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.,\textsuperscript{4} Lad\textsuperscript{5} Saper et al.,\textsuperscript{8} Aslam et al.,\textsuperscript{111} Ang et al.,\textsuperscript{211} Baer et al.,\textsuperscript{212} and Leukoch et al.,\textsuperscript{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.\textsuperscript{83} In fact, the possibility of ingesting inorganic mercury through daily food is very rare and the daily intake is estimated to be below 1μg/day.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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., Cassidy et al., and Boening 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. The BMLs of different test groups in this study were found almost proportional to the mercury concentrations in the rasasindhuram, 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.,. 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. (2) The affinity of mercury ions for thiol groups accelerates the accumulation of large amounts of inorganic mercury in the kidneys. 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.

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.,\textsuperscript{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 rasasinduram. 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.\textsuperscript{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.\textsuperscript{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, Estridge and El-Demerdash 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, Plaa and Zimmerman 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. 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.,\textsuperscript{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.\textsuperscript{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$_2$O$_2$ production by renal mitochondria is an indirect effect of inorganic mercury.\textsuperscript{38} Mercury poisoning also causes primary and secondary idiopathic membranous glomerulonephritis.\textsuperscript{239} Polycystic kidney disease is recently reported in lead intoxication.\textsuperscript{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\textsuperscript{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 McNeil et al.,WHO, Buchet et al., and Sharratt et al., 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 rasasindharum 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.,WHO, Buchet et al., and Sharratt et al.,. 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. 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 treated 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\textsuperscript{49} and Suzuki et al.,\textsuperscript{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.,\textsuperscript{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...<sup>43</sup> 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.<sup>59</sup> The affinity of mercury ions for thiol-groups accounts for the accumulation of large amounts of mercury in the kidneys.<sup>60</sup> 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.,.<sup>38</sup> He revealed that, mercury ion is known to promote oxidation of kidney cells and to disrupt renal mitochondrial function. The increased
H₂O₂ 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., and Zook. 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., Barr et al.,
Jennet et al.,\textsuperscript{235} and Guzzi et al.,\textsuperscript{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.\textsuperscript{49,50}

Hypercellular glomerulitis in the cortical area may be due to the proliferation of mesangial 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.,\textsuperscript{64} Sharratt et al.,\textsuperscript{178} and Hook et al.,\textsuperscript{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-1 group 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., Rutter and Needleman et al. 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. 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-1 group 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 throughout 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 counterparts (table and figure 3.5.1). The findings of this study are well in agreement with the earlier studies of Snowdon. 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 has reported similar results on the inhibitory effects of lead on heme synthesizing enzymes. Related findings were also reported by Fowler and Conner et al., 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. 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 hematopoietic 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. Lead also inhibits hemesynthetase enzyme, which is responsible for the introduction of iron into the tetra pyrrole porphyrine ring. 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. 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.. In human model studies, Kehoe 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. 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.

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. 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 Levi172, Plaa174 and Zimmerman175 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-hepatotoxic effect of lead as it is reported in the earlier studies of Levi172, Plaa174 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 counterparts. 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 and Zimmerman. 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., while contradictory to the earlier study reports of Levi, Plaa and Zimmerman. 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.,

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., Hook et al., and Goyer et al., 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 nagabhasam 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 nagabhasam 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.,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{49} and Zuzuki et al.,\textsuperscript{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 (nagabhasamam) 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 nagabhasamam. The earlier findings of Bogden et al.,\textsuperscript{192} and Sundstrom et al.,\textsuperscript{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 impairment 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.  

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.  

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 into cerebral edema and these findings are well supported by the earlier studies on lead induced encephalopathy in different animal models by Clasen et al.,\textsuperscript{147} Selvin-Testa et al.,\textsuperscript{148} and Zook.\textsuperscript{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.\textsuperscript{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\textsuperscript{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 nagabhasam (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 nagabhasam 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 vacuolation. The neurons and glial cells are highly sensitive to lead and the neuronal degeneration could be due to lead toxicity of the drug nagabhasam. 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 vacuolation, gliosis and necrosis of neurons in the cerebral and cerebellar 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
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.,\textsuperscript{143} and Schleapfer et al.,\textsuperscript{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.,\textsuperscript{194} and Teruo et al.,\textsuperscript{200} Fowler\textsuperscript{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.,\textsuperscript{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\textsuperscript{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.