CHAPTER - I

INTRODUCTION

INTRODUCTION

The liver can be defined as a continuous mass of par enchymal cells tunneled by vessels through which venous blood flows on its way from the gut to the heart. In mammalian liver these cellular walls are called as muralium simplex. These walls of cells are referred to as liver plates, laminae hepatis or lacunae hepatis. The specialized capillaries of the liver called sinusoids are suspended in the lacunae. They form an uniterrupted, three dimensional network contained 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.

Each branch of the portal vein is within its portal canal, accompanied by two or more aretrial branches which anastomose with each other so that a large meshed, basketlike arterial plexus surrounds each portal vein branch. The flow of blood through each territory of hepatic parenchyma, no matter how small, is controlled by specific mechanisms located at strategic points. Bulging Kupffer cells can control and steer the flow at any place in the network of sinusoids. In rat and mouse the sinusoids enter into all hepatic veins even into the largest.

The bile canaliculus is formed by two grooves in the contact surfaces of two adjoining liver cells which fit together so that a cylindrical lumen arises. Bile canaliculi do not have a wall of their own but they are lined by a condensation of cellular exoplasm. They form a 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 along with the branches of portal vein.

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

Liver plays a fundamental role in the metabolism of carbohydrate, fat and amino acids and in this tissue the metabolic pathways for these 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 it is unable to deal them here.

The hepatic portal vein carries about 70 % of the blood reaching the liver. The remainder is supplied via the hepatic artery. The hepatic portal vein drains most of the absorptive area of the gut so that apart from triglyceride, which is absorbed via

the lymphatic system almost all compounds that are 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 carbohydrate 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 is lead 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 (Kornacker and Lowenstein, 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 triglyceride, secretion of triglyceride into the blood in the form of very low density lipoproteins, 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 (Newsholme and Start, 1973).

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 carbohydrate, lipid and protein metabolism especially urea and other wastes, which are synthesized in liver and excreted through kidneys. Therefore kidney is also important organ in total liver functioning of the body.

Among the infinite number of substances occurring in nature or synthesized 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 excretary 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).

Thus whatever drug liver is metabolizing and passing it over to systemic circulation it may be excreted through kidney and hence kidney may be exposed to that drug indirectly and may be leading to renal stress or damage. Therefore kidney is also

important organ when we are considering Liver as the prime organ of study. Kidney is also involved in erythropoietin production (Naets, 1969) and therefore any stress or damage to kidney may be hazardous to the animal , as erythropoietin regulates hemopoiesis.

Considering all these reasons it seems that liver should be studied in context of kidney due to the interlinked regulatory metabolisms and their vital part in secretory and excretory functions.

Thus metabolisms of the three main constituents i.e. carbohydrates, lipids and proteins are basically carried out through liver and are regulated through adipose tissue and the waste products through the metabolisms are excreted through kidneys and thus the two organs are interrelated metabolically and are vital for normal animal physiology. Entry of any other type of material in the body may interfere the normal maintenance of carbohydrate, protein, lipid metabolism involving their effects on liver, kidney and adipose tissue or vice versa and may leading to the hepatic toxicity.

Hepatic toxicity :

There are many instances of hepatic toxicity reported even before century. It can be defined as the effects of any agent on the liver which results in a deviation from the normal function

and morphology of this organ (Mehendale, 1985). The attempts to understand the toxicology of yellow phosphorus and the haloalkane anaesthetic chloroform resulted in an appreciation of the hepatotoxic potential of chemicals (Zimmerman, 1978). Chemically inflicted hepatic toxicity has been studied remarkably. The progress in these studies have developed the understanding of the mechanisms governing many toxicological manifestations induced by toxic chemicals. In recent years it is a necessity to classify the hepatic toxicity. One such categorization is based on the type of toxic hepatic reponses (Mehendale, 1985).

Thus chemicals may lead to fatty changes, necrosis, cirrhosis or carcinoma. These toxicities are accompanied by the changes in physiological or biochemical functions of the liver. Thus the secretion of bile may be increased or decreased
Similarly intermediary metabolism of carbohydrates, lipids and protein synthesis and turnover may be altered as a consequence of many toxicological phenomena. Associated with these subcellular changes, morphological and functional changes may also be seen at the cellular level.

The great susceptibility of the liver to injury by chemical agents appears to be a consequence of the anatomical position of this organ and the central role it plays in the metabolism and disposition of foreign chemicals. Liver contains by far the highest

level of enzymatic systems capable of biotransforming foreign chemicals (Hathway et al. 1970, 1971; Krishna Murthy, 1985). Many chemicals are eliminated from the liver via the biliary route. Bile acids which play a very important role in the formation of bile fluid itself and in the solubilization of lipids in the intestine are conserved by reabsorption and enterohepatic recirculation. Foreign chemicals excreted by biliary route often enter into enterohepatic recirculation cycle thus repeatedly entering in the liver tissue. Depending upon the foreign chemical, the parent compound or its biotransformation products may accumulate in the liver.

Many chemicals are biotransformed further beyond the activated intermediates to less reactive polar metabolites and may either be eliminated by bile or by the kidney after entering the systemic circulation. It is apparent that the central role played by the liver in the removal of chemicals from the portal circulation and subsequent metabolism and disposition makes this organ especially susceptible to first and often persistent attack by these offending chemicals, culminating in toxic injury.

Recent developments have established (Gillette, 1978; Plaa, 1980) that hepatotoxicity may be inflicted by thousands of synthetic chemicals, drugs and naturally occurring chemicals such as bacterial fungal, plant and animal toxicants some toxicological responses of the liver may be exacerbated by combinations of these chemicals.

It is yet to be studied the mechanisms of toxicity governing these toxicological manifestations in an attempt to protect the host from the deleterious toxic effects of these chemicals.

The Varied Categories of Hepatic Toxicity :

The hepatic toxicity may be grouped based upon the circumstances of exposure (Zimmerman, 1978).

A) i) - Occupational Exposure

a) Routine exposure

b) Accidental exposure

Or they may be classified on the basis of the property of the toxin or the host characteristics (Zimmerman, 1978).

B) i) - The toxicity due to toxic property of drug or unusual vulnerability of the host.

a) Intrinsic hepatotoxins (Affect wide variety of species).

I) Direct acts directly e.g. CCl_4

II) Indirect - acts on particular metabolic pathway.

b) Hepatotoxins (idiosyncratics) (Action depends on unusual susceptibility of the host).

However this classification is over simplification since the total resulting hepatotoxicity depends upon many number of factors

that relate to the types of toxic chemicals, exposure situation, condition of the host, environment at the time of exposure etc. (Zimmerman, 1973).

The results of many hepatotoxins have shown a variety of morphological and biochemical lesions in liver by chemicals or biological agents.

Thus the hepatic toxicity can be acute, subchronic chronic and cirrhotic (Curtis et al, 1979; Mehendale, 1985).

a) Acute toxicity - May be used to indicate exposure to single dose but the word is often used to indicate the severity of hepatotoxicity. Acute toxicity may result in necrosis of the liver, continued exposure leading to cell death and withdrawal leading to a partial or complete recovery.

b) Subchronic toxicity - Repeated and prolonged exposure results in subchronic expression of toxiicity such as necrosis.

c) Chronic toxicity - This term indicates either repeated exposure over an extended period of time or the presence of a hepatotoxic response over an extended period of time.

d) Cirrhotic ~~toxicity~~ - Continuous repeated exposure may lead to cirrhosis.

The carcinogenicity may result from any of the above situation.

Subcellular sites of Liver toxicity :

In recent years number of toxins have been studied for their toxic effects on liver using some biochemical parameters as well as ultrastructure of the liver (Jones and Mills, 1973; Jones and Schmuckler, 1977; Slater, 1978; Dianzani, 1979; Farber and Fisher, 1980; Mehendale, 1985). They can be concluded as

i) Hepatocellular permeability may be altered by many toxic chemicals and represents a toxicant induced purterbation of the membrane functions.

ii) Elevated blood levels of many proteins and other endogenous substances has been associated with the changes in the membrane permeability of hepatocyte.

iii) Necrosis leads to high levels of certain enzymes in the blood released from the damaged liver. Clinical analysis shows elevated levels of serum transaminase which may be increased to 10 to 100 fold depending upon the damage.

iv) Many other enzymes are released from the hepatocytes as a result of a variety of hepatocellular injury. These enzymes include alkaline and acid phosphatases, lactic dehydrogenases, isocitrate dehydrogenase, sorbitol dehydrogenase and many others.

v) In many instances of hepatocellular necrosis induced by a variety of toxic chemicals and in the cholestatic injury resembling extrahepatic obstructive jaundice, many serum enzymes are indicative of the degree of the damage in such cases.

vi) In CCl_4 induced hepatocellular necrosis, accumulation of Ca^{++} has also been demonstrated indicating a change in cellular permeability.

Subcellular Changes :

i) In response to many toxic chemicals a conspicuous increase of smooth endoplasmic reticulum (SER) has been observed.

ii) The proliferation of SER on exposure to variety of inducing agents appears to be an adaptive and potentially useful phenomenon.

iii) Thus prolonged exposure to some inducing agents may lead to an ultimately decreased metabolic activity of SER despite the persistent increase in amount of SER.

iv) SER proliferation is accompanied by number of chemical and enzymatic changes indicating the adaptive nature of this change. Since SER contains many of the important enzymes involved in biotransformation.

These observations are the expressions of hepatic injury as well as the susceptibility of the liver to the subsequent challenges by other offending chemicals.

v) The observation of hyperplastic, hypoactive SER is perhaps an early manifestation of toxicity.

vi) Many chemicals and biological materials show potency to inducee mixed function oxygenases of SER.

vii) Among the specific damage to SER causing chemicals CCl_4 is one of the important in the following list (thioacetamide, dimethylnitrosaminne, phosphorus, ethionine, dimethyl aminobenzene, pyrrolizidine alkaloids and galactosamine).

viii) In CCl_4 induced hepatic injury, the formation of trichlorocarbon (CCl_3^+) free radical is formed which leads to lipid peroxidation resulting into the destruction of ER.

This is measured by decreased incorporation of amino acids into proteins and by the destruction of cytochrome P-450.

ix) The mitochondrial changes include increase in size, alteration in the matrix density, loss of cristae, decrease in number. Mitochondrial membrane becomes more permeable to Ca^{++}, which accumulates to form large granules in the matrix.

But the specificity of such changes in mitochondria is doubled since similar changes are also observed in anoxia conditions.

x) Disintegration of lysosomes and leakage of hydrolytic enzymes into the cell have been noted following experimental hepatic injury.

xi) Lysosomes are scavangers also providing the means of ridding the cell debris.

xii) Cellular autophagy results in appearance of many secondary lysosomes in sublethal hepatic toxicities.

xiii) Damage to lysosomal structure has been recorded after the administration of CCl_4.

xiv) In nuclei, progresive accumulation of intense interchromatin and peripheral heterochromatic granules is the basic feature observed after the administration of many hepatotoxins.

Though the specific actions can't be predicated for any specific hepatotoxinns still the review of the description provides the morphological, cytochemical correlation with the biochemical and physiological parameters of toxicity and the recovery also.

Experimental Models of hepatotoxicity :

Dianzani (1979) has reviewed some of the models.

A) Liposes :

i) Accumulation of lipids in the liver or fatty infiltration , fatty degeneration and liposis. The lipids predominantly include triglycerides and fatty acids.

ii) CCl_4 also induces fatty liver in addition to many antibiotics, corticosteroides, ethanol etc.

B. Necrotic Reactions.

i) Hepatic necrosis or cell degeneration leading to death may be induced by many chemicals.

ii) Along with CCl_4 many amines, alkaloids, toxins are able to induce necrosis in liver.

Hepatic necrosis may be

(a) zonal, (b) massive or diffuse.

a) Zonal necrosis -

It may be incentral, midzone or peripheral area of the lobule dependinng upon the necrotic agent, CCl_4 induce zonal necrosis which is also referred as centrolobular necrosis.

b) Massive or diffuse -

Depending upon the toxic agent the necrotic areas may appear diffused through liver structure e.g. necrosis induced by galactosamine.

C. Hepatobiliary Dysfunction.

i) In normal conditions many toxic substances are metabolized by liver by induction of microsomal enzyme system. These chemicals are eliminated via the bile

or by returning them to systemic circulation .

ii) if the hepatobiliary function is altered it leads to the consequences of the toxic injury to the liver. But this phenomenon can be used to assess the impact of toxin on the hepatobiliary function (i.e. excretory and secretary functions of liver).

iii) Many factors individually or in any other combination may affect biliary excretion.
(Klaassen, 1978; Levine, 1978; Mehendale, 1980).

a) Hepatic blood flow
b) Binding to plasma proteins
c) Permeability of sinusoidal membrane
d) Interaction with cytoplasmic binding proteins.
e) The rate and extent of biotransformation.
f) Permeability of the bile canalicular membranes.
g) Bile flow and transport of the excreted substances in the biliary tree.

D. Cirrhosis

i) It is chronic disease condition presenting morphological alterations of the lobular structure characterized by destruction and regeneration of parenchymal cells and increased tissue (Zimmerman, 1978). Septal collagen in liver is the major morphological change that is occurring in liver.

ii) Chronic administration of CCl_4 also leads to cirrhosis.

iii) Many carcinogens also induce cirrhosis.

Selection of liver as a target organ for study.

From the above review describing the normal hepatic morphology, physiology and hepatic toxicology one can get the idea that liver is a vital organ and needs to be protected in this era of pollution when all the society is constantly exposed to many known and unknown pollutants of air, water and soil.

Since all the chemicals have their metabolic path through liver it is destined to be affected at one stage or the other through the life and therefore, the liver was chosen as a target organ for studies. Though there are many hepatotoxins studied including pesticides, synthetic drugs and/alkaloids, still there is hardly any drug available in the modern therapy against many types of hepatic injuries.

But in most of the Asian countries many herbal, ethnic or traditional medicines are used for the treatment of jaundice in traditional way. In India the most ancient therapy Ayurveda refers to many herbs and composite drugs bhasmas which can be used to cure jaundice. Even one can prepare composite drugs from the properties that are described of various herbs; herbal preparations and bhasmas against any pathological conditions (Vaidya, 1981).

Since most of the Ayurvedic knowledge is based on ancient concepts of physiology, pathology and pharmacology and are interpreted as per their diagnostic methods and their nomenclatures. Their use and mechanism of actions are not easily understood to modern physicians and inspite of their traditional claims of the drugs modern physicians avoid to use these valuable drugs. Therefore, it is very necessary to test the claims and actions of these chemicals by modern methods of pharmacological studies and drug testings so that the valuable drugs can be revitalized in modern medicine, so that integrated therapy can be developed for human welfare.

Selection of CCl_4 induced hepatic injury as a model.

CCl_4 induces hepatic lesions even with a single dose. It also induces acute, subchronic or chronic hepatic lesions with prolonged treatment. In addition CCl_4 is metabolized through mixed function enzyme system in ER.During the process it leads to produce the free radical CCl_3^+ leading to lipid peroxidation and damage to ER leading to necrotic changes in hepatocytes. In addition the various morphological features of liver along with many biochemical components are well studied in CCl_4 induced hepatic injury. Therefore the results of liver protection and curation can be evaluated with the comparison to the hepatotoxic alterations.

Selection of animal albino rat as an animal model :

Many reviews on the hepatotoxicity indicated that rat, rabbit, pig and mice are the laboratory animals used for hepatotoxic studies (Popper and Schaltner, 1959; Slater, 1972; Levine, 1978; Curtis et al,1979; Paa, 1980).

The rats can be well maintained in the laboratory. They are easily bred in our animal house and one can use them at the convenient time. They can be available with specific weight and age. The required number of animals for experiments at a time can be available; so that the environmental variations and its stress on experimental animals is avoided. Only male rats are used because these results of the experiments on females have to be considered with reference to estrous cycle and that would have given another type of mode to the project. This was not the aim in the dealing of the present project.

Therefore for the reasons given above male albino rat was chosen as the animal model for the present studies.

Selection of the organs for the study.

Since the aim of the project is to test the mandur bhasma for its potency to cure CCl_4 induced hepatotoxicity, the main organ of the studies is obviously liver. As discussed earlier in this chapter the three main metabolisms of the body i.e. carbohydrate

metabolism, fat metabolism and protein metabolism are regulated through the liver.

As discussed earlier kidney is also one of the main organ that is involved in main excretion of the body along with the gut. The chemicals those affect liver they interfere with the urea formation in liver and these chemicals ought to come to kidney through systematic circulation in original form or in biotransformed form; so that they can be excreted out. Thus the hepatotoxic chemical will primarily or secondarily may affect the kidney one of the vital organ of the excretion.

At the same time the other reason to select kidney along with liver for the present study is to check the side effects if any of the drug that is used as hepatocurative on kidney so that the curative potency of drug can be evaluated on its capacity to reorganize the altered metabolism of the animal. The results prepared such way may be helpful to the Ayurvedic practitioners as well as modern physicist to decide upon the duration and dietry control in the patients to avoid stress if any on liver and kidney during curative alterations.

Selection of the parameters.

The parameters that are selected for the present studies are - (i) Histology and (ii) Lipid Peroxidation.

i) Histology :

Since CCl_4 is known to induce morphologically identifiable centrolobular necrosis in hepatocytes; it is very opt to study the histology of liver to test the curative potency of the drug mandur bhasma. It is a very convincing parameter for the recovery of liver which will establish to show total or partial recovery of hepatocytes from CCl_4 induced centrolobular necrosis.

The normal histology of the liver is well studied as well as CCl_4 induced hepatic alterations are also well studied (Rouillier, 1969; Mehendale, 1985) and therefore it is very authentic parameter to study.

ii) Lipid Peroxidation :

The concept of lipid peroxidative damage was advanced by Slater (1978) as the principal mechanism of CCl_4 induced liver injury and has found ample experimental support (Recknagel and Glende, 1978). This theory holds that CCl_4 is homolytically cleaved by a Cytochrome P-450 monooxygenase system to produce CCl_3 free radical. In the aerobic environment, the CCl_3 free-radical enters an hydrogen abstraction reaction to form an organic free radical of the fatty acid and chloroform. The cytochrome P-450 system is encased in a phospholipid membrane rich in polyenoic fatty acids. In aerobic

environment organic fatty acid radical rearranges yielding organic peroxy and hydroperoxy radicals. These radicals destroy the cytochrome P-450 hemoprotein, thus compromising the mixed function oxygenase activity. The rapid decomposition of the ER and its function is a direct result of this lipid peroxidative process.

Therefore to study the lipid peroxidation in liver and kidney homogenates will directly reflect the hepatotoxicity due to CCl_4. It will also reflect the recovery of liver and kidney also.

Why to study hepatocurative effects of the drug ?

In existing environmental situations very few time we are aware of the fact that whether we are subjected to the hepatotoxic compounds accidentaly or occupationally. We can only realize it on the clinical diagnosis of the hepatitis. The drug treatment of any kind is started after the diagnosis of the hepatic injury.

Therefore the drug that is successful in hepatoprotection may not be successful in curing the hepatic injury since the damage is comparatively severe. Sometimes dose needed may differs. Duration of treatment may vary. Therefore to study the curative effects is clinical necessity for the proposed use of the drug and for that reason present study is undertaken.

Thus in present thesis mandur bhasma an Ayurvedic preparation which has shown hepatoprotective property (Devarshi

et al., 1986) is tested for its hepatocurative properties in male albino rats after inducing hepatic injury by CCl_4 treatment; using histological recovery as main parameter. The alterations in lipid peroxidation is another important parameter which is studied.

So far we have reviewed the necessary normal and necrotic morphology of liver as well as the possible biochemical and physiological alterations during the damage. It is very appropriate to review the attempts made by other workers to study CCl_4 induced hepatotoxicity, protection or curation by any drug or chemicals or herbal products.

Mehendale (1985) has reviewed the hepatic toxicity of various toxins. Since in the present work CCl_4 induced hepatotoxicity for the testing of curative effects of drugs is used it is appropriate to give changes in liver due to CCl_4 administration.

CCl_4 induces centrolobular necrosis in liver may be probably due to the presence of more SER in the centrolobular hepatocyte which is involved in metabolism of hepatotoxins. Specific damage to ER has also been noted due to CCl_4 administration (Slater, 1978).

Isolated rat liver mitochondria incubated under hypoxic conditions with succinate and ADP activated CCl_4 to a free radical trichloromethyl free radical (Aldo et al, 1978).

CCl_4 also induced inhibition of hepatocyte lipoprotein

secretion, functional impairment of Golgi apparatus in the early phases of injury (Poli et al. 1985).

Liver regeneration with the increased levels of plasma glutamate oxaloacetate transaminase and glutamate pyruvate transaminase but with no change in thymidylate synthetase and thymidine kinase. But these increased after regeneration (Rieko, 1985).

A time scheduled course is observed in serum and liver lipid alterations produced by CCl_4 in rats (Choudhari et al, 1985). The course indicates, liver cholesterol increased for 5 days and then decreased to day 10. Phospholipids decreased by 6 hrs and stayed low. Triglyceride increased markedly by 6 hr. and stayed high. Blood serum levels increased with time and stayed high to day 10. Same is true for glutamate pyruvate transaminase and glutamate oxaloacetate transaminase. But hepatic phospholipid synthesis in rats is depressed (Gebhart, 1985).

CCl_4 being a direct toxin it affects a number of organelles such as the ER, mitochondria, lysosomes. In CCl_4 induced hepatocellular necrosis, accumulation of Ca^{++} has also been demonstrated indicating a change in cellular permeability.

CCl_4 administration initiates lipid peroxidation in liver. The concept of lipid peroxidative damage was advanced by Slater (1978) as the principal mechanism of CCl_4 induced liver injury and

has found ample experimental support reviewed by Recknagel and Glende (1973). This theory holds that CCl_4 is homolytically cleaved by a cytochrome P-450 monooxygenase system to produce CCl_3 free radical. It is more toxic than the fully chlorinated halogen CCl_4. This destructs the membrane bound cytochrrome P-450. In vitro incubation of liver microsomes with CCl_4, decrease the P-450 levels. Loss of glucose-6-phosphatase and representative monooxygenase activities, protein synthesis, capacity to form and secrete low density lipoproteins are some of the consequences of CCl_4 induced liver injury on the function of ER. There are evidences which indicate that CCl_4 toxicity may not correspond to the total cytochrome P-450 in liver tissue (Plaa, 1980). CCl_4 - induced damage to protein synthesis appears to be an irreversible effect which leads to cellular death.

The bioactivation of CCl_4 leads to CCl_3 free radical. This occurs in the ER and is catalysed by cytochrome P-450 monoxygenase system and requires NADPH. This product of homolytic cleavage becomes incorporated in to the ER and can be measured as covalently bound $^{14}CCl_4$ derived radiolable. The complete oxidation of CCl_4 may involve the formation of phosgene intermediate. Both hepatotoxicity and metabolism of CCl_4 to various products are augmented by exposure to inducing agents such as phenoborbitol (Curtis et al. 1979).

On CCl_4 administration within hours, changes in sinusoidal flow becomes discontinuous, clumping of the erythrocytes, edema, congestion hemorrhagic spots may be seen in the centrolobular area. These changes spread to other zones within the lobular structure (Mehendale, 1985).

There are attempts made to protect or recover the liver from CCl_4 induced injury to liver. liquid paraffin has shown to protect the CCl_4 induced liver damage (Moldolesi, 1969). Vaishwanar et al., (1972) tested effect of nicotinic acid and ascorbic acid on induced fatty liver. Several aminoacids also prevented liver damage induced by CCl_4 (De Toranzo, 1983). Arachidonic acid products also protect liver damage induced by CCl_4 (Guarner, et al., 1983). Inhibitors of protein synthesis like cycloheximide are known to protect the necrogenic effect of CCl_4 (Farber and Fisher, 1980; Pappas, 1986).

Phenobarbital treatment which enhances the hepatic cytochrome P-450 catalysed monooxygenase activity and SKF-52 SA diminishes the lipid peroxidation and CCl_4 induced hepatic necrosis (Mehendale, 1985). Antioxidant compounds can protect against lipid peroxidation but prior presence is required for their protective action. Especially Vitamin E, ascorbic acid and induced glutathion which stop the propagation of lipid peroxidative process. Pyrogallol and other polyols and SH-compounds, such as cysteamine are protecting the lipid peroxidation in liver, if it is given prior, simultaneously or immediately (Mehendale, 1985). Malotilate and

Metallothionin also prevented the liver damage and collagen accumulation (Kokko et al., 1987; Zacharias et al., 1990). Methoxsalen decreases metabolic activation of CCl_4 and prevents liver injury (Labbe et al., 1987). Effects of iron loadings are suppressing the lipid peroxidation induced by CCl_4 (Fletcher et al., 1989).

Thus there are many chemicals tested against CCl_4 induced hepatic injury.

In India number of herbs and their extracts are being used for the treatment of hepatitis in traditional medicine (Shastry, 1980; Upadhyaya, 1980; Sharma, 1981; Pandey, 1982). In recent years attempts to test these claims are being made on modern physiological basis as it is being revealed in the following mini review on hepatoprotective and hepatocurative alternative drugs.

Treatment of corn oil to CCl_4 treated rats inhibited rise in hepatic triglycerides (Hartman et al. 1968). Nato et al. (1968) observed decrease in tissue respiration and ribose, triose and lactate increase with simultaneous treatment of olive oil to CCl_4 treated rats. Vaishwanar et al. (1974) had shown hepatoprotective effects of Shilajeet and Eclihol showing change in serum lipoprotein and protein patterns. Vaishwanar et al (1976) further showed Shilajeet and Eclinol induced hepatoprotective alterations in liver and serum lipids. Picrorhiza purrca, Edipta alba, Solanum nigrum were tested for their hepatoprotective properties (Pandey, 1980; Mogre et al.

1981). Among the bhasmas (Ayurvedic preparation) tried kustha quaruleel (Stage horn bhasma) has been used to protect the CCl_4 induced innjury (Abdullah et al,1982).

Phenolic compounds of Arnica showed increased bile secretion and partial recovery of CCl_4 induced hepatic injury (Marchishin, 1983). Garlic extract also inhibited the enhanced peroxidation and production of lipids in CCl_4 induced liver injury (Kagawa, 1986). Among the composite drugs tested for their hepatoprotective nature are Liv-52 (Goel et al., 1991). Arogyavardhini (Dange et al., 1987). One of the components of Liv-52 (Phyllanthus emblica is also tested. (Dange et al., 1980, Rajakumari and Agarwal., 1991). Effect of Picroliv derived from root and rhizome of plant Picrorhiza kurroa have shown to prevent hepatic injury (Dwivedi et.al.,1990). It has shown to have property to act as scavengers of superoxide anions (Chander et al., 1992), and it regenerates liver (Srivastav et al.,1994) by stimulating nucleic acid and protein synthesis (Singh et al.,1992). Even hepatic protection by picroliv is also observed in Mastomys natalensis infected with Plasmodium berghei by protecting β - glutamyl cycle (Chander et al., 1994).

Our laboratory is also engaged in the testing of hepatoprotective Ayurvedic drugs on various types of CCl_4 induced hepatic injuries and using varioius types of parameters to get into details of hepatoprotection.

Mandur bhasma was used to protect the liver in the CCl_4 induced hepatic injury (3 ml/kg body wt. daily for 11 days). The hepatoprotection was evaluated on the basis of alterations in lipolytic enzymes (Devarshi et al., 1986). Kumari asav, kumari kalp, arogyavardhini and tamra bhasma were also tested against CCl_4 induced hepatic injury (3 ml/kg body wt/week for four weeks) using lipases as the parameter (Patil et al., 1989). All the above four drugs were also tested against acute hepatic injury induced by CCl_4 (3 ml/kg body wt. daily for 7 days) in albino rat using alkaline, acid, lipoprotein and harmone sensitive lipases (Patil et al., 1992). The action of these drugs was also evaluated using lysosomal enzymes, acid phosphatase and β-glucuronidase activities as parameter (Kanase et al., 1994a) on CCl_4 induced hepatic injury (3ml/kg body wt. single dose) and the same enzyme activities were also studied during hepatoprotection against acute hepatic injury (3 ml/kg body wt. daily for 7 days).

Thus the review suggests that many ayurvedic preparations are being studied for their hepatoprotective and hepatocurative properties evaluating them on the basis of modern physiological and pharmacological concepts.

As it is observed from the review of literature; search for a useful hepatoprotective and/or hepatocurative drug is very important necessity of the day. It is essential to evaluate our traditional claims of hepatoprotective and hepatocurative drugs. Keeping this in mind the present thesis is designed to test hepatocurative property of mandur bhasma (an Ayurvedic preparation) using histological recovery of liver as the main parameter of recovery. Histology of kidney is also studied so that the drug Mandur Bhasma can be tested for its total potency of physiological recovery.

Since CCl_4 is mainly inducing necrosis in liver by producing CCl_3 free radical on biotransformation; lipid peroxidation is also taken as the another parameter of recovery.

The experiments are designed to induce hepatic injury in male albino rats by CCl_4 using liquid paraffin as a vehicle as well as to test hepatocurative property of Mandur bhasma.

There were eight experimental groups used. Group I (normal rats) Group II (only CCl_4 treated rats) Group III (only liquid paraffin treated rats), Group IV (CCl_4 + liquid paraffin treated rats), Group V (only Mandur bhasma treated rats), Group VI CCl_4 + Mandur bhasma treated rats) Group VII (liquid paraffin +Mandur bhasma treated rats) , Group VIII ((CCl_4 + liquid paraffin +Mandur bhasma treated rats).

Histological data and lipid peroxidation data collected is presented in six chapters.

I - Introduction

II - Material and Methods

III - Histology

IV - Lipid Peroxidation

V - General Discussion

VI - Concluding Remarks.

I - Introduction

Deals with the reasons to select the problem, animal, drug, CCl_4 as a hepatotoxic model, curative effects etc. It further explains normal functioning of liver and regulation of metabolic activities, varioius forms of hepatotoxicity, CCl_4 induced hepatotoxicity, attempts made by various workers to search appropriate hepatoprotective medicines and approach to test the Mandur bhasma for its curative effects.

II - Material and Methods.

In this chapter detail experimental protocol is given which was designed to test the hepatocurative property of Mandur bhasma. The methods used for histological and biochemical studies are also given under this chapter.

III - Histology

Detail histological alterations in the liver and kidney in experimental animals that are used to test hepatocurative effects are given in well illustrated photomicrographic plates (six). All the observations are presented additionally in tables also.

This chapter opens with small introduction, detail observations and ends with discussion based on histological alterations.

IV - Lipid peroxidation

This chapter has small introduction to describe CCl_4 induced lipid peroxidation in liver. Detail alterations in lipid peroxidation occurring in liver and kidney are described using tables and text. Discussion based on these observations concludes the chapter.

V - General Discussion

This chapter evaluates the total alterations taking place in histology and lipid peroxidation of liver and kidney of animals of various experimental groups.

VI - Concluding Remarks.

In this chapter the conclusive comments on the present work and future plans of works are discussed.

***