Clinical Biochemistry of Hepatotoxicity

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Abstract

Liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. Liver injury caused by various toxic chemicals or their reactive metabolites (hepatotoxicants) is known as hepatotoxicity. The present review describes the biotransformation of hepatotoxicants and various models used to study hepatotoxicity. It provides an overview of pathological and biochemical mechanism involved during hepatotoxicity together with alteration of clinical biochemistry during liver injury. The review has been supported by a list of important hepatotoxicants as well as common hepatoprotective herbs.

Keywords: Hepatotoxicity; Hepatotoxicant; In Vivo models; In Vitro models; Pathology; Alanine aminotransferase; Alkaline phosphatase; Bilirubin; Hepatoprotective

Introduction

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics [1]. The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements [2,3]. Certain drugs may cause liver injury when introduced even within the therapeutic range. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature [4,5]. The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus [6]. Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool [7,8].

Liver- The Target Organ

Liver is the largest organ of the human body weighing approximately 1500 g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm. The liver performs more than 500 vital metabolic functions [9]. It is involved in the synthesis of products like glucose derived from glycogenesis, plasma proteins, clotting factors and urea that are released into the bloodstream. It regulates blood levels of amino acids. Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile aids in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them [4]. An excess of chemicals hinders the production of bile thus leading to the body’s inability to flush out the chemicals through waste. Smooth endoplasmic reticulum of the liver is the principal ‘metabolic clearing house’ for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury [4].

Models to Study Hepatotoxicity

In vivo Systems

Animal models represent a major tool for the study of mechanisms in virtually all of biomedical research [10]. They involve the complexity of the whole animal thus making the monitoring of in vivo systems quite difficult. An in vivo system fully reflects the exposing profile and the cellular function as the compounds are exposed in the successive manner through absorption from the first exposed site followed by metabolism, distribution and elimination. However, it should involve basically the same mechanism as the reactions in humans and the adverse effect must be clinically sufficiently high. Both small animals like rats, mice and guinea pigs as well as large animals like pigs, cattle, sheep and monkeys are useful and reliable for studying the hepatotoxicant effects, distribution and clearance. They may be used to elucidate basic mechanism of xenobiotic activities which will be useful in understanding their impact on human health. However, the relevance of the findings of in vivo studies using different animal models to humans may vary due to differences in drug metabolism and pathobiology in various species. Due to the lack of sufficient data to reliably assess the value of preclinical animal studies to predict hepatotoxicity in humans, the preclinical animal toxicity studies may not be sufficient as the only modelling systems used to predict hepatotoxicity [11,12]. Further, in order to reduce the use of animal in toxicity studies, there is a need for a long-term in vitro system.

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In vitro Systems

They are much easier to control and manage than the intact organism. The data of in vitro studies can be utilized for deciding appropriate doses for in vivo studies. In an in vitro system, compounds affect the cells directly and continuously until the removal of compound-containing medium [13]. These models contribute to the ‘3R’ concept [refinement, reduction and replacement] of animal experimentation which leads to reduction of animal utilization for research purposes [14]. This system is quite useful for safety evaluation in the early stage of drug discovery as they are helpful in generating sufficient results at a low cost and high speed, and with less use of animals [15]. Several in vitro human and animal liver models are available ranging from short-term to long-term cell or tissue culture systems. Generally, chemical hepatotoxicity can be studied using six in vitro experimental systems, namely, isolated perfused liver preparations, liver slices, isolated hepatocytes in suspension, isolated hepatocytes culture and co-culture, cell lines and subcellular fractions [6,12,14,16,17].

Biotransformation of Hepatotoxins

Liver plays a central role in biotransformation and disposition of xenobiotics [18]. The close association of liver with the small intestine and the systemic circulation enables it to maximize the processing of absorbed nutrients and minimize exposure of the body to toxins and foreign chemicals. The liver may be exposed to large concentrations of exogenous substances and their metabolites. Metabolism of exogenous compounds can modulate the properties of hepatotoxicant by either increasing its toxicity (toxication or metabolic activation) or decreasing its toxicity (detoxification) [6]. Most of the foreign substances are lipophilic thus enabling them to cross the membranes of intestinal cells. They are rendered more hydrophilic by biochemical processes in the hepatocyte, yielding water-soluble products that are exported into plasma or bile by transport proteins located on the hepatocyte membrane and subsequently excreted by the kidney or gastrointestinal tract [19] (Figure 1).

The hepatic biotransformation involves Phase I and Phase II reactions. Phase I involves oxidative, reductive, hydroxylation and demethylation pathways, primarily by way of the cytochrome P-450 enzyme system located in the endoplasmic reticulum, which is the most important family of metabolizing enzymes in the liver. The endoplasmic reticulum also contains a NADPH-dependent mixed function oxidase system, the flavin-containing monoxygenases, which oxidizes amines and sulphur compounds. Phase I reactions often produce toxic intermediates which are rendered non-toxic by phase II reactions. Phase II reactions involve the conjugation of chemicals with hydrophilic moieties such as glucuronic acid, sulfate or amino acids and lead to the formation of more water-soluble metabolite which can be excreted easily [6]. Another Phase II reaction involves glutathione which can covalently bind to toxic intermediates by glutathione-S-transferase [20]. As a result, these reactions are usually considered detoxification pathways. However, this phase can also lead to the formation of unstable precursors to reactive species that can cause hepatotoxicity [21,22].

The activities of enzymes are influenced by various endogenous factors and exogenous drugs or chemicals [23]. Many substances can influence the cytochrome P450 enzyme mechanism [24]. Such substances can serve either as inhibitors or inducers. Enzyme inhibitors act immediately by blocking the metabolic activity of one or several cytochrome P450 enzymes [25]. Enzyme inducers act slowly and increase cytochrome P450 activity by increasing its synthesis [26]. Certain substances may share the same cytochrome P450 specificity, thus competitively block their biotransformation activity and lead to accumulation of drugs metabolized by the enzyme. Genetic variations or polymorphisms in cytochrome P450 metabolism may also be responsible for unusual sensitivity or resistance to drug effects at normal doses among different individuals [27]. Hepatotoxicity may also arise from an adaptive immune response to proteins bound to the hepatotoxicant or its metabolites [28,29]. Random exposure to lipopolysaccharides (LPS) or other inflammatory conditions could potentiate hepatotoxicity by involving a combination of fibrin deposit-induced hypoxia and neutrophil-mediated cell damage [30].

The differences in enzyme expression and substrate specificity in species, strain or gender can produce qualitative differences or quantitative differences in the metabolic pathways involved in the bioactivation or detoxification of hepatotoxins. Hepatotoxic effect of acetaminophen differs in different species. For instance, hamsters and mice are sensitive to the hepatotoxic effects of acetaminophen whereas rats and humans appear to be resistant. This is mainly due to differences in the rate of production of toxic metabolite of acetaminophen, N-acetyl-p-benzoquinoneimine (NABQI). However, isolated hepatocytes from all the four species are equally susceptible to the toxic effects of NABQI [31]. Male rats are sensitive to the hepatotoxic effect of senecionine, a pyrrolizidine alkaloid while female rats are resistant to its hepatotoxicity. This is due to the absence of isoform of cytochrome P450 involved in the bioactivation of senecionine in female rats [32]. Further, the rate-controlling step in biotransformation reactions is cofactor supply [33]. Changes in the concentration of cofactors like NADPH and glutathione can markedly alter the sensitivity of animals to hepatotoxins. The nutritional status of animals also plays a role in the hepatic concentrations of these cofactors. Fed rats are relatively resistant to the hepatotoxic effects of bromobenzene and acetaminophen whereas an overnight fasting makes them extremely susceptible to these hepatotoxins [34].

![Figure 1: Biotransformation of hepatotoxins](image-url)
Mechanism of Hepatotoxicity

Pathology

Liver pathology serves as an important tool for identifying and characterizing liver injury whether or not clinicobiochemical changes are also identified. Main patterns of liver injury during hepatotoxicity may include zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions, neoplasm and veno-occlusive diseases (Figure 2).

Zonal necrosis: This type of injury may be caused by exogenous substances like paracetamol [35] and carbon tetrachloride [36,37]. Amatoxins cause necrosis of liver as a consequence of the cessation of protein synthesis due to the inhibition of RNA synthesis [38]. Herbal plants like Atractylis gummifera and Callilepsis laureola [39], Larrea tridentata [40] and Teucrium polium [41] also cause necrosis. Such injury is largely confined to a particular zone of the liver lobule. It may manifest as a very high level of alanine aminotransferase and severe disturbance of liver function leading to acute liver failure.

Hepatitis: This type of liver injury shows hepatocellular necrosis associated with infiltration of inflammatory cells. It may be further characterised into three categories, namely, viral, focal and chronic. Viral hepatitis, where histological features are similar to acute viral hepatitis, may be caused by halothane [42], isoniazid, acetaminophen, bromfenac, nevirapine, ritonavir, troglitazone [43] and phenytoin [4]. Reports exist for acute hepatitis caused by Chelidonium majus [44]. Focal hepatitis where scattered foci of cell necrosis may accompany lymphocytic infiltration may be caused by aspirin. Chronic hepatitis which is similar to autoimmune hepatitis clinically, serologically and histologically, may be caused by methyl dopa, diclofenac, dantrolene, minocycline and nitrofurantoin [43]. Among herbal remedies, Larrea tridentata [40] and Lycopodium serratum [45] leads to chronic hepatitis. Non-nucleoside reverse transcriptase inhibitors, especially viramune [nevirapine] are also associated with hepatitis and hepatic necrosis [46,47].

Cholestasis: This type of liver injury leads to impairment of bile flow, itching and jaundice. Kaplowitz [43] reported angiotensin-converting enzyme [ACE] inhibitors, amoxicillin, chlorpromazine, erythromycins and sulindac to be associated with etiology of cholestasis. It may be inflammatory, bland or ductal. Inflammatory cholestasis may be caused by allopurinol, co-amoxiclav or carbamazepine. Bland cholestasis without any parenchymal inflammation may be caused by anabolic steroids and androgens [4], while ductal cholestasis showing progressive destruction of small bile ducts may be caused by chlorpromazine and fluvoxacinil [48].

Steatosis: This type of liver injury may manifest as triglyceride accumulation [4,49] which leads to either small droplet [microvesicular] or large droplet [macrovesicular] fatty liver. Aspirin, ketoprofen, tetracycline, nucleoside reverse transcriptase inhibitors and valproic acid [8,43] and Scutellaria sp. plant [50] may lead to microvesicular steatosis while acetaminophen and methotrexate [51] may lead to macrovesicular steatosis. Amiodarone, chlorpheniramine and total parenteral nutrition may cause phospholipidosis [48] where phospholipid accumulation leads to pattern similar to the diseases with inherited phospholipid metabolism defects. Nucleoside reverse transcriptase inhibitors, especially zerit [stavudine], videx [didanosine], and retrovir [zidovudine] are associated with a life threatening condition called lactic acidosis [46,47]. Tamoxifen also leads to non-alcoholic steatohepatitis [8,43].

Granuloma: Hepatic granulomas are associated with granulomas located in periportal or portal areas and show features of systemic vasculitis and hypersensitivity. Drugs like allopurinol, sulfonamides,
pyrazinamide, phenytoin, isoniazid, penicillin and quinidine have been found to cause such injury [4,8].

Vascular lesions: Such condition is caused by injury to the vascular endothelium and may be caused by chemotherapeutic agents [52], bush tea [Crotalaria spp.] and anabolic steroids [53].

Neoplasm: Prolonged exposure to some medications and toxins like vinyl chloride, anabolic steroids, arsenic and thorotrast may cause neoplasms like hepatocellular carcinoma, angiosarcoma and liver adenomas [48].

Veno-occlusive: The hepatic vein becomes clogged, blocking off the blood supply to the liver. It is a non-thrombotic obliteration of small intrahepatic veins by subendothelial fibrin [54] associated with congestion and potentially fatal necrosis of centrilobular hepatocytes. The pyrrolizidine alkaloids have been associated with this type of severe liver disorder [55]. Busulfan and cyclophosphamide also cause veno-occlusive disease [43,52].

Histological findings like liver biopsy or autopsy can support the diagnosis of hepatotoxicity [56] (Benichou, 1990). Liver injury caused by hepatotoxicity can also be determined with X-rays, computerized tomography (CT) scan and endoscopic retrograde cholangiopancreatography (ERCP). Ultrastructural pathology can provide evidence for enzyme induction, mitochondrial changes, drug accumulation and early indications of histopathological symptoms.

Biochemical Mechanism

The hepatotoxic effects of chemical agents may involve different mechanisms of cytolethality [6,43] (Figure 2). These mechanisms may have either direct effect on organelles like mitochondria, endoplasmic reticulum, the cytoskeleton, microtubules and nucleus or indirect effect on cellular organelles through the activation and inhibition of signalling kinases, transcription factors and gene-expression profiles. The resultant intracellular stress may lead to cell death caused by either cell shrinkage and nuclear disassembly (apoptosis) or swelling and lysis (necrosis). Main mechanisms involved are listed below:

Direct effect of toxicant upon critical cellular systems: Hepatotoxins can attack directly certain critical cellular targets like plasma membrane, mitochondria, endoplasmic reticulum, nucleus and lysosomes thus disrupting their activity. Various chemicals and metal ions bind to mitochondrial membranes and enzymes, disrupting energy metabolism and cellular respiration [6]. Many hepatotoxins act as direct inhibitors and uncouplers of mitochondrial electron transport [25]. Covalent binding of the drug to intracellular proteins cause a decrease in ATP levels leading to actin disruption and rupture of the membrane. The mushroom toxin, phalloidin also causes increase in cell shrinkage and nuclear disassembly [apoptosis] or swelling and lysis [necrosis]. Main mechanisms involved are listed below:

Lipid peroxidation and redox cycling: These are involved in hepatotoxicity leading to cell death due to oxidative stress which is caused by an alteration in the intracellular prooxidant to antioxidant ratio in favor of prooxidants [65]. Lipid peroxyl radicals lead to increased cell membrane permeability, decreased cell membrane fluidity, inactivation of membrane proteins and loss of polarity of mitochondrial membranes. Metal ions like iron and copper participate in redox cycling while cycling of oxidised and reduced forms of a toxicant leads to the formation of reactive oxygen free radicals which can deplete glutathione through oxidation or oxidize critical protein sulphydryl groups involved in cellular or enzymatic regulation or can initiate lipid peroxidation. Excessive consumption of ethanol contributes to free radical generation, lipid peroxidation and glutathione depletion [4]. Severe α-amanitin hepatotoxicity is also contributed by a peroxidative process [66]. Halogenated hydrocarbons, hydroperoxides, acrylonitrile, cadmium, iodoacetamide, chloroacetamide and sodium vanadate are also reported to exhibit hepatotoxicity due to lipid peroxidation.

Disruption of calcium homeostasis: Calcium is involved in a wide variety of critical physiological functions. Calcium homeostasis is very precisely regulated in the cell. Cytosolic free calcium is maintained at relatively lower concentration. The calcium concentration gradient between the inside of the cell [10⁻⁷M] and the extracellular fluid [10⁻³M] is maintained by an active membrane-associated calcium and magnesium effluxing adenosine triphosphatase [ATPase] enzyme system which is an important potential target for toxicants. Chemically induced hepatotoxicity may lead to the disruption of calcium homeostasis [67,68]. Non-specific increases in permeability of the plasma membrane, mitochondrial membrane and membranes of smooth endoplasmic reticulum lead to disruption of calcium homeostasis by increasing intracellular calcium. Decline in available NADPH, a cofactor required by calcium pump may also disrupt calcium homeostasis. Disruption of calcium homeostasis may result in the activation of many membrane damaging enzymes like ATPases, phospholipases, proteases and endonucleases, disruption of mitochondrial metabolism and ATP synthesis and damage of microfilaments used to support cell structure. Quinines, peroxides, acetaminophen, iron and cadmium are some of the hepatotoxics showing this mechanism.

Biochemical Markers

The hepatotoxins produce a wide variety of clinical and histopathological indicators of hepatic injury. Liver injury can be diagnosed by certain biochemical markers like alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP] and bilirubin. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function. An elevation in transaminase levels in conjunction with a rise in bilirubin level to more than double its normal upper level, is considered as an ominous marker for hepatotoxicity [69]. Macroscopic and in particular histopathological observations and investigation of additional clinical biochemistry parameters allows confirmation of hepatotoxicity.

Hepatotoxicity can be characterized into two main groups, each with a different mechanism of injury: hepatocellular and cholestatic [1]. Hepatocellular or cytolytic injury involves predominantly initial
serum aminotransferase level elevations, usually preceding increases in total bilirubin levels and modest increases in alkaline phosphatase levels. Such injury is attributable to drugs like acetaminophen, allopurinol, amiodarone, diclofenac, isoniazid, ketoconazole, methotrexate, nevirapine, nonsteroidal antiinflammatory drugs, pyrazinamide, rifampicin, retonavir, statins, tetracyclines, trazodone, troglitazone and valproic acid [1,8]. Cholestatic injury is characterized by predominantly initial alkaline phosphatase level elevations that precede or are relatively more prominent than increases in the levels of serum aminotransferases. Such injury is associated with amoxicillin-clavulanic acid, anabolic steroids, chlorpromazine, erythromycins, estrogens, phenothiazines or tricyclics [1,8]. Generally mixed type of injuries, involving both hepatocellular and cholestatic mechanisms, occurs [70]. Azathioprine, captopril, clindamycin, ibuprofen, nitrofurantoin, phenobarbital, phenytoin, sulfonamides and verapamil are associated with causing mixed pattern liver injury [1,8,43]. The ratio ALT: ALP plays an important role in deciding the type of liver damage by hepatotoxins. The ratio is greater than or equal to five during hepatocellular damage while the ratio is less than or equal to two during cholestatic liver damage. During mixed type of liver damage, the ratio ranges between two and five. ALT and AST or in combination with total bilirubin are primarily recommended for the assessment of hepatocellular injury in rodents and non-rodents in non-clinical studies. ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST.

**Clinical Biochemistry**

The measurement of levels of substances that may be present in the blood helps in the initial detection of hepatotoxicity (Figure 3). The estimation of serum bilirubin, urine bilirubin and urobilinogen helps in knowing the capacity of liver to transport organic anions and to metabolize drugs or xenobiotics. Several enzymes that trigger important chemical reactions in the body are produced in the liver and are normally found within the cells of the liver. However, if the liver is damaged or injured, the liver enzymes spill into the blood, causing elevated liver enzyme levels. The liver enzymes like transaminases, alkaline phosphatase, γ-glutamyl transpeptidase, sorbitol dehydrogenase, glutamate dehydrogenase and lactate dehydrogenase in the blood can be measured to know the normal functioning of liver. These enzymes help in detecting injury to hepatocytes. In case of patients showing hepatotoxicity with elevated liver enzymes due to certain hepatotoxicant, the enzymes levels usually return to normal within weeks or months after stopping the exposure to the hepatotoxicant which is suspected of causing the problem. Another measurable liver function is reflected in the albumin concentration, total protein and the prothrombin time which are the markers of liver biosynthetic capacity. Biochemical markers involved in hepatotoxicity in blood plasma and serum are listed in Table 1.

**Alanine aminotransferases - the standard clinical biomarker of hepatotoxicity**

Alanine aminotransferase or serum glutamic pyruvic transaminase (SGPT) activity is the most frequently relied biomarker of hepatotoxicity. It is a liver enzyme that plays an important role in amino acid metabolism and gluconeogenesis. It catalyzes the reductive transfer of an amino group from alanine to a-ketoglutarate to yield glutamate and pyruvate. Normal levels are in the range of 5-50 U/L. Elevated level of this enzyme is released during liver damage. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver [71,72,73]. However, lower enzymatic activities are also found in skeletal muscles and heart tissue. This enzyme detects hepatocellular necrosis.

![Clinical manifestations of hepatotoxicity](image-url)
Aspartate aminotransferases

Aspartate aminotransferases or serum glutamic oxaloacetate transaminase [SGOT] is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Besides liver, it is also found in other organs like heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level [74]. Normal levels are in the range of 7-40 U/L. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury [75] as it can also signify abnormalities in heart, muscle, brain or kidney [71,72]. The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage [74].

Alkaline phosphatase- An additional conventional biomarker supplementing ALT activity

Alkaline phosphatase is a hydrolase enzyme that is eliminated in the bile. It hydrolyzes monophosphates at an alkaline pH. It is particularly present in the cells which line the biliary ducts of the liver. It is also found in other organs including bone, placenta, kidney and intestine. Several isozymes have been identified in humans and preclinical species. Normal levels are in the range of 20-120U/L. It may be elevated if bile excretion is inhibited by liver damage. Hepatotoxicity leads to elevation of the normal values due to the body’s inability to excrete it through bile due to the congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the
pancreas that empty into the duodenum [small intestine]. Increase in alkaline phosphatase and/or bilirubin with little or no increase in ALT is primarily a biomarker of hepatobiliary effects and cholestasis [4,76]. In humans, increased ALP levels have been associated with drug-induced cholestasis [77].

**γ-Glutamyl transferase- A specific biomarker of hepatobiliary injury**

**γ-Glutamyl transferase [GGT] or transpeptidase [GGTP]** is an enzyme which is found in liver, kidney and pancreatic tissues, the enzyme concentration being low in liver as compared to kidney [75]. It catalyzes transfer of γ-glutamyl groups to amino acids and short peptides. It is more useful clinically when compared to ALP. ALP is more sensitive but much less specific than GGT. The comparison of the two enzymes helps in determining the occurrence of bone or liver injury. Normal GGT level with an elevated ALP level is suggestive of bone disease as GGT is not found in bone [78] while an elevated level of both the enzymes is suggestive of liver or bile duct disease. Normal levels are in the range of 0-51 U/L. GGT is a specific biomarker of hepatobiliary injury, especially cholestasis and biliary effects [79]. It was reported as a specific indicator of bile duct lesions in the rat liver [80].

**Total bilirubin levels- Another biomarker of hepatobiliary injury**

Bilirubin is an endogenous anion derived from the regular degradation of haemoglobin from the red blood cells and excreted from the liver in the bile. It is a chemical normally present in the blood in small amounts and used by the liver to produce bile. Normal bilirubin levels in the blood range between 0.2 to 1.2 mg/dL. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a build-up of bilirubin in the blood and extracellular [outside the cells] fluid. Serum bilirubin could be elevated if the serum albumin increases and the bilirubin shifts from tissue sites to circulation. Increased levels of bilirubin may also result due to decreased hepatic clearance and lead to jaundice and other hepatotoxicity symptoms [4]. Increase in bilirubin with little or no increase in ALT indicates cholestasis. In acute human hepatic injury, total bilirubin can be a better indicator of disease severity compared to ALT [81].

Bilirubin is measured as total bilirubin and direct bilirubin. Total bilirubin is a measurement of all the bilirubin in the blood while direct bilirubin is a measurement of a water-soluble conjugated form of bilirubin made in the liver and its normal range is 0-0.3 mg/dL. Indirect bilirubin is calculated by the difference of the total and direct bilirubin and is a measure of unconjugated fraction of bilirubin.

**Urine bilirubin level**

Bilirubin itself is not soluble in water and is tightly bound to albumin and thus does not appear in urine. Under normal circumstances, a tiny amount of bilirubin is excreted in the urine. If the liver’s function is impaired or when biliary drainage is blocked, some of the conjugated bilirubin leaks out of the hepatocytes and appears in the urine, turning it dark amber. The presence of urine bilirubin indicates hepatobiliary disease [82].

**Urobilinogen level**

Hepatotoxicity may lead to an increase in the urobilinogen in urine. Increased urobilinogen has been observed during alcoholic liver damage, viral hepatitis and hemolysis [82]. Urobilinogen is a by-product of hemoglobin breakdown. It is produced in the intestinal tract as a result of the action of bacteria on bilirubin. Almost half of the urobilinogen produced recirculates through the liver and then returns to the intestines through the bile duct. Urobilinogen is then excreted in the faeces where it is converted to urobilin. As the urobilinogen circulates in the blood to the liver, a portion of it bypasses the liver and is diverted to the kidneys and appears as urinary urobilinogen. Normal urobilinogen level in urine is <1 mg/dL. Increased breakdown of red blood cells may lead to an increase in unconjugated bilirubin which undergoes conjugation, excretion in bile and metabolism to urobilinogen in intestines. More urobilinogen is reabsorbed and passed to the liver and urine thus resulting in higher level of urine urobilinogen. Low level of urine urobilinogen may be observed during obstruction of bilirubin passage into the gut or failure of urobilinogen production in the gut. Comparing the urinary bilirubin result with the urobilinogen result may assist in distinguishing between red blood cell hemolysis, hepatic disease, and biliary obstruction. Urobilinogen is increased in hemolytic disease and urine bilirubin is negative. Urobilinogen is increased in hepatic disease, and urine bilirubin may be positive or negative. Urobilinogen is low with biliary obstruction, and urine bilirubin is positive.

**Bile acids**

Bile acids are involved in many functions. They aid in the catabolism and elimination of cholesterol, regulate pancreatic secretions and contribute to the digestion and absorption of fat in the small intestine. Serum bile acid levels can be influenced by diet and fasting. Bile acids are produced from cholesterol in the liver and are stored in the gall bladder. Gall bladder contraction with feeding releases bile acids into the intestine. They are absorbed in the intestine and taken up by hepatocytes for re-excretion into bile. Measurement of bile acid concentrations is a good indicator of hepatobiliary function. The bile acids are elevated with liver injury [83,84].

**Prothrombin time**

The prothrombin time or protime [PT] is also used to differentiate between a normal and damaged liver as it evaluates the functioning of blood clotting factors that are proteins made by the liver. During hepatotoxicity, the blood clotting factors are not produced normally due to liver cell damage or bile flow obstruction and prolongation of more than 2 seconds is considered abnormal [82]. The normal range of PT is 9 to 11 seconds.

**Lactate dehydrogenase**

Lactate dehydrogenase [LDH] assists in energy production. It catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+. Normal levels are in the range of 100-220U/L. Elevated levels of this enzyme is released from damaged cells in many areas of the body, including the liver. It also helps in detecting hepatocellular necrosis [82].

**Sorbitol dehydrogenase**

Sorbitol dehydrogenase [SDH] catalyzes the reversible oxidation-reduction of sorbitol, fructose and NADH. It is primarily found in the cytoplasm and mitochondria of liver, kidney and seminal vesicles. It is a specific marker of acute hepatocellular injury [75] that functions across preclinical species like rodents, rhesus monkey and beagle dogs. Normal levels in the plasma are in the range of 1-3 U/L.

**Glutamate dehydrogenase**

Glutamate dehydrogenase [GLDH] is an enzyme that is involved
in oxidative deamination of glutamate. It is present primarily in liver with lesser amount in kidney. GLDH activity is more liver specific than transaminases and is not substantially affected by skeletal muscle damage [75]. Normal range of GLDH activity is reported to be 7-11 U/L in men and 5-6.4 U/L in women. Its activity increases with hepatocellular damage [85]. In rats, the elevations of GLDH activity reported were of much greater magnitude and persisted longer after treatment with different hepatotoxicants as compared to that of ALT [85].

**Albumin**

It is the main protein in blood and is made by the liver. Hepatotoxicity leads to decrease in albumin production [82]. Its normal range is 3.4-5.4 g/dL. It can be used as a supplementary test for hepatic biosynthetic functions.

**Total protein**

The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins like albumins and globulins are produced in the liver [82]. Normal range of total protein is 6.0 to 8.3 g/dL. Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury.

**Serum F protein or 4-hydroxyphenylpyruvate dioxygenase**

Serum F protein or 4-hydroxyphenylpyruvate dioxygenase [HPD] is a key enzyme involved in tyrosine catabolism [86]. It is produced in large amounts in liver and small amounts in kidney while circulating at low serum concentrations [0.08±0.03 U/L] in normal human subjects. There are reports showing elevations in the serum F protein of patients with hepatocellular damage [87]. They reported that the serum F protein concentration was a more sensitive and specific marker of liver damage than conventional liver function tests involving AST, ALP and GGT activities and showed a close correlation with the histological assessment of liver damage. Serum F protein is suggested as an indicator of hepatocellular dysfunction associated with anticonvulsant therapy [88]. Serum F protein is produced across a wide variety of mammalian species [89]. The correlation between elevated serum F protein and liver histopathological alterations has not yet been fully elucidated in preclinical animal models [90].

**Glutathione-S-transferase alpha**

Glutathione-S-transferase [GST] is an inducible phase II detoxification enzymes that catalyze the conjugation of glutathione with reactive metabolites formed during phase I of metabolism [75]. Induction of GST synthesis is a protective mechanism that occurs in response to xenobiotic exposure. It is released quickly and in large quantities into the bloodstream during hepatocellular injury and the elevations in its activity are more rapid than AST or ALT. Four isozymes of GST, namely, alpha, pi, mu and theta, are expressed in human and other mammals [75]. GST alpha expression is restricted to liver and kidney. Muscle necrosis is not associated with changes in serum GST alpha levels indicating that this marker may be useful in differentiating liver injury from muscle injury [75]. Normal level of GST alpha in human plasma is <4.0 µg/L. Marked hepatotoxicity and GST alpha elevations corresponding to liver histopathological findings were induced in rats by a single dose of α-naphthylisothiocyanate, bromobenzene and thioacetamide [91]. Much greater fold increases of GST alpha were observed for each compound than either serum ALT or AST activities. However, the elevation was less than those observed with GLDH, SDH or total bilirubin and bile acids. GST alpha elevations preceded histopathological necrosis in a study monitoring valproic acid induced hepatotoxicity in rats [92].

**Arginase I**

Arginase is a hydrolase that catalyzes the catabolism of arginine to urea and ornithine. There are two isozymes of arginase, cytoplasmic arginase I and mitochondrial arginase II, encoded by two different genes. Arginase I is highly liver specific compared to the other liver enzymes [93,94]. Normal value of arginase I in plasma of healthy humans is 1.8 to 30 ng/mL which increase approximately ten-folds during liver injury. Arginase I showed the earliest and the greatest increase in serum levels as compared to ALT and AST activities in thioacetamide [TAA]-induced acute and chronic histopathological injury in rats [95]. Arginase I was evaluated as a more specific test of liver function compared to traditional serum markers in humans after liver transplantation [94].

**Malate dehydrogenase**

Malate dehydrogenase [MDH] is an enzyme in the citric acid cycle that catalyzes the reversible conversion of malate into oxaloacetate utilizing NAD^+. The absolute activity in the cytoplasm is greatest in liver followed by heart, skeletal muscle and brain [96]. MDH is also a periporal enzyme that is released into the serum indicating tissue damage. Normal value of MDH in healthy human plasma is 23.5-47.7 U/L. MDH activity was utilized as a biochemical index of acetaminophen induced liver injury that coincided with histological evidence of necrosis in rats [97]. Elevations in MDH activity was found to correlate with morphological changes after dosing with thioacetamide, dimethylnitosamine and diethanolamine [98]. The measurements of MDH activity were reported to be more useful in estimating the severity of liver injury than similar AST measurements [99]. Ozer et al. [75] suggested that MDH can be utilized as a novel enzymatic serum liver biomarker.

**Purine nucleoside phosphorylase**

Purine nucleoside phosphorylase [PNP] is a key enzyme involved in the purine salvage pathway that reversibly catalyzes the phosphorolysis of nucleosides to their respective bases and corresponding 1-[deoxy]-ribose phosphate. Mammalian PNP enzyme is found in a number of tissues including liver, muscle and heart. Maximum PNP activity has been reported in rat liver with very less activity in heart and muscle [100]. The enzyme is reported to be released into hepatic sinusoids during necrosis [75]. Elevations in serum PNP activity were observed in rats after dosing with galactosamine [101]. Cellular necrosis and endothelial cell damage in hepatic sinusoids also showed increase in serum PNP activity. Ozer et al. [75] suggested that PNP can also be utilized as a novel enzymatic serum liver biomarker. Normal level of PNP in healthy human plasma is 3.2±1.4 U/L.

**Paraoxonase 1**

Paraoxonase 1 [PON1] is a calcium dependent esterase that is associated with high-density lipoprotein. It is involved in the detoxification of organophosphates in the liver. PON1 is recognized as an antioxidant enzyme and protects low-density lipoproteins from oxidative modifications. It is not a leakage enzyme and is released into normal circulation. PON1 is produced primarily in the liver. It also shows activity in other tissues like kidney, brain and lungs. Normal serum PON1 level in healthy candidates is 53-186 kU/L. Decreases in serum PON1 activity are indicative of tissue damage in liver which may be due to a reduction in PON1 synthesis and secretion by the liver [102]. PON1 activity does not appear to show high specificity for liver damage as it is linked to a number of disease conditions like atherosclerosis, vasculitis and chronic hepatic damage. However, Ozer
et al. [75] recommended its evaluation as a down regulated marker in hepatic injury.

Hepatotoxictants

Hepatotoxicity can be caused by a wide variety of pharmaceutical agents, natural products, chemicals or environmental pollutants and dietary constituents (Table 2 data included as supplementary). Exposure to these hepatotoxic agents is usually accidental, through contaminated food, water or air or from unanticipated side effects of therapeutic agents. Toxic liver diseases caused by such agents are often recognized late because their hepatotoxic potency is considered to be minimal or non-existent. Hepatotoxicity is reversible at early stages upon cessation of exposure to the toxicant. However, severe intoxication with hepatotoxic agents can lead to liver necrosis and death of the organism if left untreated.

Drugs

Drug-induced hepatotoxicity is the most important cause of acute liver failure in many countries [5,43,63]. Almost all drugs are identified as foreign substances by the body which subjects them to various biochemical transformations involving reduction of fat solubility and change of biological activity, to make them suitable for elimination [19]. Adverse drug reactions [ADRs] can be considered as Type A reactions [predictable or high incidence or pharmacological] or Type B reactions [unpredictable or low incidence or idiosyncratic; IADRs]. Type A reactions are dose-dependent and occur in a relatively consistent time-frame. All individuals are susceptible to Type A reactions which are generally a result of direct liver toxicity of the parent drug or its metabolites [103] eg acetaminophen-induced hepatotoxicity [104] or phenytoin-induced hepatotoxicity [105]. Type B reactions are unrelated to the pharmacological action of the drug [106]. They occur in a minority of individuals and occur at doses that do not cause toxicity in most individuals. They have variable latency period. Further, they have not been reproducible in animal models [5,43] eg troglitazone-induced hepatotoxicity [5] or isoniazid-induced hepatotoxicity [10]. Some of the medications causing hepatotoxicity as a potential side effect are listed below:

Anaesthesia [Halothane]: Halothane causes idiosyncratic liver toxicity by forming a reactive trifluoroacetyl chloride reactive metabolite by cytochrome P450 and suggests an immune-mediated reaction [42]. This unstable toxic metabolite binds to liver proteins causing cellular injury. Clinical investigations reveal elevated transaminases compatible with hepatitis. Patients are found to develop autoantigens and antibodies against trifluoroacetylated protein. An immune response to the oxidative metabolite of halothane can be induced in guinea pigs but no clinical toxicity was observed. The immune response did not escalate with repeated exposures suggesting the development of immune tolerance [107]. The toxicity induced in rats involves the formation of a free radical by a reducing pathway rather than trifluoroacetyl chloride by an oxidative pathway. It did not reveal the characteristics of an immune response similar to the liver toxicity observed in humans [108].

Aniline analgesics [Acetaminophen]: Acetaminophen or paracetamol is usually well-tolerated in prescribed dose but overdose is the most common cause of drug-induced hepatotoxicity worldwide. Damage to the liver is not due to the drug itself but to a toxic metabolite, NABQI, which is produced by cytochrome P-450 enzymes in the liver [61]. This metabolite is highly reactive and depletes glutathione. In normal circumstances, this metabolite is detoxified by conjugating with glutathione in phase II reaction. However, during overdose, a large amount of the toxic metabolite is generated which overwhelms the detoxification process and leads to liver cell death and hepatocellular necrosis. Administration of acetylcysteine, a precursor of glutathione, can limit the severity of the liver damage by capturing the toxic metabolite [109]. Hydroalcoholic extract of Aerva lanata has been reported to possess hepatoprotective activity against paracetamol induced hepatotoxicity in rats [110].

Aniline antibiotics [Sulfonamides]: Sulfonamides are aromatic amines associated with a wide range of adverse reactions including hepatotoxicity. Higher incidence of hepatotoxicity was observed in patients with advanced HIV infection which was probably caused by increasing the oxidation to toxic metabolites by the P 450 system [1,111]. Similar symptoms were observed in dogs and humans [112]. Larger breeds especially Dobermans, were at higher risk than small breeds. But the ethical and practical issues involved in experimentation with large-dog breeds, limit the practical usefulness of this animal model.

Anticoagulants: Oral anticoagulants like warfarin, ximelagatran, enoxaparin, acenocoumarin, phenprocoumon and heparin are being used for prevention of stroke and venous thromboembolism [113]. Anticoagulants induced hepatotoxicity has been found to be associated with asymptomatic elevation of serum transaminases, clinically significant hepatitis and fatal liver failure. Elevation of alkaline phosphatase was reported with dabigatran, ximelagatran and warfarin. Jaundice was reported only with ximelagatran and warfarin [113]. Heparin hepatotoxicity involved direct toxicity, hepatocyte membrane modification and immune-mediated hypersensitivity reaction [114]. Phenprocoumon hepatotoxicity caused direct damage of hepatocytes by reactive metabolites which resulted in augmented antigenicity and consequent immunogenic reaction. It also involved high energy reactions involving cytochrome P-450 enzymes, causing decline of adenosine triphosphate levels, loss of ionic gradients, cell swelling and rupture [115].

Anticonvulsants or antiepileptic drugs: Some of the anticonvulsants may give rise to hepatotoxicity. Chloral hydrate, clonazepam, diazepam, primidone and sultiam are not considered to induce serious liver disease. Sodium valproate is an effective anticonvulsant involving less risk of hepatotoxicity [116]. Valproate is transformed to valproyl adenosine monophosphate and valproyl coenzyme A in the mitochondrial matrix. The valproate induced depletion of coenzyme A affects the mitochondrial pool of this cofactor and thus impairs mitochondrial enzymes involved in β-oxidation of fatty acids [42]. Patients who take phenytoin often have transaminase elevation up to three times the upper limit of normal (ULN) but liver biopsies do not reveal significant pathology [117]. The usage of felbamate was markedly reduced because of its association with aplastic anemia and hepatotoxicity in some patients [5]. Phenobarbital is rarely known to cause hepatic damage including hepatocellular and cholestatic liver injury and also hypersensitivity reaction.

Anti-hyperlipidemic drugs: The pattern of injury from anti-hyperlipidemics is typically hepatocellular or mixed in nature with rare instances of pure cholestatic hepatitis [118, 119]. Atorvastatin and lovastatin-related hepatotoxicity has been associated with a mixed pattern of liver injury typically occurring several months after the initiation of the medication [120]. Simvastatin hepatotoxicity is hypothesized to occur due to drug-drug interactions [121]. Provastatin has been reported to cause acute intrahepatic cholestasis [122]. Fenofibrate may very rarely instigate an autoimmune hepatitis type reaction especially when taken in combination with statin medications.
[123]. Ezetimibe that lowers cholesterol by inhibiting its intestinal absorption at the brush border of the small intestine rarely causes hepatotoxicity in the form of severe cholestatic hepatitis and acute autoimmune hepatitis [124].

**Antimalarial drugs:** Antimalarial drugs like amodiaquine can cause hepatotoxicity in humans by oxidation to a reactive metabolite, iminoquinone, by liver microsomes and peroxidases [60]. The reactive metabolites can irreversibly bind to proteins which lead to direct toxicity by disrupting the cell function. Such patients were found to have antidrug IgG antibodies [125]. Amodiaquine can induce immune response in rats analogous to that in humans but it is not sufficient to result in clinical toxicity [126].

**Anti-tuberculosis:** There are reports regarding the hepatotoxic effects of three classes of anti-tuberculosis drugs, namely, nucleoside reverse transcriptase inhibitors [NRTIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs] and protease inhibitors [PIs] [48]. They may lead to hepatotoxicity by different mechanisms, namely, mitochondrial damage by nucleoside analogs like didanosine and stavudine, hypersensitivity reactions by nevirapine, efavirenz, or abacavir, direct liver injury by using full doses of ritonavir and immune reconstitution phenomena, mainly in severely immunosuppressed patients with underlying chronic hepatitis B virus [HBV]. Hypersensitivity reactions are the most common with anti-tuberculosis drugs [184]. Nucleoside analogs [NRTIs], especially zidovudine [zidovudine], and retrovir [didanosine], are associated with lactic acidosis and hepatic steatosis [46]. Steatohepatitis accelerates the progression of liver fibrosis in patients with chronic hepatitis C virus [HCV] infection. NNRTIs, especially viramune [nevirapine] are associated with hepatitis and hepatic necrosis. They cause liver damage by hypersensitivity reactions or by direct toxic effects. Nevirapine is more hepatotoxic than efavirenz [127]. The presence of underlying chronic HCV infection enhances the risk of developing liver enzyme elevations. Most protease inhibitors have been associated with episodes of liver toxicity, with lopinavir/low-dose ritonavir, fosamprenavir/low-dose ritonavir and nelfinavir being less hepatotoxic [128] and tipranavir/low-dose ritonavir most hepatotoxic [129]. Low-dose ritonavir used as booster for other protease inhibitors does not cause hepatotoxicity. Patients with chronic HCV infection have an increased risk of liver enzyme elevations following exposure to most antiretroviral drugs. The management of hepatotoxicity should be based on the knowledge of the mechanisms involved for each drug. Treatment of HCV infection may reduce the chances for further development of liver toxicity in these patients.

**Anti-tuberculosis drugs:** Anti-tuberculosis drug-induced hepatotoxicity [ATDH] is a serious problem and main cause of treatment interruption and change in treatment regimen during tuberculosis treatment course [130]. ATDH causes substantial morbidity and mortality. Asymptomatic transaminase elevations are common during anti-tuberculosis treatment but hepatotoxicity can be fatal when not recognized early and when therapy is not interrupted in time. Anti-tuberculosis drugs like isoniazid, rifampicin and pyrazinamide have been found to be potentially hepatotoxic [130]. There has been a report of ethambutol-induced liver cholestatic jaundice, with unclear circumstances. The risk of anti-tuberculosis drug-induced hepatotoxicity has been found to increase by various factors like high alcohol intake, older age, pre-existing chronic liver disease, chronic viral infection, advanced TB, female sex, concomitant administration of hepatotoxic drugs, inappropriate use of drugs and nutritional status [19, 130]. Anti-tuberculosis drug-induced hepatotoxicity has been defined as a treatment-emergent increase in serum alanine aminotransferase or aspartate aminotransferase greater than three or five times of the ULN, with or without symptoms of hepatitis and/or jaundice, respectively [4]. Detoxification of drugs and metabolites are related to the activities of liver enzymes. Polymorphism of these enzymes can cause variation of hepatotoxicity by anti-tuberculosis drugs [8]. The exact mechanism of ATDH is still unknown. Most anti-tuberculosis drugs are liposoluble and they are transformed into water soluble compounds by hepatic phase I and phase II biotransformation enzymes.

Isoniazid-induced hepatotoxicity is considered idiosyncratic i.e. reactive toxic metabolites [hydroxide, mono acetylhydrazide] rather than the parent drug are responsible for hepatotoxicity [62,63]. No animal model has been able to reproduce the characteristics of isoniazid-induced hepatotoxicity in humans [10]. A much more rapid onset of toxicity was observed in rabbits treated with isoniazid at 3-hour intervals for 2 days [62]. Some of the animals showed increased levels of transaminases that peaked at 36 hours as well as focal areas of liver necrosis. The mechanism of rifampicin-induced hepatotoxicity is unknown and there is no evidence for the presence of a toxic metabolite [131]. The combined use of rifampicin and isoniazid has been associated with an increased risk of hepatotoxicity [132]. Rifampicin induces isoniazid hydrolyse, increasing hydrazine production when rifampicin is combined with isoniazid thus explaining the higher toxicity of the combination. The mechanism of pyrazinamide-induced hepatotoxicity is also unknown. It is not clear whether hepatotoxicity is caused by pyrazinamide or its metabolites. In a rat study, pyrazinamide inhibited the activity of several cytochrome P450 isoenzymes [133] but a study in human liver microsomes showed that it has no inhibitory effect on the cytochrome P450 isoenzymes [134]. A hepatoprotective effect of N-acetylcysteine [135] and silymarin [136] on ATDH has been shown in rats.

**Arthritis medications:** It is not considered common but when it occurs it can be potentially serious. In patients treated for rheumatoid arthritis with methotrexate, microscopic evidence of liver injury has been found for any transaminase elevation above the ULN [1,137].

**Chemotherapy:** Chemotherapy uses toxic chemicals or drugs like tyrosine kinase inhibitors, alkylating agents, antimetabolites, antitumor antibiotics, platinum, biologic response modifiers and androgens to destroy cancer cells [52,138]. But during treatment, if the toxins build up in the body faster than the liver can process them, hepatotoxicity may occur [139]. Chemotherapeutic agents alone or in combination may cause hypersensitivity reactions or direct hepatic toxicity [52].

**Corticosteroids or glucocorticoids and anabolic androgenic steroids:** Glucocorticoids promote glycosogen storage in the liver. An enlarged liver is a rare side effect of long-term steroid use in children [140]. Steatosis may be observed both in adult and children upon prolonged use [141]. Anabolic androgenic steroids being marketed as dietary supplements are a cause for serious hepatotoxicity [4,142].

**Non-steroidal anti-inflammatory drugs [NSAIDs]:** Hepatotoxic effects of non-steroidal anti-inflammatory drugs like acetylsalicylic acid range from asymptomatic elevations of serum transaminases and alkaline phosphatase to acute cytolytic, cholestatic or mixed hepatitis. Increases in serum transaminases and alkaline phosphatase are useful parameters to monitor as early warning sign. In more severe cases, there may be accompanying signs and symptoms of anorexia, nausea, vomiting, abdominal pain, weakness and jaundice besides increases in bilirubin and prothrombin time. Little is known about the mechanism of NSAID-induced hepatotoxicity. Both dose-dependent and idiosyncratic reactions have been documented [143]. Two main mechanisms are considered responsible for injury, hypersensitivity...
and metabolic aberration. Hypersensitivity reactions often have significant anti-nuclear factor or anti-smooth muscle antibody titres, lymphadenopathy and eosinophilia. Metabolic aberrations can occur as genetic polymorphisms and alter susceptibility to a wide range of drugs [144]. Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity. Ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin are associated with idiosyncratic reaction. The clinical and biochemical features of diclofenac hepatotoxicity in humans and rats relates both to impairment of ATP synthesis by mitochondria, and to production of active metabolites, particularly N,N-dihydroxydiclofenac, which causes direct cytotoxicity. Mitochondrial permeability transition (MPT) has also been shown to be important in diclofenac-induced liver injury, resulting in generation of reactive oxygen species, mitochondrial swelling and oxidation of NADP and protein thiols [144].

Alcohol-induced hepatotoxicity
Excessive consumption of alcohol leads to hepatotoxicity which is a major health care problem worldwide [145]. Oxidative stress may play a major role in the ethanol-mediated hepatotoxicity. It induces cytochrome P450 which promotes metabolism of ethanol itself, acetaminophen and others. Ethanol metabolism yields acetaldehyde which contributes to glutathione depletion, protein conjugation, free radical generation and lipid peroxidation [4]. Findings have also demonstrated that ethanol feeding impairs several of the multiple steps in methionine metabolism that leads to progressive liver injury. Ethanol consumption has been reported to predominantly inhibit the activity of a vital cellular enzyme, methionine synthase, involved in remethylating homocysteine. In some species, ethanol can also increase the activity of the enzyme, betaine homocysteine methyltransferase which catalyzes an alternate pathway in methionine metabolism by utilizing hepatic betaine to remethylate homocysteine and form methionine and maintain levels of S-adenosylmethionine, the key methylating agent. Under extended periods of ethanol feeding, however, this alternate pathway cannot be maintained. This results in a decrease in the hepatocyte level of S-adenosylmethionine and increases in two toxic metabolites, S-adenosyl homocysteine and homocysteine. Betaine has been reported to have a protective effect against the clinical problems caused by ethanol-induced vitamin A depletion and peroxidative injury in a variety of experimental models of liver disease [146,147,148]. Betaine, by virtue of aiding in the remethylation of homocysteine, removes both toxic metabolites [homocysteine and S-adenosylhomocysteine], restores S-adenosylmethionine level, reverses steatosis, prevents apoptosis and reduces both damaged protein accumulation and oxidative stress.

Natural products
There are two groups of toxicologically different compounds, amatoxins and phalloitoxins in the hepatotoxic mushrooms of the Amanita species, primarily of Amanita phalloides [149]. Reports exist for the toxicity cases of exposure to Amanita bisporigera [150]. They are cyclopeptides containing a tryptophan residue substituted at position 2 of the indole ring by a sulfur atom which is critical for their toxicity [151]. α-Amanitin is a powerful natural hepatotoxin that belongs to the amatoxins isolated from deadly poisonous Amanita phalloides mushroom. The basic molecular mechanism of their toxicity was attributed to inhibition of RNA polymerase II of the eukaryotic cells [152]. Earlier, in vitro experiments demonstrated that α-Amanitin could act either as an antioxidant or as a prooxidant depending on the treatment conditions and toxin concentration [151,153]. Zhelueva et al. [66] have hypothesized that a peroxidative process [free radical reactions] in hepatocytes might be contributing to the severe α-Amanitin hepatotoxicity. At present, the most effective clinical antidote to acute Amanita phalloides mushroom poisoning is silybin, an antioxidant possessing free radical scavenger activity and inhibiting lipid peroxidation, stabilizing membrane structure and protecting enzymes under conditions of oxidative stress. The mushroom toxin, phalloidin binds to actin thus disrupting the cell cytoskeleton, resulting in increased plasma membrane permeability [57].

Likewise, aflatoxins, which are fungal toxins, cause both acute hepatotoxicity and liver carcinoma in exposed humans and animals [29,154,155]. They are produced by the fungi, Aspergillus flavus and A. parasiticus, which are common contaminants of grain foods. There have been several reports of acute aflatoxicosis resulting in death in humans [154]. They have also reported that bacterial lipopolysaccharide [LPS] enhances the acute hepatotoxicity of aflatoxins in rats by a mechanism that depends on tumour necrosis factor α (TNF α).

Toxic freshwater cyanobacteria are very common worldwide and have been responsible for animal [156,157] and human intoxications [158,159] due to the hepatopaneutaxin called microcystins as well as pentapeptide hepatotoxins called nodularins. Cyanobacteria [Microcystis aeruginosa, Anabaena spp., Anabaenopsis spp., Nostoc spp., Planktothrix spp., Hapalosiphon spp.] have been reported to commonly occur in natural lakes, reservoirs and large slow flowing rivers. Aquatic animals like edible molluscs, fish and crayfish may be killed by microcystins but in many cases the toxicity is sub lethal and so the animals can survive long enough to accumulate the toxins and transfer them along the food chain and pose a risk for human health [159]. These cyanobacterial toxins are reported to cause death by liver haemorrhage within a few hours of the acute doses in mouse bioassays [160]. The mammalian toxicity of microcystins and nodularins is mediated through their strong binding to key cellular enzymes called protein phosphatases [161,162]. Nagata et al. [163] have reported the protective effect of specific monoclonal antibodies on microcystin induced hepatotoxicity under both in vitro and in vivo conditions in mice.

Ecteinascidins [ETs] are marine natural products isolated from extracts of the tunicate, Ecteinascidia turbinata with potent cytotoxic activity [164,165]. The preclinical studies revealed hepatotoxicity in rats, the females being more susceptible than the male rats [166]. The studies of Reid et al. [167] did not predict major gender-dependent differences in the toxicity of ET743 based on metabolism. Fumonisins are a group of naturally occurring mycotoxins produced primarily by fungi, Fusarium verticillioides, F. moniliform and F. proliferatum, which frequently are found in corn. Experimental administration of fumonisins cause dose-dependent hepatotoxicity in all species including cattle, pigs, horses, primates, sheep, rabbits, swine and rats [168]. The backbone of the fumonisin molecule resembles that of the sphingoid bases, sphinganine and sphingosine, two important precursors of sphingolipids. Sphingolipids are essential components of cell membranes, and sphingoid bases play an important role in signal transduction. Fumonisin B1 inhibits sphinganine [sphingosine]-N-acetyltransferase, a critical enzyme in the biosynthesis of sphingolipids and this fumonisin-induced alteration in sphingolipid biosynthesis in endothelial cells lead to increased permeability of the cell layer [169]. He [170] reported that fumonisin B1 disrupts sphingolipid metabolism by inhibiting ceramide synthase and induces expression of cytokines including TNFα in liver leading to perturbation of cell signaling.

Phomopsin is a hexapeptide mycotoxin produced by the fungus, Phomopsis leptostromiformis which grows on lupins after autumn rains [171,172]. The most affected animals are sheep, cattle, horses and pigs.
Rubratoxin is a mycotoxin produced by the fungus, Penicillium rubrum and P. purpurogenum which is most commonly found on cereal grains. Symptoms vary depending on the degree of exposure and hence extent of the liver damage or injury. Symptoms may be acute, sub acute or chronic depending on the severity of the exposure. Factors such as age, race, gender, overall health and underlying liver problems may also influence a person’s risk of developing liver problems and the severity of the symptoms. The hepatotoxic substance from the cultures of P. rubrum produced toxic hepatitis and body hemorrhages in white mice, guinea pigs, rabbits, and dogs [173,174]. Neubert and Merker [175] described several biochemical and histological observations in hepatic cells of rats injected with this material.

Pentacyclic triterpenoids are the focus of attention for drug research for anti-cancer, anti-AIDS, antiinflammatory and antimicrobial activities. The toxic compounds of Lantana camara, lantadenes, are also pentacyclic triterpenoids [176,177]. Both ruminants like cattle, buffaloes, sheep and goats, and non-ruminant animals like rabbits, guinea pigs and female rats are susceptible to the hepatotoxic action of lantana toxins [178,179]. Likewise, a number of species of Eupatorium are toxic to grazing animals [180]. They are also reported to contain many bioactive constituents that can be exploited for drug discovery. Freeze-dried E. adenophorum powder is reported to cause hepatic injury in mice [181]. Katoch et al. [182] reported that freeze-dried E. adenophorum leaves caused hepatotoxicity and cholestasis in rats as evident by their observations on elevated bilirubin level, increase in the activities of plasma enzymes and hepatic lesions. The hepatotoxicant of E. adenophorum has been characterized as 9-oxo-10,11-dehydroageraphorone [ODA], a cadinene sesquiterpene [183]. High tannin concentration in American cranesbill, bayberry, bilberry, buckthorn, cola tree, lady's mantle, oaks, poplar, walnut, wild iris, quercus and rosemary are also a potential hepatotoxicant [184].

Industrial toxins

The rapidly increasing levels of environmental chemicals, especially heavy metals like mercury, lead and arsenic are matters of increasing concern. Several natural, industrial and anthropogenic processes have been implicated for their higher environmental levels in various parts of the world. Exposure to even low levels of these heavy metals is known to have potential hazardous effect in animals as well as humans [185]. Mercury intoxication has been a public health problem for many decades [186]. Mercury has been one of the most dramatic and best documented examples of bio-accumulation of toxins in the environment, particularly in the aquatic food chain. Ezeku et al. [187] showed that mercuric chloride is highly toxic to the liver functions in rats. They reported increase in bilirubin concentration thus indicating that bile is not being excreted and/or that too much hemoglobin is being destroyed and/or that the liver is not actively treating the hemoglobin, it is receiving and could therefore lead to jaundice. They also reported protective action of Zingiber officinalis on mercuric chloride induced hepatotoxicity. Toxicity of lead is closely related to its accumulation in many tissues inside the body and its interference with the bioelements that will hamper several physiological and biochemical processes [188]. In vivo studies in lead exposed animals and workers showed the generation of reactive oxygen species, stimulation of lipid peroxidation and decreased antioxidant defense system [189]. Elingera eliator has been found to have a powerful antioxidant effect against lead-induced hepatotoxicity [185]. Likewise, environmental exposure to arsenic also imposes a big health problem worldwide. Oxidative stress has been suggested as a contributory factor in the development of arsenic induced hepatotoxicity. The metal chelating effect of sinapic acid, a phenylpropanoid compound found in various herbs and high-blan cereals has been reported to possess a protective role against arsenic induced toxicity in rats [190].

Carbon tetrachloride is said to induce hepatotoxicity in rats, rabbits and humans after being metabolised to trichloromethyl free radical which causes peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes [36,37,191]. Trichloromethyl free radicals elicit lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. These events lead to liver damage by loss of cell membrane integrity. Based on the studies with isolated perfused rat liver, Masuda [59] suggested that covariant binding of carbon tetrachloride metabolites rather than lipid peroxidation has a significant role in the production of centrilobular necrosis following carbon tetrachloride administration. The ethanol extract of Spirulina laxisima West [Pseudanabaenaceae] has been found to have a protective effect against carbon tetrachloride-induced hepatotoxicities in rats [192]. Similarly, polyphenolic extracts from Ichnocarpus frutescens leaves have been found to have a protective effect on experimental hepatotoxicity of carbon tetrachloride [193]. Prakash et al. [194] have reported the hepatoprotective activity of leaves of Rhododendron arboreum in carbon tetrachloride induced hepatotoxicity in rats. They proposed that the flavonoids and phenolic compounds present in the leaves may have the potential hepatoprotective properties.

The hepatotoxicity of chloroform was reported to be due to phosphogene-mediated cellular glutathione depletion or increased amounts of covalent binding to hepatoceallar macromolecules [195,196].

1,1-Dichloroethylene is also reported as a remarkable hepatotoxic. It is more potent, faster acting and has a far more precipitous dose threshold for liver injury in the fasted rat than carbon tetrachloride or 1,2-dichloroethylene [197,198]. Animals with diminished glutathione levels are more vulnerable to liver injury by 1,1-dichloroethylene [199]. Various groups have reported the hepatotoxicity of various olefins including vinyl chloride, trichloroethylene and 1,1-dichloroethylene [200,201]. 5-Nitro-o-toluidine is reported to cause hepatocellular carcinogenicity in rats [202]. The compound when taken orally as a sweetener caused liver failure. Similarly, 4-nitro-2-aminotoluene, used as an artificial sweetener, has also shown liver toxicity. Hepatotoxic effects of various polybrominated biphenyls in rats have also been reported [203,204]. However, Schanbacher et al. [205] reported that no hepatotoxicity was observed in cattle by polybrominated biphenyls. Deng et al. [206] reported the toxic effects of di- and tri-nitro toluenes and amino-nitrotoluenes and proposed that the liver toxicity could be a secondary effect of primary hematological toxicities caused by these compounds. They found that hypoxia signalling could be an important pathway affected by the compounds.

Herbal and alternative remedies or dietary supplements

Since ancient times, many herbs are known to play an important role in the treatment of various ailments. Now-a-days, the consumption of herbal remedies in industrialised and developing countries is gaining popularity. These are generally recognised as safe and effective but some of these herbal remedies have been found to contain hepatotoxic constituents [39]. Very few herbal remedies have received adequate medical and scientific evaluation. Further, they may be contaminated with excessive amount of banned pesticides, microbial contaminants, heavy metals, chemical toxins adulteration with synthetic drugs [207,208,209]. The liver injury from herbal remedies.
has ranged from mild elevations of hepatic enzymes to fulminant liver failure requiring liver transplantation. Complete absence of potential idiosyncratic reactions in any herbal therapy cannot be guaranteed. Intake of herbal supplements can cause adverse effect on lives of people with normal functioning livers and no history of prior liver disease. New patterns of liver injury continue to emerge among known herbal hepatotoxins. The varied manifestations of liver injury include steatosis, acute and chronic hepatitis, hepatic fibrosis, zonal or diffuse hepatic necrosis, bile duct injury, veno-occlusive disease, acute liver failure requiring liver transplantation and carcinogenesis. Potential interactions between herbal medicines and conventional drugs may also interfere with patient management [210,211]. Pyrrolizidine alkaloids (PA) are found in many herbs belonging to Asteraceae and Boraginaceae families and their toxicity is well-documented [212,213]. Any herb containing pyrrolizidine alkaloids is potentially hepatotoxic. Hepatotoxicity due to PA can result from either small amounts ingested over long periods of time or from large amounts ingested over a short period of time. This hepatotoxicant has been found in approximately 350 different plant species. Some of the most toxic of these herbs containing PA are Tussilago farfara [Coltsfoot], Borago officinalis [Borage], Symphytum spp. [Comfrey], Eupatorium purpureum [Queen of the meadow], Petasites spp. [Butterbur], Senecio spp. [Lifefoot], Heliotropium and Crotalaria species. Pyrrolizidine poisoning is common in Africa and Jamaica, two areas of the world where herbal teas containing this substance are consumed as folk remedies for a number of ailments. PAs have been associated with a severe type of liver disorder known as veno-occlusive disease. This can result in abdominal pain, vomiting, ascites, hepatomegaly, edema, cirrhosis, liver failure, and even death due to extensive liver damage.

All preparations containing germander [Teucrium chamaedrys], as a weight-loss remedy, were prohibited for human use in France and Canada following the reports of several cases of hepatitis [214,215]. Furan-containing neoclerodane diterpenoids from germander have been found to show hepatotoxicity in rat hepatocytes [215].

Several reports of hepatotoxicity were made for Larrea tridentata [Chapparal], proclaimed to be an aging retardant [212]. It has been reported to cause jaundice, fulminant hepatitis, subacute hepatic necrosis, cholestatic hepatitis and acute liver failure [216,217]. It may cause liver injury by inhibition of cyclooxygenase or cytochrome P450 activity or through an immune-mediated response. Acorus spp. and Asarum spp. contain α-asarone, a volatile allylbenzene which can form a hepatotoxic epoxide metabolite when activated by hepatic microsomal enzymes [212]. Acute hepatitis was reported following intake of greater celandine [Chelidonium majus], widely used to treat gallstone disease and dyspepsia [44,218]. The hepatotoxicity caused by Kava [Piper methysticum rhizoma] is also well-documented [70,219,220]. Cinchicigua racemosa rhizome [black cohosh, root] is also reported to be hepatotoxic [221]. Margosa oil is reported to induce microvesicular steatosis [42]. Marijuana [Cannabis sativa] and hashish [Cannabis indica] commonly cause abnormalities of aspartate aminotransferase, alanine aminotransferase or alkaline phosphatase but serious hepatotoxicity has not been reported [42]. Cocaine can also cause ischemic necrosis of the liver.

Many Chinese herbal remedies like Ma-huang, an alkaloid derived from plants of the Ephedra species [222] and Sho-wu-pian have also been found to be hepatotoxic. Jin Bu Huan [Lycopodium serratum] typically used as an herbal sedative, has been reported to cause acute hepatitis [45]. It was reported to contain tetrahydrodopalmatine, an alkaloid that was used for alleviating pain and promoting sleep. Dictamnus dasycarpus and D. baixiangi, one of the most commonly used Chinese herbs for treatment of eczema, was also found as a potential culprit in the liver toxicity cases in England [223]. The herb has not shown up as a liver toxin in laboratory animal testing, and it is not reported in the medical literature from other countries as being suspected for causing adverse liver reactions.

### Treatment

The treatment of hepatotoxicity is dependent upon the causative agent, the degree of liver dysfunction and the age and general health of

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**Table 3. Common medicinal plants having hepatoprotective activity.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Active component(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Achillea millefolium</td>
<td>Caffeic acid</td>
<td>[226]</td>
</tr>
<tr>
<td>2</td>
<td>Andrographis paniculata</td>
<td>Andrographolide</td>
<td>[226,229]</td>
</tr>
<tr>
<td>3</td>
<td>Anoectochilus formosanus</td>
<td>Kinsenoside</td>
<td>[229]</td>
</tr>
<tr>
<td>4</td>
<td>Bacopa monniera</td>
<td>Bacoside A</td>
<td>[229]</td>
</tr>
<tr>
<td>5</td>
<td>Cassia tora</td>
<td>Ononitol monohydrate</td>
<td>[228]</td>
</tr>
<tr>
<td>6</td>
<td>Cassia fistula</td>
<td>Ethanolic extract</td>
<td>[226]</td>
</tr>
<tr>
<td>7</td>
<td>Cichorium intybus</td>
<td>Alcoholic extract, flavonoids</td>
<td>[227]</td>
</tr>
<tr>
<td>8</td>
<td>Colchicum autumnale</td>
<td>Cocchine</td>
<td>[229]</td>
</tr>
<tr>
<td>9</td>
<td>Curcuma longa</td>
<td>Curcumin</td>
<td>[228]</td>
</tr>
<tr>
<td>10</td>
<td>Eclipta alba</td>
<td>Ethanolic extract</td>
<td>[226,229]</td>
</tr>
<tr>
<td>11</td>
<td>Equisetum arvense</td>
<td>Oniline, Kaempferol-3-o-glucoside</td>
<td>[226]</td>
</tr>
<tr>
<td>12</td>
<td>Foeniculum vulgare</td>
<td>Essential oil</td>
<td>[228]</td>
</tr>
<tr>
<td>13</td>
<td>Garcinia mangostana</td>
<td>Gargicine</td>
<td>[228]</td>
</tr>
<tr>
<td>14</td>
<td>Glycyrrhiza glabra</td>
<td>Glycyrrhizin</td>
<td>[230]</td>
</tr>
<tr>
<td>15</td>
<td>Jatropha curcas</td>
<td>Methanolic extract</td>
<td>[228]</td>
</tr>
<tr>
<td>16</td>
<td>Phyllanthus amarus (Bhualamala)</td>
<td>Lignans, alkaloids, bioflavonoids</td>
<td>[226,229,230]</td>
</tr>
<tr>
<td>17</td>
<td>Picrorhiza kuruoa (Katuka)</td>
<td>Irridioid glycoside mixture (Picroliv)</td>
<td>[230]</td>
</tr>
<tr>
<td>18</td>
<td>Protonum hepatophyllum (Arnecega)</td>
<td>α- And β- Amyrin</td>
<td>[226]</td>
</tr>
<tr>
<td>19</td>
<td>Silkybum mariarnun (Milk thistle)</td>
<td>Flavonolignan (Silymarin)</td>
<td>[227]</td>
</tr>
<tr>
<td>20</td>
<td>Solanum nigrum (Makoi)</td>
<td>Aqueous extract</td>
<td>[227]</td>
</tr>
<tr>
<td>21</td>
<td>T. catappa (Jangli erandi)</td>
<td>Punicalagin and punicalin</td>
<td>[229]</td>
</tr>
<tr>
<td>22</td>
<td>Trigonella foenum graecum (Chandrika)</td>
<td>Polyphenolic extract</td>
<td>[228]</td>
</tr>
<tr>
<td>23</td>
<td>Wedelia calendulacea (Peela Bangra)</td>
<td>Alcoholic extract</td>
<td>[227]</td>
</tr>
</tbody>
</table>

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the individual. There is no effective treatment other than stopping the causative medication or removal from the exposure to the causative agent and providing general supportive care. The best way is to discontinue the use of any medicinal drug that may put excess stress on the liver and use an alternate medication that helps to diminish or manage the side effects of hepatotoxicity. Alternatively the dosage of current drug may be changed. Abstinence from alcohol use may also reduce the risk of hepatotoxicity. Prompt use of N-acetylcysteine after acetaminophen overdose [224] and intravenous carnitine for valproate-induced hepatotoxicity [225] has been reported for the treatment of acute liver injury. Diuretics or water-pills like furosemide and hydrochlorothiazide may also be prescribed as they work to prevent or treat fluid accumulation in the body. Cholestyramine and ursodeoxycholic acid may be used for alleviation of pruritus. Nutrient supplements like taurine, methionine, S-adenosylmethionine, arginine, polyenylphosphatidylcholine, α-lipoic acid, vitamin B, antioxidant vitamins [A,C,E] and methylsulfonylmethane that support phase I and phase II activities also serve as hepatoprotective agents. There are many herbs and herbal drugs which are reported to have antioxidative, anti lipid peroxidative, anti-inflammatory, membrane herbal hepatoprotective agent which prevents damage to the liver by

There are many herbs and herbal drugs which are reported to have antioxidative, anti lipid peroxidative, anti-inflammatory, membrane herbal hepatoprotective agent which prevents damage to the liver by antioxidative, anti lipid peroxidative, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanism [236]. Glycyrrhiza glabra, Picrorhiza Kurroa and Phyllanthus amarus have also been proved scientifically to possess hepatoprotective effect [227]. Drugs like troglitazone, bromfenac, ticrynafen, benoxaprofen, bromfenac, trofoxacin, ebrodine, nimesulide, nefazodone, ximelagatran and pemoline have been withdrawn due to hepatotoxicity [231,232,233]. Preclinical tests of various therapeutic agents should be done scientifically. Public should be properly educated about the probability of various hepatotoxicants. Due emphasis should be given on the possibility of drug interactions and sharing knowledge of newly reported hepatotoxins. All the therapeutic agents should be properly labelled and the dose standardized. Due importance should be given to quality control. The individuals should adhere to recommended doses. They should report unexpected liver symptoms. Regular monitoring of liver function tests should be done.

Chaotic use of many herbal remedies is a growing medical, scientific and public health problem. The vast biodiversity of nature is an abundant source of many bioactive compounds that may be useful in the fight against chronic diseases. Many studies are being carried on a vast number of herbs to evaluate their biological activity. The plant bioresource needs to be screened appropriately for various therapeutic activities for the discovery of new drugs. Such systematic studies are also needed so that the herbal remedies can be used with much more security. Because of the liver’s important role in biotransformation of drugs and toxins, drug-induced hepatotoxicity should be a major concern in drug development and chronic drug therapy.

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