1. INTRODUCTION
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Uncontrolled environmental pollution, poor sanitary conditions, xenobiotics, alcoholic intoxication and the indiscriminate use of potent drugs predispose the liver to a vast array of disorders. However, infection by virus still remains as the major cause of liver disease. Global estimates indicate that there are about 18,000 deaths every year due to liver cirrhosis caused by hepatitis. Hepatocellular carcinoma ranks among the top ten common tumours of the world, with an average of over 2,50,000 new cases being reported every year.

The liver, weighing between 1200-1500 grams is the largest solid organ in the body. Essentially the liver has four quite distinct functions:

1. It supplies bile salts and bicarbonates to assist in digestion.
2. It acts as a buffer between the gut and the systemic circulation maintaining stable levels of amino acids and glucose.
3. It synthesises a large number of specialised proteins, carbohydrates and lipids.
4. It is a major excretory pathway for the larger and more hydrophobic metabolites, foreign substances and drugs.

Didactically liver disorders may be classified as hepatitis (inflammation of the liver), hepatotosis (non-inflammatory disorders or degeneration of the liver parenchyma), chronic hepatitis and liver cirrhosis. However there is no strict hepatological delineation of these disorders, making a similar classification of the hepatoprotective agents virtually impossible.

Jaundice or icterus is a condition characterised by the increase in the bilirubin
of the blood. When the increase is slight (less than 2mg/100ml), its presence can often be detected only by serum analysis and latent jaundice is then said to exist. With greater increase, there is visible yellow coloration of the skin, sclerae and mucous membranes. The main types of jaundice are haemolytic jaundice, hepatocellular jaundice and obstructive jaundice. Haemolytic jaundice causes excessive destruction of the red blood cells resulting in increased bilirubin formation. This type of jaundice is mild except in the newborn.

Hepatocellular jaundice is usually associated with damage to the parenchymal cells of the liver by toxic or infective agents, the power to transfer bilirubin from the blood to the biliary canaliculi being diminished as a consequence. The cellular degeneration and necrosis permit the diffusion of bilirubin that has reached the canaliculi into the blood.

Obstructive jaundice occurs when there is a block to the pathway between the site of conjugation of bile in the liver cells and the entry of bilirubin into the duodenum. Stasis occurs within the dilated bile ducts and canaliculi. Conjugated bilirubin and other constituents are retained and this enters the blood stream.

Parenchymal disease of the liver may be acute, sub-acute or chronic. Acute parenchymal disease is the result of hepatic cell degeneration or necrosis. This is brought about largely by the action of infective or toxic agents. Idiosyncrasy, allergy and nutritional factors may also play a part. Certain xenobiotics or hepatotoxins when administered to animals are metabolised by the liver giving rise to toxic metabolites, thereby leading to liver injury$^{4,5}$. 
### Table 1

**TYPES OF HEPATOTOXIC AGENTS**

**INORGANIC AGENTS**

| Metals and Metalloids | antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorus, selenium, tellurium, thallium, zinc |

**Hydrazine derivatives** | Iodides |

**ORGANIC AGENTS**

| Natural plant toxins | albitocin, cycasin, icterogenin, indospicine, lantana, agaione, nutmeg, pyrrolizidines, saffrole, tannic acid |

| Mycotoxins | aflatoxin, cyclochlorotine, ethanol, luteoskryrin, ochratoxins, rubratoxins, sterignatocystins, griseofulvin, sporidesmin, tetracycline and other antibiotics |

| Bacterial toxins | exotoxins (*C.diphtheria, Cl.botulinus, Str. hemolyticus*), endotoxins, ethionine |

| Synthetic Non-medicinal | Haloalkanes and haloolefins Nitroalkanes Chloroaromatic compounds, Nitroaromatic compounds, Organic amines, Azo compounds, Phenol and derivatives, Various other organic compounds |

| Medicinal agents | over 100 drugs used for treatment and diagnosis |
The agents listed in this table vary considerably in their potential for causing hepatic injury.

**Common Hepatotoxins**: Chemical hepatic injury\(^6\) is encountered in a variety of circumstances (Table 1). Some natural toxins like the peptides of *Amanita phalloides*, the pyrrolizidine alkaloids, the toxin of the cycad nut, and other phytotoxins are hazards posed by the environment that are ingested in ignorance of their toxicity or are taken as folk medicines\(^7,8,9\).

Domestic exposure to hepatotoxins include the accidental or suicidal ingestion of CCl\(_4\) and elemental phosphorus\(^{10}\) or of huge overdoses of medicinal agents (acetaminophen\(^{11}\), ferrous sulfate\(^{10}\)). Ingestion of hepatotoxic mushrooms remains an important cause of toxic liver injury. The chief domestic exposure to a known hepatotoxin however, remains the intake of excessive amounts of ethanol.

A grave threat at present is the potential for hepatotoxicity for man and animals posed by the contamination of environment by industrial byproducts and wastes, and by insecticides, and by the presence of these toxic materials in the food chain. The demonstrated presence\(^{12}\) of CCl\(_4\) in bodies of water from which domestic water supplies derive and of chlorinated biphenyls in water and food products illustrates the threat. Recent reports suggest the presence of carbon tetrachloride as a contaminant of chlorine used to disinfect drinking water\(^{13}\). The chlorinated hydrocarbons which have been widely employed as versatile solvents and reagents in the course of industrial operations\(^{14,15}\) are now used with increased caution.

Liver damage due to peroxidised lipids in food has now come to be regarded as a serious problem for human health. Fatty oils, which are a daily necessity in human life, when exposed to air generate peroxidised lipids, thereby exposing man to the
constant danger of liver lesion by lipid peroxides\textsuperscript{16}. It has been reported that vegetable oils which contain linoleic acid yield peroxides by heating and/or aeration, exhibiting the said toxicity\textsuperscript{17}.

However, although liver disorders are induced by innumerable hepatotoxins, the main cause of liver disease is known to be the virus. In certain types of viral hepatitis, transformation to chronic hepatitis by continuous infection of the virus, brings the complications of morbidity and difficulty of healing. The mechanism of onset of viral hepatitis and of its transformation to chronic hepatitis is now recognised to involve immune responses of the host rather than the direct action of the virus\textsuperscript{18}.

Clinical and experimental observations have led to the general agreement that there are two main categories of substances that produce hepatic injury (Table 2)

One group consists of agents that are intrinsically toxic ie. their hepatotoxicity is a fundamental property to which most exposed individuals are susceptible. These are called intrinsic, true or predictable hepatotoxins. The other group consists of agents that produce hepatic injury only in unusually susceptible humans ie their toxic effects result from the special vulnerability of the affected individual rather than the intrinsic toxicity of the incriminating agent. This form of chemical hepatic injury is called non predictable or idiosyncratic\textsuperscript{19,20,21}.

The most prevalent clinical forms of acute parenchymal disease of the liver include viral hepatitis (acute infective hepatitis and serum hepatitis) and leptospirosis. Here, the damage to the liver falls into the category of either acute zonal necrosis in which necrosis occurs in the same zone of all the lobules of the liver, the central zone being the most affected or acute massive necrosis in which widespread necrosis affects
### TABLE-2

**CLASSIFICATION OF HEPATOTOXIC AGENTS AND MAJOR CHARACTERISTICS OF EACH GROUP**

<table>
<thead>
<tr>
<th>Category of Agent</th>
<th>Mechanism</th>
<th>Histological Lesion</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTRINSIC TOXICITY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>Direct physicochemical distortion and destruction of structural basic cell metabolism</td>
<td>Necrosis (Zonal) and/or steatosis</td>
<td>CCl₄ CHCl₃ Phosphorus</td>
</tr>
<tr>
<td>Indirect</td>
<td>Interference with specific metabolic pathways leading to structural injury</td>
<td>Steatosis or Necrosis</td>
<td>Ethionine Mycotoxins</td>
</tr>
<tr>
<td><strong>CHOLESTATIC</strong></td>
<td>Interference with hepatic excretory pathways leading to cholestasis</td>
<td>Bile casts</td>
<td>Icterogenin C-17 alkylated anabolic and contraceptive steroids.</td>
</tr>
<tr>
<td><strong>HOST IDIOSYNCRASY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>sulfonamides, PAS, Halothane</td>
</tr>
<tr>
<td>Metabolic abnormality</td>
<td>Production of hepatic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Iproniazid Isoniazid Halothane</td>
</tr>
</tbody>
</table>
the entire hepatic lobules, through large expanses of the liver leaving other areas relatively unharmed.

Chronic parenchymal diseases of the liver include chronic hepatitis and liver cirrhosis and may be attributable to a previous episode of acute hepatic disease in some cases. In others, the condition is insidious in onset and no frank history of acute liver disease can be elicited.

**Carbon tetrachloride as a hepatotoxin**

During the early part of this century CCl₄ was first found to produce hepatic injury in man and experimental animals. The intervening years have seen thousands of reports devoted to this agent. Poisoning with CCl₄ has been a well-accepted and widely used model to study the pathophysiology of inflammation, liver injury or hepatic inflammation. In the course of unravelling the mechanism by which it produces fatty liver, CCl₄ has served to elucidate the pathogenesis of fatty metamorphosis induced by other etiological factors. While it can lead to damage of a number of tissues, it is particularly damaging to the liver and kidneys of many species. The agent is a potent hepatotoxin. Single doses lead promptly to centrizonal necrosis and steatosis. Within a few minutes there is injury to the endoplasmic reticulum, which leads to functional defects of the hepatocyte and multiple biochemical manifestations of hepatic injury.

Carbon tetrachloride because of its high lipid solubility is well distributed in the body, but produces toxic effects that are largely confined to the liver and kidneys. The toxicity is increased by agents (e.g. phenobarbitone) which induce microsomal drug metabolizing enzymes and reduced by the inhibitors of microsomal enzymes. The microsomal mixed function oxidase system withdraws an electron from CCl₄ leaving the
reactive trichloromethyl radical $\text{CCI}_3$. This free radical has a life time of only about 100 microseconds and so has time to diffuse for only a short distance within the liver cell before undergoing secondary reactions. The secondary reactions which are responsible for the biochemical damage may be of various kinds.

(a) oxidation of thiols to disulphide bonds.

(b) saturation of double bonds in lipids, proteins or nucleotides, resulting in covalent attachment of the free radical group of those sites and

(c) lipid peroxidation reaction in which polyunsaturated membrane lipids are converted to peroxide derivatives and eventually to aldehydes and other products leading to a further cascade of reactions, which results in irreversible membrane damage.

Prolonged administration of $\text{CCl}_4$ can lead to cirrhosis$^{31}$ and to hepatic carcinoma$^{32}$. Most of the acute and chronic hepatic injury appears to result from the action of a metabolite of the toxin.$^{23}$ Chemically $\text{CCl}_4$ is a simple, strongly non polar molecule$^{14}$ which undergoes metabolism in the smooth endoplasmic reticulum. A chemical characteristic of relevance to the hepatotoxic potential of $\text{CCl}_4$, emphasised by Recknagel and Glende$^{23}$ and Slater$^{33}$, is the relatively low energy for the C-Cl bond. The bond association energy is progressively higher in less toxic haloalkanes and tends to be lower in more toxic haloalkanes$^{23,29,33}$.

**Experimental models for the evaluation of antihepatotoxic activity**

Experimental hepatotoxic states provide essential models for the study of the physiologic and biochemical reflections of hepatic disease. The classical agent for the study of the effects of hepatic necrosis is $\text{CCl}_4^{34}$, although many other necrogenic
substances have also been used. Galactosamine has been described\textsuperscript{35} to produce a lesion in experimental animals which resembles that of viral hepatitis. Thioacetamide induced liver injury has also been used though less frequently to evaluate the hepatoprotective activity. It is reported to cause inhibition of the respiratory metabolism of the liver due to the uncontrolled entry of Ca\textsuperscript{++} ions, resulting in the inhibition of oxidative phosphorylation\textsuperscript{36}. Acetaminophen (paracetamol) is a non-toxic drug in the usual therapeutic dose. In overdose, however, as a suicide attempt, it is a cause of severe hepatic necrosis\textsuperscript{37,38}. Necrogenic doses have ranged from 7-70 g and in most cases exceeded 15 g. It has recently been reported to produce liver injury through the cholestatic effect\textsuperscript{39}. Lately an immunological method has been described by Mizoguchi and coworkers\textsuperscript{40}. In this model trinitrophenylated liver macromolecular fractions have been used to induce hepatic injury through an immunological mechanism. Recent \textit{in vitro} methods include using peroxide induced cytotoxicity in primary cultured hepatocytes\textsuperscript{41} and complement mediated cytotoxicity in primary cultured hepatocytes\textsuperscript{42}.

The studies of the effects of toxic agents in the liver have utilised whole animals or various \textit{in vitro} preparations. The use of the whole animals is essential for the demonstration that an exogenous agent has a true adverse effect on the liver in a setting of physiologic significance. Whole animals also elucidate the effect of various factors and manipulations on the mechanisms of injury and for the pathophysiologic impact of the hepatic injury. The \textit{in vitro} models however, may be employed to elucidate specific aspects of the mechanism of injury.

**Whole animal as a model**

The studies performed during the past 100 years have employed a variety of species. Most popular have been the rats because of their size and relatively low cost.
Accordingly most of the information on experimental hepatotoxicity applies to the rat. To a varying degree mice, hamsters, guinea pigs, rabbits, dogs, cats, cattle, swine, horses, sheep and several species of birds have been employed. During the recent years, primates have come to use, for the obvious reason of greater presumed relevance to diseases of humans.

**Parameters of injury**

The utilisation of the whole animal has involved selection of measures of hepatic injury. These include lethality, histologic changes seen by light and electron microscopy, chemical changes in the liver and physiologic and biochemical tests that measure the functional status or that reflect the type of intensity of hepatic injury.

**Lethality**

Death as a measure of hepatotoxic potency is applicable mainly to known hepatotoxins. The employment of LD$_{50}$ or other measures of lethal potency permit the comparison of hepatotoxic agents.

**Histology**

The light microscope is the traditional method for demonstrating toxic hepatic injury and categorizing its type.$^{19,21}$

The electron microscope provides the earliest histological evidence of injury. It demonstrates the subtle features of a lesion too subtle to be appreciated by the light microscope. It also yields clues to the mechanism of injury.

**Chemical changes in hepatic tissue**

The analysis of chemical changes in the liver has served to quantitate liver injury
and to clarify the mechanisms of injury. For agents that produce fatty metamorphosis without necrosis and without much changes in serum enzymes, the chemical measurement of fat and the microscopic demonstration of fat are reliable techniques for detection of injury. Similarly, by the measurement of increased hepatic contents of dienes and malonic dialdehyde, the products of lipid peroxidation after CCl₄ injury, were concluded to be via lipid peroxidation⁴⁵. Measurement of the lipids in the liver permit relating the intensity of adverse effect of agents like ethionine, phosphorus and tetracycline. GSH (Glutathione) appears to play a protective role against chemical injury by some agents. Hepatic injury results from covalent bonding to macromolecules which occurs when the binding capacity of the available GSH is exceeded⁴⁶.

The measurement of cytochrome p-450 has served to identify the site and nature of injury produced by hepatotoxins⁴⁷.

**Physiological and Biochemical Measures of Hepatic Functions and Injury**

The ability of the liver to synthesise urea, cholesterol and its esters, and plasma proteins including clotting factors, and to maintain normal blood levels of glucose and amino acids, provide a group of time-honoured reflections of hepatic injury in experimental animals. Deviation from normal, of these functional responsibilities served the early studies of chemical hepatic injury. These measures however are too insensitive, non-specific or cumbersome to be useful in monitoring the degree of injury, comparing the relative toxicity of different agents, or assessing the potentiating or protective effect of various treatments on the toxicity of an agent. Metabolism of drugs, excretion of foreign dyes and serum enzymology are more useful⁴⁸.

**Bilirubin levels**

Elevation of bilirubin levels of the serum accompanies sufficiently severe
parenchymal injury, but it is a relatively insensitive measure of chemical hepatic injury. For the demonstration of hepatocellular injury, the clearance of sulfobromophthalein (BSP or ICG) is simpler than bilirubin clearance, and measurement of blood levels of enzymes released by the injured liver is even simpler. Thus far, there appears to have been little application of these tests to experimental hepatotoxicity.

**Serum enzymology**

Serum enzymology has become the standard approach to the measurement of hepatic injury in the recent past. Studies during the 1930s and 1940s demonstrated abnormal serum levels of alkaline phosphatase and pseudocholinesterase in liver damage. But it was not until the recognition in 1955, that transaminase (aminotransferase) levels of the serum are sensitive measures of hepatic injury, that the use of serum enzyme assay, as a tool for experimental hepatotoxicology, came into its own. It is useful for the detection of early damage, for demonstration of hepatic injury without sacrifice of animals and for serial measurements especially in larger animals. Serum enzymology as a parameter of injury, has been applied to the study of possible hepatotoxicity of unknown agents, to the comparison of the toxicity of known agents, to the demonstration of onset of injury, and to the study of the potentiation or inhibition of the toxic phenomena by physiologic manipulations or administration of various agents.

The parenchymal cells of liver contain certain enzymes in large amounts and these may be released into the plasma when the cell is damaged. Of these, the most useful for the study of the experimental toxic hepatic injury are alanine aminotransferase (ALT) also known as serum glutamic pyruvic transaminase (SGPT) and aspartate aminotransferase (AST) or serum glutamic oxalacetic transaminase (SGOT). In virtually
all mammalian species, the SGOT is a sensitive measure of acute hepatic necrosis. In the rat, the SGPT is almost as sensitive as the SGOT. Clinically SGOT and SGPT are of immense value to distinguish hepatocellular jaundice from obstructive jaundice.

The serum alkaline phosphatase is an enzyme excreted by the hepatic parenchymal cells into the biliary canaliculi and eventually into the intestine. It is useful to measure the alteration in function brought about either by parenchymal cell damage or obstruction of the bile ducts. In acute or chronic parenchymal liver disease the elevation is only moderate, whereas in obstructive jaundice and extrahepatic cholestases, the enzyme levels record a marked increase.

**In Vitro Models**

The past few years have witnessed the development of a number of in vitro models for the study of hepatotoxicity. These include the perfused liver, liver homogenates and slices, suspension of hepatocytes freshly isolated from the liver or grown in tissue or organ culture, or isolated organelles from hepatocytes. The problem has been approached by pretreating the intact animal with the agent under study prior to sacrifice and removal of the liver for the in vitro studies, or by adding the agent under study to the perfusate or to the medium containing hepatic tissue, cells or organelles. The use of microorganisms, as in studies of the effects of aflatoxins, ethionine or carcinogens on bacteria offers the convenience of in vitro models, although strictly speaking microorganisms are, of course, in vivo models.

**Plants as Hepatoprotective Agents**

Despite tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help the
regeneration of the parenchymal cells. While corticosteroids and immunosuppressive agents, the side effects of which are alarming, are the only drugs of choice in modern medicine for the management of liver ailments, plants and natural products are proving to be good hepatoprotectants. This is evident from the voluminous work published on their hepatoprotective activity. The importance of plant products in modern medicine even in a highly advanced society as that of the U.S.A. can be seen from the data of national surveys, wherein it was found that 25% of all the prescriptions dispensed contained crude plant materials or crude plant extracts. About 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess liver protective activity and about 600 commercial herbal formulations with claimed hepatoprotective activity are being sold world-wide. Of these, about forty patent polyherbal formulations, representing various Indian herbs are available in the Indian market. For centuries, indigenous drugs, either alone or in combination have been advocated in the traditional systems of medicine especially ayurveda for the treatment of liver disorders.

It was the isolation of silymarin, a flavonolignan from Silybum marianum that kindled widespread world research on hepatoprotective agents. Other important antihepatotoxic drug discoveries from plant sources include cynarin from Cynara scolymus, and schizandrin from Schizandra spp. which have been included under the literature review. The discovery of diverse chemical compounds from the natural products and synthetic compounds used in protective liver therapy such as phospholipids, sugar alcohols, pyrimidine, purine derivatives, vitamins, cysteine, glutathione, corticoids, androgens, penicillamine, ricinin etc. does not confine the activity to any particular class of compounds but emphasises once again the complexity of liver disorders in addition to the different action mechanisms of different pharmaceuticals.
preparations. However, it may be noted that many of the antihepatotoxic compounds mentioned in literature are phenolic and phenol propane derivatives. Systematic pharmacological studies are therefore well conceived and justified especially in the lignans, neolignans, higher condensed flavonoids and cinnamic acid derivatives.