent and timely observations were done for each drug model for three days. The post drug administration symptoms were recorded systematically. The symptoms taken into consideration were anorexia, oliguria, diarrhea, emaciation (general weakness), weight gain/loss, paresis (hyperactivity) and sluggish movements, hair loss (alopecia), pruritis, gingivitis (gingival inflammations and lesion), scabby lesion around mouth, anus etc…

## 2.9: Collection of Blood Samples after Drug Administration Period for the Estimation of Mercury and Lead by AAS.

Blood Sample Preparation: -

Immediately after drug administration period (14 days), 0.5 ml to 1 ml of blood was collected from drug treated and control animals by tail cannulation technique. Acid digested samples were prepared according to the protocols mentioned above. The colourless samples were stored in labeled glass bottles (mercury and lead free) for AAS analysis as per laboratory norms.

## 2.10: Estimation of Delta-aminolaevulinic Acid (δ-ALA) by Ion Exchange Chromatography

### 2.10.1: Principle of Ion Exchange Chromatography.[219]

In ion exchange chromatography, solutes in a sample are separated by their differences in sign and magnitude of ionic charge. In practice, ionic analytes are selectively eluted from ion exchange resins by varying pH and or ionic strength of the mobile phase.

Types of resins**: -**

(1) Cation exchange resins: - Contain covalently bonded, negatively charged functional groups. These can be of two types, strong acidic such as sulphonate ions, or widely acidic groups such as carboxylate ions or sulphoethyl, carboxy methyl phosphate, sulphomethyl or sulphopropyl. This technique is most useful for the separation of organic and inorganic ions, aminoacids, nucleotides and proteins.

(2) Anion exchange resins: - Characterized by strongly basic quaternary amines, such as triethylaminoethyl groups or weakly basic groups such as aminoethyl, diethylaminoethyl, guanidoethyl, and epichlorhydrin triethanolamine groups, which can bear a positive charge.

**2.10.2: Clinical Application of Ion Exchange Chromatography.**

Ion exchange chromatography is clinically used for the separation of hemoglobin-variants, isoenzymes of creatine kinase or lactate dehydrogenase and amino acids. Ion exchange chromatography is also used to remove interfering ions from mixtures; for example, urinary porphobilinogen and delta-aminolaevulinic acid can be retained on ion exchange resins, while interferences are eluted. Porphobilinogen and δ-ALA can then be eluted with acid, and then reacted with Ehrlich's reagent and quantified. In this study, the binding property of ion exchange resins (dowex-2x8 and dowex-50x8) to delta-aminolaevulinic acid was used to estimate δ-ALA in urine.

Requirements and apparatus: -

(1) Chromatography columns----2

Size of columns used: 30 cm x 0.7cm (Borosil)

Reagents**:** -

(1) Dowex 2 x 8, 200-400 mesh size (Zigma-Aldrich fine chemicals, Product No. 9049-11-9)

(Strong basic anion exchangers)

Active group: Dimethyl ethanol benzyl ammonium.

Cross linkage: 8%, Moisture content: 34-40%

Ionic form: Chloride.

Effective pH: 0-14.

Capacity: 1.2 mE /ml wet resin.

Maximum operating temperature: $99^0$C.

(2) Dowex 50 x 8, 200-400 mesh size (Zigma-Aldrich fine chemicals, Product No. 69011-20-7)

Type of resin: Strong Acidic Cation Exchangers, gel form

Active group: Sulphonic acid

Cross linkage: 8%, Moisture content: 50-58

Ionic form: Hydrogen

Effective pH: 0-14.

Exchange Capacity: 1.7mE/ml

Maximum operating temperature: $150^0$C.

(3) Sodium acetate (Standard grade, BDH Chemical Ltd. Poole, England) 3N, 408gms of trihydrate per liter of solution (double distilled water used)

(4) Silver nitrate solution (AR grade, Zigma-Aldrich Biochemicals & Reagents, product No. S-0139). Dissolved a small quantity of $AgNO_3$ in 50 ml double distilled water then added 1 ml Nitric acid.

(5) Sodium hydroxide 2N (AR-grade, Zigma Biochemicals&Reagents Product No. S-505-8)

(6) Hydrochloric acid 2N (AR grade Product No. 33, 925-3, Zigma-Aldrich)

(7) Sodium acetate-M, 136 gms of trihydrate per liter of solution.

(8) Acetyl acetone (2, 4-Pentanedione, Product No.A-3511, Zigma-Aldrich)

(9) Acetate buffer pH. 4.6 dissolved 54mLglacial acetic acid (AR-grade, Product No. A 6283, Zigma-Aldrich) and 136 gms of sodium acetate (trihydrate) in about 500 ml of distilled water and diluted to a liter with distilled water.

(10) Ehrlich's reagent. Dissolved one gram of p-dimethyl benzaldehyde, (Dimetyl aminobenzaldehyde, Product no. D2004, Zigma-Aldrich) in about 30 ml glacial acetic acid and 8 ml perchloric acid then made to 50 ml with more glacial acetic acid. Prepare on the same day of the experiment.

### 2.10.3: Protocols for the Preparation of Ion Exchange resins [219]

(1) Dowex 2 x 8 columns:

To remove finer particles, stirred the resin in water, allowed to stand for one to two hours, decanted the supernatant and repeated the washing until a clear supernatant is obtained. Used a glass tube of 30 cm x 0.7 cm with an indentation 10 cm from the bottom, above which a plug of glass wool is knitted. Enough quantity of slurry (resin) was added to get a column height of 2.0 ± 0.1 cm, when it has settled. A flow rate of about 0.3ml per minute must be observed. The column was washed with 3N sodium acetate. Tested this washed liquid with silver nitrate solution and continued washing until the liquid became chloride free. Then washed free of acetate with double distilled water, then the column was ready for use.

(2) Dowex 50 x 8 Column:

The dowex 50x8 resin was washed as above to remove the finer particles. To convert the resin to sodium form, kept the resin in a closed beaker with 2N sodium hydroxide for over night. The second column was prepared with the alkaline resin as column-1. Then washed until neutral with

distilled water and converted to the acid form by treating with 4N Hydrochloric acid. Then successively treated with six volumes of the 2N hydrochloric acid and then with double distilled water. The column was kept in twice its volume of double distilled water.

### 2.10.4: Determination of Urine Delta-aminolaevulinic acid (δ-ALA)

1. Collection of urine samples from the test and control animals.

After fourteen days of drug administration, the lead drug treated and their control animals were kept in labeled metabolism cages. The twenty-four hours' urine samples were collected and stored as per laboratory norms for the estimation of δ-ALA.

2. Procedure for the estimation of δ-ALA.

For the determination of δ-ALA, applied 1 ml urine to the dowex 2 x 8 column. Washed twice with 2 ml. distilled water, then applied the combined elutes to the dowex 50 x 8 column and washed with 16 ml. water to remove urea. To elute the δ-ALA, first added 3-ml. 0.5 M sodium acetate. Transferred this elute to a 10 ml stoppered, graduated cylinder, added 0.2 ml. acetyl acetone and made to 10 ml. with the acetate buffer with pH. 4.6; then placed the glass stoppered cylinder in a boiling water bath for 10 minutes. Cooled to room temperature, then added 2 ml of Ehrlich's reagent, mixed and read the resulting colour 15 minutes later at 555 nm against a blank of 0.5 M sodium acetate treated in the same way as the eluate.

Micro gram of δ- amino laevulinic acid/ml = Extintion of unknown x 47.

## 2.11: Postmortem Studies.

### 2.11.1: Postmortem Studies of Animals died during Drug Administration Period.

Some experimental rats were dead before the drug administration was completely over. The animals died during drug administration period were subjected to postmortem studies as per standard procedures.[220] The observations and findings of postmortem were recorded as per laboratory norms.

### 2.11.2: Postmortem Studies of Sacrificed Animals.

After fourteen days of drug administration and three days of post-drug administration period, all the test and control animals were sacrificed. Blood samples were collected from each test and control animal by jugular vein puncture. The blood samples were labeled and kept for serum analysis. Postmortem studies of the sacrificed animals were done as per standard protocols.[220] The observations and findings were recorded as per norms. During autopsy, the specimens (kidney, liver and brain) to be subjected for AAS analysis and histopathological studies were collected and stored.

## 2.12: AAS Analysis of Tissue Samples for Mercury and Lead.

The tissue samples of kidney, liver and brain collected during postmortem studies were subjected to di-acid digestion. The colourless samples were stored in labeled glass bottles free from mercury and lead. The AAS analysis of tissue samples were done at Indian Institute of Spices Research (IISR), Chelavoor, Calicut and Central Institute of Fisheries Technology (CIFT), Matsyapuri, Cochin.

## 2.13: Serum Analysis for Biochemical Parameters.

Blood samples collected during postmortem studies were subjected to centrifugation for serum separation. The serum samples were used to estimate bilirubin, total protein, liver enzymes (AST, ALT and ALP), creatinine, urea, uric acid and serum calcium.

### 2.13.1: Determination of Serum Bilirubin (Total & Conjugated)

(Malloy and Evellin[221] method modified by Lo SF[222])

Serum bilirubin and sulphanilic acid in the presence of nitrous acid undergo a diazo reaction, resulting in the formation of pink coloured azobilirubin compounds with absorbance directly proportional to the bilirubin concentration. Direct bilirubin being a water soluble substance, directly react in acid medium; however indirect or unconjugated bilirubin is solubilized using a surfactant (methanol) and then reacted similar to direct bilirubin. The absorbance of the test solutions are measured at 540 nm against blanks.

### 2.13.2: Determination of Total Protein.

(Biuret method-modified by Doumas et al.,[223])

The peptide bonds of protein react with copper ions in alkaline solution to form blue-violet complex, (the Biuret reaction) each copper ion complexing with five or six peptide bonds. Tartarate is added as stabilizer whilst iodide is used to prevent auto reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and measured at 540 nm.

### 2.13.3: Determination of Serum Albumin by BCG-Dye method [224]

Albumin binds with Bromocresol green (BCG) at pH 4.2 causing a shift in the absorbance of yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin present, which is measured photometrically at 620nm.

### 2.13.4: Determination of Aspartate Amino Transferase by Kinetic Method [225]

Aspartate aminotransaminase (AAT/AST) catalyses, the transfer of amino group from aspartate to α-ketoglutarate forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH measured at 340 nm by means of a malate dehydrogenase (MDH) coupled reaction.

$$\text{Aspartate} + \alpha - \text{ketoglutarate} \xrightarrow{\text{AAT}} \text{oxaloacetate} + \text{glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

### 2.13.5: Determination of Alanine Amino Transferase by Kinetic Method [225]

Alanine amino transaminase (ALT) catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate. The pyruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH. The rate of decrease in NADH is measured photometrically at 340 nm and this is proportional to the catalytic concentration of ALT present in the sample.

$$\text{L} - \text{alanine} + \alpha - \text{ketoglutarate} \xrightarrow{ALT} \text{glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{LDH} \text{Lactate} + \text{NAD}^+$$

### 2.13.6: Determination of Serum Alkaline Phosphatase by Kinetic Method [226]

Alkaline phosphatase (ALP) catalyses the transfer of the phosphate group from 4-nitrophenyl phosphate to 2-amino, 2-methyl, 1-propanol (AMP), and liberating 4-nitrophenol in alkaline medium. The catalytic concentration is determined from the rate of 4-nitrophenol formation measured at 405 nm.

$$4-\text{nitrophenyl phosphate} + \text{AMP} \xrightarrow{ALP} \text{AMP} - \text{phosphate} + 4-\text{nitrophenol}$$

### 2.13.7: Determination of Serum Urea by Kinetic Method (Initial rate) [227]

Urease converts urea to carbondioxide and ammonia. The evolved ammonia reacts with α-ketoglutarate in the presence of enzyme glutamate dehydrogenase (GLDH). In this reaction NADH is converted to NAD. The rate of change of NADH is monitored at 340 nm and is directly proportional to the urea concentration in the sample. The overall reaction is summarised as: -

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2 \text{ (Urease)}$$

$$NH_3 + \alpha\text{-Ketoglutarate} + \text{NADH} \longrightarrow \text{Glutamate} + \text{NAD (GLDH)}$$

GLDH: Glutamate dehydrogenase

### 2.13.8: Determination of Serum Creatinine by Kinetic Method [228]

Creatinine reacts with alkaline picrate to produce a reddish colour (Jaffe's reaction). Specificity of the reaction is improved by the introduction of initial rate method. The absorbance of orange yellow colour formed is directly proportional to the creatinine concentration and is measured photometrically at 500-520 nm.

### 2.13.9: Determination of Serum Uric Acid by Uricase Method [229]

Uricase converts uric acid to allantoin and hydrogen peroxide which further reacts with a phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a coloured dye complex. Intensity of the colour is directly proportional to the amount of uric acid present in the sample and the absorbance is measured at 510 nm. The overall reaction is summerised as: -

$$\text{Uricacid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenolic compound} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + H_2O$$

### 2.13.10: Determination of Serum Calcium by Arsenazo-III Method [230, 231]

Calcium reacts with arsenazo-III under natural conditions to form a purple coloured complex, which has maximum absorbance at 650 nm. The intensity of the colour formed is directly proportional to the calcium concentration in the sample.

## 2.14: Tissue processing and slide preparation for histopathological studies.

Some portions of the internal organs (kidney, liver and brain) collected during postmortem studies were subjected to tissue processing. The detailed procedures involved in histopathological slide preparations are given below.

### 2.14.1: Protocols for Histological Slide Preparations[232]

Fixation: -

The specimens (kidney, liver and brain) dissected and collected during postmortem studies were kept in 40% formalin for twelve hours for fixation.

The formalin fixed tissues were processed by paraffin wax embedding technique. This process consists of four different steps (1) dehydration (2) clearing (3) impregnation in paraffin and (4) embedding or blocking

1. Dehydration: -

The tissues are serially kept in 60% ethanol for one hour, 80% ethanol for one hour, 90% ethanol for 1 hour and two changes of absolute alcohol for one hour each. The absolute (water content) nature of the last change of the alcohol was tested with anhydrous $CuSO_4$ (any water content in the alcohol will change the white anhydrous $CuSO_4$ to blue crystals).

2. Clearing: -

For doing clearing, the tissues were kept in chloroform for overnight (during clearing replacement of alcohol takes place). Three changes have been done, the first change in one hour, the second change after two hours and the third change after another two hours.

3. Impregnation with paraffin wax: -

Melted paraffin wax had been kept in oven at $58^{o}$c in three stainless steel beakers for three days. The tissues were bathed in the first beaker for 30 minutes, in the second and third for the same duration of time.

4. Embedding (blocking) with paraffin: -

L-blocks were laid on a glass plate and adjusted the size according to the size of the specimen. Fresh molten wax was poured into the mould to form a 3mm thick layer in the bottom of the mould and gently pressed the surface of the tissue against the solid layer in the bottom. When the surface layer of the block cooled sufficiently, immersed the block in cold water to cool it rapidly. When the wax did set quiet hard, the blocks were removed

from the mould and trimmed the wax block leaving 1-1.5 mm around the embedded tissue.

### 2.14.2: Cutting of Paraffin Sections[232]

The trimmed blocks were fixed to the block holder with the aid of heat and fitted to the microtome. The feed mechanism of the microtome was fully turned back to move the mechanism at maximum range. Inserted the rough knife in the knife holder and screwed tightly. It was ensured that, the whole surface of the block would move parallel to the edge of the knife. Tightened all the adjusting screws of the microtome to about 15µ and trimmed the block with rough knife until a complete through-through section of the tissue was cut. Replaced the rough knife with the sharp knife, checked the screws again and took 2-3 stripes to cope up with the difference in knife. Applied ice blocks to the surface of the block for a few seconds and wiped out the surface of the block to remove wetness. The microtome gauge was set according to the nature of the specimen (for kidney, liver and brain, the gauge adjusted to get 6µ- thickness). Operated the microtome and collected the ideal slices by the help of camel hairbrush.

The slightly compressed or creased slices are corrected (normalized) by floating the slices on warm water in a water bath with $56^0$C ($2^0$C below the melting point of wax). The sections are carried in clean albuminized slides (Mayer's egg albumin- prepared by taking 5 ml egg white, 5ml glycerin and one crystal of thymol mixed well and filtered and stored in refrigerator).

### 2.14.2: Staining Procedure[232]

1. Placed the sections in xyline for 2-5 minutes to remove the paraffin.
2. The sections were taken from the xylene and transferred to absolute alcohol for one minute.
3. Kept the sections in 60% of alcohol for one minute.
4. Brought the sections to running water for 5 minutes.
5. Placed the slide in haematoxyline (Mayor's) for 15 minutes.
6. The stained sections were dipped into acid-alcohol for a few seconds.
7. The haematoxylin-stained slides were drained and transferred to running tap water for blueing for 10 minutes.
8. The blued sections were transferred to 1% aqueous eosine for 2-4 minutes.
9. The sections were thoroughly washed in water.
10. Sections were transferred to 60% alcohol for 1minute.
11. Kept the sections in absolute alcohol for 1 minute.
12. Kept the sections in xylene for 1 minute.
13. A second change was done until get clear sections. To ensure the clarity, the sections were held against a dark background with light falling in them; unclear areas will have a milky appearance. Such sections can be returned to absolute alcohol and then again to xylene
14. Mounted in a dry mounting media like DPX (dextrine polysterene xylene mountant).

## 2.15: Statistical Methodology[233]

Analysis of variance (ANOVA) is used to test hypothesis about differences between two or more means. The results of the ANOVA are presented in an ANOVA table, followed by the 'F' statistic and associated P value. If the P value is less than 0.05, then we can accept the hypothesis that there is an influence of the qualitative factor on the dependent data, or that the means of at least two of the subgroups differ significantly. The standard error of a statistic is the standard deviation of the sampling distribution of that statistic. Standard errors are important because they reflect how much sampling fluctuation a statistic will show. The standard error of a statistic depends on the sample size. The mercury and lead levels in the samples are mentioned in Mean ± Standard Error (SE). The experimental data were analysed using SPSS-12.0 version (statistical package for social studies) for microcomputers.

Chapter 3

# RESULTS

## 3.1: AAS analysis Results of Ayurvedic/patented herbal drugs

### Table 3.1.1: Mercury and lead concentrations in Ayurvedic and patented herbal preparations

| Number | Name of the drug | Average mercury levels in ppm | Average lead levels in ppm |
|---|---|---|---|
| 1 | Kasthuryadi tablet | 749 | Not Detected |
| 2 | Agnikumararasam tablet | 289 | ND |
| 3 | Vettumaran tablet | 33000 | ND |
| 4 | Chandraprabha tablet | 112 | ND |
| 5 | Swasanandam tablet | 28070 | ND |
| 6 | Rasasindhuram powder | 38275 | ND |
| 7 | Suryaprabha tablet | 25251 | ND |
| 8 | Abrakabhasmam powder | ND | ND |
| 9 | Kanthasinthuram powder | ND | ND |
| 10 | Shankubhasmam powder | ND | 59 |
| 11 | Annabhedichurnam powder | ND | ND |
| 12 | Lohasavam liquid | ND | ND |
| 13 | Lohabhasmam powder | ND | ND |
| 14 | Kumaryasavam liquid | ND | ND |
| 15 | Dasamoolarasayanam | ND | ND |
| 16 | Parushakadilehyam | ND | ND |
| 17 | Rasakarpurabhasmam powder | 39348 | ND |
| 18 | Mahavilvadi lehyam | ND | ND |
| 19 | Hingula bhasmam | 45281 | ND |
| 20 | Jathiphaladi tablet | 18984 | ND |
| 21 | Swasakudararasam powder | 38982 | ND |
| 22 | Chavyanaprasam capsule | 546 | ND |
| 23 | Chavyanaprasam lehyam | ND | ND |
| 24 | Gulguluthikthakam ghritham | Not Detected | Not Detected |
| 25 | Gulguluthikthakam liquid | ND | ND |
| 26 | Kajjali powder | 655538 | ND |
| 27 | Chayilyam (cinnabar) powder | 655848 | ND |

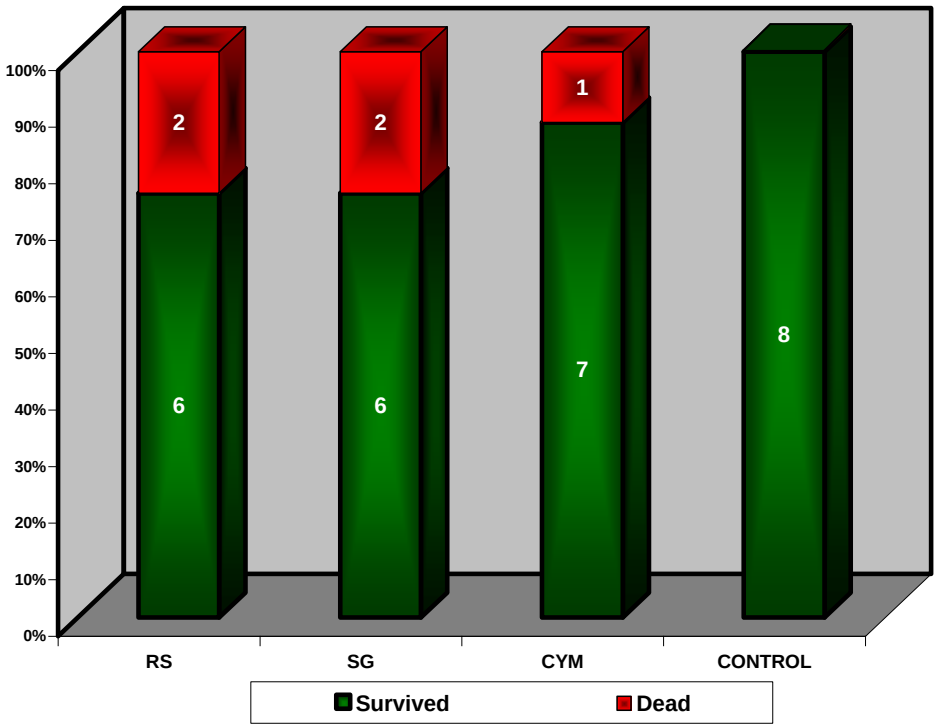
| Number | Name of the drug | Average mercury levels in ppm | Average lead levels in ppm |
|---|---|---|---|
| 28 | Nagabhasmam powder | ND | 844977 |
| 29 | Gorochanadi tablet | ND | 798 |
| 30 | Patented herbal drug-1 | 10000 | 79327 |
| 31 | Patented herbal drug-2 | ND | 14 |
| 32 | Patented herbal drug-3 | 10000 | 55827 |
| 33 | Patented herbal drug-4 | 24 | 43 |
| 34 | Patented herbal drug-5 | 15 | ND |
| 35 | Patented herbal drug-6 | 112 | 100 |
| 36 | Patented herbal drug-7 | ND | 38 |
| 37 | Patented herbal drug-8 | 42 | 121 |
| 38 | Patented herbal drug- 9 | 702 | ND |
| 39 | Patented herbal drug-10 | ND | 55 |
| 40 | Patented herbal drug-11 | ND | 62 |

Atomic Absorption Spectrophotometric results show the presence of mercury and lead in different Ayurvedic/patented herbal drugs.

Mercury and lead concentrations in certain commonly available ayurvedic/patented herbal drugs were studied. The results are given in the table-3.1.1. In addition to the Aurvedic and patented herbal drugs, one mineral of mercury (chayilyam) and a treated mineral (*kajjali*) have also been included in the study. A total of forty samples, including thirty eight drugs and two raw materials for mercurial drug preparations, were analysed by Atomic Absorption Spectrophotometry. Fourteen drugs out of forty tested samples contained substantial amounts of mercury (15-45281ppm). Seven samples contained lead (14-844977ppm) and five samples contained both mercury and lead. The mineral of mercury, chayilyam and the *kajjali* (chayilyam treated with equal amounts of sulphur) contained above 65% of mercury.

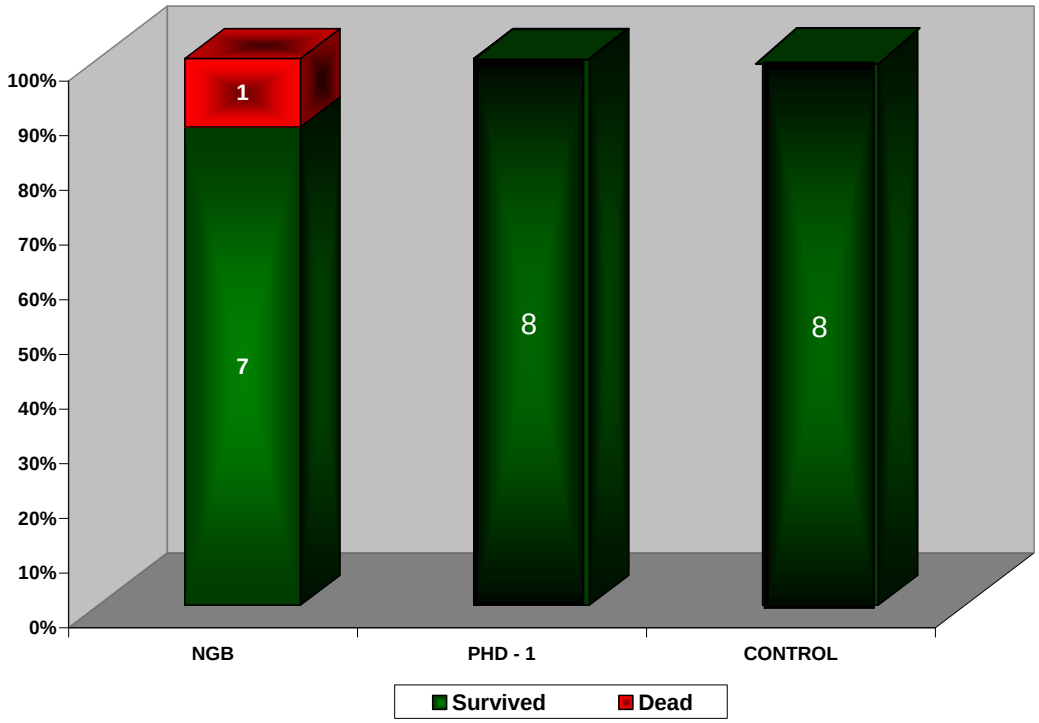
### 3.2: Mortality/Survival Rates in Drug Treated and Control Groups.

**Figure 3.2.1: Percentage of mortality/survival in mercurial drug treated and control animal groups.**




The graph shows percentage of mortality in rasasindhuram (RS), swasanandam (SG), and chayilyam (CYM) drug treated and control animal groups during drug administration period.

**Figure 3.2.2: Percentage of mortality/survival in lead drug treated and control animal groups.**




The graph shows the percentage of mortality in nagabhasmam (NGB), Patented Herbal Drug-1 (PHD-1) treated and control animal groups during drug administration period.

## 3.3: Post Drug Administration Symptoms in Mercurial Drug Treated and Control Groups.

### 3.3.1: Rasasindhuram (RS) treated group

1. Six out of eight animals were survived after drug administration.
2. Excessive salivation was noted in three animals and the remaining three were appeared normal.
3. Anorexia (loss of appetite) was very common in four animals. Food intake was normal in the remaining animals.
4. Oliguria was noted in three animals, the remaining animals' urine output was normal.
5. Emaciation and weight loss were observed in four animals.
6. Stiff legged walk was noticed in three animals, but the remaining animals showed normal walk.
7. Alopecia with pruritis was observed in five animals, the remaining animals appeared with normal hair distribution.
8. Scaby lesions around mouth and anus was noted in four animals but this type of symptoms were absent in others.
9. Tendered gingival tissues with inflammation were observed in four animals. The remaining animals appeared normal.
10. Chronic diarrhea was noted in four animals, but the remaining two with normal stool.

### 3.3.2: Swasanandam (SG) treated group

1. Six out of eight animals were survived after drug administration.
2. Excessive salivation was noted in four animals, the remaining two were appeared normal.
3. Anorexia was observed in three animals. Food intake was normal in the remaining animals.
4. Oliguria was noted in three animals, the remaining animals' urine output was normal.
5. Emaciation and weight loss were observed in three animals.
6. Stiff legged walk was noticed in three animals, but the remaining animals showed normal walk.
7. Alopecia with pruritis was observed in four animals, the remaining animals appeared normal hair distribution.
8. Scaby lesions around mouth and anus was noted in three animals but this type of symptoms were absent in others.
9. Tendered gingival tissues with inflammation were observed in three animals. The remaining animals appeared normal.
10. Chronic diarrhea was noted in three animals, but the remaining three with normal stool.

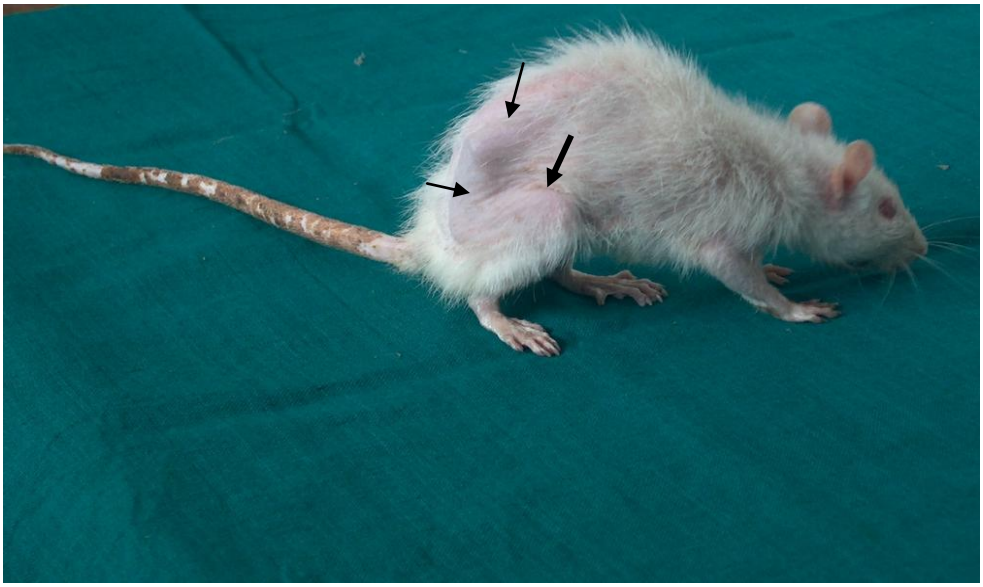
### 3.3.3: Chayilyam (CYM) treated group

1. Seven out of eight animals were survived after drug administration.
2. Excessive salivation was noted in three animals, the remaining four were appeared normal.
3. Anorexia was observed in three animals. The remaining animals in the group took normal amount of food
4. Oliguria was noted in two animals, the remaining animals' urine output was normal.
5. Emaciation and weight loss were observed in three animals.
6. Stiff legged walk was noticed in three animals, but the remaining animals showed normal walk.
7. Alopecia with pruritis were observed in two animals, the remaining animals appeared normal hair distribution.
8. Scaby lesions around mouth and anus were noted in two animals, the remaining animals appeared normal.
9. Tendered gingival tissues with inflammation were observed in two animals. The remaining animals appeared normal.
10. Chronic diarrhea was noted in two animals, but the remaining five with normal stool.

### 3.3.4: Control group

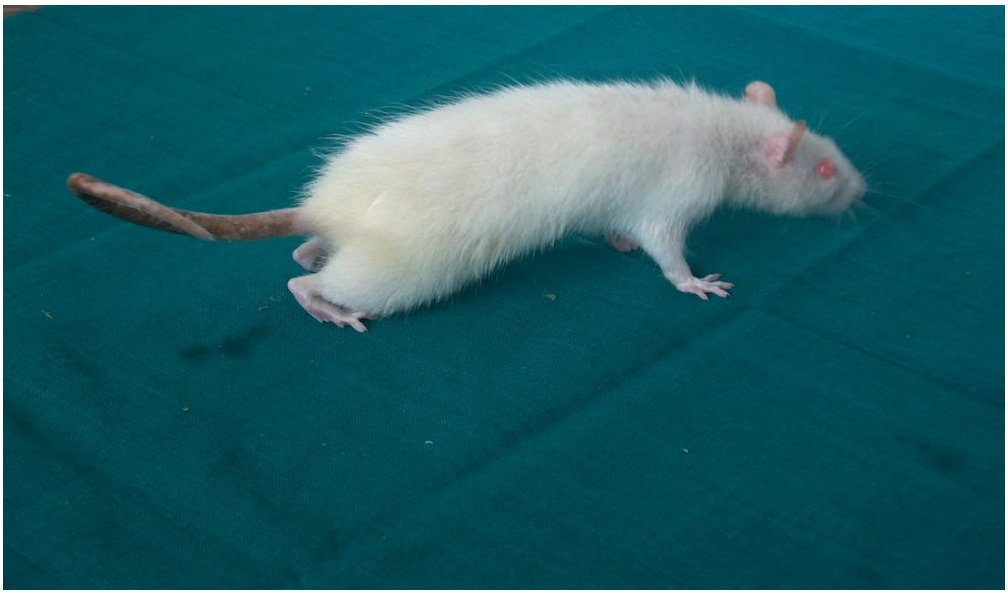
Control animals were appeared healthy and normal, after the anupaana drava administration.

**Figure- 3.3.5: Rasasindhuram treated rat with alopecia.**



Thin arrows in the picture indicate alopecia. The bold arrow indicates pruritis in the skin. The rat also appeared as emaciated with severe sick posture.

**Figure- 3.3.6: Control animal administered with anupaana dravas.**



Picture shows a healthy control rat with normal skin and hairs. The healthy posture is also noticeable.

## 3.4: Post-drug Administration Symptoms of Lead-drug Treated and Control Groups.

### 3.4.1: Nagabhasmam (NGB) treated group

1. Seven out of eight animals survived after drug administration.
2. Excess salivation and tendered gingival tissue with inflammation were not observed in any animal.
3. Anorexia was noticed in one animal only; the remaining animals took normal amount of food.
4. Oliguria was noticed in four animals, the remaining animals' urine volume was normal.
5. Diarrhea observed in two animals and the rest of the animals were appeared normal after the drug administration period.
6. Emaciation was noticed in two animals, the remaining animal appeared normal.
7. Alopecia with pruritis were noticed in one animal only, the remaining animals appeared with normal skin and hair.
8. Scabby lesions around mouth and anus were not observed in any one.
9. Hyperactivity was observed in four animals, the remaining animals were in normal movements.
10. Weight gain (10±2gm) was noted in two animals,
11. Weight loss (5gm) in one and the remaining animals appeared normal.

### 3.4.2: Patented herbal drug-1(PHD-1) treated group

1. All the animals were survived after drug administration.
2. Excess salivation and tendered gingival tissue with inflammation were not observed in any animal.
3. Anorexia was noticed in two animals only; the remaining animals took normal amount of food.
4. Oliguria was noticed in two animals, the remaining animals' urine volume was normal.
5. Diarrhea observed in two animals and the rest of the animals were appeared normal after the drug administration period.
6. Emaciation was noticed in two animals, the remaining animal appeared normal.
7. Alopecia with pruritis was not observed in any animal.
8. Scabby lesions around mouth and anus were not observed in any one.
9. Hyperactivity was observed in five animals, the remaining animals were in normal movements.
10. Weight gain (10±5gm) was noted in five animals.
11. Weight loss (5-8gm) in two animals and the remaining one appeared normal.

### 3.4.3: Control group

Control animals were appeared normal and healthy after the anupaana drava administration.

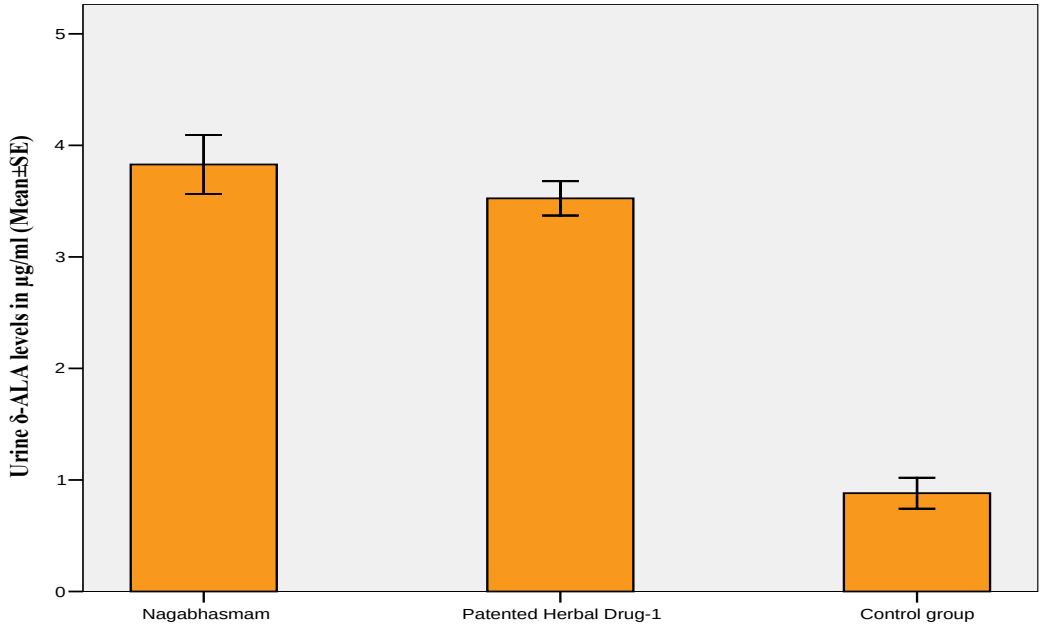
## 3.5: Estimation of Delta-aminolaevulinic Acid in the Urine Samples of Lead Drug Treated and Control Groups.

**Table-3.5.1: Urine δ-ALA levels in lead drug treated and control groups.**

| GROUPS | Urine δ-aminolaevulinic acid levels in µg/ml (MEAN±SE) |
|---|---|
| **NGB (n=7)** | 3.82 ± 0.26** |
| **PHD-1 (n=8)** | 3.52 ± 0.15** |
| **CON (n=8)** | 0.88 ± 0.13 |

** P<0.0001

**Figure- 3.5.1: δ–aminolaevulinic acid levels**




The graph shows δ-aminolaevulinic acid levels in the urine samples of nagabhasmam (NGB) and patented herbal drug-1 (PHD-1) treated and control animal groups.

The urine delta-aminolaevulinic acid levels of treated and untreated animals are given in the table-3.5.1 and figure-3.5.1. In the drug treated group, the urine δ–ALA levels were found highly elevated compared to the normal counter parts. The urine delta-aminolaevulinic levels were observed as higher in the group exposed to nagabhasmam while the values were found slightly less in the group treated with PHD-1. This is expected since the lead content was higher in the drug nagabhasmam, compared to PHD-1. The control groups showed almost normal values of urine δ–aminolaevulinic acid levels.

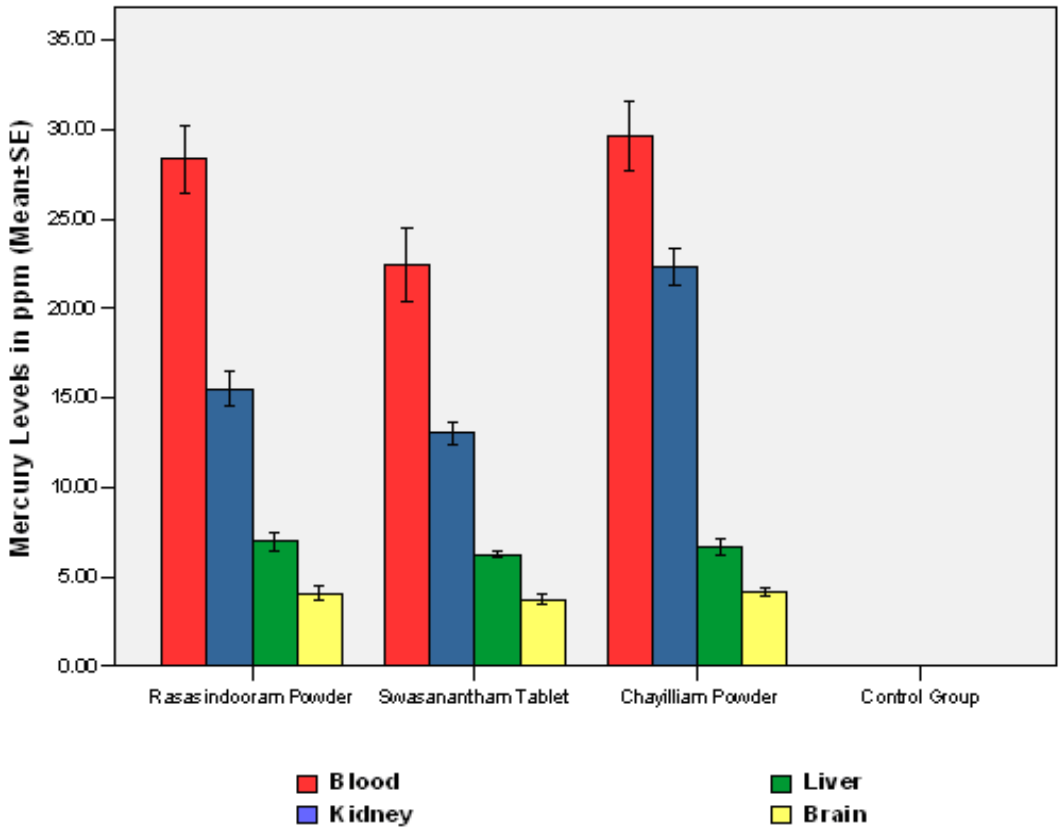
### 3.6: AAS Analysis of Tissue Samples for Mercury.

**Table- 3.6.1: Tissue mercury levels in treated and control groups.**

| GROUPS | Tissue mercury levels in ppm (Mean ± SE) | | | |
|---|---|---|---|---|
| | Blood | Kidney | Liver | Brain |
| RS (n=6) | 28.33±1.89** | 15.50±0.96** | 6.95±0.46** | 4.08±0.40** |
| SG (n=6) | 22.47±2.06** | 13.02±0.61** | 6.25±0.18** | 3.73±0.29** |
| CYM (n=7) | 29.64±1.98** | 22.36±1.02** | 6.67±0.45** | 4.19±0.25** |
| CON (n=8) | 0.08±0.01 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 |

**P<0.0001

**Figure-3.6.1: Tissue mercury levels in treated and control groups.**



The mercury levels in blood, kidney, liver and brain of the treated and untreated animal are given in the table-3.6.1 and figure-3.6.1. In the drug treated animals, the tissue levels of mercury were highly elevated compared to the normal counterparts. The maximum levels of mercury in the tissue were observed in the animals which received chayilyam and the minimum levels were noted in the group treated with swasanantham tablet. This is expected as the mercury content in the drug was higher in chayilyam followed by rasasindhuram and swasanantham. The mercury levels in the control group were negligible and the mild presence may be due to the mercurial contamination in the animal feed.

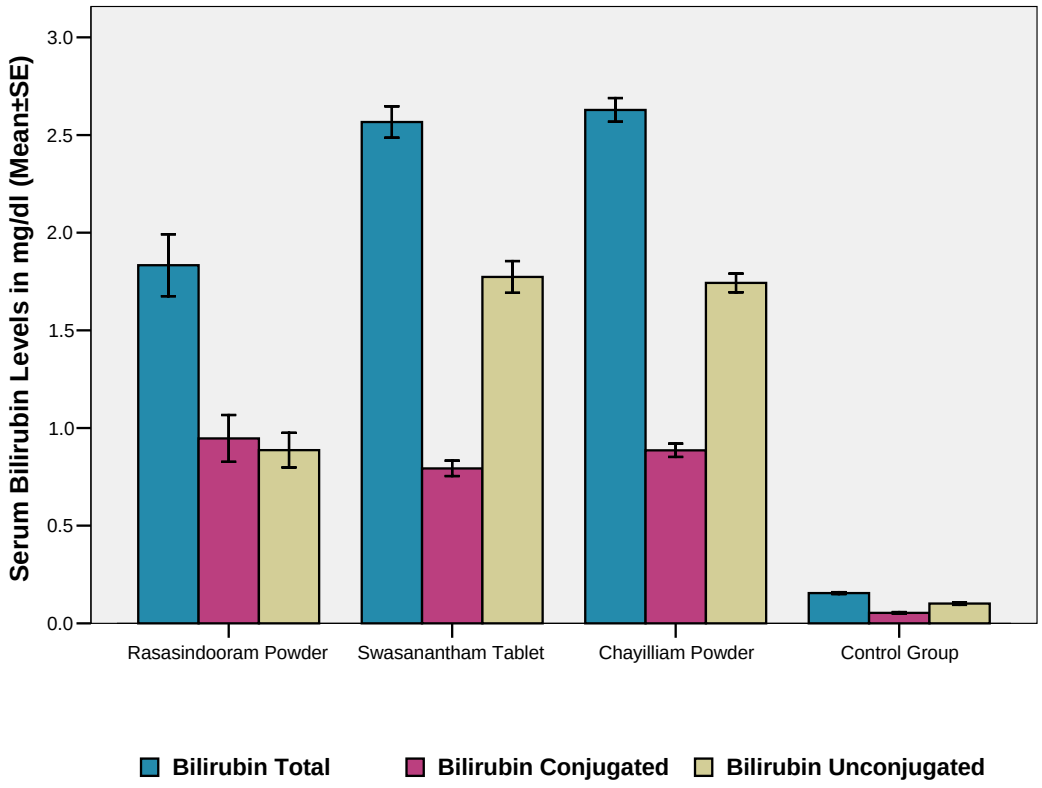
## 3.7: Serum Analysis of Mercurial-drug Treated and Control Groups for Biochemical Parameters.

**Table-3.7.1: Serum bilirubin levels in drug treated and control groups.**

| GROUPS | Serum bilirubin levels in mg/dl (Mean ± SE) | | |
|---|---|---|---|
| | Bilirubin total | Bilirubin Conjugated | Bilirubin Unconjugated |
| RS (n=6) | 1.83±0.16** | 0.95±0.12** | 0.89±0.09** |
| SG (n=6) | 2.57±0.08** | 0.79±0.04** | 1.77±0.08** |
| CYM (n=7) | 2.63±0.06** | 0.89±0.03** | 1.74±0.05** |
| CON (n=8) | 0.16±0.01 | 0.05±0.00 | 0.10±0.01 |

** P<0.0001

**Figure-3.7.1: Serum bilirubin levels in treated and control groups.**



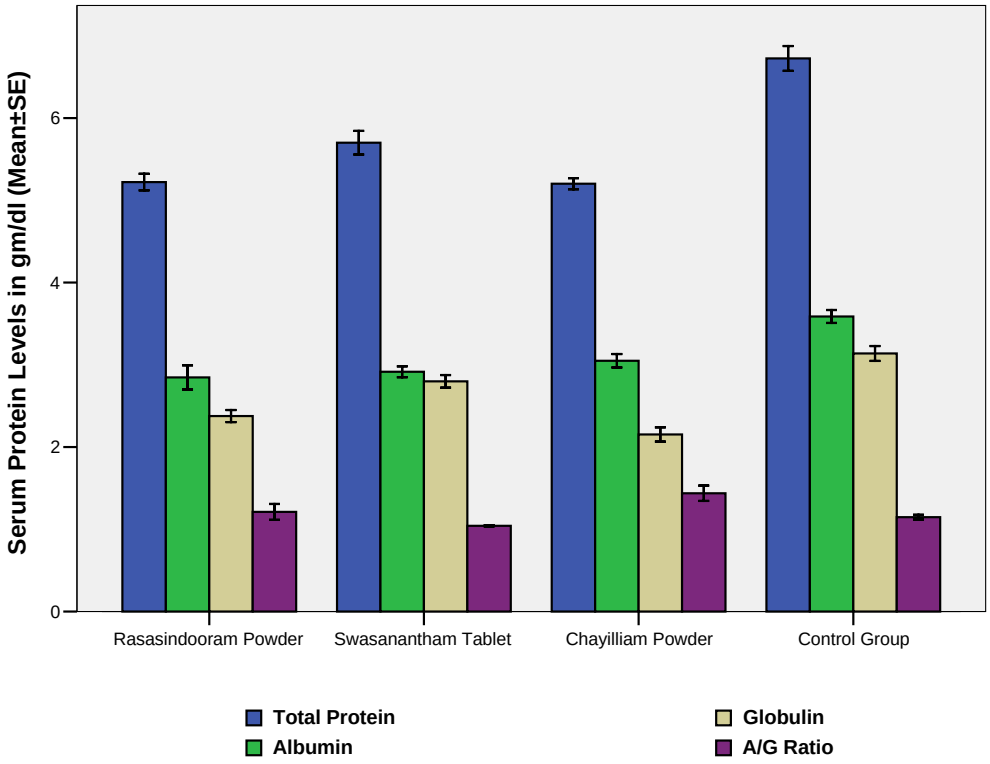
Serum total bilirubin, conjugated bilirubin and unconjugated bilirubin of the treated and untreated control animals are given in the table-3.7.1 and figure-3.7.1. In the drug treated groups, the serum bilirubin profiles were highly elevated compared to the normal counterparts**.** The maximum elevations in bilirubin, both total and conjugated, were observed in the animals which were received chayilyam and the minimum was in the group treated with rasasindhuram powder. This is expected, because the Mercury level is the highest in chayilyam group, next in the rasasindhuram group and least in the swasanandam group. The control group showed almost normal values of bilirubin profiles.

**Table-3.7.2: Serum protein levels in treated and control groups.**

| GROUPS | Serum protein levels (Mean ± SE) | | | |
|---|---|---|---|---|
| | Total protein Gm/dl | Albumin gm/dl | Globulin gm/dl | A/G Ratio |
| RS (n=6) | 5.22±0.10** | 2.85±0.15** | 2.38±0.07** | 1.21±0.10 |
| SG (n=6) | 5.7±0.14** | 2.92±0.07** | 2.80±0.08* | 1.04±0.01 |
| CYM (n=7) | 5.20±0.07** | 3.05±0.08** | 2.15±0.09** | 1.44±0.09* |
| CON (n=8) | 6.73±0.15 | 3.59±0.08 | 3.14±0.09 | 1.15±0.03 |

** P<0.0001; * P<0.05

**Figure-3.7.2: Serum protein levels in treated and control groups.**



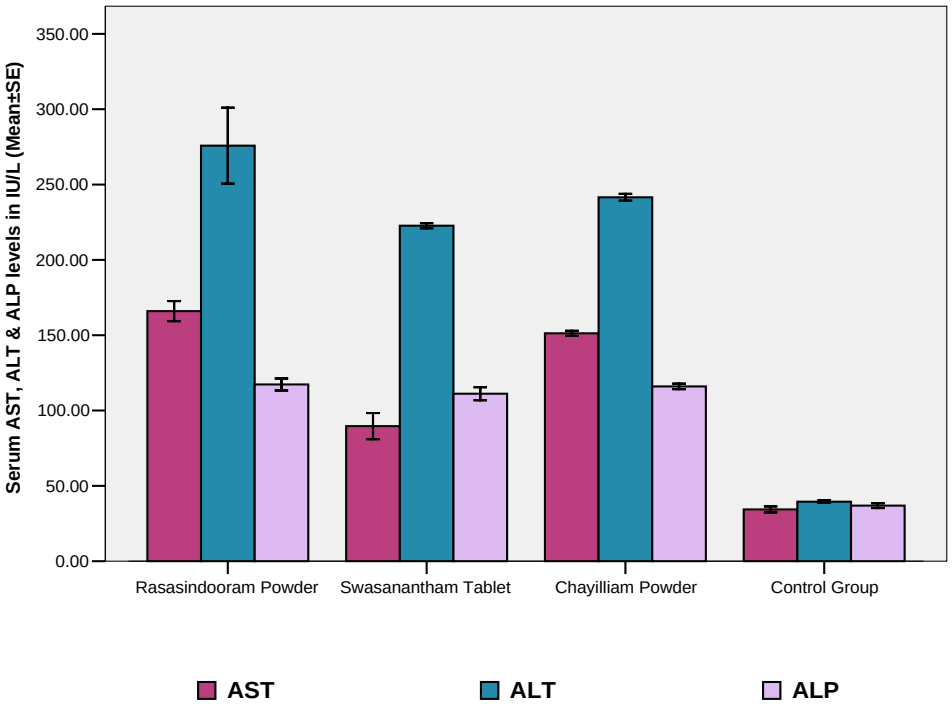

The serum total protein, albumin and globulin levels of the treated and untreated control groups are given in the table-3.7.2 and figure-3.7.2. In the drug treated groups, the serum protein levels were highly significant compared to the normal control counterparts. The chayilyam group showed the lowest levels of proteins, where as the swasanandam group presented with maximum levels of protein in the treated group. The decreases of protein levels were proportional to the mercury levels in the drugs. The alterations in the A/G ratios were not significant.

**Table-3.7.3: Serum enzyme levels in treated and control groups**

| GROUPS | Serum AST, ALT and ALP levels in IU/L (Mean ± SE) | | |
|---|---|---|---|
| | AST | ALT | ALP |
| **RS(n=6)** | 166±6.66** | 275.83±25.20** | 117.33±3.98** |
| **SG(n=6)** | 89.67±8.67** | 222.67±1.71** | 111.17±4.32** |
| **CYM(n=7)** | 151.29±1.67** | 241.57±2.26** | 116±1.85** |
| **CON(n=8)** | 34.38±1.99 | 39.50±0.85 | 36.88±1.59 |

****P<0.0001**
**AST**- Aspartate Amino Transferase, **ALT**- Alanine Amino Transferase
**ALP**- Alkaline Phosphatase

**Figure-3.7.3: Serum enzyme levels in treated and control groups**



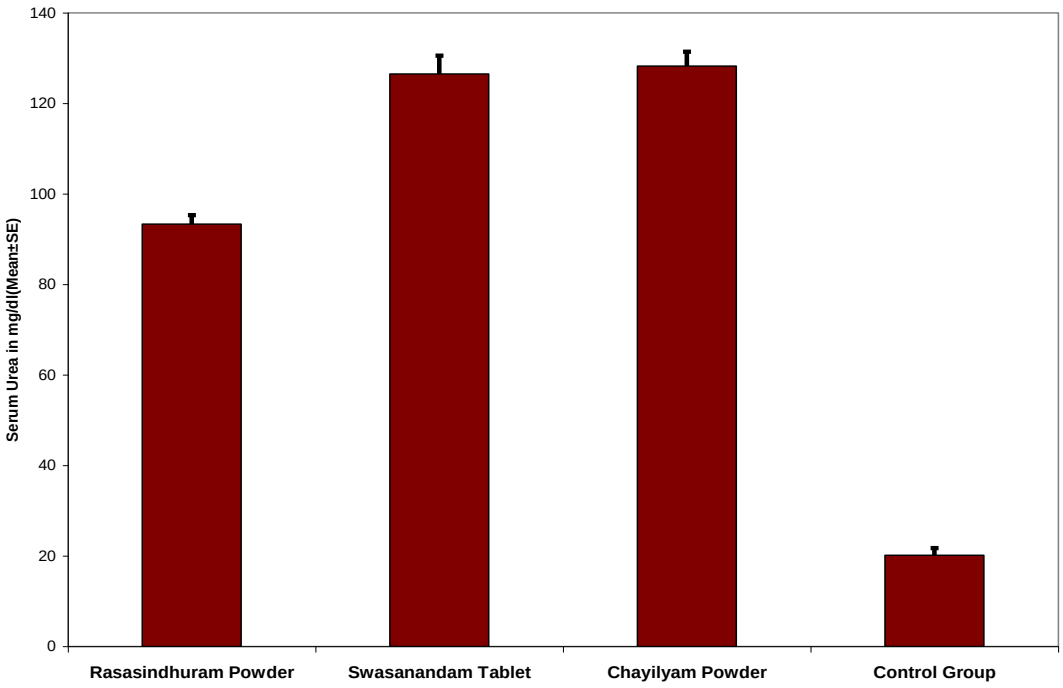

The serum aspartate amino transferase, serum alanine amino transferase and serum alkaline phosphatase levels of the treated and untreated control animals are given in the table-3.7.3 and figure-3.7.3. In the drug treated groups, the serum AST, ALT and ALP levels were highly elevated compared to the normal counterparts**.** The maximum levels of serum AST, ALT and ALP were observed in the animals treated with rasasindhuram powder and the minimum was in the group treated with swasanandam tablet. The control groups showed normal values of liver enzymes.

**Table-3.7.4: Serum urea in treated and control groups**

| GROUPS | Serum urea levels in mg/dl (Mean ±SE) |
|---|---|
| **RS(n=6)** | 93.33±2.01** |
| **SG(n=6)** | 126.50±4.02** |
| **CYM(n=7)** | 128.29±3.12** |
| **CON(n=8)** | 20.13±1.63 |

** P<0.0001

**Figure-3.7.4: Serum urea in treated and control groups.**



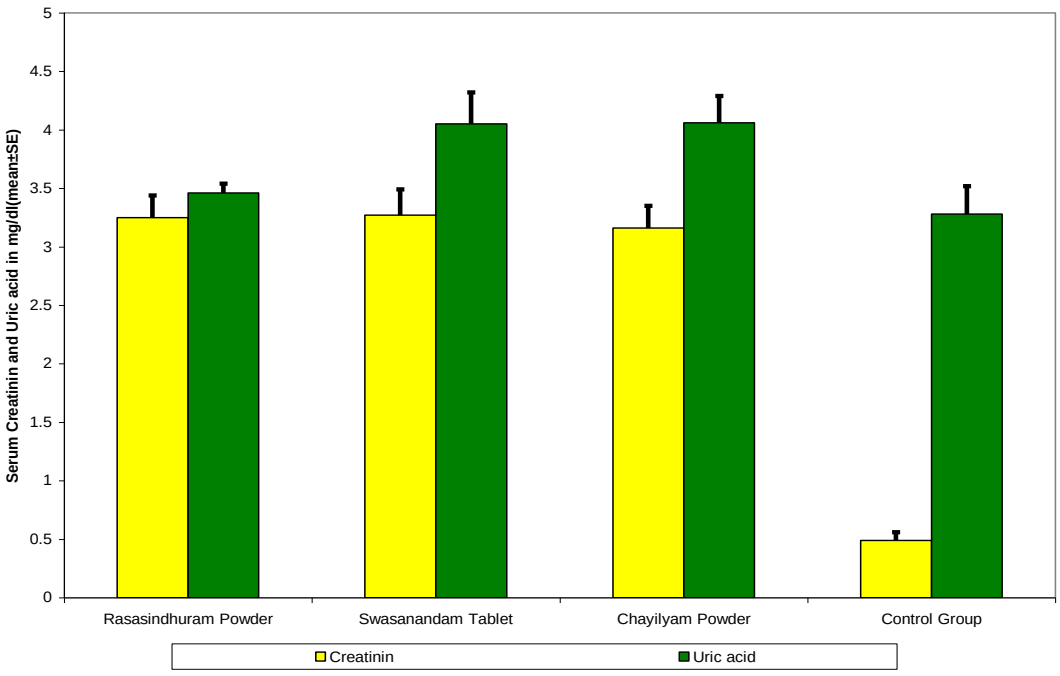

The serum urea levels of the treated and untreated animals are given in the table-3.7.4 and figure-3.7.4. In the drug treated groups, the serum urea levels were found elevated compared to the normal counterparts. The maximum elevation of serum urea was observed in the chayilyam group and minimum was noticed in the group of animals treated with rasasindhuram powder.

**Table-3.7.5: Serum creatinine and uric acid levels in treated and control groups.**

| GROUPS | Serum creatinine and uric acid levels In mg/dl (Mean ± SE) | |
|---|---|---|
| | Creatininie | Uricacid |
| RS(n=6) | 3.25±0.19** | 3.46±0.08 |
| SG(n=6) | 3.27±0.22** | 4.05±0.27 |
| CYM(n=7) | 3.16±0.19** | 4.06±0.23 |
| CON(n=8) | 0.49±0.07 | 3.28±0.24 |

** P<0.0001

**Figure-3.7.5: Serum creatinine and uric acid in treated and control groups.**



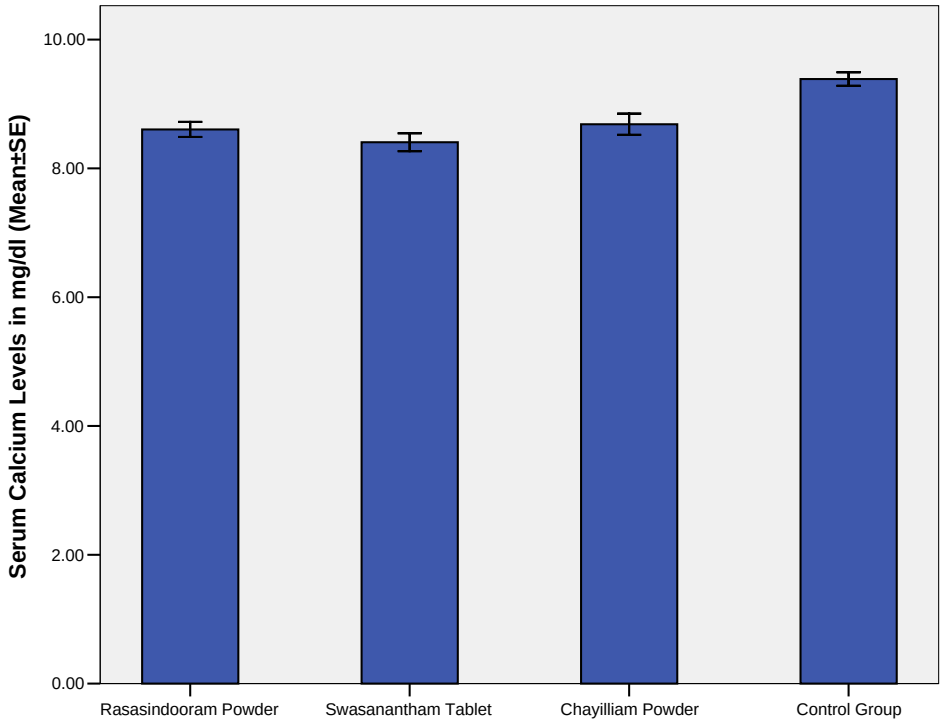

The serm cretinine and uric acid levels are given in the table-3.7.5 and figure-3.7.5. In the drug treated groups the serum creatinine levels were found elevated compared to the normal counter parts. The maximum levels of serum creatinine were observed in the animals which received swasanandam tablet and the least amount was noticed in chayilyam powder treated group. The serum uric acid level was found insignificant compared to the control group.

**Table-3.7.6: Serum calcium levels in treated and control groups.**

| GROUPS | Serum calcium level in mg/dl | |
|---|---|---|
| | MEAN±SE | Range |
| RS(n=6) | 8.61 ± 0.12* | 8.10 – 8.90 |
| SG(n=6) | 8.41 ± 0.14* | 8.10 – 9.00 |
| CYM(n=7) | 8.69 ± 0.16* | 8.00 – 9.20 |
| CON(n=8) | 9.39 ± 0.11 | 9.00 – 9.80 |

*P<0.05

**Figure- 3.7.6: Serum calcium levels in treated and control groups.**




The serum calcium levels of the treated and untreated animals are given in the table-3.7.6 and figure-3.7.6. In the drug-treated groups, the serum calcium levels were decreased compared to the normal counterparts, but the decrease was not significant. The lowest levels of serum calcium were observed in the swasanandam group, whereas the chayilyam group showed maximum amount of serum calcium. The range of calcium level of all the treated groups manifested lower values than control group, and which never overlapped the calcium range of the control animals.

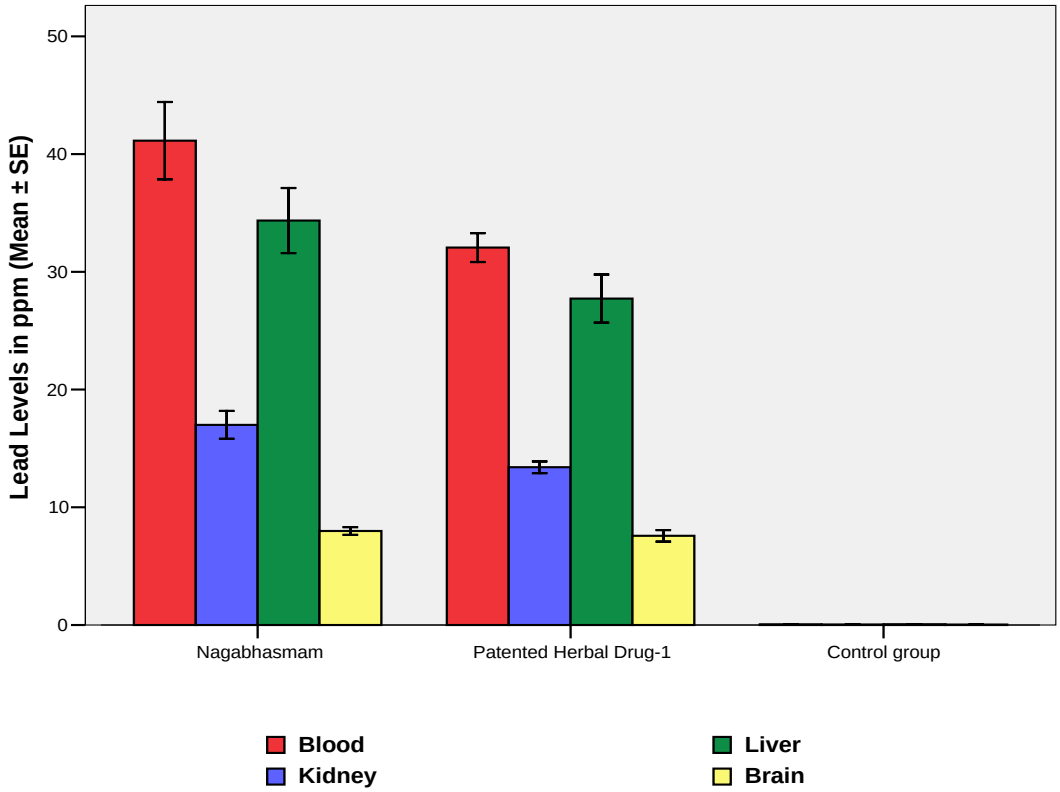
## 3.8: AAS Analysis of Tissue Samples for Lead.

**Table-3.8.1: Tissue lead levels in treated and control groups**

| GROUPS | Tissue lead levels in ppm(Mean ± SE) | | | |
|---|---|---|---|---|
| | Blood | Kidney | Liver | Brain |
| NGB(n=7) | 41.14±3.28** | 17.00±1.18** | 4.36±2.77** | 7.99±0.33** |
| PHD-1(n=8) | 32.06±1.22** | 13.40±0.50** | 27.73±2.04** | 7.58±0.49** |
| CON(n=8) | 0.05±0.02 | 0.04±0.02 | 0.07±0.01 | 0.03±0.02 |

** P<0.0001

**Figure-3.8.1: Tissue lead levels in treated and control groups**




The lead levels in blood, kidney, liver and brain of the treated and untreated animal are given in the table-3.8.1 and figure-3.8.1. In the drug treated animals, the tissue levels of lead were highly elevated compared to the normal counterparts. The maximum levels of lead in the tissue were observed in the animals, which received nagabhasmam, and the minimum levels were noted in the group treated with PHD-1. The lead levels in the control groups were negligible and the presence may be due to the mercurial contamination in the animal feed.

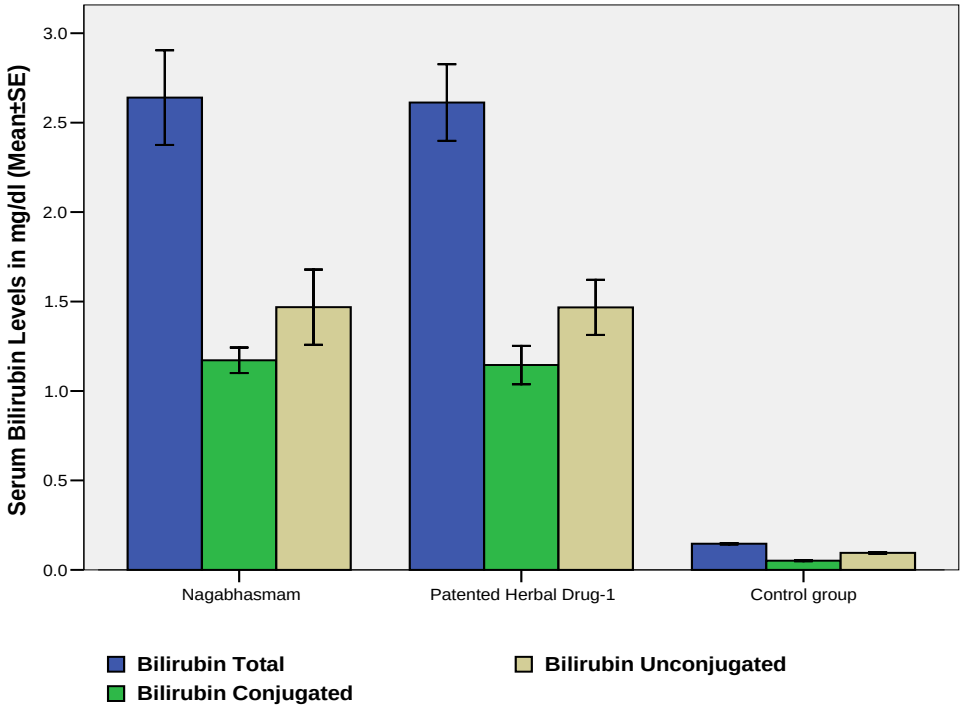
## 3.9: Serum Analysis of Lead Drug Treated and Control Groups for Biochemical parameters.

**Table-3.9.1: Serum bilirubin levels in treated and control group.**

| GROUPS | Serum bilirubin levels in mg/dl(Mean ± SE) | | |
|---|---|---|---|
| | Bilirubin total | Bilirubin Conjugated | Bilirubin Unconjugated |
| NGB(n=7) | 2.64±0.26** | 1.17±0.07** | 1.47±0.21** |
| PHD-1(n=8) | 2.61±0.21** | 1.15±0.11** | 1.47±0.15** |
| CON(n=8) | 0.15±0.00 | 0.05±0.00 | 0.10±0.01 |

** P<0.0001

**Figure-3.9.1: Serum bilirubin levels in treated and control group**

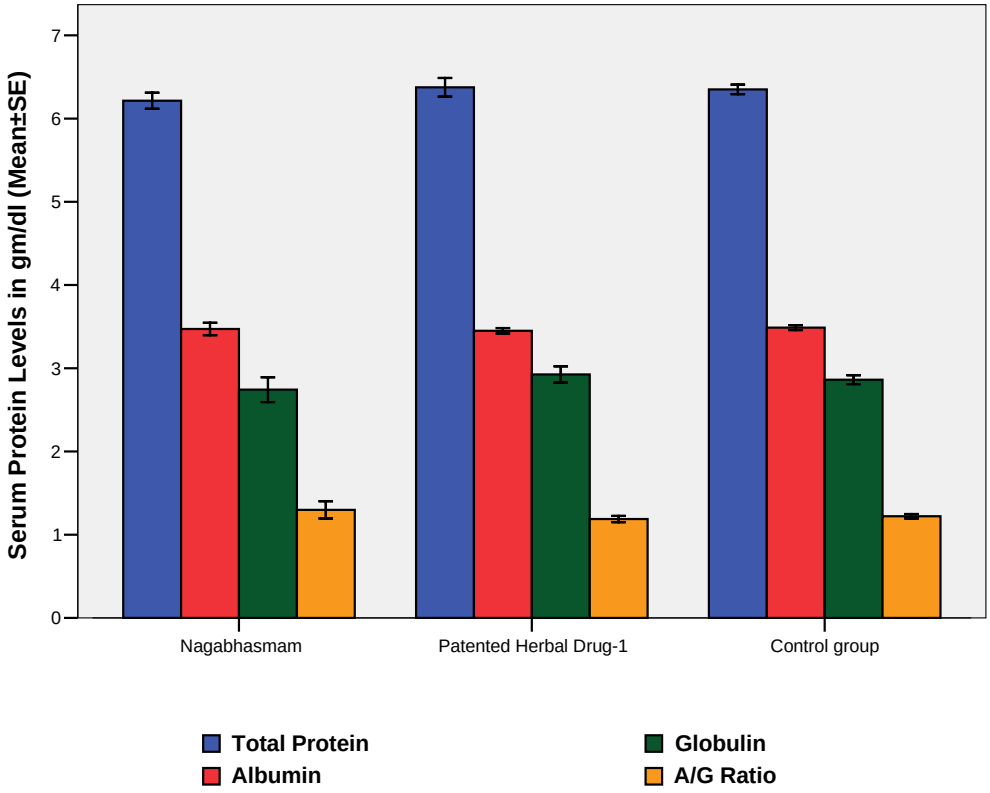



The serum total bilirubin, conjugated bilirubin and unconjugated bilirubin of the treated and untreated control animals are given in the table-3.9.1 and figure-3.9.1. In the drug treated groups, the serum bilirubin profiles were highly elevated compared to the normal counterparts**.** Higher elevations in bilirubin both total and conjugated were observed in the animals, which were received nagabhasmam compared to the group treated with PHD-1. The control group showed almost normal values of bilirubin profiles.

**Table-3.9.2: Serum protein levels in treated and control groups**

| GROUPS | Serum protein levels (Mean ± SE) | | | |
|---|---|---|---|---|
| | Totalprotein Gm/dl | Albumin gm/dl | Globulin gm/dl | A/G Ratio |
| PHD-1(n=7) | 6.21±0.10 | 3.47±0.07 | 2.74±0.15 | 1.30±0.10 |
| NGB(n=8) | 6.38±0.11 | 3.45±0.03 | 2.93±0.10 | 1.19±0.04 |
| CON(n=8) | 6.35±0.06 | 3.49±0.03 | 2.86±0.05 | 1.22±0.03 |

**Figure-3.9.2: Serum protein levels in treated and control groups**



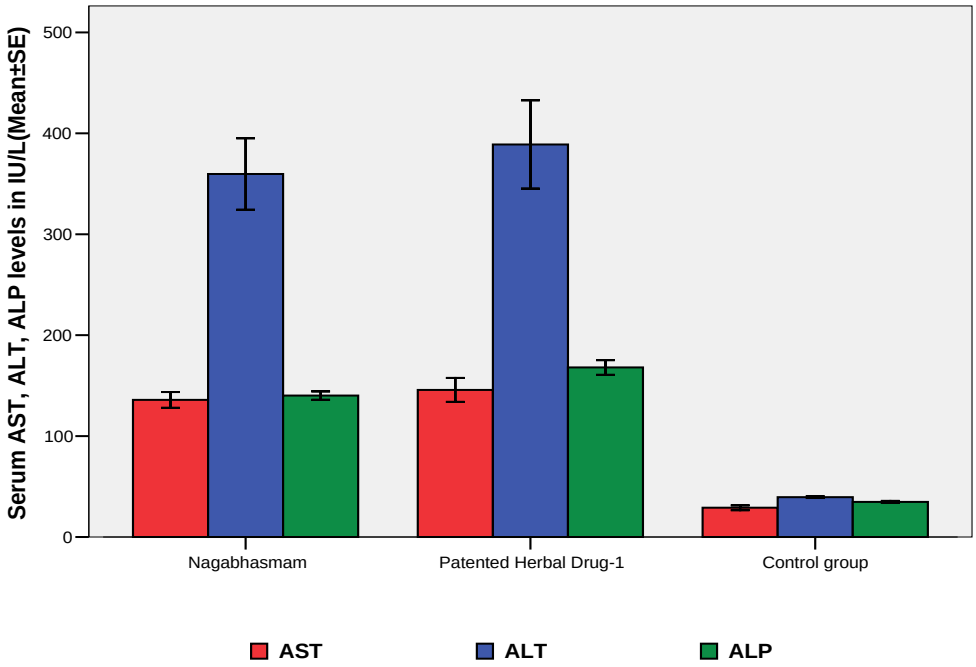

The serum total protein, albumin and globulin levels of the treated and untreated control groups are given in the table-3.9.2 and figure-3.9.2. In the drug treated groups, the serum protein levels were insignificant compared to the normal counterparts. The nagabhasmam group showed the lower levels of proteins, whereas the PHD-1 group presented with slightly higher levels of protein in the treated group. The alterations in the A/G ratios were not significant.

**Table-3.9.3: Serum enzyme levels in treated and control groups**

| GROUPS | Serum AST, ALT and ALP levels in IU/L (Mean ± SE) | | |
|---|---|---|---|
| | AST | ALT | ALP |
| NGB(n=7) | 135.86±7.82** | 359.71±35.42** | 140.14±4.17** |
| PHD-1(n=8) | 145.75±11.89** | 389.00±43.80** | 168.00±7.31** |
| CON(n=8) | 29.00±2.41 | 39.50±0.85 | 34.75±0.98 |

****** P<0.0001
**AST**- Aspartate Amino Transferase, **ALT**- Alanine Amino Transferase
**ALP**- Alkaline Phosphatase

**Figure-3.9.3: Serum enzyme levels in treated and control groups**



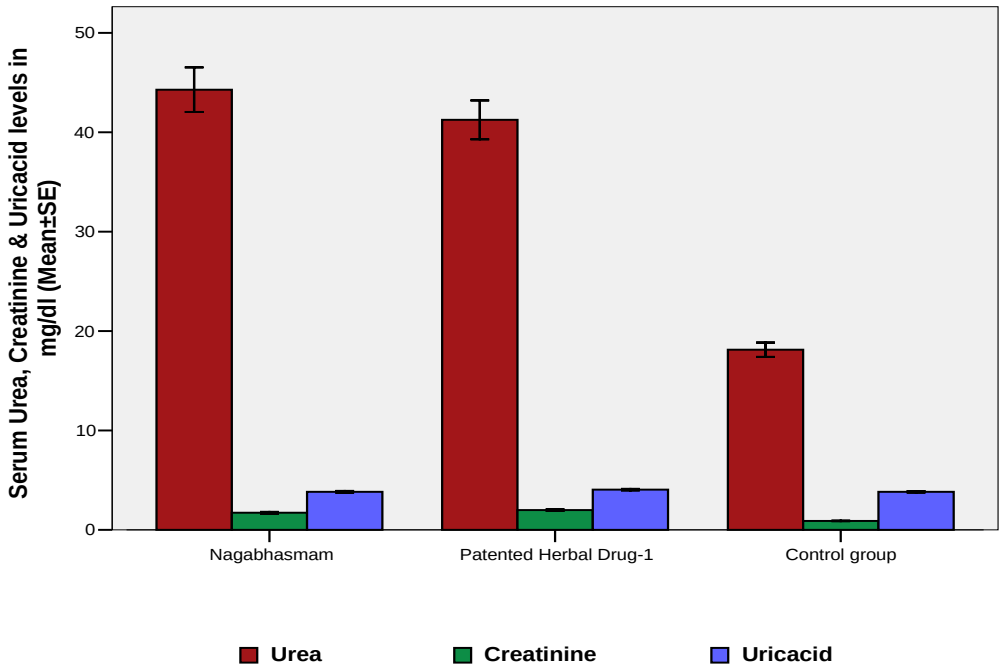

The serum aspartate amino transferase, serum alanine amino transferase and serum alkaline phosphatase levels of the treated and untreated control animals are given in the table-3.9.3 and figure-3.9.3. In the drug treated groups, the serum AST, ALT and ALP levels were highly elevated compared to the normal counterparts**.** Comparatively higher levels of serum AST, ALT and ALP were observed in the animals treated with PHD-1 than that treated with nagabhasmam. This is contrary to the concentration of lead in the drugs; nagabhasmam contained higher amounts followed by PHD-1.

**Table-3.9.4: Serum urea, creatinine and uric acid levels in treated and control groups.**

| GROUPS | Serum urea, creatinine and uric acid levels in mg/dl (Mean ± SE) | | |
|---|---|---|---|
| | Urea | Creatininie | Uricacid |
| NGB(n=7) | 44.29±2.24** | 1.72±0.08** | 3.82±0.08 |
| PHD-1(n=8) | 41.25±1.95** | 1.98±0.07** | 4.05±0.07 |
| CON(n=8) | 18.00±0.71 | 0.90±0.02 | 3.82±0.06 |

** P<0.0001

**Figure-3.9.4: Serum urea, creatinine and uric acid levels in treated and control groups.**



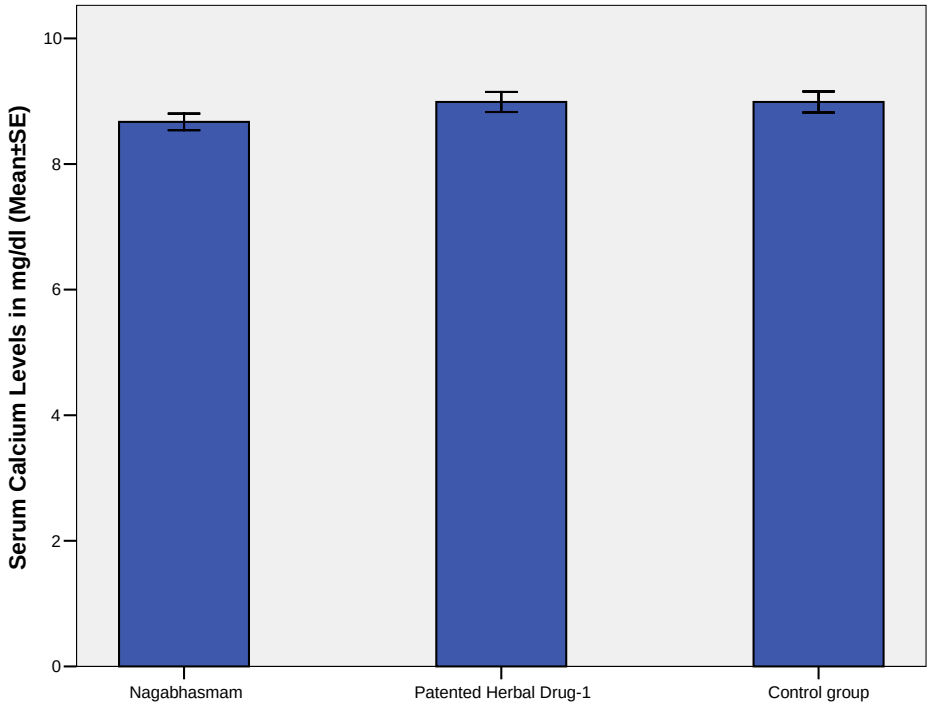

The serum urea, creatinine and uric acid levels of the treated and untreated animals are given in the table-3.9.4 and figure-3.9.4. In the drug treated groups the serum urea and creatinine levels were elevated compared to the normal counterparts. Slightly higher levels of serum urea were observed in the nagabhasmam group compared to the group of animals treated with PHD-1. The level of serum creatinine was observed as little higher in the animals which received PHD-1 compared to that was noticed in the nagabhasmam treated group. The serum uric acid levels were not significant in both the test groups compared to control group.

**Table-3.9.5: Serum calcium levels in treated and control groups.**

| GROUPS | Serum calcium level in mg/dl | |
|---|---|---|
| | MEAN±SE | Range |
| NGB(n=7) | 8.67 ± 0.13 | 8.00 - 9.00 |
| PHD-1 (n=8) | 8.98 ± 0.15 | 8.50 - 9.50 |
| CON(n=8) | 8.98 ± 0.16 | 8.20 - 9.50 |

Serum Calcium levels in treated groups are not significant compared to control groups.

**Figure-3.9.5: Serum calcium levels in treated and control groups.**



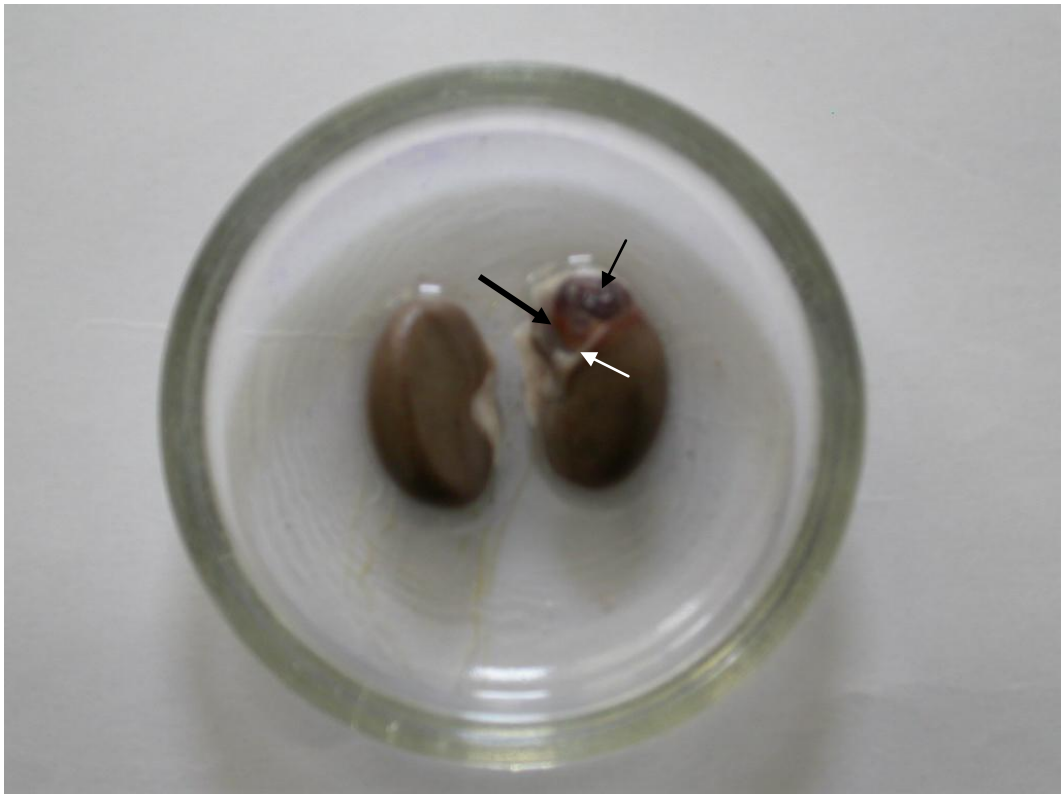

The serum calcium levels of the treated and untreated animals are given in the table-3.9.5 and figure-3.9.5. The serum calcium levels of the drug treated groups were not significantly different from the normal counterparts. The range of calcium level of the lead drug treated groups did not show any significant change compared to normal. The ranges of serum calcium are also overlapping with control groups.

## 3.10: Postmortem Findings of Animals Died during Drug Administration Period.

### 3.10.1: Mercurial drug treated group.

1. In rasasindhuram drug model two rats were found dead on the10$^{th}$ and 12$^{th}$ day of drug administration period.
2. In swasanadam drug model two animals were found dead on the 9$^{th}$ day of drug administration period.
3. In chayilyam drug model one animal was found dead on the 13$^{th}$ day of drug administration period.
4. 30±5 gm weight loss was noticed in each dead animal.
5. Inflammation was noticed in the mouth and around the anus.
6. Alopecea was noticed.
7. Foul and abnormal odour was noticed, while opening the visceral cavity.
8. Liver and kidneys were abnormally swollen and heavy.
9. Large fluid filled cystic lesions were present in the kidneys of two animals.
10. The large fluid filled cysts were located in the cortical region of the kidney and observed degenerative coagulative necrotic tissues in the lesions (figure-3.10.2).
11. The stomach was empty, food materials were totally absent. In two cases, the stomach was filled with gastric fluid.
12. Ulceration was noticed in the mouth, stomach, small intestine and also in the rectal region of the large intestine. Extensive areas of coagulative necrosis were found at many areas of gastrointestinal tract.
13. Lungs were clogged with mucous and congested; heart was found edemic and brain appeared in normal size.

**Figure 3.10.2: Cystic lesions in the kidney.**



Picture shows large and multiple cystic lesion in the kidney of swasanandam tablet treated animal (died during the drug administration period). Thin arrow indicates large multiple cyst in the right kidney. Bold arrow indicates broken cystic lesion and the white arrow indicates coagulative necrosis.

### 3.10.3: Lead-drug treated groups

1. In nagabhasmam drug, treated group one animal was found dead on the tenth day of drug administration.
2. In PHD-1 drug treated group, all animals appeared almost normal during the drug administration period.
3. No inflammation was noticed in the mouth and anus, but a greyish colour was noticed in the gingival tissues.
4. Body weight was found almost normal.
5. Hairs were found normal on the skin.
6. Normal odour was felt while opening the visceral cavity.
7. Liver was abnormally swollen and had yellow patches.
8. Kidneys were appeared normal in size and colour.
9. In the stomach food materials were found.
10. Ulceration was not seen in the stomach and other parts of GI tract.
11. Other internal organs except brain were found normal; brain appeared as swollen with edema.

## 3.11: Postmortem Findings of Sacrificed Animals in the Mercurial Drug Treated and Control Group.

### 3.11.1: Mercurial drug treated group.

1. Six animals out of eight were survived, in both rasasindhuram and swasanandam groups. Seven out of eight were survived in the chayilyam group.
2. Majority of animals appeared as emaciated and weight loss was also observed.
3. Tendered gingival tissues and inflammation in the mouth were noticed in several animals.
4. Abnormal hair loss (alopecia) with pruritis were noticed in majority of animals.
5. Foul and abnormal odour was observed, while opening the abdominal cavity.
6. Liver appeared normal but the kidneys were abnormally swollen and heavy.
7. Large fluid filled cystic lesions were seen in the kidneys of five animals. A few manifested with degenerative lesions of coagulative necrosis.
8. The fluid filled cysts were in the cortical region and had a size of one third or above the size of the kidney, majority of cysts were protruded out ward (figure-3.11.3).
9. Food materials were found in the stomach.

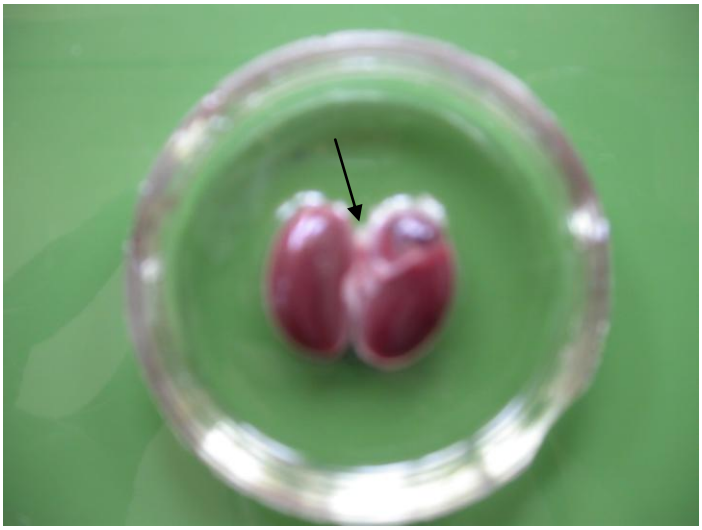
10. Ulceration was noticed in the mouth, stomach, small intestine and in the rectal region of the large intestine. Gastrointestinal tract manifested coagulative necrosis.

11. Lungs were clogged with mucous and appeared congested. Heart was found edemic and brain appeared in normal size.

### 3.11.2: Control group

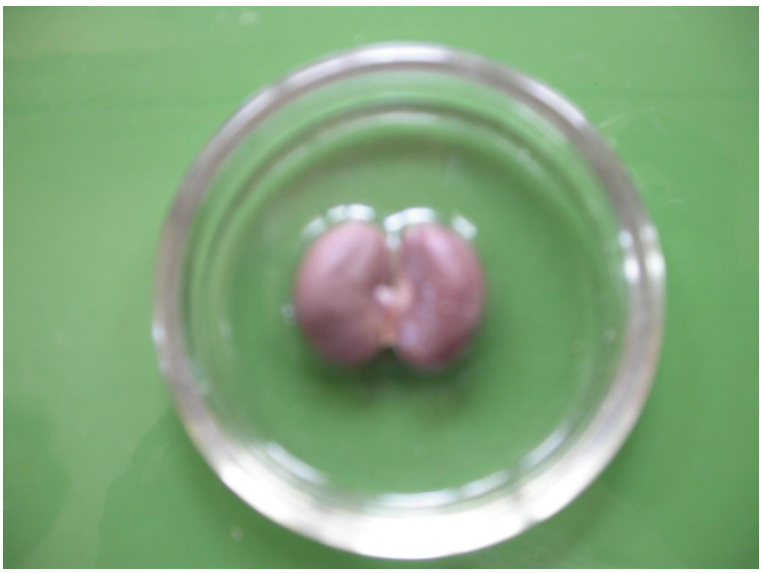
Control animals were appeared normal, with out much change in weight. The internal organs were observed with normal size, contour and colour.

**Figure-3.11.3: Cystic lesion in the kidney**



Picture shows cystic lesion in the kidney of rasasindhuram treated rat during postmortem. Thin arrow indicates large fluid filled cyst in the right kidney. Left kidney appears normal. The right kidney is slightly swollen.

**Figure-3.11.4: Normal kidneys of the control rat**



Picture shows normal kidneys of the control rat. Kidneys appear normal in colour, size and weight.

## 3.12: Postmortem Findings of Sacrificed Animals in the Lead Drug Treated and Control Groups.

### 3.12.1: Lead-drug treated groups

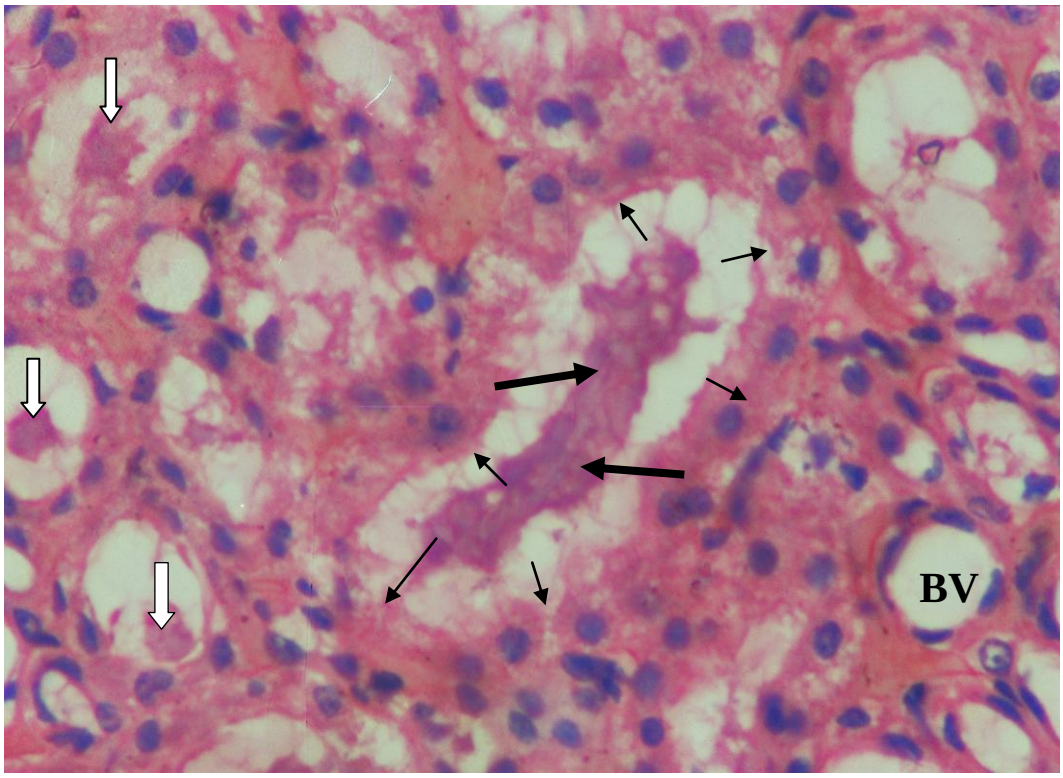
1. Seven animals out of eight were survived in nagabhasmam drug group and eight out of eight were survived in PHD-1 drug group.
2. Majority of animals appeared with normal size.
3. Mouth was appeared normal, inflammation was not obvious.
4. Skin appeared normal with normal distribution of hairs.
5. Normal odour was noticed, while opening the abdominal cavity.
6. In majority of animals, liver were appeared swollen and heavy.
7. Kidneys were appeared with normal size and colour.
8. Food materials were found in the stomach.
9. Mouth and gastrointestinal tract were found normal, ulceration was not obvious.
10. Lungs were appeared normal, heart was found normal and brain appeared swollen in majority of cases.

### 3.12.2: Control group

Control animal were appeared normal with out any significant change. The internal organs were observed with normal size and colour.

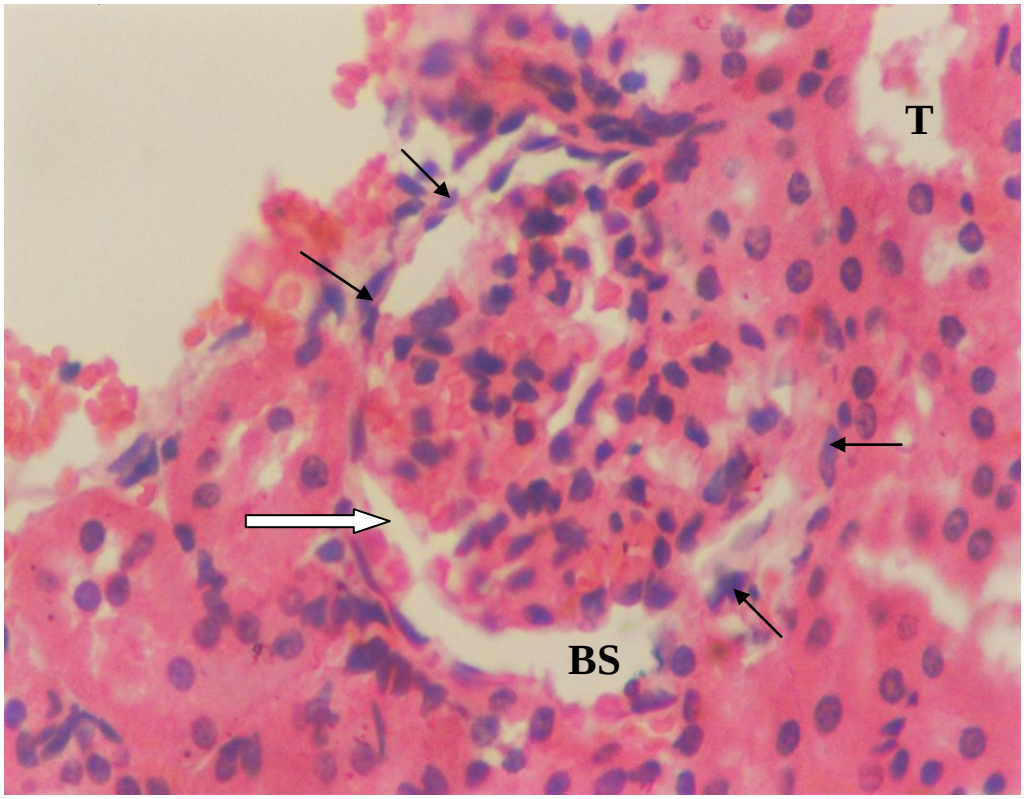
## 3.13: Histopathology of the Kidney in Swasanandam Exposed Rats.

**Figure-3.13.1: Kidney of swasanandam drug treated rat (400 X, H and E)**



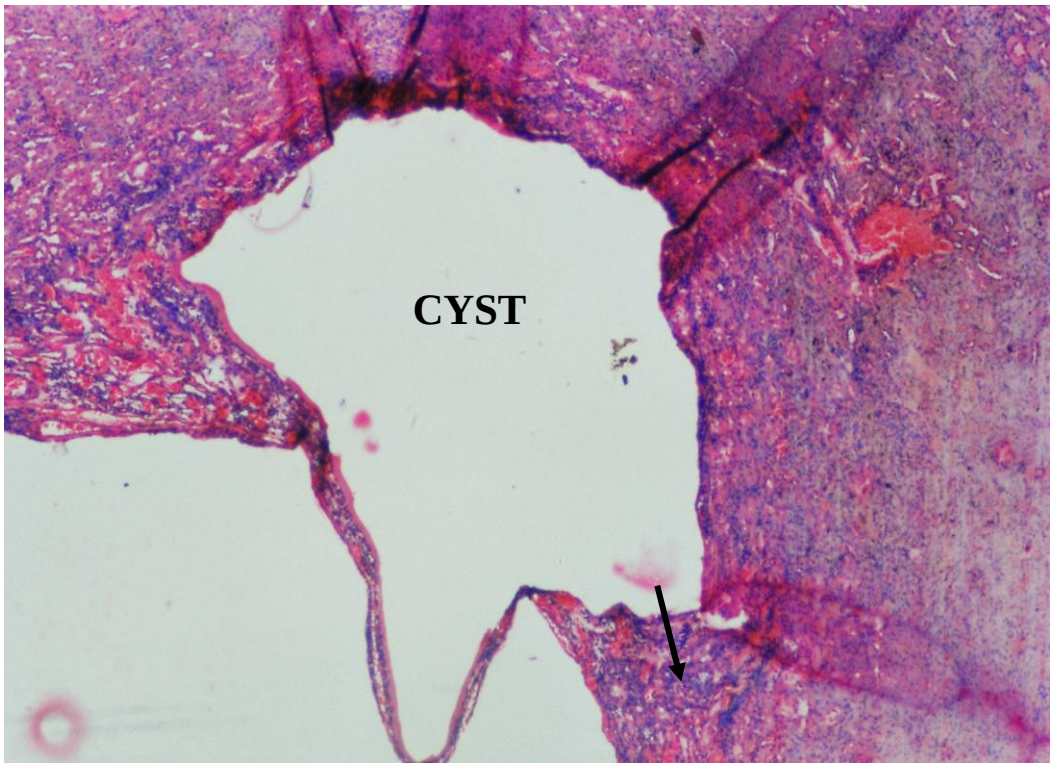

Photomicrograph shows renal tubular cell necrosis. White-block arrows indicate cellular debris in the proximal convoluted tubules (PCT). Thin arrows mark the out line of the PCT. Bold arrows indicate necrotic cells. BV marks a blood vessel

**Figure-3.13.2: Kidney section of swasanandam treated rat (400 X, H and E)**



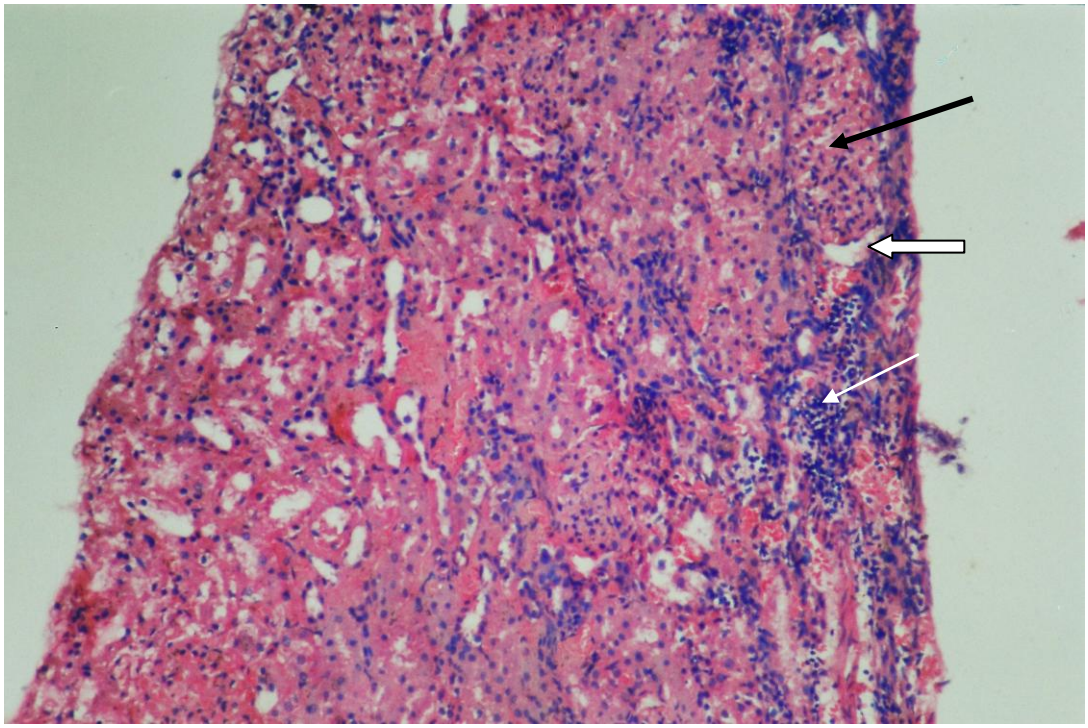

Photomicrograph shows hyper-cellular glomeruli in the renal cortex, which is in close apposition to the Bowman's capsule or synechae (white arrow). '**BS**' indicates Bowman's Space. There are changes associated with glomerulitis. '**T**' indicates tubule and thin arrows indicate distracted epithelial layer of Bowman's capsule.

**Figure-3.13.3: Kidney of swasanandam treated rat (40 X, H and E)**



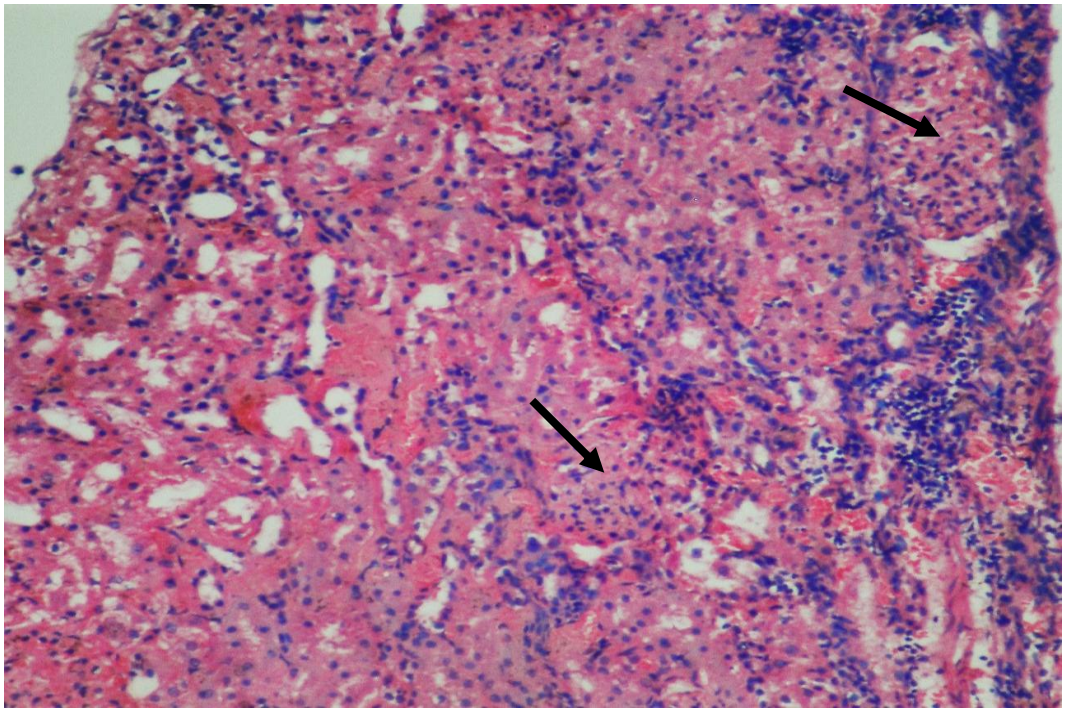

Photomicrograph shows abnormally large renal cystic lesions. Arrow indicates compressed renal tissue. Magnified photomicrograph of the out lined area is given on the next page.

**Figure-3.13.4: Wall of the renal cyst (100 X, H and E)**



Photomicrograph shows compressed renal tissue with one hyper-cellular glomerulus. Bold arrow indicates hyper cellular glomeruli. Thin (white) arrow shows interstitial round cell infiltration. White-block arrow indicates Bowman's space. Magnified photomicrograph of the outlined area is on the next page.

**Figure-3.13.5: Hyper cellular glomeruli in the renal cortex (100 X, H and E)**



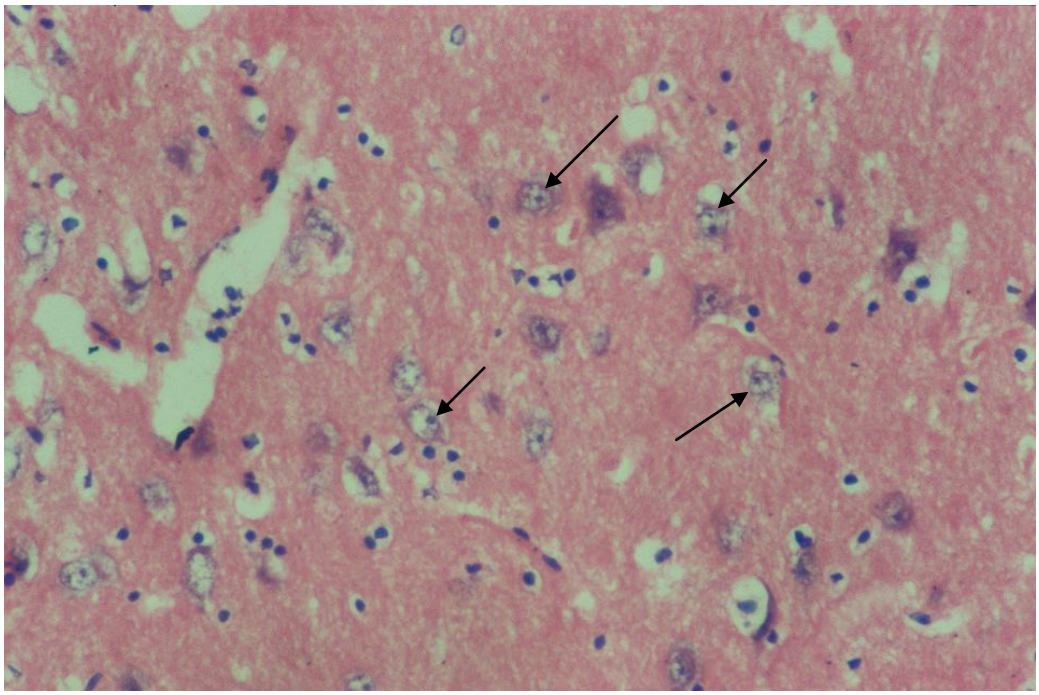
Photomicrograph shows compressed tissues in the renal cortex. Arrows indicate hypercellular glomeruli.

**Table-3.13.6: Histopathological findings of mercurial drug (swasanandam) treated and control rats' kidney.**

| Histological Observations | Drug (Swasanandam) Treated Group | Control Group |
|---|---|---|
| Renal tubular necrosis | Severe and obvious | Normal |
| Cellular debris in the PCT | Massive presence observed | Absent |
| Necrotic cells in the PCT | Present | Absent |
| Epithelium of basal membrane and glomerulus | Distracted and adhered together | Appeared normal |
| Prominent synechiae | Obvious | Absent |
| Bowman's space | Abnormally widened | Normal size |
| Glomerular cyst | Large cyst present | Absent |
| Hypercellular glomeruli | Present | Absent |
| Interstitial round cell infiltration | Massive infiltration occurred | Absent |
| Degeneration of parenchymatous cells | Present | Absent |

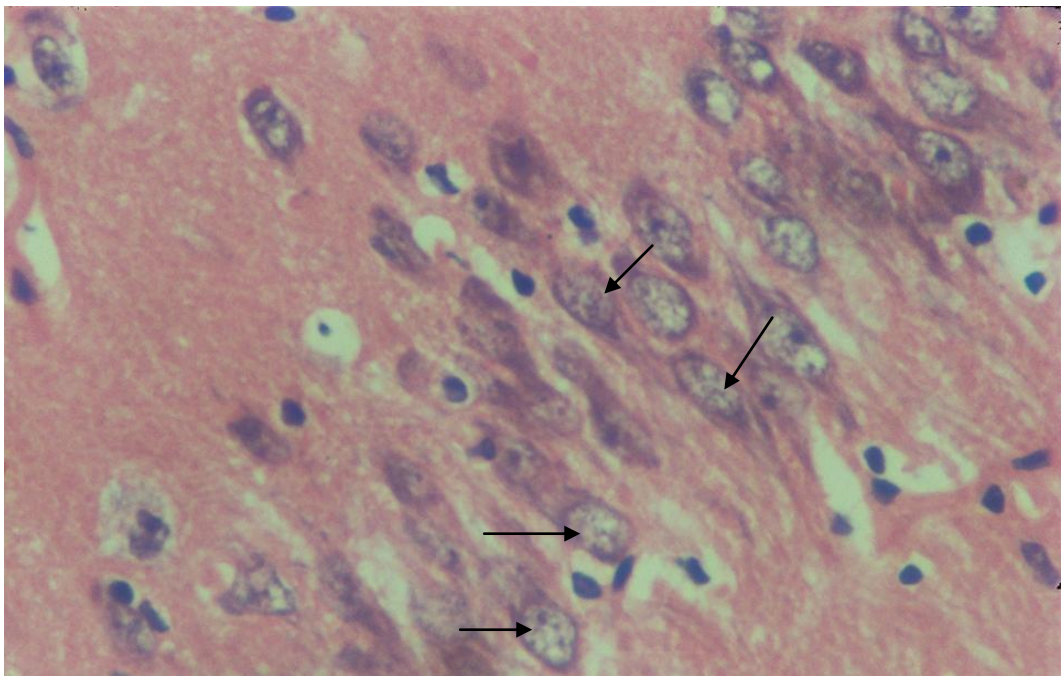
## 3.14: Histopathology of the Brain in Nagabhasmam Exposed and Control Rats.

**Figure-3.14.1: Section of control rat's cerebellum and dentate nucleus (40X, H and E)**



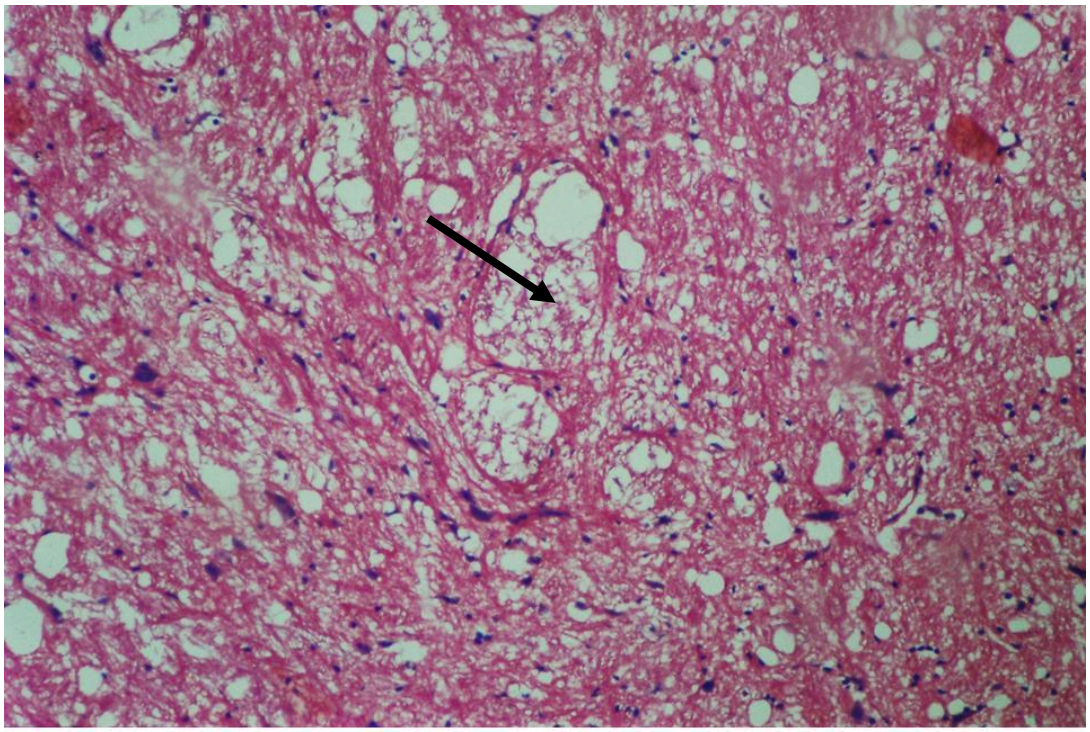
Photomicrograph shows L.S of brain including cerebellum and dentate nucleus. Thin arrows indicate normal astrocytes (glial cells). The L.S appeared normal in structure and histology. Glial cells per unit area and its morphology also appeared normal.

**Figure-3.14.2: Section of cerebellum and dentate nucleus of control rat (100X, H and E)**



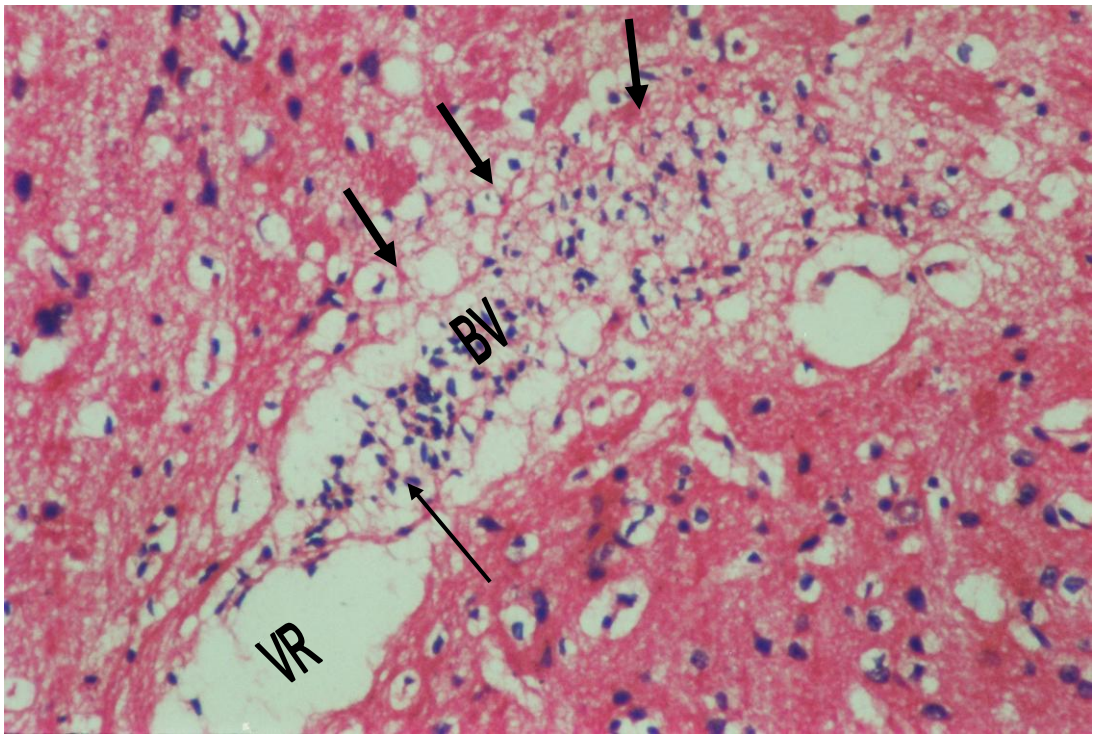
Photomicrograph shows normal neurons in the cerebellum and dentate nucleus (thin arrows). The glial per neuron cell ratio is normal. The histology also found normal.

**Figure-3.14.3: Cerebellar edema in nagabhasmam treated rat (100 X, H and E)**



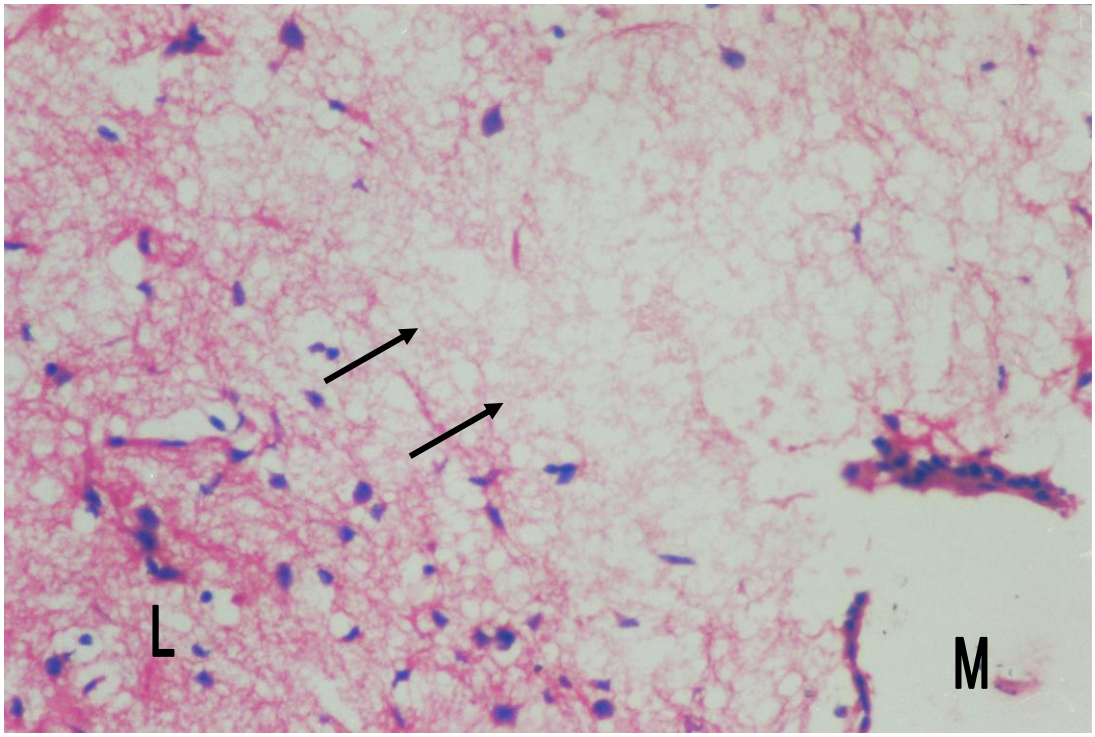
Photomicrograph shows cerebellar edema at peduncular region. The arrow points microcystic changes at peduncular region.

**Figure-3.14.4: Photomicrograph of brain of nagabhasmam treated rat (100X, H and E)**



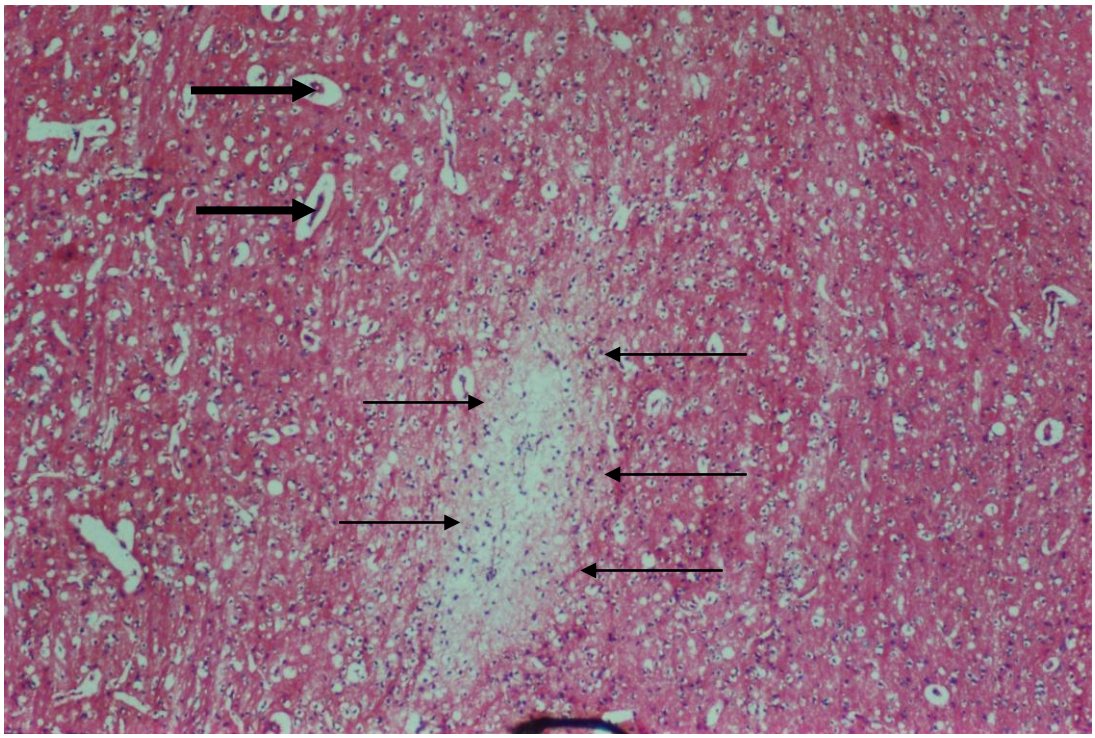

Photomicrograph shows perivascular edema in the cerebellar area. Bold arrows indicate perivascular edema. VR denotes Virchow-Robin space (VR-Space). BV marks blood vessel. Thin arrows indicate round cell infiltration.

**Figure-3.14.5: Brain (cerebellum) edema in nagabhasmam treated rat (100 X, H and E)**



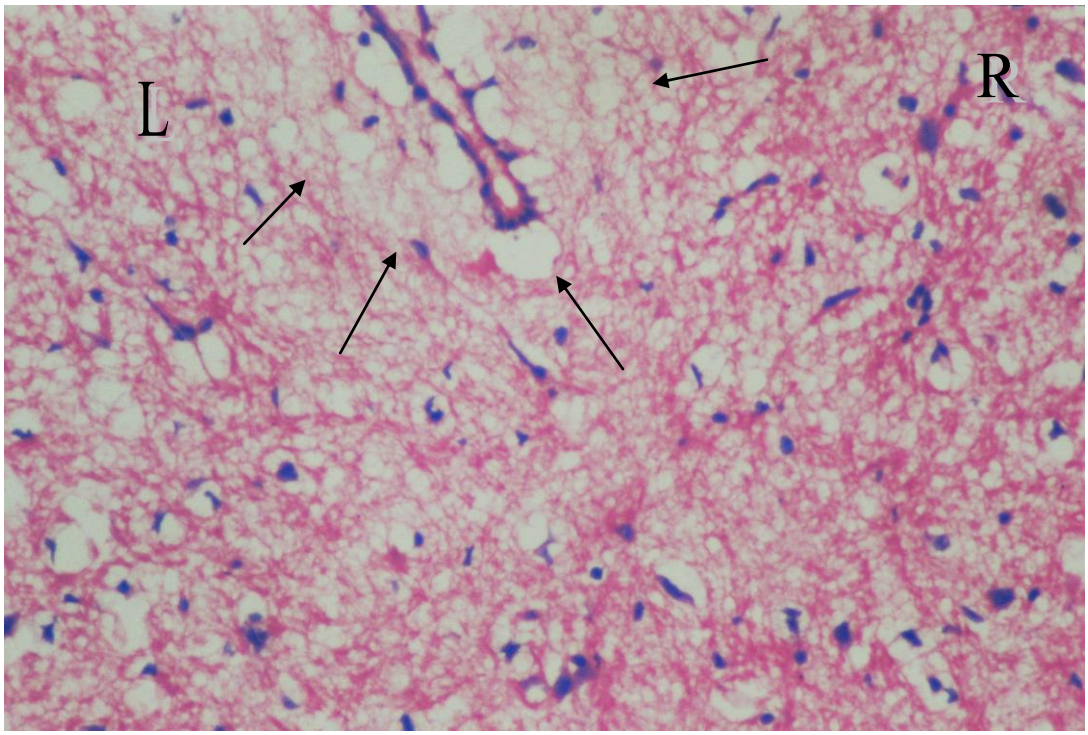

Photomicrograph shows edema of brain. Bold arrows in the picture indicate sparsely cellular edematous tissues in the cerebellar region. 'L' marks less-edematous area. 'M' denotes micro-cystic changes.

**Figure-3.14.6: Perivascular edema in nagabhasmam treated rat (40X, H and E)**



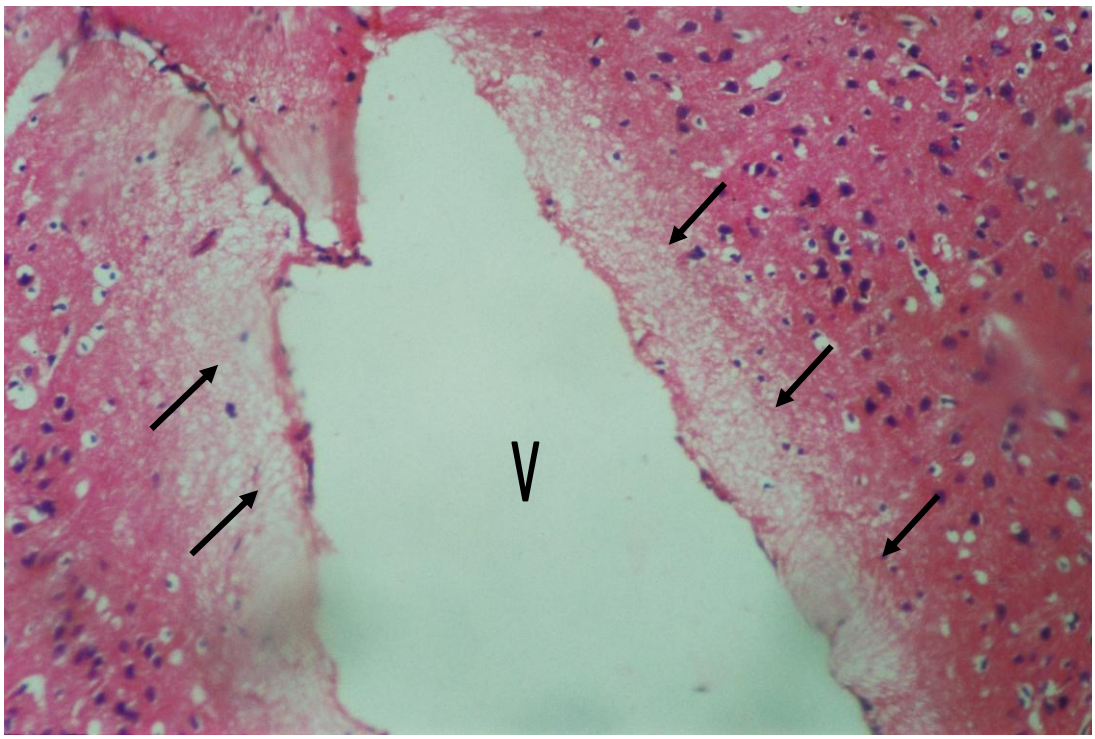
Photomicrograph shows Perivascular edema (scanner view). The thin arrows indicate perivascular edema and round cell infiltraton. Bold arrows indicate normal blood capillaries.

**Figure-3.14.7: Brain edema in nagabhasmam treated rat (100 X, H and E)**



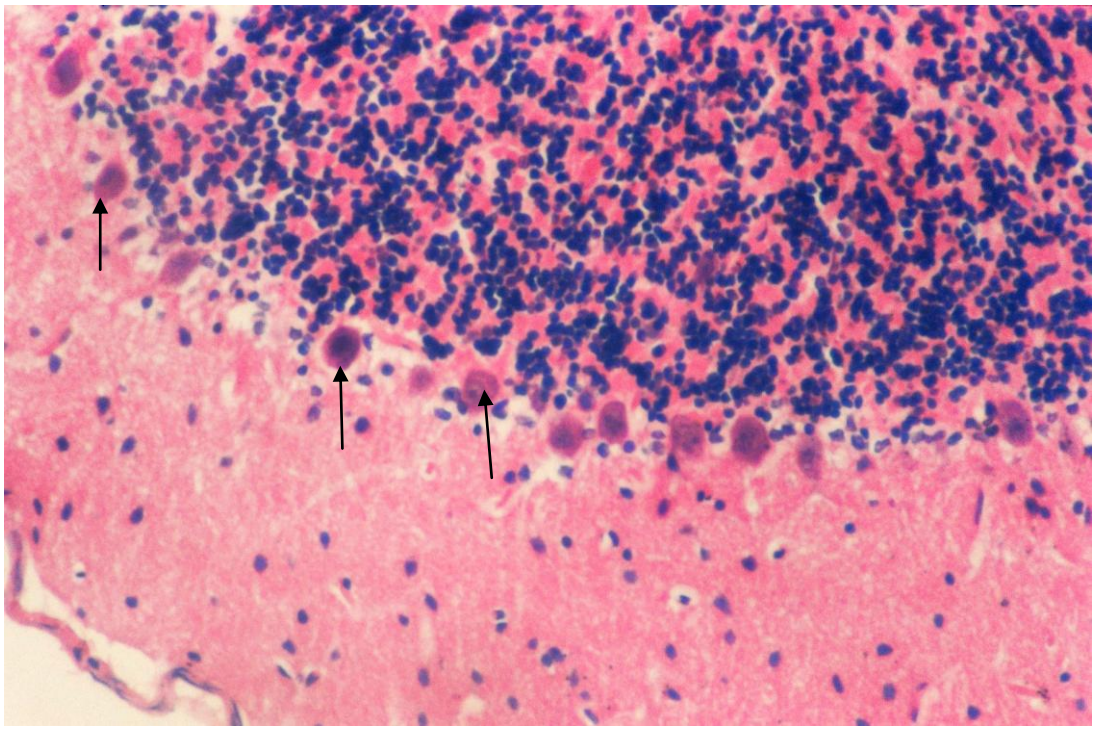

Picture shows brain edema.'**L**' marked in the picture indicates edematous tissue and '**R**' indicates non-edematous tissue in the brain. Arrows indicate edematous area.

**Figure-3.14.8: Periventricular edema in nagabhasmam treated rat (100X, H and E)**



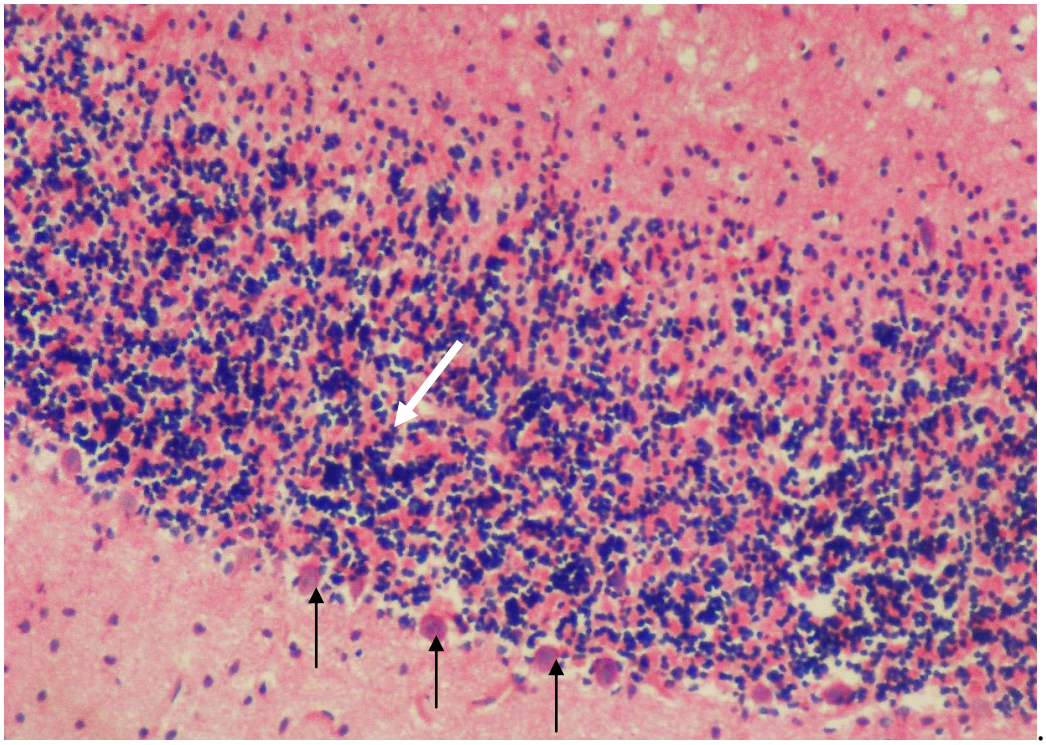

Photomicrograph shows Periventricular edema. Arrows indicate periventricular edema. 'V' marks abnormally large ventricle. Lighter staining area is an indicative of more fluid accumulation (edemic tissue). The uniformly and thickly stained areas indicate normal tissues.

**Figure-3.14.9: Cerebellum of the control rat (100 X, H and E)**



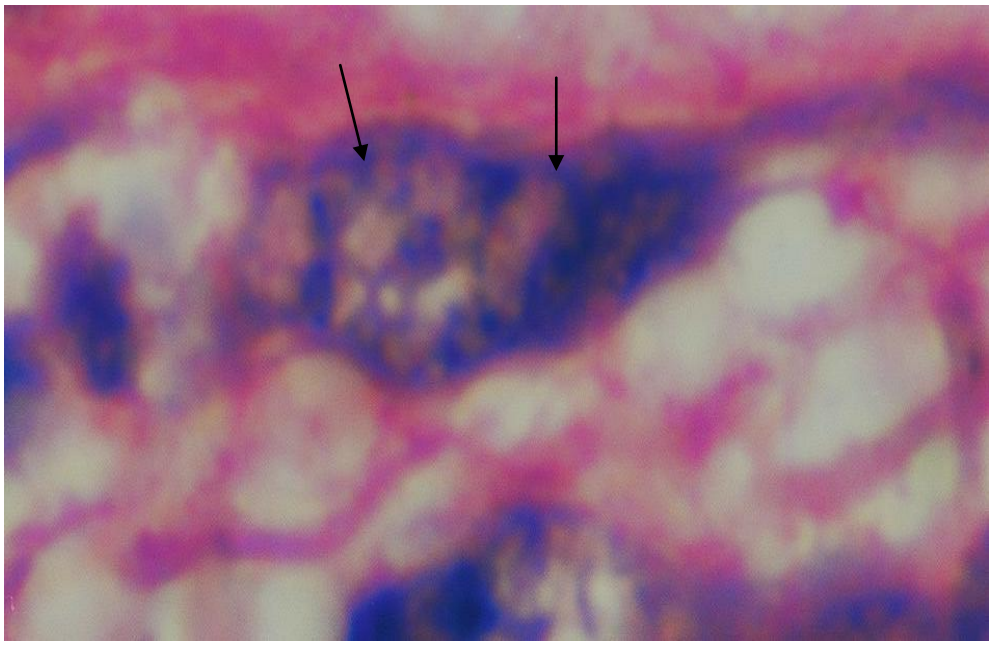
The picture shows normal number and distribution of Purkinje cells in the cerebellum. Arrows indicate normal Purkinje cells.

**Figure-3.14.10: Cerebellum of nagabhasmam treated rat (40X, H and E)**



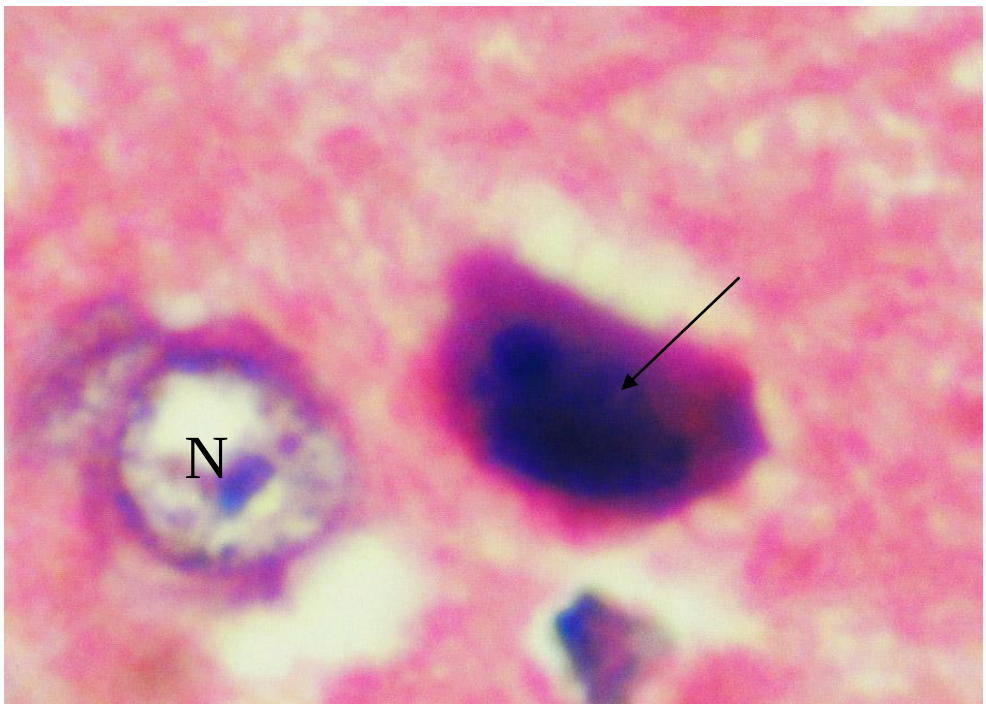
Photomicrograph from the cerebellum showing numerical decrease of Purkinje cells. The white arrow points granular layer of cerebellar grey matter. The thin arrows indicate typical purkinjee cells.

**Figure-3.14.11: Brain section (cerebral basal ganglia) of nagabhasmam treated rat (1000X, H and E).**



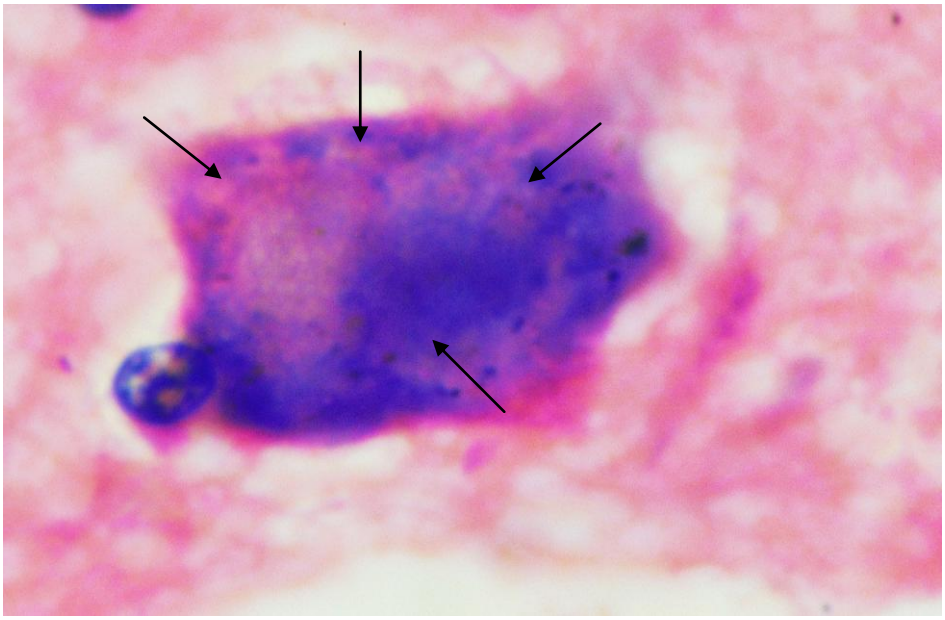
Photomicrograph shows the neurons with early degenerative changes. Arrows indicate peripheral clumping of chromatin and nuclear vacoulation.

**Figure-3.14.12: Basal ganglia of nagabhasmam treated rat (1000X, H and E).**



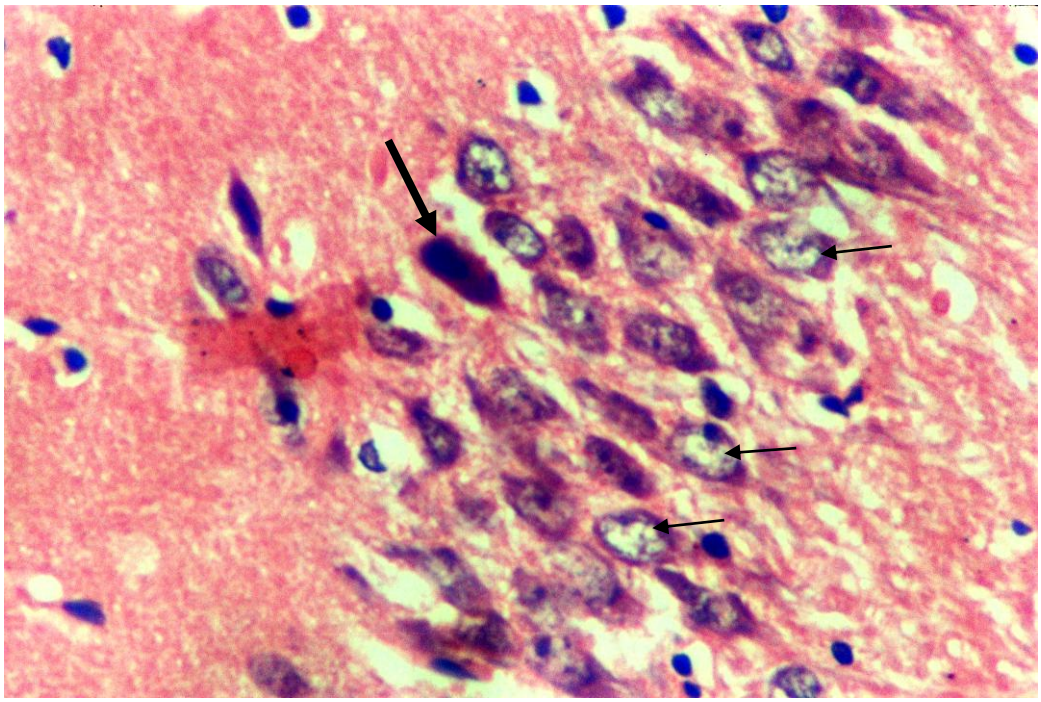

Photomicrograph shows section of cerebral basal ganglia cells. Thin arrow shows aggregation of nuclear chromatin, resulting in the formation of homogenous hyperchromatic mass. 'N' indicates normal nucleus

**Figure-3.14.13: Brain section (cortex) of nagabhasmam treated rat (1000X, H and E).**



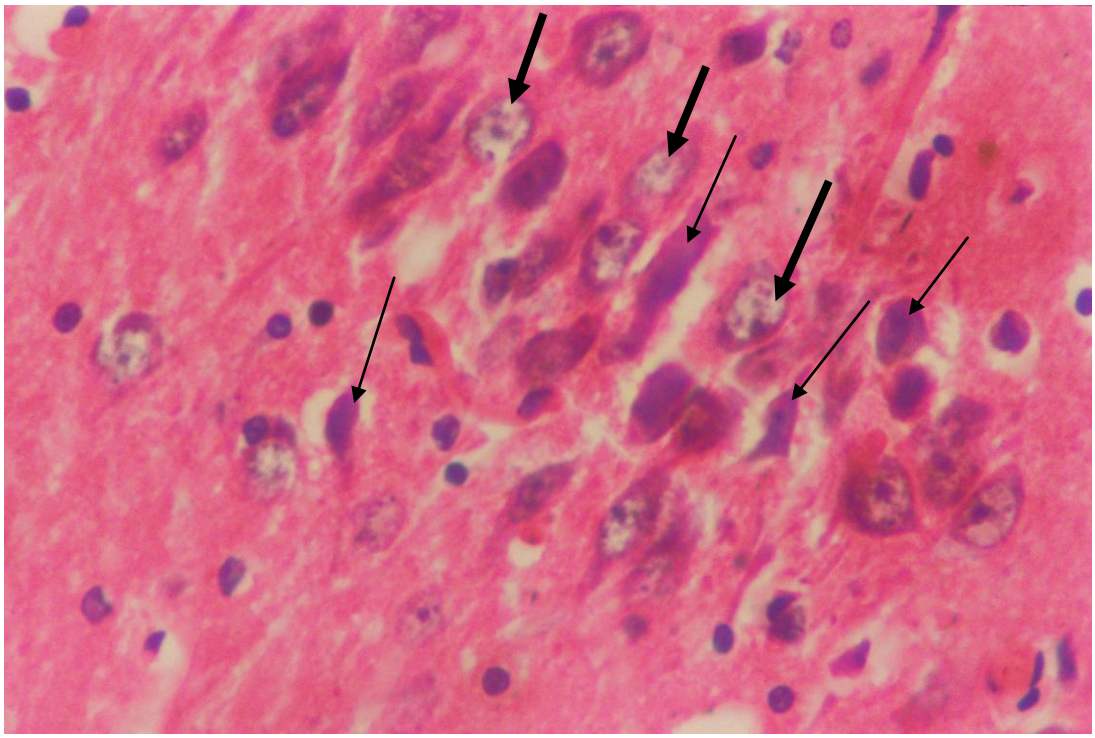
Photomicrograph shows degenerative changes in the Pyramidal cells. The thin arrows show late changes of karyorhexis and nuclear fragmentation in neurons (apoptosis).

**Figure-3.14.14: Brain section of control rat (100X, H and E).**



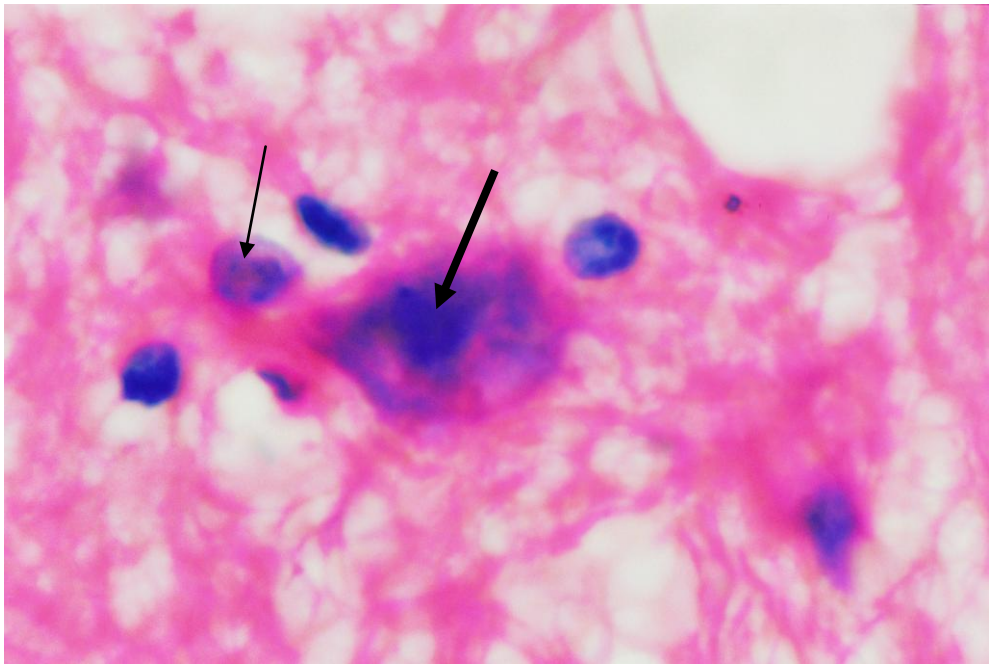
The photomicrograph shows normal and healthy astrocytes in the brain of control rat. Thin arrows indicate normal astrocytes in the cortex and bold arrow indicates one smudged cell, which is sometimes seen in healthy and normal brain.

**Figure-3.14.15: Cerebral cortex of nagabhasmam treated rat (100X, H and E)**



The photomicrograph shows numerous degenerating neurons in the cortical region. The bold arrows indicate normal neurons. The thin arrows indicate smudged nucleus of neurons.

**Figure-3.14.16: Ccerebral cortex of nagabhasmam treated rat (1000X, H and E)**



The photomicrograph shows degenerating neurons in the cortical region. The bold arrow indicates cells with intra-nuclear inclusion bodies. The thin arrow indicates normal nucleate neuron.

**Table-3.14.17: Histopathological Findings of the Brain Tissues of Test and Control Animals.**

| Histology observations | Drug (nagabhasmam) treated Group | Control group |
|---|---|---|
| Morphology of astrocytes | Abnormal | Normal |
| Glial cells per unit area | Less in number | Normal |
| Glial cells per neuron ratio | Less in number | Normal |
| Perivascular edema in the cerebellum | Severe and obvious | Absent |
| Virchow-Robin space in the cerebellum | Abnormally widened | Normal |
| Round cell infiltration | Massive | Normal |
| Microcytic changes | Present | Absent |
| Purkinjee cells distribution | Numerically decreased | Normal |
| Periventricular edema | Abnormally widened | Normal |
| Degenerative changes in the neurons | Abnormally numerous | Normal |
| Intra-nuclear inclusion bodies | Present in many neurons | Absent |

Chapter 4

# DISCUSSION

## 4.1: Mercury and Lead in some Ayurvedic Drugs.

In this study, forty Ayurvedic preparations both generic and patented were procured from local market and tested for the presence of mercury and lead using atomic absorption spectrophotometry. Fourteen drugs contained substantial quantities of mercury. Seven out of forty drug preparations contained heavy amounts of lead while five samples contained both mercury and lead. These findings are well in agreement with the earlier findings of Gogtay et al.,[4,] Lad[5,] Saper et al.,[8,] Aslam et al.,[111,] Ang et al.,[211,] Baer et al.,[212] and Leukoch et al.,.[213] Whose studies were on the qualitative and quantitative analysis of heavy metals in Ayurvedic and patented herbal drugs with toxicological studies in animal and human subjects. The presence of heavy amounts of mercury and lead in these Ayurvedic/patented herbal medicines questions the efficacy of Ayurvedic protocols for drug preparation.

The estimation of these metals in the Ayurvedic and patented herbal drugs shows that the concentration of mercury ranged between 15ppm to 45281ppm (table 3.1.1). Surprisingly, in ten drugs the mercury contents were above 10,000ppm, whereas the mercury concentration exceeded several lakhs of ppm in the mineral of mercury (chayilyam) and in the *kajjali* (a compound occur by heating mercury with sulphur), by which majority of mercurial drugs are processed. This is when the allowed concentration of mercury in food is only less than 50 μg/kg.[83] In fact, the possibility of ingesting inorganic mercury through daily food is very rare and the daily in take is estimated to be below 1μg/day.[84,85] Developed countries are on the track of mercury hunting in different drugs. In the recent past, thimerosal (sodium ethyl mercury thiosulphate) induced autism has been reported in children from many countries.[237] Even though minute quantities of thimerosal are used as a preservative in vaccines, due to its autism connection the compound has been removed from all vaccines in the United States.[238]

Similarly, in the case of lead-drugs, the concentration of lead was found to be ranging between 14ppm to 844977ppm (table 3.1.1). According to WHO, The Provisional Tolerable Weekly Intake (PTWI) of lead in adult is 50 μg/kg body weights and in children it is only 25 μg/kg.[128] As per a study conducted in USA, most of the food items, drinks, water etc… contain lead in some microgram quantities. In contradictory to this fact, in our study, some drugs contained lead at a concentration of about 844977ppm. These details disclose the threat of mercury and lead toxicity that can come through the consumption of Ayurvedic and patented herbals drugs in our day today life.

The study further reveals that rasasindhuram powder and swasanandam tablet contained substantial concentrations of mercury and the patented herbal drug-1 and nagabhasmam powder contained high amounts of lead. Even though the drugs had been processed by the laborious and time-consuming protocols of Ayurveda, we quantitatively estimated high concentrations of mercury and lead. Therefore, the claims and clarifications of the drug manufacturers and Ayurveda practitioners have to be suspected. It is very necessary to confirm through scientific means, whether the protocols prescribed in the Ayurveda manuals are effective to remove the toxicity of the drugs. Sometimes for economical reasons, the manufacturers might have omitted or neglected certain steps in the official procedure for drug preparation. Faith based protocols for drug preparation with prejudiced anticipations on drug products and their therapeutic efficiency do not have scientific background. Therefore, further scientific studies are needed on this matter.

## 4.2: Mercury Drug Models

### 4.2.1: Post-Drug Administration Symptoms

In the mercury drug model, two mercurial drugs and a mineral of mercury were subjected for animal studies. The post-drug administration symptoms in rats treated with rasasindhuram and swasanandam were found almost similar. However, in the chayilyam group the post-drug administration symptoms were moderate compared to that of other two drug groups. Even though swasanandam is prepared from Chayilyam, the mineral of mercury, the former manifested more toxic effects than the latter. The post-drug administration (clinical) symptoms in the test groups of animals were found almost similar, which indicates that the mercury content in all the three drugs manifested the similar kind of toxic effects in test animals. The most prevalent symptoms observed in the test animals were excessive salivation, anorexia, oliguria, diarrhea and weight-loss. Most of the treated animals were manifested with skin disorders like scabby lesions around mouth and anus, alopecia and pruritis. Weight loss with emaciated appearance and stiff legged walk were common in majority (Figure-3.3.5). However, the control animals were appeared healthy and normal after the anupaana drava (diluted juice of ginger) administration period (figure-3.3.6). The post-drug administration symptoms observed in the treated categories are matching with the findings of Jalali et al., [39,] Cassidy et al., [43] and Boening.[67] on the toxicological studies of inorganic and organic mercury compounds in different animal models.

### 4.2.2: Tissue Mercury Levels

The tissue mercury levels of test groups are significantly ($P<0.0001$) higher compared to the control group as shown in the table-3.6.1 and figure-3.6.1. The ingested inorganic mercury, after digestion and absorption, gained access to the blood circulation and this can be assessed as blood mercury level

(BML). The BML can be considered as an index of mercury absorption.[92] The BMLs of different test groups in this study were found almost proportional to the mercury concentrations in the rsasindhuram, swasanandam and chayilyam drug groups. This is why chayilyam group showed the highest BML and swasandnam group showed minimum blood mercury level.

The circulating mercury was deposited in the tissues in the following order of decreasing concentration: kidney, liver and brain. This observation is well in agreement with the previous studies of Sin et al.,.[92] The level of mercury in kidney was highest in chayilyam group and lowest in the swasandam group. According to the results, kidneys accumulated highest amounts of inorganic mercury; it might be due the following reasons. (1) The uptake of inorganic mercury by kidney cells occurs through active transport, but mostly by diffusion.[59] (2) The affinity of mercury ions for thiol groups accelerates the accumulation of large amounts of inorganic mercury in the kidneys.[60] The elevated concentration of mercury in renal tissues may show evidences of impaired function within a few minutes after the poison reaches the circulation. Among higher vertebrates including humans, inorganic and alkoxyalkyl compounds cause kidney damage, which usually leads to death.[59]

The liver mercury levels in the different drug treated groups are not proportional to the corresponding BML and mercury concentration in the drugs. Rasasindhuram treated animals showed slightly more mercury levels in the liver tissues than swasanandam treated animals. Unlike in kidney, the mercury level of liver is not proportional to its concentration in blood and in the drug also.

The brain mercury levels were found to be proportional to the blood mercury levels and to the mercury concentrations in different drugs. The brain mercury levels were found to be significantly elevated in the test groups compared to the control animals ($P<0.0001$). It is observed that brain

accumulates least amount of mercury and the observations are well in agreement with the earlier findings on tissue mercury levels in mercury exposed animals by Sin et al.,.[92]

### 4.2.3: Biochemical Studies of Serum

The chronic exposure to inorganic mercury has caused different types of organ toxicities in experimental animals. The hepatotoxic and nephrotoxic effects of inorganic mercury were well established in the present study. The drug treated group showed elevated levels of both total bilirubin and conjugated bilirubin. The bilirubin values are found to be significantly elevated in the test groups compared to the control group ($P<0.0001$). The maximum elevation in total and conjugated bilirubins was observed in the animals received chayilyam and minimum in the group treated with rasasindhuram. The toxic effect of organic mercury (methyl mercury) on the formation of bilirubin in the liver has been well documented in the earlier studies of Winship.[36] But the effect of inorganic mercury on the synthesis of bilirubin in the liver has not been reported anywhere. The swasanandam treated animals showed maximum elevation of bilirubin levels, in spite of its minimum mercuric content. This might be due to the presence of some other hepatotoxic substances in the drug, other than mercury.

Liver may manifest the hepatotoxic effects by organic or inorganic mercurials as decreased synthesis of protein. In our study, the mercurial drug treated animals showed decreased serum protein levels, which were significantly ($P<0.05$) lower compared to their normal counterparts. The serum protein levels of the test groups were proportional to the mercury levels in the corresponding drugs. The impaired synthesis of serum proteins in the test groups might be due to the hepatotoxic effect of mercury in the drugs. Similar findings were also reported by El-Demerdash.[66] In his studies, he

reported that, mercuric chloride treated rats manifested biochemical alterations like decreased protein levels in serum, liver and brain.

The abnormal elevation of liver enzymes such as ALT (alanine aminotransferase), AST (aspartate aminotransferase) and ALP (alkaline phosphatase) can be taken as an index for liver injury or disease. In the present study, the serum AST, ALT and ALP levels were found to be significantly ($P<0.0001$) elevated in the treated groups compared to control category. The maximum levels of liver enzymes were observed in the rasasindhuram group and minimum levels were noted in the swasanadam group. The elevation of AST, ALT and ALP were proportional to the mercury levels in the corresponding drugs. The hepatotoxic effect of inorganic mercury is manifested as the abnormal elevation of liver enzymes. These findings are well in agreement with the earlier studies of Dufour[46], Estridge [48] and El-Demerdash[66] on the hepatotoxic manifestations of toxic substances. El-Demerdash had specifically observed the increased serum AST, ALT and ALP levels in rats treated with inorganic mercury (mercuric chloride). In contrary to the present findings, Levi[172], Plaa[174] and Zimmerman[175] reported that, mercury and lead do not generally cause hepatotoxicity and liver injury. May be due to such reporting, mercury and lead are not yet included in the list of hepatotoxic agents, which cause liver necrosis and fatty liver, cholestasis (drug induced), hepatitis and carcinogenesis.

The first response of mercury poisoning in the kidney may be as diuresis, due to the suppression of tubular reabsorptive function; soon the renal damage becomes so extensive and that results in oliguria and finally anurea.[236] The same findings were noticed in the mercurial drug treated groups in the present study. Two animals in each, rasasindhuram and swasanandam groups were died during drug administration. Postmortem studies revealed the toxic effects of inorganic mercury on kidneys. Large

cysts with coagulative necrotic lesions (figure-3.10.2) were found in the kidneys and it might be due to the heavy accumulation of inorganic mercury in the renal tissues. These findings are matching with the study results of Sin et al.,[92] as if kidney accumulates highest amount of mercury during toxic exposure.

Acute inorganic mercury poisoning affects proximal tubule and causes vesiculation and exfoliation of brush border membrane followed by calcium influx and finally cell death. Under chronic toxicity, the size of the kidney may be affected. The initial stages manifest with interstitial edema, inflammatory infiltration with lymphocytes and tubular cell changes such as necrosis.[235] These findings justify the formation of cystic lesions with necrotic tissues in the cortical area of the kidneys observed in our study. The nephrotoxic effects of mercury ions can be reasoned as the metallic ions are known to promote oxidation of kidney cells and to disrupt renal mitochondrial function. The increased $H_2O_2$ production by renal mitochondria is an indirect effect of inorganic mercury.[38] Mercury poisoning also causes primary and secondary idiopathic membranous glomerulonephritis.[239] Polycystic kidney disease is recently reported in lead intoxication.[240] But in the present study, we observed polycystic lesions in the kidneys of mercurial drug treated animals. These findings are not in agreement with the recent studies of Wortman[240,] on polycystic kidney disease due to lead intoxication. In our study, it was found that, inorganic mercury poisoning could also cause polycystic kidney disease and this finding can be considered as a new revelation.

The abnormal elevation of serum urea and creatinine may be considered as good markers for renal insufficiency. Mercury in all forms (elemental, organic and inorganic) causes renal toxicity. The nephrotoxic effects of mercury are well established in the present study. In the drug treated

group, the serum urea levels were found to be elevated significantly ($P<0.0001$) compared to the normal counter parts. The creatinine levels were also found significantly ($P<0.0001$) increased compared to the control group. These findings are well in agreement with the previous studies of Mc Neil et al.,[40], WHO[61], Buchet et al.,[62] and Sharratt et al.,.[178] on the abnormal elevation of serum urea, creatinine and uric acid in organic and inorganic mercury exposed human and animal models. In the test group, the serum urea was found highest in the chayilyam group and lowest in rasasindhuram exposed animals, instead of swasanandam group, which contained lowest levels of mercury. That is, serum urea levels were not proportional to the blood mercury levels and mercury concentrations in the corresponding drugs.

The uric acid levels were not found significant ($P>0.05$) compared to the control group and this is not in agreement with the previous studies of McNeil et al.,[40,] WHO[61,] Buchet et al.,[62] and Sharratt et al.,.[178] Elevated serum urea and creatinine levels in the test animals reveal renal deficiency for certain extent, but the serum uric acid levels found almost normal, when compared to the normal counter parts. This might be due to the counter effect of some herbal ingredients in the drug.

In the drug treated group the serum calcium levels were found to be significantly ($P<0.05$) lower compared to the normal counter parts. Hepatotoxicity due to heavy metals normally leads to the impaired synthesis of proteins especially the albumin. The nephrotoxic effect of mercury may be manifested with albuminuria via glomerulitis. This condition may finally lead to the depletion of albumin in the blood.[61, 62] Serum albumin is very essential for maintaining the normal serum calcium levels. Significant decrease of total protein especially albumin was observed in the liver function tests and it might be the reason for manifesting lower serum calcium level. The lowest levels of serum calcium were observed in the swasanandam trated animals

whereas the maximum amounts of serum calcium were noticed in the animals treated with chayilyam. The range of serum calcium levels were not overlapped by the range of serum calcium values of the control group, which indicated the effect of mercurial drugs on serum calcium levels. In the present study, it is noted that the decrease of calcium is inversely proportional to the blood mercury levels and the mercury concentrations in the drugs. These findings are in disagreement with previous studies of Synder[49] and Suzuki et al.,.[50] on the unaltered blood electrolyte levels during mercury toxicity in human and animal models.

### 4.2.4: Postmortem Findings

The postmortem studies of the sacrificed animals revealed the toxic effects of mercury in the internal organs. The typical characteristics of inorganic mercury poisoning observed were tender gingival tissues with inflammation, ulceration in the gastro-intestinal tract, petechial hemorrhages of the internal organs. The kidneys found edemic and had large fluid filled cystic lesions (figure-3.11.3). Swollen liver, mucous clogged lungs and edemic heart were also observed. The brain appeared with normal size and contour in all test animals. Majority of these findings are well in agreement with the earlier studies of Cassidy et al.,.[43] on the histological changes of internal organs under chronic and acute inorganic mercury poisoning. However, the fluid filled multi-lobed cystic lesions found in the kidneys of the treated animals are a new revelation that has not been previously reported in any other studies on mercury toxicity. Similar postmortem findings had been observed for animals died during drug administration period (figure-3.10.2) also.

Rasasindhuram and swasanandam treated groups showed 25% of mortality rate, whereas it was 12.5% in the chayilyam group (figure-3.2.1), even though it contained about 65% of mercury. This shows that the mortality

rates of the test groups were not in proportional to the concentration of mercury present in the drug ingested. During post drug administration period, more toxic effects were observed in the swasanandam group than in its parent compound chayilyam treated test group. Number and size of the cystic lesions in the kidneys (figure-3.10.2 and 3.11.3) and extension of coagulative necrotic lesions in the gastro-intestinal tracts appeared severe in the animals died during drug administration period. Polycystic kidneys were observed only in mercurial drug treated groups whereas their control animals had normal kidneys (figure-3.11.4). These findings confirm that, the death happened during drug administration period may be due to the toxic effect of mercurial drugs. The findings are well in agreement with that of Cassidy et al.,.[43] The postmortem findings of the control animals were found to be normal.

### 4.2.5: Histopathological Changes in the Kidney

The histopathological studies of renal tissues revealed the nephrotoxic effect of mercurial drugs in the exposed animals. According to the Atomic Absorption Spectrophotometric results, kidney accumulated the highest amounts of inorganic mercury. This might be due the uptake of inorganic mercury in the kidneys, by both active and passive (diffusion) mechanisms.[59] The affinity of mercury ions for thiol-groups accounts for the accumulation of large amounts of mercury in the kidneys.[60] The abnormal deposition of inorganic mercury was manifested as renal dysfunctions. The first response of the kidney to mercury toxicity was diuresis, which might be due to suppression of tubular re-absorptive function. Very soon, the renal damage became so extensive and that resulted in oliguria and finally anuria. These symptoms were observed in the drug administration period and during the post drug administration period too. Similar findings were reported earlier by Miller et al.,.[38] He revealed that, mercury ion is known to promote oxidation of kidney cells and to disrupt renal mitochondrial function. The increased

$H_2O_2$ production by rat renal mitochondria is an indirect effect of inorganic mercury, which in turn causes renal damage and finally leads to anuria.

The renal function test results of the animals treated with mercurial drugs had shown abnormal elevation of serum urea and creatinine. Significant decrease of serum proteins and serum calcium levels were also observed in this study. The nephrotoxic and hepatotoxic effects of mercury were discussed in the corresponding sessions of this study and all these findings firmly support the histopathological observations. Histopathological sections (longitudinal) of kidneys of rats exposed to swasanandam were prepared and analyzed. Renal tubular necrosis and the proximal tubules filled with cellular debries are illustrated in the figure-3.13.1. The necrotic cells are also well marked in the same figure. The findings are well in agreement with the earlier histopathological studies of Cassidy et al.,[43] and Zook.[208] The authors established the toxic effects of inorganic mercury on renal tissues as proximal tubular necrosis in different types of animals.

In figure-3.13.2 a Bowman's capsule is illustrated. The basal membrane (epithelium) of the Bowman's capsule appeared distracted; the Bowman's space (BS) became abnormally widened and distributed irregularly. The disrupted epithelial linings in the basal membrane and epithelium of the glomerulus adhered together and caused to form synechiae in the capsular space. This blocked the flow of glomerular filtrate, accumulated in the Bowman's space, and finally resulted in the formation of glomerular cyst (figure-3.13.3). The disruption of epithelium of the basal membrane might be extended to the proximal convoluted tubule. This condition led to the suppression of reabsorption, and caused diuresis, followed by oliguria and anuria, in the later stages glomerulitis. These findings are in agreement with the earlier studies of Kibukamusike et al.,[64] Barr et al.,[65]

Jennet et al.,[235] and Guzzi et al., [239] on inorganic mercury induced glomerulitis and associated renal disorders in human beings and also in animals.

The enlarged view of the outer wall of the cyst is shown in figures-3.13.4 and 3.13.5, which illustrate the compressed renal tissues with a hypercellular glomerulus. Round cell infiltrations in the interstitial spaces, with fatty degeneration of parenchymatous cells are also seen.[49,50] Hypercellular glomerulitis in the cortical area may be due to the proliferation of mesengial cells or endothelial cells or polymorpho nuclear neutrophils. When this condition progressed, the compression of afferent and efferent arterioles might have occurred and this might have blocked the glomerular circulation, resulting in oliguria and in the later stage anuria. All these pathological manifestations were observed during drug administration period and in the post drug administration period. These findings are well in agreement with the earlier studies on renal failure (animal and human models) due to heavy metals toxicity by Kibukamusike et al.,[64] Sharratt et al.,[178] and Hook et al.,.[179] In the present study, the histopathological results confirm the toxic effects of inorganic mercury on experimental rats.

## 4.3: Lead Drug Model

### 4.3.1: Post-drug Administration Symptoms

The post-drug administration (clinical) symptoms observed in the nagabhasmam (NGB) and patented herbal drug-1(PHD-1) groups were found milder than the mercurial drug treated groups. In the post-drug administration period (three days), some of the lead drug treated animals manifested the symptoms like anorexia, emaciation, oliguria, diarrhea, hyperactivity and weight gain or weight loss. The symptoms were more severe in the nagabhasmam group than PHD-1group and had death of one animal, on the tenth day of drug administration period. The mortality rate observed in

nagabhasmam group was 12.5%. Mean while in the PHD-1 group, all animals were survived after the drug administration period and observed no mortality (figure-3.2.2). Most of the lead toxicity symptoms manifested after the post-drug administration period are well in agreement with the earlier findings of Holtzman et al.,[145], Rutter[150] and Needleman et al,..[151] on the lead toxicity symptoms in children and adult.

In this study, we observed some post drug administration symptoms such as diarrhea and weight gain, contradictory to the earlier findings.[145,150,151] Majority of the animals in the PHD-1 group showed slight increase in body weight, while weight increase was limited to only two animals in the NGB group. The weight gain attained in the PHD-1group might be due to the effect of some other ingredients in the drug. The control group did not manifest any of the above symptoms; they appeared normal and healthy through out the experimentation period.

### 4.3.2: Hematotoxic Effects of Lead

In the present study, the urine delta-aminolaevulinic acid levels of both the test and control groups were estimated. The delta-aminolaevulinic acid levels in the test animals were found to be elevated significantly ($P<0.0001$) compared to their normal counter parts (table and figure 3.5.1). The findings of this study are well in agreement with the earlier studies of Snowdon.[195] He used organic lead (lead acetate) as the test drug and observed abnormal elevation of delta-aminolaevulinic acid. In the present study, same findings were observed with inorganic lead (in the drugs) exposure. In human studies, Hamond[159] has reported similar results on the inhibitory effects of lead on heme synthesizing enzymes. Related findings were also reported by Fowler[160] and Conner et al.,[161] in fishes.

The hematotoxic effect of lead is an elaborately studied topic in lead toxicology. Lead inhibits nearly all enzymatic steps involved in heme synthesis.[17] During heme synthesis lead inhibits aminolaevulinic acid dehydratase (ALAD) by binding with the sulfhydryl groups of the enzyme and prevents the conversion of delta-aminolaevulinic acid to porphobilinogen. The inhibition of ALAD is manifested as elevated δ-aminolaevulinic acid excretion in the urine. The toxic effect of lead on haematopoietic systems can be detected by estimating the urine levels of delta-aminolaevulinic acid. In human beings, elevated δ-ALA excretion is a more sensitive and specific index of lead exposure.[17] Lead also inhibits hemesynthetase enzyme, which is responsible for the introduction of iron into the tetra pyrrole porphyrine ring.[160, 161] The inhibition of heme synthesizing enzymes lead to anemia and it is a classic indication of lead toxicity.

### 4.3.3: Tissue Lead levels

The tissue lead levels of test groups are significantly ($P<0.0001$) higher compared to the control group as shown in the table-3.8.1 and figure-3.8.1. The ingested lead, after digestion and absorption, gained access to the blood circulation and this can be assessed as blood lead level (BLL). The definitive test for lead toxicity is measurement of blood lead level.[234] Lead content in the circulating blood is first deposited in the soft tissues and finally in the hard tissues like bone which is considered as the final reservoir for lead. The tissue levels of lead were measured by Atomic Absorption Spectrophotometry and the findings are found well in agreement with the earlier studies of Rabinowitz et al.,.[166] In human model studies, Kehoe[176] reported that, a dose of 0.62mg lead per day is sufficient to bring about slight accumulation of lead in human body. Through Ayurvedic drugs, people ingest heavy amounts of lead everyday; therefore, lead accumulation in such cases is a matter of fact. For instance, the prescribed dose for nagabhasmam is 100 to 200mg twice a

day and usually it continues for 14 to 21 days. This drug contains eighty-four percentage of lead and the consumer of this drug ingests 84 to 168mg of lead per day. In the present study, we could establish the accumulation of lead in the tissues of lead-drug treated animals.

In our study we found that, the retention of lead in the soft tissues was greater in the liver, followed by kidneys and brain in decreasing order of lead concentrations. This observation is well in agreement with the previous findings of Whanger.[168] He claimed that, the retention of lead in soft tissues is greatest in the liver, followed by aorta, muscle and brain in decreasing order. Even though liver accumulates the highest quantity of lead, the innate regenerating property of liver protect the organ from lead toxicity for some extent.[172]

It is observed that, although the test drugs contained substantial quantities of lead (84% in nagabhasmam and 7.9% in Patented Herbal Drug-1) the tissue lead levels were found very low. This might be due to the high excretion level of lead through urine and feces.[151] The tissue lead levels of the two different test drug groups were found proportional to the lead concentrations in the corresponding drugs. For Example nagabhasmam powder contained about 84% of lead and each animal of this group had received 3.33 mg/kg per day; the tissue lead levels in these animals were found more than that of PHD-1 group. The animals in the nagasbhasmam treated group not only showed more tissue lead levels but manifested severe lead toxicity symptom also. The mortality rate in the nagabhasmam group was 12.5%, against the 0% in the PHD-1 group.

### 4.3.4: Biochemical Studies of Serum

In experimental animals, the chronic exposure to lead causes different types of organ toxicities. The hepatotoxic, nephrotoxic and neurotoxic effects

of lead are well established in the present study. The drug treated group showed elevated levels of both total and conjugated bilirubin. The bilirubin levels were found to be significantly ($P<0.0001$) elevated in the test group compared to the control group. The maximum elevation in total and conjugated bilirubins was observed in the animals, which were receiving nagabhasmam powder and minimum in the group treated with the drug PHD-1. The abnormal elevation of bilirubin levels due to the hepatotoxic effect of lead is well in agreement with the earlier studies on lead poisoning in human model through Ayurvedic drugs by Dunbabin et al.,.[190]

The findings of this study on hepatotoxic effects of lead are in contradiction to the earlier studies of Levi[172], Plaa[174] and Zimmerman[175] to some extent. They claimed that, mercury and lead are not generally causing hepatotoxicity and liver injury because liver has high regenerating property. Even though the serum bilirubin levels of the test groups were found elevated in the histopathological analysis, the liver tissues were found almost normal as that of the control group. The normal histopathological observations of the liver tissues of the lead drug treated animals in the present study justify the non-hepato toxic effect of lead as it is reported in the earlier studies of Levi[172,] Plaa[174] and Zimmerman.[175]

During heavy metal toxicity, the synthetic functions of liver may be compromised. In such conditions, impaired synthesis of proteins and some enzymes may occur. However, in our study, we did not notice any reduction in protein synthesis in the drug treated category. The serum protein changes of test animals were not significant ($p>0.05$) compared to the normal counter parts. These findings are not in agreement with the reports of Niesink et al., on the inhibition of protein synthesis by heavy metal toxicity.[173] Regeneration capability of liver might have protected from the hepatotoxic effects of lead for certain extent and resulted almost normal serum protein levels.[172] The

unchanged serum protein levels in the test groups, even under lead toxicity are supported by the earlier findings of Plaa[174] and Zimmerman.[175] They had claimed that, mercury and lead are not generally causing hepatotoxicity and liver injury, hence they are not included in the list of hepatotoxic agents, which cause liver necrosis, fatty liver, cholestasis (drug induced), hepatitis(drug induced) and carcinogenesis.

Atomic Absorption Spectrophotometric results have shown that, the liver tissues accumulated highest levels of lead among the soft tissues. This might be the reason for the production of elevated levels of liver enzymes. In the present study, the serum levels of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of the drug treated and control groups were studied. The liver enzyme levels of the test groups were found to be significantly ($P<0.0001$) elevated compared to the control animals. Maximum elevations of liver enzymes were observed in the animals, which were receiving PHD-1 and minimum in Nagabhasmum group. These findings are well in agreement with the observations in human studies by Dunbabin et al.,[190] while contradictory to the earlier study reports of Levi[172,] Plaa[174] and Zimmerman.[175] Abnormal elevation of serum ALT level above 100 times is considered as a specific marker for hepatotoxicity and this condition may lead to toxic hepatitis. In this study, the high-level increase of ALT observed may be due to the hepatotoxic effect of lead. These findings are well in agreement with the previous studies on abnormal liver enzyme synthesis due to toxic hepatitis by Estridge et al.,.[48]

The nephrotoxic effects of lead drugs were well established in the present study. In the drug treated groups the serum urea and creatinine levels were found to be elevated significantly ($P<0.0001$) compared to the normal counter parts. These findings are well in agreement with the previous studies of Sharratt et al,.[178], Hook et al,.[179] and Goyer et al,.[182] As they had claimed,

during heavy metal toxicity, acute renal failure may be manifested with uremic syndrome. Under this condition, elevated blood urea, nitrogen and creatinine are considered as typical measures of lead induced renal failure. The serum urea levels were found maximum in nagabhasmam treated group and minimum in PHD-1 group. It is also observed that the serum urea levels were proportional to the lead concentrations in the corresponding drugs and also in the tissues. However, the serum creatinine levels were observed as maximum in the PHD-1 group, which contained comparatively low lead concentration, and minimum in the nagabhasmam treated group.

The findings of our study (the abnormal elevation of serum urea and creatinine) are not in agreement with the study results of Aviv et al.,[196] because they had claimed that, in renal function tests the blood urea-nitrogen and serum creatinine did not increase in rats receiving lead in drinking water. In contrary to this, we observed significant elevation of serum urea and creatinine in the test animals and this might be due to the nephrotoxic effects of lead in the Ayurvedic drug.

The serum uric acid levels in the test animals were found not to be significant ($P>0.05$) compared to the control animals. These findings disagree with the earlier studies of Goyer.[181] He reported that lead content in the blood forms protein-lead complexes in the renal tubules as dense accumulations and causes increased reabsorption of uric acid to blood, hence increased serum uric acid levels in the blood. In renal function test of the present study, why serum urea and creatinine showed remarkable elevation and uric acid became almost normal in both test groups is yet to be found out. In previous studies on the nephrotoxic effects of lead, the serum urea, creatinine and uric acid levels showed remarkable elevation. Hence these have been considered as indices of renal toxicity.[181,196]

In the drug treated group, the serum calcium levels were not found significant (P>0.05) compared to the normal counter parts as it is represented in the table-3.9.5 and figure-3.9.5. Hepatotoxicity due to heavy metals normally leads to the impaired synthesis of proteins especially, albumin. Nephrotoxic effect of lead can be manifested as albuminuria via glomerulitis and leads to the depletion of albumin levels in the blood. Serum albumin is very essential for maintaining the normal serum calcium levels in blood. In the present study, the serum protein and albumin levels were found to be normal in the test animals compared to the control group. There is no significant decrease of serum calcium levels in the test animals compared to the control counterparts. It is also observed that the range of serum calcium levels in the test groups were overlapped by the range of calcium levels in the control group. These findings are well in agreement with the earlier studies on the toxicological effects of methyl mercury on blood electrolytes by Synder[49] and Zuzuki et al.,.[50]

### 4.3.5: Postmortem Studies

The post mortem studies revealed the toxic effects of lead in the internal organs. The postmortem findings of the sacrificed animals and those that died during drug (nagabhasmam) administration period were almost similar. The obvious postmortem finding in both categories was brain edema, especially in the cerebellar region. The death of one animal, during the drug administration might be due to the neurotoxic effect of lead in nagabhasmam. The earlier findings of Bogden et al.,[192] and Sundstrom et al.,[193] on lead induced encephalopathy, support the findings of the present study. The postmortem findings of the control animals were found to be normal.

### 4.3.6: Histopathological Changes in the Brain

In the present study, histopathological analysis of brain tissue was done and it revealed the neurotoxic effects of lead on rat's nervous system. The brain section (longitudinal section of cerebellum) of the rats treated with nagabhasmam is shown in the figure-3.14.3. In this photomicrograph, edema in the peduncular region of the cerebellum is clearly evident. Microcystic changes can also be noted in the peduncle. Perivascular edema in the cerebellum is illustrated in figure-3.14.4. The edematous area around the blood vessel is infiltrated with round cells and the Virchow-Robin space appeared as abnormally widened. A blood vessel with disrupted endothelium is illustrated in the same picture. The disrupted endothelium of the blood vessel with damaged astrocytes constitutes an impairement of the blood brain barrier and this could be the reason for edema in the cerebellum. The perivascular edema due to the compromised blood-brain-barrier system is explained in literature (Holtzman et al.,[145]). The direct mechanism by which the blood brain barrier and blood vessels that compose the barrier mechanism may be compromised and this might be due to the astrocytes appearing to be vulnerable to the toxic effects of lead. The astrocytes cover the vascular walls of the brain vessels, and lead can injure these structures. Therefore, it is very likely that lead toxicity is the reason for perivascular edema.

The brain sections of control animals are shown in the figures-3.14.1 and 3.14.2, which illustrate the normal distribution of astrocytes and neurons. The cerebellar edema is absent in these sections, when compared to the animals treated with the drug, nagabhasmam. This suggests that the perivascular edema in the cerebellum of lead-drug treated animals could be due to the compromised function of blood brain barrier. The findings are in agreement with the earlier studies of Holtzman et al., [145] and Bradbury[146] on lead induced encephalopathy.

The neurotoxic effect of lead is a well-researched topic in lead toxicology because brain tissues are highly susceptible to lead. The most serious manifestation of lead poisoning is lead encephalopathy. In human beings, the early symptoms of lead encephalopathy are laziness, vertigo, ataxia, falling, chronic headache, insomnia, restlessness (hyperactivity in children) and irritability. As the disease progresses, the patient may first become excited and confused, then the last stage manifests as delirium with repeated colic and tonic convulsions or lethargy and finally the coma stage.[149]

The encephalopathy induced by lead toxicity is most likely to occur due to a compromise in the blood brain barrier, which is constituted by blood vessel endothelium and astrocytes. Brain edema occurs in the interstitial area due to decreased blood vessel integrity. The elevated lead levels disrupt the blood-brain-barrier system and plasma proteins such as albumin enter the interstitial spaces, as do some ions also. This increases osmotic pressure, and in response to this phenomenon, water accumulates. This edema causes an increase in intracranial pressure and restricts blood supply to the brain tissues, resulting in brain ischemia. The aberrations in the blood supply to the vital areas of the brain results in symptoms of encephalopathy.[146]

Cerebellar edema is illustrated in the figure-3.14.5. Sparsely cellular edematous tissues with microcystic changes are seen in the affected area. Cerebellar edema is found in continuation with perivascular edema. The series of events in brain edema starts with the disruption of endothelium and astrocytes in the blood brain barrier. Perivascular edema in the cerebral cortex is illustrated in the figure-3.14.6. The edematous areas show round cell infiltration, and normal blood capillaries are seen in the non-edematous areas. Edematous cerebral area is clearly differentiated from non-edematous area (figure-3.14.7). One capillary with disrupted endothelium and perivascular edema is also illustrated in the same photomicrograph. This photograph

substantiates the evolution of perivascular edema in to cerebral edema and these findings are well supported by the earlier studies on lead induced encephalopathy in different animal models by Clasen et al.,[147], Selvin-Testa et al.,[148] and Zook. [208]

Physiologically, the cerebral edema is due to several reasons; a disrupted blood-brain barrier system effectively increases the permeability of albumins and other electrolytes. The electrolytes escape from the blood capillaries to the interstitial spaces and gradually increase the osmotic pressure. This increased osmotic pressure promotes the entry of water into the interstitial area and results in cerebral edema. The lack of lymphatic structures in the central nervous system means that the fluid flows into the cerebral ventricles. This condition may lead to increased intracranial pressure, which results in compression of brain and blood vessels causing the decreased blood supply to the brain tissues and finally manifested as encephalopathy.[147,148]

Periventricular edema is well illustrated in the figure-3.14.8. The ventricle appeared abnormally widened and filled with excess cerebrospinal fluid. The abnormal accumulation of cerebrospinal fluid in the ventricle increases the intra-ventricular pressure, and compression of cerebral cortex may occur impairing the vital functions of cortex. The permeability of the blood vessels in the brain is limited by the blood brain barrier system, which is constituted by vascular endothelium and astrocytes. The astrocytes are highly sensitive to lead and this alters the barrier system. A collapsed blood brain barrier system allows free passage of electrolytes, and finally in edema. This pathogenesis is likely in the lead drug treated group; and the findings are well in agreement with the earlier studies of Bradbury.[146] on lead induced encephalopathy due to impaired blood-brain barrier system.

The cortical and sub cortical sections of cerebellum of control rats are shown in figure-3.14.9. The number and distribution of Purkinje cells and

glial cells appeared to be normal in the control group, but the numerical decrease of Purkinje cells are observed in the corresponding brain sections of animals exposed to nagabhasmam (figure-3.14.10). These findings are in agreement with the earlier research works of Krigman et al.,[197] and Basha[198] on the effect of lead intoxication in the postnatal growth of rat nervous system. Their histological studies revealed the reduction of grey matter and thinner cortical mantle in the brain sections. Reduced or delayed subdivisions of dendrites, and axons were also reported in the same study. The toxic effects of lead on Purkinje cells in the cerebellum are well established in this study because the numerical decrease of neurons is very much evident in the test group compared to the control brain sections.

The brain section (basal ganglia) of the rats treated with nagabhasmam is illustrated in the figure-3.14.11. This microphotograph shows the neuron with early degenerative changes, which start with peripheral clumping of chromatin and nuclear vacoulation. The neurons and glial cells are highly sensitive to lead and the neuronal degeneration could be due to lead toxicity of the drug nagabhasmam. The findings of this study are in agreement with the findings of Zook[208,] on the effect of accidental lead exposure in dogs. The neuro-histopathology of lead-exposed dogs had revealed lesions in the brain, which involved vascular damage consisting of dilation 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 vacoulation, gliosis and necrosis of neurons in the cerebral and cerbellar cortex.

In lead exposed rats, it is observed in histology that, the neurons are highly susceptible to lead toxicity. The degeneration of neurons starts with peripheral clumping of chromatin called the early degenerative changes

(figure-3.14.11). The degenerative changes proceed with aggregation of nuclear chromatin and results in the formation of homogeneous hypochromatic mass of nuclear material (figure-3.14.12). The degenerative changes in the cerebral cortex further progress to late changes of karyorhexis and nuclear fragmentation in neurons and cell death (figure-3.14.13). These changes occur in the cerebral cortex and are in agreement with the earlier studies of Costa et al., [143] and Schleapfer et al.,.[194] They claimed degenerative changes of neurons followed by cell death in lead exposed animals models.

The astrocytes and neurons observed in the brain sections (cerebral cortex) of control rats are found healthy and normal (figure-3.14.14). Nevertheless, the brain section of test animals, showed several degenerating neurons in the cortical area (figure-3.14.15). The degenerating neurons in the cortical sections are numerous in the treated category when compared to the control group. The degenerating neurons show intra-nuclear inclusions (figure-3.14.16). These changes in the cortical neurons are due to lead toxicity and are well in agreement with the findings of Sclaepfer et al., [194] and Teruo et al.,.[200] Fowler[160] also supports the findings of the present study, in which the protein binding property of lead in the brain causes the deterioration of astrocytes.

We observed the presence of intra-nuclear inclusion bodies in some neurons and our findings are in agreement with the earlier study reports of Teuro et al.,[200] They claimed the presence of lead induced intra-nuclear inclusion bodies in the neurons and astrocytes of lead exposed rats. Meanwhile in another study, the intra-nuclear inclusion bodies were observed in the renal tubular cells in lead toxicity[199,] but in our study, we did not observe significant histological changes in the renal tissues. The histopathological studies of the brain sections have revealed the neurotoxic

effects of lead in the Ayurvedic drug, similar to changes/observations in lead toxicity.

Health is in the center stage of man's thinking and gets maximum attention of individuals and nations. Health is considered as wealth; the promotion of health, restoration of health and prevention of disease are the greatest task before the nation. The deterioration of health, physical and mental fitness is alarmingly on the rise. The causes for this situation are plenty, ranging from change in food habit to change in life style. In fact both of these are closely interlinked and complementary to each other.

The medical practice today in the world in general and in India particular is in a very confused state. It stands on a crossroad. A drug used today may be declared as poison tomorrow. People's faith in different systems of medicine is oscillating from one to another. Now people are more restless, they easily get tempted to switch over from one system of drugs to another. Self-medication is another dangerous sign. In this background, it is obligatory to make it known the beneficial and adverse effects of drugs, of any system, including Ayurvedic drug preparations. It is important to unfurl the myth that Ayurvedic drugs are cent percent safe. Our study has shown the definite adverse effects of Ayurvedic drugs mainly due to heavy metals, from which they produced. A long-term study is needed to expand this knowledge. It will be beneficial to the Ayurvedic pharmaceutics formulation and betterment of health of future generation.

Chapter 5

# SUMMARY & CONCLUSIONS

## 5.1: Summary

The main aim of the present study was to evaluate the opinion that Ayurvedic drugs are cent percent safe and free from adverse side effects.

- In the present study, forty Ayurvedic/patented herbal drugs were tested. It was attempted to estimate the heavy metal contents; mercury and lead in some common Ayurvedic drugs used in and around Kozhikkode area.
- Concentration of mercury and lead in forty drugs were estimated using Atomic Absorption Spectrophotometry.
- Four drugs were selected for animal studies – Two each rich in mercury and lead.
- One native mineral of mercury; the chayilyam (cinnabar) was also used for animal studies.
- Albino rats of Wistar strain were used for animal model experiments.
- Post-drug administration (clinical) symptoms were observed.
- Mercury and lead levels in different tissues (blood, kidney, liver and brain) were estimated by using AAS analysis.
- Hematotoxic effects of lead were studied by estimating delta-aminolaevulinic acid.
- Hepatotoxic, nephrotoxic and neurotoxic effects of mercury and lead were studied.
- Tissue injuries by heavy metal toxicity were evaluated by histopathological studies.

The major findings of our study can be summarised as follows: –

- Twenty six out of forty drugs contained mercury or lead or both.
- The test animals manifested the typical symptoms of heavy metal toxicity.
- The mortality rate of mercury treated groups (RS and SG) was 25%.
- The mortality rate of lead treated group (NGB) was 12.5%.
- The blood concentration of heavy metals significantly rose.
- Accumulation of mercury was highest in kidneys.
- Accumulation of lead was highest in liver.
- Abnormal liver functions were observed due to hepatotoxicity of heavy metals.
- Abnormal kidney functions were noted because of the nephrotoxic effects of heavy metals.
- Histopathological studies confirmed the renal tissue injury by mercury and nervous tissue injury by lead.
- Histopathological findings substantiate the pathophysiology behind abnormal liver and kidney function tests.

### 5.1.1: Conclusion

- Mercury and lead content in certain popular Ayurvedic drugs are alarmingly high.
- This heavy metal toxicity of drugs caused severe damage to the animal tissues mainly, kidney, liver and brain.

- It is expected that these drugs in human beings under similar circumstances may manifest similar toxic effects.
- Therefore, any Ayurvedic drugs should not be considered as cent percent safe with out any adverse side effects.
- Use and practice of these drugs should be subjected to thorough scientific evaluation.
- Toxicological, pharmacokinetic, animal and human trial studies must be made mandatory for every non- bioderived Ayurvedic drugs.
- Ingredients, side effects and contraindications of the drugs must be declared on the label.
- Availability of non-bioderived medicinal preparations as Over-The-Counter (OTC) drugs and self-medication should be discouraged.
- Attempt should be made to promote the therapeutic effects of Ayurvedic and herbal medicines on a strong scientific foundation with minimizing the adverse side effects.

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# THE TOXICITY STUDIES OF MERCURY AND LEAD IN AYURVEDIC PREPARATIONS

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# SYNOPSIS

## Introduction

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.[1] Our people consider the preparation, use and practice of Ayurvedic drugs as part and parcel of Indian life. Besides the spiritual and traditional dimensions, people believe that Ayurvedic drugs are absolutely safe, devoid of side effects and residual toxicity, because they are prepared from plants and animals. Interests in herbal medicines have also been facilitated by certain other factors, including the perception that pharmaceutical medications are expensive, over prescribed and may often be hazardous. Alternatively, herbal medicine is often perceived as being "natural" and therefore is considered to be safe. Most of all Ayurvedic preparations are easily available in pharmacies as OTC (over the counter) drugs. Nowadays, herbal supplements are receiving increasing exposure through media, including internet, in lay journals and more recently in 'scientific' press. Our Ayurvedic therapy and herbal treatment systems are attracting a lot of tourists to this country, especially to Kerala. Ayurvedic firms in our state are effectively utilising this situation as a means for their income generation. On the other hand, entrepreneurs in this field are exploiting people's craze to this traditional system in a very unhealthy way.

While the system enjoys wide acceptance among people, drug experts have estimated approximately 6000 medicines in the 'Ayurvedic Formulary' (pharmacopoeia for Indian medicines) which intentionally contain at least one 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.[1] Currently, heavy metal toxicity through the consumption of Ayurvedic, Siddha or patented herbal drug preparations is an ignored but a prevailing problem in India and other countries. Due to the easiness in procuring Ayurvedic drugs from the market and the generally

prevailing concept that they are risk free, there is an indiscriminate and unscientific use of Ayurvedic drugs among people. The use of heavy metal based Ayurvedic preparations may lead to serious heavy metal toxicity. As mercury and lead are the most widely used heavy metals for drug preparation in Ayurveda[2,] the toxicity due to these metals may become very common.

**Objectives**

In Ayurveda, the Indian system of medicine, there is a belief that, through Ayurvedic protocols any spurious substance can be changed into valuable medicines. Hence, people judge that, Ayurvedic drugs are completely safe and devoid of side effects. But this is a tall claim without any strong scientific support, since studies carried out in this line are few. Most of the Ayurvedic drugs available in the market are with undeclared compositions (the contraindications, side effects etc… are also not mentioned). When there are several reports that many Ayurvedic drugs are prepared from heavy metals and other toxic elements, and the protocols for drug processing are not effective to remove their toxicity, it is logical to doubt whether Ayurvedic preparations are free from heavy metals like mercury and lead. According to 'The Ayurvedic Formulary of India' mercury and lead are the most widely used heavy metals in this drug industry.[2] As these metals are potent nephrotoxic, hepatotoxic, neurotoxic and hematotoxic agents, the toxicity can be very much expected with the use of heavy metal drugs.

This study was designed to detect and estimate mercury and lead in some commonly used Ayurvedic and patented herbal drugs by atomic absorption spectrophotometry. Animal experiments have been done in *Wistar albino* rats using five drugs, three drugs containing mercury and two drugs, rich in lead. It was also proposed to study the mortality rate, post-drug administration symptoms (clinical), autopsy, tissue (blood, kidney, liver and brain) levels of mercury and lead, liver functions, renal functions and histopathology. Statistical analysis was also done to evaluate the significance of results.

## Methodology

The study was conducted in the Physiology division of Department of Life Sciences, University of Calicut, Kerala during the period from August 2001 to June 2006. The toxic effects of mercury and lead present in some Ayurvedic and patented herbal preparations on experimental animals were proposed to be studied. For this purpose, forty Ayurvedic preparations including some patented herbal drugs used for common ailments or as health supplements and aphrodisiacals were sorted out. The drugs were procured from pharmacies and general supermarkets around Kozhikkode and Calicut University areas as 'Over The Counter (OTC) drugs. The collected drugs were subjected to atomic absorption spectrophotometry (AAS) for estimating mercury and lead content. Four drugs (two each containing mercury and lead) and a mineral of mercury have been chosen for animal model studies. *Wistar albino* rats were used as animal models, every test and control group consisted of eight animals each.

The mercury drug models consisted of three test groups, rasasindhuram (RS), swasanandam (SG) and chayilyam (CYM), whereas the lead-drug model consisted of two test groups, nagabhasmam (NGB) and patented herbal drug-1 (PHD-1). Control groups were restricted as one for each drug model to promote minimum sacrifice as per SPCA norms. The test groups were ingested with corresponding drugs (drugs mixed with anupana dravas) and control groups with anupana dravas (conjuvants) only for fourteen days. The toxicity symptoms (clinical) during drug administration and post drug administration periods were observed. After fourteen days of drug administration, blood samples were collected from both test and control animals by tail cannulation technique. The di-acid digested blood samples were subjected to AAS analysis for estimating mercury and lead content. The hematotoxic effects of lead were studied; for this purpose, twenty-four hours' urine samples were collected from lead drug treated animals and their controls by using metabolism cages. The urine samples were subjected to ion-exchange chromatography for estimating delta-aminolaevulinic acid.

After three days of post-drug administration period, the test and control animals were sacrificed by jugular vein puncture. During this process, maximum blood volumes were collected to separate serum. The serum samples were subjected to liver and renal function tests. The animals died during drug administration period and the sacrificed animals were subjected to autopsy. During postmortem, the internal organs like kidney, liver and brain specimens were collected for AAS analysis and histopathological studies. AAS analysis of kidney, liver and brain tissues were done to estimate mercury and lead. The internal organs (kidney, liver and brain) were subjected to tissue processing and slide preparation for histopathological evaluations. Statistical analysis was done by using SPSS-12.0 version software for microcomputers. ANOVA test was used to access statistical significance.

**Results and Discussion**

In this study, forty Ayurvedic drugs including some patented herbal preparations have been analysed for mercury and lead contents by AAS. Among the tested samples, fourteen drugs contained substantial quantities of mercury. Seven out of forty drugs contained heavy amounts of lead, while five samples contained both mercury and lead. An aggregate of 65% of tested samples contained substantial amounts of mercury, lead or both. The occurrence of heavy metals in Ayurvedic and herbal medicinal preparations was established previously by many scientists.[3,4]

In the mercurial drug samples, the concentration of mercury ranged between 15ppm to 45281ppm. Surprisingly, in ten drugs the mercury contents were above 10,000ppm. In fact, the possibility of ingesting inorganic mercury through daily food is very rare and the daily intake is estimated to be below 1μg per day.[4] Similarly in the case of lead-drugs, the concentration of lead was found to be ranging between 14ppm to 844977ppm. According to WHO, the Provisional Tolerable Weekly Intake (PTWI) of lead in adult is 50 μg/kg body weights and in children it is only 25 μg/kg.[6] As per a study conducted in USA, most of the food items, drinks, water etc… contain lead in some microgram quantities.[6] In spite of this fact, we estimated 844977ppm lead in nagabhasmam. These details unveil the threat of mercury and lead toxicity

that can come through the consumption of Ayurvedic and patented herbal drugs in the users.

After a few days of drug administration, the test animals started to manifest toxicity (clinical) symptoms of mercury and lead. The important clinical symptoms observed were excessive salivation, anorexia, oliguria, diarrhea, weight-loss, emaciation, scabby lesions around mouth and anus, alopecia and pruritis. These symptoms were more severe in mercurial drug groups than lead drug groups. Weight loss with emaciated appearance and stiff legged walk were common in majority of mercurial drug models, whereas hyperactivity and weight gain were observed in some animals in the lead drug models. During drug administration period, we observed 25% mortality in mercury drug models (RS and SG groups) and 12.5% in lead drug model (NGB group). However, the control groups did not manifest any of the above symptoms; they appeared normal and healthy throughout the experimentation period. The clinical symptoms in the test groups clearly match the toxicity symptoms of mercury and lead reported in the earlier studies.[7,8]

The toxic effect of lead on haematopoietic systems can be detected by estimating the urine levels of delta-aminolaevulinic acid (δ-ALA). In human beings, elevated δ-ALA excretion is a more sensitive and specific index of lead exposure. In our study, we observed increased levels of δ-ALA in the lead drug treated rats. The δ-ALA levels in the test animals were found to be elevated significantly ($P<0.0001$) compared to their normal counter parts. The elevated levels of δ-ALA in the test animals revealed the hematotoxic effect of lead. These findings are supported by the previous studies on lead induced hematotoxicity in rats and also in human beings.[9,10]

AAS results of drug treated animals showed the presence of mercury and lead in blood, kidney, liver and brain tissues. The concentration of mercury and lead in the circulating blood is known as blood mercury and lead levels (BML and BLL) respectively. BML and BLL are considered as the definite tests for mercury and lead toxicity.[11,12] In the mercury drug models, mercury was found deposited mostly in the kidneys followed by liver and

brain, whereas in lead drug treated animals, the highest amount of lead was observed in liver followed by kidney and brain. The accumulation of mercury and lead in the vital organs might be the reason for clinical symptoms and mortality in the test groups. The tissue mercury and lead levels of test groups were significantly higher, compared to their control groups ($P<0.0001$). Our observations are supported by the earlier study findings of Sin et al.,[11] and Whanger.[13]

In heavy metal toxicology, liver function test can be taken as relevant method for assessing the intensity of mercury and lead toxicity. In this study mercury and lead drug exposed animals showed elevated levels of serum bilirubins (conjugated and unconjugated) and liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Decreased synthesis of serum proteins were also observed in mercury drug models. The liver function parameters of the test groups were found to be statistically significant compared to the control groups. These observations are well in agreement with the previous studies carried out by Dufour et al.,[14] and El-Demerdash.[15] Renal function tests (serum urea, creatinine and uric acid) of test animals revealed the toxic effects of mercury and lead in experimental animals. Serum urea and creatinine levels of the test groups were found to be elevated significantly compared to the control groups ($P<0.0001$). These findings are supported by the previous studies on mercury and lead induced uremic syndrome.[16,17]

Autopsy studies revealed the toxic manifestations of mercury and lead in the internal organs of rats. The remarkable changes observed in the mercurial drug groups were, severe ulceration in the gastro-intestinal tract, edemic kidneys with fluid filled lesions. However, the lead drug groups showed large edemic brain with abnormal contour. The present postmortem findings are supported by the earlier studies of Cassidy et al.,[18]

Histopathological studies confirmed the nephrotoxic and neurotoxic effects of mercury and lead respectively. Renal tubular necrosis, glomerular cysts and hypercellular glomerulitis were observed in the mercurial drug groups and these histological changes might be the reasons for diuresis,

followed by oliguria and anuria. Histopathological findings also support the abnormal elevation of serum urea and creatinine in the test groups. Our observations are supported by the earlier studies.[19] The most serious manifestation of lead poisoning is brain edema (encephalopathy). The lead induced encephalopathy is most likely to occur due to a compromise in the blood brain barrier. Brain edema at different brain areas and numerical decrease of neurons in the cerebellum are well illustrated in the histopathological studies. The present observations are similar to the earlier histopathological studies.[20,21]

**Conclusion**

- Mercury and lead content in certain popular Ayurvedic drugs are alarmingly high.
- This heavy metal toxicity of drugs caused severe damage to the animal tissues mainly, kidney, liver and brain.
- It is to be expected that these drugs in human beings under similar circumstances may manifest similar toxic effects.
- Therefore, no Ayurvedic (non-bioderived) drug should be considered as cent percent safe with out any adverse side effect.
- Use and practice of these drugs should be subjected to thorough scientific evaluation.
- Toxicological, pharmacokinetic, animal and human trial studies must be made mandatory for every non-bioderived Ayurvedic drug.
- Ingredients, side effects and contraindications of the drugs must be indicated on the label itself.
- Availability of non-bioderived drugs as Over-The-Counter (OTC) drugs and self-medication should be discouraged.
- Attempts should be made to promote the therapeutic effects of Ayurvedic and herbal medicines on a strong scientific foundation by minimizing the adverse side effects.

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