Chemical Induced Liver Injury: Types, Mechanisms and Biomarkers

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ABSTRACT

Liver is a primary organ involved in biotransformation of foods and drugs. Liver diseases are a major worldwide problem; Hepatic disorders are mainly caused by toxic chemicals, e.g. - alcohol, carbon tetra chloride, anticancer agent, analgesic, anti-inflammatory drugs, anti-tuberculosis agent and heavy metals. Various risk factors for liver damage include age, gender, alcoholism, nutrition and genetic polymorphisms of cytochrome P450 have also been considered. The present review enumerate various hepatic diseases, risk factors and chemicals induced hepatic injury via different mechanical pathway as well as numerous biochemical changes viz. serum biomarkers, proteomics biomarkers, genomic biomarkers, metabolic biomarkers and micro RNA. This review could be immensely useful for researchers especially for pharmacologists, toxicologist working on hepatotoxicity and drug research organization.

Keywords: Liver; Biotransformation; Risk Factors.

1. Introduction

Liver, the largest organ of the human body located between the absorptive surface of gastrointestinal tract offer wide range of functions including protein synthesis, detoxification and production of biochemicals necessary for digestion[1]. It is central target of the toxicity of drug, xenobiotic and oxidative stress because of an important role in metabolism and relationship to the gastrointestinal tract. The frequent cause of hepatic injury is drug but it also depends upon its anatomical location and its biochemical and physiological function[2]. Drug and its active metabolite induced different appearance on liver at cellular level as well as genetic level Extensive use of drugs even at therapeutic level damage liver in susceptible individuals[3] [Figure 1, Figure 2].

Figure 1: Number of death due to cancer (Total=8.2 million death).

Figure 2: Figure illustrate Common factors affecting liver damage OTC (over the counter); BH-3 (B-cell lymphoma-2 homology-only members of the B-cell lymphoma-2 protein family).

2. Types of Hepatotoxicity

2.1 Hepatitis

Hepatitis is the most common disease of liver inflammation. Hepatitis viruses are the most common cause of hepatitis in the world but other infections can also cause hepatitis such as toxic substances (e.g. alcohol,
certain drugs), and autoimmune diseases. There are mainly 5 hepatitis virus assigned as A, B, C, D and E among which particularly due to B and C, more than 1 million people die each year (WHO, 2014). Viral infection generally causes inflammatory reaction marked by release of cytokines and chemokines which may lead to cancer development[5]. Inflammation induced oxidative stress acquire Kupffer cells to promote stellate cells activation via NF-κB and AP1. Continual activation of these genes results in cirrhosis, fibrosis and severe liver damage leading to development of HCC[5, 6].

2.2 Cirrhosis
Cirrhosis of liver is an advanced and consequence stage of liver diseases. Excessive use of alcohol and chronic infection with hepatitis viruses (such as hepatitis B and hepatitis C) are the most common causes of cirrhosis.

2.3 Non-alcoholic fatty liver disease (NAFLD)
NAFLD is metabolic disorder commonly observed in obese and diabetic patients[7]. Insulin resistance is a key pathogenic factor resulting in hepatic fat accumulation. The exact mechanism of hepatic triglyceride accumulation and subsequent hepatocellular damage are incompletely understood. Hepatic triglyceride accumulation subsequently leads to hepatic insulin resistance by interfering with tyrosine phosphorylation of insulin receptor substrates 1 and 2[8]. This may exacerbate overall insulin resistance[9, 10].

2.4 Cholestasis
Disruption or failure of bile formation is a pathophysiological process termed as cholestasis. Cholestasis can be defined by three ways like: biochemical, physiological and morphological. In biochemical condition, altered serum constitute observed in cholestasis e.g. hyperbilirubinemia, bile academia and elevated enzymes such as alkalinephosphatase and gamma glutamyl transpeptidase. In physiological condition, reduced bile flow is observed. Morphologically, cholestasis characterized by presence of greenish yellow–orange waxy plugs in hepatocellular canaliculi most evident in the centrilobular areas in many species. This change is often observed by deformation and loss of canalicularmicrovilli[11, 12].

2.5 Steatosis
Abnormal retention of lipid within the liver cells lead to generation of Steatosis. Type 2 diabetes, obesity and Steatosis are closely related with each other[9]. Multiple factors worked together for the development of fatty liver disease. The mechanism of lipid accumulation is not fully understood but probably relate to alterations of the pathways of uptake, synthesis, degradation, or secretion in hepatic lipid metabolism resulting from insulin resistance. Acute exposure of many chemicals e.g. carbon tetra chloride and several drugs e.g. aspirin as well as alcohol can induce Steatosis[9, 13, 14].

3. Risk Factors
3.1 Age
Generally old age is relatively at high liver damage risk than other ages. The increased risk of drug induced liver damage in elderly carries some biological changes in absorption, distribution, metabolism and elimination. Older age was a predictor of cholestatic expression of drug induced liver damage regardless of the type of drug involved[15, 16] [Figure 2].

3.2 Gender
The research studies clearly pointed towards the women at higher risk for drug induced liver diseases. A study conducted in Japan and Sweden clearly support the percentage of liver injury is 8.7 % in children ranging from 3 to 17 years[17, 18] [Figure 2].

3.3 Alcohol
Several studies reported the risk of developing liver damage accelerate with the increased alcohol consumption. A lot of studies support the positive relationship between alcohol intake and liver cancer yet its exact role is not fully understood[19-21] [Figure 2].

3.4 Medication interaction
Concomitant administration of drugs sometime results in interaction which is complex, challenging and complicates causality assessment[22, 23]. The administration of drugs simultaneously may have mutual effects such as drugs can be synergistic or antagonistic for liver damage. Antibiotic are the most common cause of liver injury in the United States and Europe[24] [Figure 2].

3.5 Nutrition
Deficiency of nutrition may initiate liver disorder as reported in patients with HIV, tuberculosis or alcoholism. It may be due to reduced hepatic glutathione in liver tissues of these patients[25, 26] [Figure 2].
3.6 Genetic polymorphism
Genetic polymorphism of cytochrome enzyme and protein involved in the metabolism of drugs are important predisposing factors in liver diseases. Slow acetylation status has increased and severity of anti-tubercular drug induced hepatotoxicity\[27, 28\][Figure 2].

3.7 Pre-existing liver disease
Previous studies showed that there is no risk of drug induced liver disease in patients having chronic liver disease. The study performed in Spain and United State have not shown that alcohol consumption increase the severity or chronicity of idiosyncratic drug induced liver disease\[29\] but recent data suggest that presence of fatty liver disease or chronic viral hepatitis may stimulate the drug induced liver disease\[30\][Figure 2].

4. Chemicals induced Hepatotoxicity
4.1 Alcohol
Consumption of chronic heavy alcohol developed serious health problems including severe liver diseases including fatty liver, alcoholic hepatitis, fibrosis/ cirrhosis, and hepatocellular carcinoma\[31\]. In a report published by WHO, 70 % of mortality due to liver disease is directly related to alcohol. Alcohol consumption leads to liver injury mainly through endotoxin, oxidative stress and inflammation. The metabolism of alcohol in liver occurred by oxidation mainly supported by alcohol dehydrogenase. Since liver is mainly responsible for metabolizing ingested alcohol; therefore it is more susceptible to alcohol related injury\[32\]. Chronic alcohol consumption inhibits hepatic alcohol dehydrogenase and induced biochemical changes mainly cytochrome P450 2E1 isozyme. Both, ADH- and CYP2E1 catalyzed oxidation of ethanol are shown to be associated with generation of acetaldehyde (a reactive aldehyde that binds to cellular proteins and DNA) and/or reactive oxygen species (ROS) causing peroxidation of unsaturated lipids and oxidation of proteins and DNA. Such reactions alter systemic redox balance by reducing anti-oxidative capacity [such as glutathione (GSH) depletion] resulting in enhanced oxidative stress. However, it is not well understood whether generation of acetaldehyde and ROS, and depletion of GSH, or both are responsible for oxidative stress in liver disease related to chronic alcohol consumption\[33, 34\].

4.2 CCl₄
Carbon tetra chloride in one of the most extensively used toxicant for inducing liver injury for mutagenicity and DNA damage study in animals. Hepatic microsomal enzyme (CYP2E1) metabolized carbon tetra chloride to degraded metabolites, trichloromethyl (CCl₃) and trichloromethyl peroxyl (CCl₃O₂) which is mainly responsible for hepatotoxicity\[35\]. These metabolites are unstable radicals and show strong binding affinity towards protein and lipids in the cell membrane or removing a hydrogen atom from an unsaturated lipid, there by triggering lipid peroxidation and causing liver damage\[36, 37\].

4.3 NSAIDs
NSAIDs are the most commonly prescribed drug for the treatment of rheumatic arthritic disease and other chronic inflammatory disorders\[38\]. NSAIDs which mostly influence to produce toxicity are nimesulide, diclofenac and sulindac. NSAIDs are associated with idiosyncratic hepatotoxicity about 19/100,000 treated individual with elevation of serum transaminase to hepatocellular or cholestatic injury and occasionally to fatal hepatitis. Drug induced liver injury is commonly classified in to intrinsic like idiosyncratic hepatotoxicity which is further classified as allergic and non-allergic and other clinical classification differentiate e.g. hepatocellular, cholestatic or mixed liver enzyme patterns, histological criteria, acute vs. chronic onset, or severity\[39, 40\] [Table 1].
Table 1. Clinical and pathological symptoms of drug-induced liver diseases

<table>
<thead>
<tr>
<th>Associated Drugs</th>
<th>Signature disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate, Tetracycline, Valproic Acid,</td>
<td>Steatosis</td>
<td>78</td>
</tr>
<tr>
<td>Paracetamol, Acetaminophen, Isoniazid, Methyldopa, Troglitazone,</td>
<td>Hepatitis</td>
<td>79, 80</td>
</tr>
<tr>
<td>Thioacetamide, Amiodarone, tamoxifen, valproic acid, Perhexiline Maleate,</td>
<td>Cirrhosis</td>
<td>80</td>
</tr>
<tr>
<td>Methotrexate, Corticosteroids, Colchicine, Angiotensin inhibitors, Tocopherol,</td>
<td>Steatohepatitis</td>
<td>78, 80</td>
</tr>
<tr>
<td>Amoxicillin/Clavuante, Clopidogrel, Estrogen, Oral contraceptive, Erythromycin,</td>
<td>Cholestasis</td>
<td>82</td>
</tr>
<tr>
<td>Statins, Isoniazid, Acetaminophen, Aspirin, Allopurinol, Ciprofloxacin, Rifampin,</td>
<td>Hepatocellular</td>
<td>83</td>
</tr>
<tr>
<td>Tocopherol, Amiodarone, Estrogens, Calcium channel blockers</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.4 Paracetamol (PCM)
PCM is most widely used analgesic and antipyretic agent which induced liver injury in dose dependent manners. The reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) covalently binds to glutathione protein to form conjugate which lead to irreversible hepatocyte injury and necrosis by different mechanism. NAPQI bind to sulfydryl group of glutathione causing depletion of hepatic anti-oxidative capacity and oxidative damage of various cell components which result in necrosis ultimately result in death[41, 42] [Table 1].

4.5 Thioacetamide
In many experiments, Thioacetamide is used for induction of liver fibrosis and cirrhosis. It induced liver injury by oxidative stress and mainly due to formation of toxic metabolite Thioacetamide-S-oxide, formed during its biotransformation by the microsomal flavine adenine dinucleotide (FAD)–containing monoxygenase[43, 44]. Oxidative bioactive Thioacetamide is the toxic metabolite responsible for protein covalent binding that leads to toxicity[45] [Table 1].

4.6 Azathioprine
Azathioprine is prodrug for mercaptopurine, used as an immunosuppressive drug used in organ transplantation and autoimmune disease like refractory severe rheumatoid arthritis, systemic lupus erythematosus, psoriasis and inflammatory bowel disease[46]. Although it has several beneficial effects yet its use is limited due to harmful effect on liver and bone marrow. The mechanisms of azathioprine include depletion of GSH due to free radical formation leading to mitochondrial injury, depletion of ATP and finally lead to death by necrosis thus causing liver injury[47] [Table 1].

4.7 Anti-tubercular agent
The three key tuberculosis drug isoniazid, pyrazinamide and rifampicin play a central role in inducing hepatotoxicity. A meta-analysis of studies involving the use of combinations of antituberculosis drug regimens mainly in adults has shown an incidence rate of liver toxicity of 2.6% with isoniazid and rifampicin co-administration Isoniazid is a prodrug which is activated by catalase-peroxidase enzyme[48]. This drug is mainly metabolized by the microsomal enzyme, CYP2E1 by acylation in liver. The principal metabolite, N-hydroxyacetetyl hydrazine undergoes further dehydration to form toxic metabolite, acetyl diazine which further break in to acetylonium ion, acetyl radical and ketenes which bind to hepatic macromolecules and induce liver injury[49] [Table 1].

Rifampicin is another drug used in treatment of tuberculosis, also cause liver injury is metabolized to desacetyl rifampicin by desacetylation and further hydrolyzed to 3-formyl rifampicin which is the main inducing agent for liver injury[50].
5. Mechanisms of Hepatotoxicity

Since liver received blood majorly from gastrointestinal viscera, which also bring drugs and xenobiotics in concentrated form. Because of an important target of metabolism and unique relationship with gastrointestinal tract, it’s become a target for toxicity of drugs, xenobiotics and oxidative stress. Hepatic injury may cause due to direct cell toxicity of chemicals or metabolic. Hepatic injury occurred by different mechanism include bile acid induced apoptosis during cholestasis, pathophysiological effect of mitochondrial dysfunction and cell damage by reactive oxygen species and nitrogen species.

5.1 Bile acid induced hepatocyte apoptosis

Bile acid only synthesized by liver, important for metabolism of fatty acid and lack of bile acid lead to a pathophysiological headway termed as cholestasis. Accumulation of bile acid during cholestasis leads to hepatocyte apoptosis due to stimulation of magnesium ion dependent endonuclease. Hydrophobic bile acids are especially hepatotoxic, and they accumulate in the liver in cholestatic disorder. The failure to secret bile acids in to the bile results in liver injury, cirrhosis and death from liver failure\(^{[51]}\). Apoptosis mainly occurred by two path ways (1) death receptor pathway and (2) the mitochondrial pathway. To determine if death-receptor pathways contribute to bile acid-mediated apoptosis, hepatocytes from tumor necrosis factor-receptor 1 (TNF-R1) and Fas-deficient mice were exposed to GCDC. TNF-R1 and Fas are the predominant death receptors expressed by hepatocytes. Hepatocytes from Fas-deficient lpr mice were resistant to GCDC-mediated apoptosis, whereas TNF-R1-deficient hepatocytes readily underwent apoptosis. Unexpectedly, hepatocytes from Fas ligand-deficient mice were also sensitive to GCDC stimulated apoptosis. These data implicate ligand-independent Fas-mediated apoptosis as a contributing mechanism for bile acid-related liver injury. To further test this concept, the bile ducts of wild type and Fas-deficient mice were ligated to produce severe extrahepatic cholestasis. Caspase 8, an initiator cysteine-aspartate protease in apoptosis, was activated in wild type animals but not Fas-deficient mice. Bile duct ligated Fas-deficient animals also had less apoptosis, decreased liver injury, and improved survival as compared to wild type mice. Thus, Fas activation appears to play a dominant role in bile acid cytotoxicity\(^{[52, 53]}\)\(^ {\text{[Figure 3,4]}}\).

Figure 3: Bile acid-induced hepatocyte apoptosis; Bile acids are normally secreted rapidly from hepatocytes by transporters located in the canaliculamembrane. In cholestasis, secretion is impaired, resulting in elevated concentrations of toxic bile acids (TBA) within hepatocytes. At pathophysiologic concentrations, toxic bile acids trigger translocation of intracellular Fas bearing vesicles to the plasma membrane where they self-aggregate in the absence of ligand. Activated Fas receptor complexes on the plasma membrane then cause caspase 8 activation and an apoptotic cascade.

Figure 4: Drug induced mitochondrial dysfunction APAF (Apoptotic protease activating factor):FADD (Fas-Associated protein with Death Domain).

5.2 Drug induced mitochondrial dysfunction

Finding the mechanism of drug induced liver injury is a challenge because it always involves several mechanism, regulatory system and risk factor complex interaction. Drug induced liver injury involve intrinsic and extrinsic pathway emphasizing the central role of mitochondria for the mechanisms leading to apoptosis. Drug
or its active metabolites create direct cell stress by which it target mitochondrial function. Reactive metabolites can exert initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH), or binding to enzymes, lipids, nucleic acids and other cell structures. When hepatocyte liver injury occurs, the liver abundant ALT will leak in to the extracellular space and enter the blood, wherein it shows a slow clearance rate with a half-life of approximately 42 hr. The typical reference range is 7-35 IU/L in females and 10-40IU/L in males. An elevation of serum ALT activity is often reflective of liver damage. Unfortunately, extra-hepatic injury such as muscle injury can also lead to elevation in ALT making ALT not entirely hepato-specific. In addition, fenofibrate was found to increase ALT gene expression in the absence of apparent liver injury and hepatotoxin microcystin-LR was reported to suppress ALT gene expression [56, 57].

6. Biomarkers of Hepatotoxicity

6.1 Serum biomarker

6.1.1 ALT

The most widely used clinical biomarker of liver disease in preclinical species and humans is ALT. Alanine is present in liver in higher concentration and ALT is responsible for its metabolism (transamination). When hepatocyte liver injury occurs, the liver abundant ALT will leak in to the extracellular space and enter the blood, wherein it shows a slow clearance rate with a half-life of approximately 42 hr. The typical reference range is 7-35 IU/L in females and 10-40IU/L in males. An elevation of serum ALT activity is often reflective of liver damage. Unfortunately, extra-hepatic injury such as muscle injury can also lead to elevation in ALT making ALT not entirely hepato-specific. In addition, fenofibrate was found to increase ALT gene expression in the absence of apparent liver injury and hepatotoxin microcystin-LR was reported to suppress ALT gene expression [56, 57].

Figure 5: Biochemical indicators of hepatotoxicity (↑) increased value during hepatotoxicity, (↓) decreased value during hepatotoxicity: ALP (Alanine phosphate); AST (Aspartate aminotransferase); CH (cholesterol); γ-GGT (γ-glutamyl transpeptidase); GDH (glutamate dehydrogenase); HDL (high density lipoprotein); LDL (low density lipoprotein); MDH (malate dehydrogenase); SDH (sorbitol dehydrogenase); TB (Total bilirubin); TG (triglyceride); VLDL (very low density lipoprotein).

Figure 6: Figure illustrate cellular, molecular, immunological and biochemical alteration during hepatotoxicity induced by various factors. ALT (Alanine aminotransferase); AST (Aspartate aminotransferase); LDL (low density lipoprotein); HDL (high density lipoprotein); TB (Total bilirubin); TG (triglyceride); JNK (C-Jun N-terminal kinase); IL (interleukins); TNF-α (Tumor necrosis factor-α); ER (endoplasmic reticulum); TRAIL (Tumor necrosis factor-related apoptosis inducing ligand),
<table>
<thead>
<tr>
<th>Bio-marker</th>
<th>Specific function</th>
<th>Tissue Localization</th>
<th>Injury</th>
<th>Specific damage marker</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>Transamination in alanine cycle</td>
<td>Primarily localized in liver</td>
<td>Elevated in blood due to liver necrosis and with heart and skeletal muscle injury (necrosis)</td>
<td>Hepatocellular necrosis</td>
<td>Commonly used to assess hepatocellular injury</td>
<td>55, 56, 57</td>
</tr>
<tr>
<td>AST</td>
<td>Catalyzes the reversible transfer of an α-amino group between aspartate and glutamate</td>
<td>Localized in heart, brain, skeletal muscle and liver</td>
<td>Elevated in blood due to liver or extrahepatic tissue injury</td>
<td>Hepatocellular necrosis</td>
<td>Less specific than ALT</td>
<td>56, 58</td>
</tr>
<tr>
<td>TBL</td>
<td>Main physiologic role as an antioxidant</td>
<td>Taken up, conjugated in liver and secreted in bile</td>
<td>increased markers of hepatobiliary injury and liver function, due to hemolysis</td>
<td>Cholestasis, biliary, liver function</td>
<td>Conventional biliary injury, in conjunction with ALT, better binder of disease severity in humans</td>
<td>56, 57, 58</td>
</tr>
<tr>
<td>ALP</td>
<td>Remove phosphate group from molecules such as proteins, nucleotides etc.</td>
<td>Broad tissue localization</td>
<td>Marker of hepatobiliary injury</td>
<td>Cholestasis</td>
<td>Conventional biliary injury, associated with drug induced cholestasis in humans</td>
<td>60</td>
</tr>
<tr>
<td>GGT</td>
<td>Involve in transfer of amino acids across the cellular membrane and glutathione metabolism</td>
<td>Activity localized to kidney &gt; liver, pancreas</td>
<td>Marker of hepatobiliary injury</td>
<td>Cholestasis, biliary</td>
<td>Conventional biliary injury, high sensitivity in humans, elevation caused by alcohol/heart disease</td>
<td>57</td>
</tr>
<tr>
<td>Albumin</td>
<td>Regulate colloidal osmotic pressure of blood</td>
<td>Main constituent of serum total protein</td>
<td>Decreased in blood with chronic liver disease</td>
<td>Liver function</td>
<td>Liver fails to synthesize enough protein, especially albumin</td>
<td>84</td>
</tr>
<tr>
<td>Cholesterol/triglycerides</td>
<td>Build and maintain cell membrane as well as fluidity of membrane</td>
<td>Cell membrane localization</td>
<td>Increased in blood due to the failure of bile elimination</td>
<td>Liver function</td>
<td>Liver fails to remove them to bile ducts</td>
<td>85</td>
</tr>
</tbody>
</table>
Table 2. Summary of current clinical biomarkers of liver toxicity

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Urobilinogen</td>
<td>Low level in urine due to biliary obstruction</td>
<td>Liver function</td>
<td>Colorless product of bilirubin reduction, similar role to bilirubin</td>
<td>86</td>
</tr>
</tbody>
</table>

**6.1.2 Aspartate amino transferase (AST)**

In the last 30 years, AST has also been proved as a standard biomarker for identification of severity of various liver diseases.[56, 58] Similar to ALT, AST is responsible for metabolism (transamination of aspartate). Even though the sensitivity of the AST test is believed to be lower than that of ALT because of low mitochondrial enzyme, it is still a widely used liver biomarker.[57] [Figure 5, 6] [Table 2]

**6.1.3 Alkaline phosphate activity (ALP)**

ALP is mainly present in cell membranes in multiple tissues mainly in hepatocytes. At alkaline pH, it hydrolyzes monophosphates. Several isoenzymes have been identified in different organs like intestinal, kidney and placental forms. Identification of bile duct blocked when ALP concentration increased. It is also identified as major diagnostic biomarker as recommended as FDA guidance and by clinicians.[57] [Figure 5, 6] [Table 2]

**6.1.4 Total bilirubin**

Total bilirubin is a composite of indirect (nonheaptic) and direct (hepatic) bilirubin. This product of hemoglobin degradation is a marker of hepatobiliary injury, especially cholestasis and biliary effects.[59] In acute human hepatic injury, total bilirubin can be a better indicator of disease severity compared to ALT. Bilirubin may also be increased due to non-hepatic causes such as hemolysis. Analysis of indirect compared to direct bilirubin does not necessarily add information in routine assessment when compared to total bilirubin.[60] [Figure 6] [Table 2].

**6.1.5 Gamma-glutamyl transferase activity (GGT)**

Gamma-glutamyl transferase (GGT) activity is localized to liver, kidney, and pancreas tissues, yet enzyme concentration in liver is low compared to kidney GGT has multiple functions including catalytic transfer of gamma-glutamyl groups to amino acids and short peptides hydrolysis of GSH to a gamma-glutamyl moiety and cysteine glycine in GSH and GSH conjugate catalolism. GGT activity is a marker of hepatobiliary injury, especially cholestasis and biliary effects.[61] [Figure 6] [Table 2].

**6.1.6 Bile acid**

Bile acids functionally contribute to the catabolism and elimination of cholesterol; are the primary determinant of bile flow; regulate pancreatic secretions; and release of GI peptides, and contribute to the digestion and absorption of fat (and indirectly fat-soluble vitamins) in the small intestine. Total bile acids are also implicated in various signal transduction pathways and are elevated with liver injury and functional change; it can be influenced by diet and fasting.[62, 63] [Figure 6] [Table 2, 3].
### Table 3. Summary of emerging biomarkers of liver toxicity

<table>
<thead>
<tr>
<th>Biomarker candidate</th>
<th>Bio-fluid evaluated</th>
<th>Origin</th>
<th>Proposed indication</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-1</td>
<td>Plasma</td>
<td>Produced by a variety of cells</td>
<td>Cellular response to tissue damage</td>
<td>87</td>
</tr>
<tr>
<td>Glutathione S-transferase P-form</td>
<td>serum</td>
<td>Present in the hepatocytes</td>
<td>Hepatocellular injury</td>
<td>88</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>serum</td>
<td>Primarily found in the liver and to a lesser degree in the kidney and skeletal muscle</td>
<td>Hepatocellular necrosis</td>
<td>88, 88</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>serum</td>
<td>Localized in the mitochondria and extra mitochondrial compartment, found primarily in the liver but also in skeletal muscle, heart and brain</td>
<td>Hepatocellular necrosis</td>
<td>88</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>serum</td>
<td>Primarily in the liver but also present in heart muscle and brain; mainly in the cytoplasm of endothelial cells, kupffer cells, and hepatocytes</td>
<td>Hepatocellular necrosis</td>
<td>46</td>
</tr>
<tr>
<td>Paraoxanase 1</td>
<td>serum</td>
<td>Produced primarily in the liver but also found in the kidney, brain and lung</td>
<td>Hepatocellular necrosis</td>
<td>20</td>
</tr>
<tr>
<td>Glutathione S-transferase alpha</td>
<td>serum</td>
<td>Liver specific</td>
<td>Hepatocellular necrosis</td>
<td>81</td>
</tr>
<tr>
<td>Apopipoprotein E</td>
<td>serum</td>
<td>Produced in the liver but also found in the brain and kidney</td>
<td>Hepatocellular necrosis</td>
<td>56</td>
</tr>
<tr>
<td>Bile acids</td>
<td>Urine, serum</td>
<td>Synthesized primarily in the liver</td>
<td>Liver dysfunction including intrahepatic cholestasis</td>
<td>89</td>
</tr>
<tr>
<td>Steroids</td>
<td>Urine, serum</td>
<td>Metabolites of Cholesterol</td>
<td>Oxidative stress and liver damage</td>
<td>90</td>
</tr>
<tr>
<td>Acylcarnitines</td>
<td>Urine, serum</td>
<td>Located in the heart, muscle, brain, liver and kidney</td>
<td>Failure of fatty acid oxidation</td>
<td>91</td>
</tr>
<tr>
<td>miRNA-122</td>
<td>Plasma/Serum</td>
<td>Liver specific expression</td>
<td>Viral-, alcohol- and chemical induced liver injury</td>
<td>92</td>
</tr>
<tr>
<td>miRNA-192</td>
<td>Plasma/Serum</td>
<td>Liver specific expression</td>
<td>Chemical induced liver injury</td>
<td>92</td>
</tr>
<tr>
<td>miRNA-291a-5p</td>
<td>Urine</td>
<td>Unknown</td>
<td>Chemical induced liver injury</td>
<td>93</td>
</tr>
</tbody>
</table>

#### 6.2 Genomic approach:

Genomics is a discipline in genetics that applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes (the complete set of DNA within a single cell of an organism)\(^{[66]}\). In the identification of drug induced liver injury, the use of genomic approach to determine patterns of changes in
mRNA transcripts, introduced as toxicogenomics grab the attention\(^{(65)}\) [Table 4].

<table>
<thead>
<tr>
<th>Types of hepatotoxicity</th>
<th>Drug associated with hepatotoxicity</th>
<th>Types of Human study</th>
<th>Enzyme/HLA allele</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Efavirenz</td>
<td>Open-label trial (n=156)</td>
<td>CYP2B6</td>
<td>94</td>
</tr>
<tr>
<td>Drug</td>
<td>Anti-tuberculosis drug</td>
<td>Cohort study (n=89)</td>
<td>SLCO1B1 &amp;SLC10A1</td>
<td>95</td>
</tr>
<tr>
<td>Drug</td>
<td>Anti-tuberculosis drug</td>
<td>Case study (n=445)</td>
<td>IL-4 and IL-10</td>
<td>92</td>
</tr>
<tr>
<td>Drug</td>
<td>Isoniazid-containing antituberculosis drug regimen</td>
<td>Case study (n=33)</td>
<td>cytochrome P4502E1</td>
<td>96</td>
</tr>
<tr>
<td>Drug</td>
<td>Rifampin</td>
<td>Open study (n=273)</td>
<td>SLCO1B1 15 haplotype</td>
<td>97</td>
</tr>
<tr>
<td>Drug-induced idiopathic hepatitis</td>
<td>Chlorpromazine</td>
<td>Case study (n=71)</td>
<td>HLA-D R6</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4. Select examples of genetic polymorphisms associated with a possible increased risk of hepatotoxicity from specific drugs

6.3 Proteomics evaluation:

The qualitative and quantitative proteomics evaluation is an important step to differentiate the protein expression for better understanding of novel protein biomarker in diverse biological function\(^{(66, 67)}\) [Table 5].

<table>
<thead>
<tr>
<th>Disease</th>
<th>Manifestation</th>
<th>Biomarker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver disease</td>
<td>Apoptosis</td>
<td>Cytokeratin-18</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Oxidative stress</td>
<td>Malonaldehyde</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBARS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxidised (LDL)</td>
<td>66</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Hyaluronic acid</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Type IV collagen S</td>
<td>Fibronectin,</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>TIMP1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Procollagen III N peptide</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Select examples of proteomics biomarker along with hepatotoxicity

TNF-α (Tumor necrosis factor-α), TBARS (thiobarbituric acid reactive substances), LDL (low density lipoprotein), IL-6 (Interlekin-6), TIMP1 (tissue inhibitor of metalloproteases)

6.4 Metabolomics approach:

Metabolomics involved the measurement of the metabolite pool that exists within a cell or tissues under a particular set of conditions. The metabolic profile is greatly influenced by both genetic and environmental factors, thereby providing phenotypic-specific data that can be evaluated in a longitudinal manner. Metabolomics analyses focus on the discovery of novel, clinically relevant biomarkers in easily obtained biofluids such as urine and serum\(^{(68)}\). As hepatotoxicity is the major cause for drug related adverse events, metabolomics has been employed in multiple preclinical studies to identify more selective markers of drug induced liver injury. Metabolites from several major pathways have been reported in multiple studies\(^{(69)}\) [Table 3].

6.5 Micro RNA as biomarker:

Micro RNAs are short approx 22 nucleotide, noncoding RNAs which have been recently identified as vital post transcriptional regulators of gene expression in most eukaryotic genome\(^{(69, 70)}\). The human genome is predicted to encode ~1000 mi RNAs and it is assumed that they can regulate approximately one third of all human transcripts\(^{(71)}\). Recent several studies proved miRNA as useful noninvasive diagnostic marker and its unique stability with unique position in biofluid including blood and urine in liver diseases\(^{(72, 73, 74, 75)}\) [Table 6].
<table>
<thead>
<tr>
<th>miRNA biomarker</th>
<th>Etiology</th>
<th>Inference</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-224</td>
<td>HCC (n=19)</td>
<td>Up-regulation of miR-224 reflect HCC</td>
<td>100</td>
</tr>
<tr>
<td>miR-500</td>
<td>HCC (n=40)</td>
<td>an oncofetal miRNA, highly expressed in fetal liver aberrantly expressed in HCC</td>
<td>101</td>
</tr>
<tr>
<td>miR-338</td>
<td>HCC (n=20)</td>
<td>Down regulation of miR-338 in HCC metastasis</td>
<td>102</td>
</tr>
<tr>
<td>miR-122</td>
<td>Hepatitis B viral infection (n=83)</td>
<td>Increase of miR-122 in Hepatitis B viral infection</td>
<td>71, 103</td>
</tr>
<tr>
<td>miR-25, -92a, let7f, miR-375</td>
<td>Hepatitis B viral infection (n ≥ 150)</td>
<td>miR-375 is Hepatitis B viral infection specific and predicts HCC</td>
<td>73</td>
</tr>
<tr>
<td>miR-122, miR-34a</td>
<td>Chronic Hepatitis C viral infection (n=34) and Non-alcoholic fatty liver disease (n=34)</td>
<td>Both display liver damage and fibrosis</td>
<td>104</td>
</tr>
<tr>
<td>miR-885-5p</td>
<td>HCC, Liver cancer and Chronic hepatitis B (n=100)</td>
<td>specifically predict HCC</td>
<td>104</td>
</tr>
<tr>
<td>miR-122, -222, -223, miR-21</td>
<td>HBV patients without HCC (n=48) and HBV patients with HCC</td>
<td>miR-122 up-regulation in HBV patients with HCC</td>
<td>105</td>
</tr>
<tr>
<td>miR-29, miR-133a</td>
<td>HCC</td>
<td>miR-29 increase while miR-133a down-regulation reflect the liver fibrosis</td>
<td>106</td>
</tr>
<tr>
<td>miR-122, miR-192</td>
<td>Acetaminophen-induced acute liver injury</td>
<td>Up-regulation reflect liver damage</td>
<td>107</td>
</tr>
<tr>
<td>miR-21, miR-122, miR-223</td>
<td>Hepatocellular carcinoma, Chronic hepatitis B virus (n ≥ 150)</td>
<td>All increased in Hepatocellular carcinoma, Chronic hepatitis B virus</td>
<td>108</td>
</tr>
<tr>
<td>miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801</td>
<td>Hepatitis B Virus-Related HCC (n =800)</td>
<td>Clearly differentiate between health Hepatitis B Virus and Hepatocellular carcinoma</td>
<td>109</td>
</tr>
<tr>
<td>miR-122</td>
<td>Hepatitis C Virus (n=68)</td>
<td>Correlate the necro-inflammation with chronic hepatitis C virus infection</td>
<td>110</td>
</tr>
<tr>
<td>miR-122, miR-148a, miR-194</td>
<td>Liver transplant</td>
<td>Show the rejection and hepatic injury after liver transplantation</td>
<td>111</td>
</tr>
<tr>
<td>miR-571, miR-652</td>
<td>Hepatitis C induced liver cirrhosis or Alcoholic patients</td>
<td>reflect their putative roles in the mediation of fibrogenic and inflammatory processes in distinct cellular compartments involved in the pathogenesis of liver cirrhosis</td>
<td>106</td>
</tr>
<tr>
<td>miR-21</td>
<td>HCC, chronic hepatitis (n =166)</td>
<td>A promising biomarker for HCC</td>
<td>112</td>
</tr>
<tr>
<td>miR-106b ,miR-181 b</td>
<td>Hepatitis B Virus (n =62)</td>
<td>Show high diagnostic accuracy for liver cirrhosis</td>
<td>90</td>
</tr>
<tr>
<td>miR-197-3p, miR-505-3p</td>
<td>Primary biliary cirrhosis (n=10), Hepatitis B Virus (n=5), Hepatitis C Virus (n=5)</td>
<td>Down-regulation of both these markers serve as clinical biomarker of primary biliary cirrhosis</td>
<td>113</td>
</tr>
<tr>
<td>miR-483-5p</td>
<td>HCC (n=69)</td>
<td>Highly expressed in HCC tumor tissues</td>
<td>114</td>
</tr>
<tr>
<td>miR-20a and miR-92a</td>
<td>Hepatitis C Virus (N=58)</td>
<td>Sensitive and cost-effective biomarkers for early detection of HCV infection.</td>
<td>115</td>
</tr>
<tr>
<td>miR-122</td>
<td>Chronic Hepatitis C Virus-induced fibrosis (n=164)</td>
<td>Reflect the liver injury and inflammation</td>
<td>116</td>
</tr>
<tr>
<td>16 miRNA panel</td>
<td>Hepatitis B Virus in children(n=60)</td>
<td>Reflect Hepatitis B Virus in children</td>
<td>117</td>
</tr>
<tr>
<td>microRNA-122, microRNA-22</td>
<td>Hepatitis B virus (HBV) (n=198)</td>
<td>miR-122 and miR-22 levels were elevated in acute or chronic HBV infection</td>
<td>118</td>
</tr>
</tbody>
</table>
7. Conclusion
Liver being a dynamic and vital organ participates actively in multi-metabolic functions of foods, chemicals, biological and xenobiotic as well as detoxification of viral and bacterial products. The present review focus on types of liver injury and damage elicited by various factors along with serum, genomics, proteomics, and metabolomics biomarkers. This review also focuses on new emerging biomarkers miRNA appeared in different liver diseases. The various risk factors such as age, gender, alcohol, medication interaction, genetic factors and nutrition are also been outlined. All the biomarkers considered show specificity and sensitivity which is useful tool in understanding the liver injury. Furthermore the review should be helpful for researchers pursuing in field of Hepatology, hepatic disorders and Hepatoprotective drugs.

Author Contribution
The first author of the manuscript has given a frame to manuscript while work has done by corresponding author.

Conflict of interest
There is no conflict of interest with anyone.

Acknowledgments
There is no funding or sponsorship.

References
14. Powell EE, Jonsson JR, Clouston AD. Steatosis:


34. Saravanan S, Pandikumar P, Pazhanivel N, et al. Hepatoprotective role of abelmoschus esculentus (Linn.) moench, on carbon tetrachloride-induced liver injury. Toxicology Mechanisms and Meth-
55. Amacher DE. Toxicologist's guide to biomarkers
68. Coen M. Metabolic phenotyping applied to pre-clinical and clinical studies of acetaminophen metabolism and hepatotoxicity. Drug Metabolism Reviews 2015; 23:1-16.
81. Dawwas MF, Aithal GP. End-stage methotrexate-related liver disease is rare and associated with features of the metabolic syndrome. Alimentary Pharmacology & Therapeutics 2014; 40:938-948.
85. Poynard T, Ratziu V, Naveau S, et al. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. See comment in PubMed Commons below Comp. Hepatol 2005; 23:4-10.
95. Chen YJ, Zhu JM, Wu H, et al. Circulating mi-
98. Targher G. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. Journal of Hepatology 2006; 45:879-881.

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