Introduction

Bhasmas are derived from metals and their ores, numerous physicians (other than those practising Ayurveda) always look at their use doubtfully. Testing of the claims of these drugs in laboratory animals helps to know the physicians of modern disciplines about drug dependent metabolism in the modern pharmacological, physiological and histological concepts. Since they are tested directly in living animals many of the concepts of the *in vivo* drug metabolism can be tested and analysed.

This type of approach will evaluate the claims of Ayurvedic system of medicine and diagnosis that is age old and time tested. This will help to extend use of many drugs that are used against liver disorders, blood pressure, anaemia, diabetes *etc.* This will also help to develop the integrated medicine for the welfare of the human being.

1) Reasons to take the problem:

In recent years the extending population, increasing industrialisation coupled with the various type of effluents are adding to the already polluted air and water. Tremendously increased and ever expanding populations of the cities, compelling to live 2\3rd of the

population under poor hygienic and health conditions. While the problems of wastewater management, drinking water, use of heavy fertilizers and pesticides in villages are also affecting the health of the people.

Poor people are suffering due to their financial problems and ignorance while others are suffering due to addictions and unhealthy habits. The demand of medicines and physicians is increasing so also the prices of diagnostic processes and medicines. Large part of the population in developing or non developing country are not able to afford numerous types of therapeutics, while on the other hand in spite of increasing research in this field some diseases and disorders are not under control.

Almost every society has the heritage of traditional therapies that are mostly Eco-friendly, derived from the natural products which are available in the surroundings. Thus for the preparations of the drug it is compulsory to grow the plants. Most of these medicines are still used by traditional practitioners successfully against the diseases and disorders most common in that region.

WHO has realised their importance in achieving the goal of the global healthy society and has accepted all the types of ethnic and

traditional medicines. These drugs are easily accessible, affordable financially, result oriented and time proven.

Among the traditional and ethnic medicines Chinese medicine and Ayurveda have long histories of thousands of years.

Ayurveda is time tested oldest therapy compatible to all the sorts of modern medicines. It provides its own pharmacology, diagnostic methods and therapeutics. Due to some social stigma and British rule this system of therapy was discontinued. But numerous traditional practitioners' families kept the therapy and literature alive.

There are numerous composite drugs in Ayurveda, which can be used against almost all kinds of diseases and disorders. All these drugs should be popularised among all the types of physicians, so that the integrated system of medicine can be developed. The ignorance about the medicines and therapy can be removed if the physicians are convinced on the basis of modern physiology and pharmacology. Similarly the toxicological studies will help the safety use of the ayurvedic medicines.

2) Why the toxicity testing?

Nag bhasma is one of the medicines used in Ayurved and Siddha, which is prepared from lead through various processes described in the literatures of these medical systems. In the survey of the clinical

toxicity of commercial products under Asian herbal remedies some Ayurvedic, siddha and Unani drugs that contain lead are considered toxicity causing (Gosselin *et al,* 1984). In therapeutic sense a larger dose of any medicine (including modern medicines) or larger dose to a chronic patient can be a condition of toxicity looking at their common use in ethnic societies it is necessary to evaluate its toxicity and therapeutic claims.

Nag bhasma is used against diabetes, disorders related to eye, rectum, enlarged spleen, haemorrhages and leucorrhea, disorders of liver and gastrointestinal tract, acidity, disorders of lymphatics, anaemia and T. B. It is also used as general tonic to maintain the health (Ayurveda Sarsangrah, 1971; Bodas, 1981). It is not only used independently, but also with other drugs (Ayurveda Sarsangrah, 1971)

In spite of its use against numerous disorders and diseases; physicians avoid its use in therapy due to its lead origin, since the lead toxicity is well studied and properly publicised. Any type of drug that is marketed is tested for toxicity; although Nag bhasma is traditionally used and time tested it is necessary to test its safety for use. Additionally people hardly know that there are some of the methods described in Ayurvedic texts that assure the purity of bhasma (Ayurveda Sarsangrah, 1971; Bodas, 1981). In modern days if its use to be extended to

physicians following different medicines, so that it can be available to the physicians who prefer to use integrated medicine. Therefore this is an attempt to test the toxicity of the Nag bhasma. The results may provide the toxicity if any, or any other side effects the bhasma may show or the results may provide some interesting observations that may help to design new drugs or may reveal some other interesting metabolisms.

Since the possibility of toxicity of lead from Nag bhamsa is in probability; the different markers has to be selected on the basis of the lead toxicity and hence it is necessary to review the literature concerned with it. In the present review due to fear of extensive expansion the restrictions were imposed to limit it by the work based on toxicity experiments that involve rats or other animals.

3] Review on Lead toxicity:

Rabinowitz (1998) reviewed the measurable biomarkers of human lead exposure and toxicity. These are influenced by biotransformational metabolisms of lead and the appearance of some of the key products or their metabolisms associated with organ specific biological component may be enzymes, lipids, carbohydrates, proteins nucleic acids or histology, histochemistry, ultrastructure *etc.* (Mushak, 1998).

Physiologically based models to predict the health effects are necessary. Numerous works have been carried out and are being carried out (especially the works on laboratory animals and particularly that on rats). The works reviewed deal in detail with the numerous parameters that can be of use in toxicity monitoring or diagnosis (Mahaffey, 1998).

Lead Metabolism:

Lead is non-essential element that is relatively abundant in the body of mammals. Although Schwarz (1974) reported some evidence for possible essential function for lead, under certain unusual conditions lead is stimulatory and enhanced protein synthesis, increased erythropoiesis, respiration, DNA synthesis, cell replication and reproduction (Luckey, 1975_b). Small doses of intraperitonealy injected lead stimulate DNA Synthesis in rat kidney (Choie and Richter, 1974).

Numerous reviews on lead toxicity indicate encephalopathy, renal toxicity and toxicity to hemopoietic tissue, reproductive system, foetal development and bones. (Venugopal and Luckey, 1978; Skerfving, 1987; Hook *et al*, 1979; Rabinowitz, 1998).

The metabolism and toxicology of lead is extensively studied and reviewed by researchers in different, but related Scientific disciplines

(Venugopal and Luckey, 1978; Skerfving, 1987; Hook *et al*, 1979; Glossen *et al*, 1984).

Absorption of lead salts from the gastrointestinal tracts of the mammals is poor about 1.5% over a dosage range of 2 - 108 mg/day. Rate of absorption is not influenced by dosage in cattle, sheep and rabbits (Venugopal and Luckey, 1978). Absorption of insoluble lead sulphate, sulphide or chromate is poor, but that of lead acetate and lead nitrate is slightly greater.

In rats absorption of lead from gastrointestinal tracts is influenced by age (Forbes and Reina, 1972), the age-absorption curve of lead is similar to calcium (Taylor *et al*, 1962). Other factor influencing lead absorption is the levels of dietary calcium and vitamin D and the motor activity of the digestive tract. High calcium and increased peristalsis of the digestive tract lowered lead absorption and high dietary vitamin increases it. Soluble lead salts administered intraperitonealy, intramuscularly and subcutaneously are not fully absorbed and part is retained at the injection sites (Choie and Richter, 1972). Intravenously injected lead salts are either sequestered in erythrocytes or deposited on erythrocyte stroma membrane about 5 % remains in the plasma mostly as colloidal lead phosphates and diffusible lead peptides or lead organic acid complexes. The absorption of inhaled lead is more complete and

rapid (90 %) than by any other route. Respiratory tract absorbs lead retained in the lung if the particles range from 0.01 to 0.1 μm size. Absorbed lead is deposited mostly in bone and some soft tissues. Lead accumulates in the body to varying degrees depending upon the nature of lead compound and the environmental load of lead. Intravenously administered lead is distributed mostly in liver and bone, while ingested lead is distributed as follows - bones 60 %, liver – 25 %, kidney – 4 %, reticuloendothelial system – 3 %, intestinal wall 3 % and other tissues in traces. The kidneys contain a high concentration of lead/g tissue under conditions approximating the steady state *i. e.* with about 400 μg Pb^{++} being absorbed daily. The retention of the lead in mammalian tissues is as follows – liver > kidneys > aorta > muscle > brain. Lead permeates the placental barrier (Horiuchi *et al*, 1959).

Lead treatment activated peroxisomal catalase in liver but it was lowered in kidney indicating stabilisation of peroxisomes (Popova and Popov, 1998). The subcellular distribution of lead within the kidneys and other tissues was determined by injecting Pb^{-203} intraperitonealy into rats (Barltrop *et al*, 1971). Most of the lead was found in the lysosomes. Reductions in number of lysosomes and vacuolar dilation of the intercellular space were noted on lead acetate administration (Schroeer *et al*, 1982). Renal cell organelles contained lead in the following order

Supernatant > microsomal > mitochondrial = nuclear. Rats already exposed to lead showed 35 % reduction in uptake of radiolabeled lead. Inorganic lead is excreted in both faeces and urine. Faecal lead represented the unabsorbed portion of ingested lead and lead from bile. About 80 % of intravenously injected lead were found in bile through the entero-hepatic pathway. Lead is excreted through the kidneys by both glomerular filtration and Trans lobular flow. It is also eliminated through sweat and milk, although such excreted quantities are small. Urinary excretion of lead is increased in acidosis and after vitamin D, parathyroid hormone or iodide administration. The kidneys can excrete large amounts of lead without excessive damage to the renal tubular lining by keeping the renal lead in a non-diffusible, non-toxic inclusion body from within a the renal cell and excreting the inclusion body (Goyer, 1971a). Increase in excretion of sodium and to a lesser extent also that of potassium (Saketa *et al*, 1979a) seems to be coupled with decreased Na^+–K^+ potassium dependent ATPase and potassium-dependent phosphatase activity (Saketa *et al*, 1979a). Impaired reproductive capacity is also noted (Venugopal and Luckey, 1978; Skerfving, 1987; Hook *et al*, 1979).

On the basis of the data accumulated through these years experimental model of lead nephropathy has been proposed to correlate

between renal functional changes and haematological indices of lead toxicity (Khalil – Manesh *et al* 1994).

The toxic effects of lead on the hemopoietic system were studied extensively and reviewed (de Bruin, 1971; Arena, 1974). Anaemia is not serious consequences in lead toxicity and can be recovered. The toxic effects of lead on hemopoiesis are : a] Abnormal circulating erythrocytes, b] Impairment of the production of haemoglobin owing to lead activity on the bone marrow and c] stimulation of erythropoiesis in bone marrow.

Lead readily binds to, SH^-, $PO4^{3-}$, and $COOH^-$ containing ligands of the erythrocyte membrane to increase the mechanical but decreased the osmotic fragility of erythrocytes. Lead inhibits aminolevulinic acid dehydrates which is involved in haem synthesis (Waldron, 1966) by shifting the pH to more acidic values (Wigfield *et al*, 1986) and increases its excretion through urine. The synthesis of this enzyme is also increased. The toxicity is also expressed through abnormal red cells with inadequate haemoglobin (de Bruin, 1971), decreased haemoglobin levels, hematocrits and electrophoretic mobility of RBCs (Terayama 1986).

Lead toxicity to the kidneys is less dramatic than lead induced anaemia and neurological effects but prolonged treatment scars and shrinks the kidneys in rats. The kidney is considered as a target organ

for effects of inorganic lead. Kidney accumulates lead. Influence on kidney function occurs by bound-lead levels (Ramirez-Cervantes *et al,* 1978; Lillis *et al,* 1979; Skerfving, 1987). Short term and intense exposure to rat causes functional break down. Kidney malfunctioning results into structural damage to mitochondria and the proximal tubules at a blood concentration of lead as 150 μg / 100 ml. It also results in loss of amino acids, glucose and phosphates in urine. Reduction in mitochondria, reduced cytochrome content resulting in respiratory abnormalities (Rhyne and Goyer 1968; Cardona *et al,* 1971). In recent years reduction in serum levels of 1, 25-di-OH-cholecalciferol (the major active form of vitamin D (Mahaffey *et al,* 1982). Meyer *et al* (1984) observed the excretion of tubular enzyme N-acetyl-β-glucosaminidase.

Lead toxicity is mainly the result of the concentration of diffusible lead in the soft tissues, the organism detoxifies it by converting it into a non-diffusible form to protect the cellular organelles and metabolites. The large intranuclear inclusion bodies that are formed in the proximal tubular cells of the kidneys have the function of binding lead into a non-diffusible form (Goyer *et al,* 1970; Oskarson and Flower, 1985). This protein is evidently synthesised in the cytoplasm and moves into the nucleus, during the course of transtubular flow a portion of the lead enters the nucleus where it forms a lead–protein complex. The

induction or sequestration of a unique protein is noted in lead- protein complex (Shelton, 1982). Early appearance of intranuclear inclusions in the segments of renal proximal tubules of rats following ingestion of lead (Murakami *et al,* 1983; Stiller and Friedrich, *et al,* 1983) was noted. Lead forms complexes with the phosphate group of nucleotides, nucleic acids, and catalyses a nonenzymatic hydrolysis of nucleoside triphosphates particularly ATP (Rosenthal *et al,* 1966). Even radiolabelled phosphate uptake is reduced in RNA (Sroczyn *et al,* 1967).

In most recent review the gene influencing the intoxication have been considered in details of which are being defined (Ava *et al,* 2000). The most well known gene in this regard is the ALAD gene, which has been highly studied in human populations and animal models. This genetic polymorphism appears to have strong influence on lead absorption and bioaccumulation but its role in neurotoxicity is still unclear. The role of the VDR polymorphism and the HFE gene are even less clear. Both of them are involved in transport and bioaccumulation of other divalent ions.

Effects of lead on liver :

Oral/ I. P. Administration of 150-mg lead acetate/kg body wt resulted in the marked reduction in the number of lysosomes (Schroeer

et al, 1982). $Pb(NO_3)_2$ treatment for 24 hours in rats (Scoppa *et al*, 1974) showed increased liver weight and decreased protein concentration, with inhibition of oxidative phosphorylation and release of lysosomal enzymes. In the same experiment cytochrome P-450 synthesis was decreased but its degradation was increased. Lead induced (Tondon *et al*, 1993) hepatic metallothionine, when it was with iron deficiency enhanced the normal synthesis of renal and intestinal metallothionine lowered under the influence of reduced body iron status. The antibody raised against lead induced thionine was cross reactive with the antibody raised against Zn++ induced thionine (Ikebuchi *et al*, 1986). Lead administration (Satiji and Vij, 1995) resulted in depression of D–aminolevulinic acid dehydratase and uroporpyrinogen I synthetase activity in liver while the metabolism of pentobarbitone was also reduced.

In cultured hepatocytes the studies showed cell function mediated by calcium ions as a second messenger is affected by Pb^{++} intoxication (Pounds *et al* 1982). *In vitro* metabolisms of drugs aminopyrine, aniline and p–nitroionisole by liver homogenates (Satiji and Vij, 1995). In liver homogenate, large granule fraction showed rapid release of latent acid phosphatase.

Effects of lead on kidney:

Lead administration (i.v.) to rat indicated that Pb^{++} uptake might be influenced by energy and metabolism dependent mechanisms (Craan *et al* 1984). Lead acetate (0.05, 0.15, 0.3 mM Pb/kg) increased urinary V-glutamyl transferase in males at highest dose, which is indicative of mild tubular and glomerular disturbances (Huguet *et al* 1982). One per cent lead acetate through drinking water *ad libitum* (for 3 weeks and 10 weeks resulted in increased kidney weights due to increase in fluid content of cells or cloudy swelling Robert and Goyer, 1968). It showed positive correlation with the degree of amino-aciduria suggesting a transport defect for water and cations as well as amino acids. Electron microscopic observations of proximal tubules showed intranuclear inclusions, mitochondrial swelling and mitochondrial lesions (after 10 weeks) and clumped mitochondrial matrix granules. Long term administration of lead through drinking water (Duvenkamp, 1984) resulted in the intranuclear inclusions which were PAS +ve and were composed of filaments and granules lacking any kind of surrounding membrane Increase in nuclear volume was noted well before the Pb^{++} deposition initiates

I. $Pb(NO_3)_2$ administered (i.v.) showed altered serum lipoproteins by stimulation of hexose monophosphate shunt (Pani *et al*, 1984). Lead

acetate treatment to female Egyptian goats increased serum alkaline phosphatase and bilirubin (Gouda *et al* 1985). The increase in the serum GOT was higher and for a longer period than the increase of GPT but serum total lipids and cholesterol were lowered in the first 3 months. Lead (20 mg/kg body weight intragastricaly) given weekly for 7 weeks to male buffalo rats resulted in lowered total and HDL cholesterol levels coupled with increase in free cholesterol and hypertriglyceridemia (Skoczynska and Smolik, 1994). In rats oral administration increased SGOT, SGPT levels and further increased on fasting (Hyashi *et al*, 1993).

II. Effects of lead on lysosomes:

Lysosomes are important compartment of the cell, which are involved in the degradation of intracellular and extracellular materials. They process the incoming materials into metabololically acceptable forms to cellular compartments and recycle the building blocks of the cells. The lysosomal acid hydrolysing enzymes are involved in degradation of numerous compounds incoming through endocytosis by heterophagy or those ware out in cell through autophagy. The lysosomal membranes act as compartmental barrier and trasporter of the degraded material. This role of lysosomes brings numerous incoming materials through lysosomes. The compounds may affect lysosomes in two ways.

1). Indigestable material may reach lysosomes and accumulate there causing enlargement and interference in cellular function.

2). Compounds may cross the lysosomal membrane and be trapped as a result of the difference in pH between lysosomes and cellular compartment.

The pH of the interior of lysosomes is below 5.00 whereas the cytosol is essentially neutral with pH 7.20. As a result weak bases may be trapped in lysosomes as they concentrate water enters in lysosomes resulting in marked distension in lysosomes which may result in impairment of lysosomal degradation activities. This is reflected through activities of lysosomal enzymes. Some of the compounds may be trapped in membrane due to pH barrier this may also alter the transport properties of the lysosomes finally affecting the activities of lysosomal enzymes. These are main recycling pathways of the cell and on its non-functioning numerous alterations may be leading to pathological conditions. This metabolism of lysosomes is true in all types of cells (Albert *et al*, 1992).

In lead induced toxicological effects, studies on lysosomes are restricted. The subcellular distribution of lead within the kidneys and other tissues was determined by injecting Pb^{203} intraperitonealy into rats

(Barltrop *et. al*, 1971). Most of the lead was found in the lysosomes. Reductions in number of lysosomes and vacuolar dilation of the intercellular space were noted on lead acetate administration (Schroer *et. al* 1982). Lead and zinc activated acid phosphatase activity in liver homogenates' large granular fractions (Popova and popov, 1998). The transplacental effect of lead compounds on newborn rats showed increased inorganic pyrophosphatase activity in kidney (Tsafaris and Alexaki, 1992). In the lead treated rats dose dependent release of lysosomal enzymes was observed in post mitochondrial fraction (Scopa *et al*, 1974). Marked reduction in number of lysosomes was also noted ultrastructurally (Schroeer *et al*, 1982) on Pb^{++} administration (oral/ i.p.).

Hook *et al* (1979) have reviewed the nephrotoxicity under which they have considered heavy metal toxicity also which included toxicity related to lysosomes. Following low dose exposure significant quantity of metal is found in renal tissue even prior to the development of physiological sign of toxicity. Renal lysosomal sequestration may be a means by which heavy metals are prevented access to essential macromolecules. This phenomenon is stimulated by chronic low level exposure to metals and may result from one or more of the following: lysosomal endocytosis of a metal protein complex, autophagy of intoxicated organelle such as mitochondria and/or binding of the metal

to acidic lipoproteins within the lysosomes. Metabolism for accumulation is specific and metal specific possibly the detoxification of the metal may inactivate the enzyme systems of ER.

Effects of lead on Histology and ultrastructure:

Lead intoxicated Egyptian goats showed significant histopathological alterations in liver (Gouda *et al*, 1985). Liver pathology is hardly studied in detail but some ultrastructural observations were noted (Schroeer *et al*, 1982) which reported the alterations in mitochondria, peroxisomes and lysosomes which revealed reduction in number of lysosomes. Rats fed on diet containing lead acetate for three weeks showed two distinct histological features- i) intranuclear inclusions and ii) mitochondrial swelling. These changes were confined to cells of proximal tubules. The number of inclusions increased with the increased ingestion of lead. Maximum mitochondrial lesions appeared on 10 weeks (Robert and Gover, 1968) of administration.

4] Animal selection for *in vivo* toxicity testing:

Numerous reviews and articles are routinely published and are reviewed in books, where rat are used for toxicological studies (Hodgson *et al*, 1979; Venugopal and Luckey, 1978; Bryan *et al*, 1995). Breeding of Albino rats and maintaining them in animal houses is well established.

Farris and Griffith (1971) and Melby and Altman (1977) have reviewed the literature.

The albino rats required for experimental work were used from the departmental animal house, where they were bred and provided rat mesh and water *ad libitum*. The other ideal animal house conditions were maintained. Albino rats were chosen for *in vivo* testing as they are bred easily. Being small they can be handled easily. Their maintenance is financially affordable, besides their breeding cycle being small sets of animals of same sex, age and weights can be available to run the comparative study. For present work, male albino rats were used

5) Selection of organs for studies:

The reviews on lead toxicity indicate that the toxicity is more related to the levels of diffusible lead and to the lead content of soft tissues such as liver, kidneys and brain than to the lead content of total body. Lead absorbed from the blood plasma is distributed to different organs. Among the soft tissues the liver and the kidney attain the highest concentrations (Barry, 1975; Brune *et al*, 1980; Skerfving, *et al*, 1983 Skerfving, 1988). In animal experiments (Aungust *et al*, 1981; Hietanen *et al*, 1980; Skerfving, 1988), there is no constant relationship between blood and soft tissues. Thus accumulation in liver and kidney is higher

than in blood and need to study the toxicity in these organs becomes essential. In oral route of entry of lead, liver becomes more important due to its anatomical position, since main concentration of lead often is to reach liver primarily. The reviews have also indicated that studies on lead poisoning have shown the kidney as the main organ that shows toxic effects. Chronic exposures in high doses of lead produce marked increase in the incidences of renal adenocarcinoma in rodents (IARC monograph 1980; US EPA-Air quality criteria for lead). In recent work the role of lead binding proteins in renal cancer is reviewed (Fowler *et al*, 1994).

Thus on the basis of the survey of the literature it was decided to select liver and kidneys for the studies. At this stage to have a brief review on liver and kidney will provide us a rear view of these organs.

Liver :

Liver is a continuous mass of parrenchymal cells tunneled by vessels through which the venous blood flows on its way from the gut to the heart. The cellular walls/muralium simplexes are called as liver plates/laminae hepatis/lacunae hepatitis. The specialised capillaries of the liver sinusoids are suspended in lacunae and form an

uninterrupted three dimensional network content in the vast labyrinth of lacunae.

Under normal blood pressure conditions one can notice a radial arrangement of sinusoids and liver plates around the smallest roots of hepatic veins, which are called as central veins. The vaguely defined territory around, surrounding such central veins is called as hepatic lobule.

A large basketlike arterial plexus surrounds each portal vein branch. The blood flow is controlled by specific mechanisms located at strategic points, Kupffer cell carry out these functions at any place in sinusoids. In rat and mouse the sinusoids enter all hepatic veins even into the largest. The bile canaliculus is formed by two groves in the contact surfaces of two adjoining liver cells, which fit together so that a cylindrical lumen arises. Bile canaliculae do not have a wall of their own, but they are lined by a condensation of cellular exoplasm. They form polygonal meshes throughout the muralium hepatic cord. All of them open in small ductules, which join to form common bile duct in rats. The bile ducts branches along as it runs with branches of portal vein.

The trafficking cells of blood that visit the hepatic muralium are usually called Kupffer cells their presence is also an important feature of the mammalian liver (Roullier, 1969).

Liver plays a fundamental role in the metabolism of the carbohydrate, fat and amino acids and in this tissue the metabolic pathways for three compounds are closely integrated and regulated (Newsholme and Start, 1973).

The number of metabolic reactions involved in carbohydrate metabolism within the liver is very large and is beyond the scope of the present project. The hepatic portal carries 70 % of the blood reaching the liver. The remainder supplied via hepatic artery. The hepatic portal vein drains most of the absorptive that are of the gut, so that apart from triglycerides, which absorbed via the lymphatic system almost all compounds absorbed from the gut pass through the liver. Therefore the liver is favourably situated to function as the initial regulator of the blood levels of many compounds that enter the body through the gut. In higher animals the ingested carbohydrates enters the blood as glucose. Which is produced from digestive degradation of starch, glycogen *etc.* The liver possesses the facility for removing large quantities of glucose from the portal vein, when the concentration exceeds the normal. This depends upon the presence of the enzyme

glucokinase, which catalyses the phosphorylation of glucose to glucose–6-phosphate. This hexose monophosphate leads to four paths (1) Back to glucose (2) glycogen (3) pentose phosphate (4) pyruvate through glycolysis. Fructose feeding increases the rate of hepatic lipogenesis and elevated plasma triglyceride levels in rat (Konacker and Loweinstein, 1965), but without any effect on adipose tissue (Naismith, 1971). Control of glucose uptake and release by the liver is well known. Similarly control of glycolysis and gluconeogenesis in liver and kidney cortex is also well studied. Liver is able to carry out most of the known reactions involved in fatty acid metabolism. Such reactions constitute the pathways of fatty acid synthesis from acetyl–CoA, esterification of fatty acids and storage of triglycerides, secretion of triglycerides, secretion of triglycerides into blood in the form of Low Density Lipoproteins (LDL) synthesis of phospholipids and cholesterol esters, lipolysis of triglycerides, oxidation of fatty acids and the formation of ketone bodies, Liver also possesses the capacity to increase the chain length and or desaturate certain fatty acids (Newsholm and and Start, 1973).

The main features of lead toxicity are accumulation of lead in various soft parts of body and in long term toxicity deposition of lead even in bones was noted. The symptoms that are observed in human

survey dominate neuropathy, encephalopathy, nepheropathy and anemia (Valle and Ulmer, 1972; Venugopal and Luckey, 1978). Other transitions elements are capable of causing liver damage (Powell, 1985). The metals induce binding proteins called metallothionines, which sequester the metals resulting in their inactivation and in the case of the liver exit and in case of lead its accumulation preferably in nucleus, mitochondria and lysosomes (Valle and Ulmer, 1972; Venugopal and Luckey, 1978)

Kidney :

Similar to the liver, kidney is the important metabolic organ, which is involved in important reactions of excretion and the metabolic wastes formed out of carbohydrates, lipid and protein metabolism especially urea and other wastes, which are synthesised in liver and excreted through kidneys. Therefore kidney and liver are important organs in total functioning of the body.

Kidney is divided into cortex and medulla anatomically. Cortex is major part and receives most of the blood supply and hence the nutrients. The functional anatomical unit of the kidney is nepheron. All nepherons have their vascular structure, the glomeruli in kidney cortex along with proximal tubules. The remaining tubular part extends in

medulla and deep cortex. The details of structure and function of kidney are reviewed by Seldin and Giebisch (1985) and Brenner and Rector (1986).

Among the infinite number of substances occurring in nature or synthesised in chemical laboratories many are known to induce kidney changes. This is due to the unique functional role of the kidney, which is not merely perfused by low concentrations of the chemicals but acts as their principal excretory organs. In the course of this process the chemical agents are separated from their protective protein binding, concentrated to often extremely high levels and brought in direct contact with the parenchymal kidney cells while passing through the tubular system and while being secreted or reabsorbed (Zbinden, 1969). Kidney is also involved in erythropoietin production (Naets, 1969) and there fore any stress or damage to kidney may be hazardous to the animal, as erythropoietin regulates hemopoiesis.

Lead is probably the most abundant nephrotoxic metal. Lead induced nepherotoxicity is characterised morphologically by the presence of intranuclear bodies, karyomegaly, cytomegaly and alterations in mitochondria these are coupled with impairing of functioning (Goyer, 1982; Lock, 1995).

Since in lead toxicity kidney is a target organ and liver is also damaged by lead; it was decided to study both of them in present project

6) Plan of toxicity testing of Nag bhasma.

The Nag bhasma is a therapeutic drug and is not toxic substance. It has been used and is being used to treat the patients. For this reason the classical methods used in toxicity testing to determine the doses or other related principles of toxicity testing are not applicable. In recent years physiologically based models are developed (Hon-Wing Lung, 1995). The model for therapeutic drugs was first introduced by Himmelstein and Lutz (1979) and it was further developed for environmental chemicals (Menzel, 1987). Considering the probable action/metabolism of drug the parameters and doses may be designed and this makes the studies more meaningful. The same principle is used in the toxicity testing.

In the present project toxicity of Nag bhasma was tested since it is lead originated and lead toxicity is doubted, may be primarily or may be as the consequences of metabolism/s by users or marketing persons. For above discussed reasons the following approach was adapted in the present work.

- To ensure the purity of Nag bhasma preparations, it was prepared in the laboratory as per Ayurveda Sarsangrah (1964).

- Since Nag bhasma is given orally to the patients; in *in vivo* animal testing the drug was fed orally.

- Three doses of Nag bhasma were selected from the therapeutical doses used in human beings. To the patients about 60 mg maximum dose is used depending upon the conditions of the patient. The highest dose of 90 mg was used in addition to two more doses of 30 mg and 60 mg. In lead toxicity concentration of lead also decides the mode of toxicity hence low doses are used.

- Selection of different intervals for the three doses used was based on the Ayurvedic principles again. For the primary treatment to check the response the medicine it is given for 7 days or saptak. In present project three intervals for each dose treatment were selected for studies. One week, two weeks and three weeks for each of the dose were the schedules used for intervals.

- In lead toxicity kinetic models based on studies of bone and cartilage on the basis of human lead toxicity (Lansdown, 1995). Lansdown has stated that in routine toxicity studies; cartilage and bone tend to be less frequently damaged as compared to liver,

kidney and endocrine organs. For this reasons to test the toxicity in present project, liver and kidneys were used as the main organs of testing. Role of liver in normal secretory and excretory functions is well reviewed by Talwar (1980) and Guyton (1992). Liver is also the main xenobiotic metabolising organ (Timbrell, 1995). The products of xenobiotic metabolism are excreted through kidney and hence kidney was also used for toxicity testing. The structure and function of kidney is reviewed by Talwar (1980) and Guyton (1992).

- Since the lead toxicity was to be tested, the control animals were used for the comparison of damage in liver and kidney. $Pb(No3)_2$ was used as the source of lead. The treatment of $Pb(NO_3)_2$ was given orally, since the Nag bhasma treatment was also given orally. The dose selected was 20 mg, and given once daily for 7, 14 and 21 days. On 21 days (largest interval used) caused histologicaly detectable foggy necrosis in the $2/3^{rd}$ part of the kidney or induced nephritis. This was considered as the lowest oral toxic dose to induce lead toxicity in male rats.

- Kidney damage was used as the toxicity indicator since most heavy metals are potent nephrotoxins (Hook *et al,* 1979). 1% lead acetate through drinking water *ad libitum* (Robert and Goyer, 1968) for 3 weeks and 10 weeks resulted in increased kidney weights due to

increase in fluid content of cells or cloudy swelling. It showed positive correlation with the degree of aminoaciduria suggesting a transport defect for water, cations as well as amino acids. In the three intervals studied, foggy necrosis appeared on treatment of 21 doses. The early two intervals remain the intermittent stages of foggy necrosis development. The alterations can be compared with the toxicity even with the smallest of the effective concentration of lead. Therefore above stated doses and intervals were used.

6) Selection of different parameters for toxicity studies :

A. Diagnostic tests/Serological parameters

i) *Haemoglobin content* :-

a) Whole blood was used for the haemoglobin bioassays.

b) Cytological demonstration of haemoglobin in RBCs.

ii) *Liver function tests* - Liver being the main drug metabolizing organ, its functional tests are important to judge the physiological stress. Modern physicians use these tests as diagnostic tests and hence they are included in the studies.

iii) *Kidney function tests* - Kidney being another important organ that is involved in the excretion of blood borne waste which also includes metabolites originated as the products of biotransformation of drugs/xenobiotics, therefore to adjudge the physiological status of kidney these tests were included. They are of diagnostic importance to physicians.

iv) Pb^{++} demonstration in liver and kidney :-

Venu Gopalan and Lucky (1976); Rabinowitz (1998) reviewed Pb^{++} toxicity. The observations had shown accumulation of Pb^{++} in prolonged intake. For this reason Pb^{++} was demonstrated by histochemical methods from Liver and kidney

B. HISTOLOGY

The histology of the liver and kidney was studied as the conclusively deciding parameter of drug effects on the organs. Liver histology and kidney histology are well known and reviewed by (Roullier, 1963) and Roullier and Muller (1969). Since histological structures reflect the functioning, the alterations there in reflect the effects of the drug metabolism. The earlier works from the department have used histology as parameter not only to test the various claims of the drugs,

but also for the toxicological studies (Devarshi *et al.* 1986, Kanase *et al* 1992, 1994, 1995, 1997, 1998, Patil *et al*, 1989, 1992, $1993_{a,b}$, 1996, 1998, 1999).

C. Biochemical parameters :

ii) Lysosomal enzyme activities :- In the biochemical parameters, lysosomal enzymes *viz.* Acid phosphatase, β-glucuronidase and Cathepsin D, from liver and kidney were studied. Lysosomes are the cellular organelles that are primarily involved in cellular defence, in metabolisms of materials entering the cells through endocytosis, in the autophagy to destruct the warned out cell organelles and recycle the building blocks. They accumulate the materials that are resistant to the array of hydrolytic enzymes or the products of their digestion (Alberts *et al.* 1992). Thus, they are one of the cell organelles that are involved in numerous metabolic activities and hence alterations there in are the indications of altered metabolisms. Their role is well studied in tissue injuries and various pathological conditions (Holtzaman, 1975). Their status may reflect the effects of drugs at cellular level. For these reasons lysosomal enzymes were studied in bioassay.

Selection of lysosomal enzymes:

1) Acid phosphates activities :- Alterations in acid phosphates activities are indicators of general phosphate metabolism related to lysosomal turn over.

2) β-Glucurnoidase:- The activities are involved in the glucuronide metabolism which is the part of the excretory metabolism of the liver.

2) Cathapsine D :- It is a lysosomal protease. The activities are involved in protein degradation and hence are indicators of lysosome dependent protein metabolism. The metals are moved with specific binding protein for metabolism, *e.g.* Lead induced hepatic metallothionein (Tondon *et al*, 1993) and may influence the protein metabolism.

The work carried out is presented in the thesis in different chapters.

I. - Introduction: It includes the reasons to take the problem, selection of doses, intervals of treatment and other features of the problem, reviews of relevant work on lead toxicity and metabolism, liver, kidney *etc* .It gives brief idea about the work.

II. - Material and Methods: Under this chapter all the details of experimental protocol are given. The parameters and techniques used are given in detail.

III. - Haemoglobin

IV. - Diagnostic tests : Serological Studies - The results of liver and kidney function tests are presented here and discussed.

V. – Histology : The alterations in histological picture of the liver and kidney are presented along with the probable discussions.

VI. - Lysossomal enzymes: The enzyme activities of acid phosphatase, β-glucuronidase, Cathepsin D, are presented with the probable discussion.

VII. - General discussion: All the results obtained are considered together to conclude the results. Which is followed by bibliography.