INTRODUCTION

Liver

Liver is a major organ crucial for detoxification of various xenobiotics (Abdel-Daim et al., 2013). It is also involved in plasma protein synthesis and purification of blood (Altamirano and Bataller, 2011). It plays a major role in metabolic regulation, excretion of cholesterol, bile and bile pigments.

Liver is constantly exposed to a number of insults through drugs and environmental pollutants leading to injury (Rehm et al., 2009). Liver damage is associated with cellular necrosis, plasma membrane damage and depletion in glutathione level (GSH) (Paula et al., 2010). Long-standing hepatic injury leads to hepatic steatosis, fibrosis, life-threatening liver cirrhosis and hepatocellular carcinoma (Ramachandra Setty et al., 2007).

Anatomy of the liver

Figure 1. Anatomy of the liver (LeCluyse et al., 2012)
Liver weighs approximately 1.3 to 1.5 kg. It is located beneath the diaphragm. Falciform ligament divide sit into 2 lobes- left and right. The anterior extension of the peritoneal folds connects the liver to the diaphragm and anterior abdominal wall. Two smaller lobes are found on the posterior surface (caudate lobe) and the inferior surface (quadrate lobe) of the right lobe. Riedel’s lobe is an anatomical extension of the right lobe of the liver and consists of a projection that may feel like a mobile tumor in the right abdomen. Liver has a rich blood supply from portal vein and hepatic artery. Each supplies approximately half of the oxygen reaching the liver, making it highly resistant to infarction. The venous drainage from the liver converges into the hepatic veins that exit at its posterior surface to join the inferior vena cava near its entry into the right atrium (Zakim and Boyer, 1996).

Cells of the liver

The periportal area surrounding the afferent blood vessels, lymphatic vessels, bile ducts and nerves, is highly complex and consists of dense matrix of collagen. Spaces within the matrix contain a variable cell population, such as fibroblasts, hematopoietic cells, inflammatory cells, epithelial and endothelial cells (LeCluyse et al., 2012). The liver lobule consists mainly of plates of hepatocytes and sinusoids, with collagen matrix in between the two that harbors kupffer cells (resistant macrophages of liver) and fat-storing stellate cells. The interior of the hepatic sinusoids are lined by the endothelial, Kupffer and stellate cells. Additionally pit cells, liver specific NK T cells are also seen in the sinusoidal lumen (Kmiec, 2001). The main parenchymal mass is normally that of hepatocytes. In rat, the liver is made up to about 60% of hepatocytes and 40% non-parenchymal cells. Extracellular space forms approximately 23% of its volume (Bioulac-Sage et al., 2007).

Functions of the liver

It plays a vital role in digestion, metabolism, detoxification and excretion of waste materials from the body. Nutrients entering the liver from GI tract through hepatic artery are processed, modified and stored. Most major plasma proteins are either mainly or exclusively synthesized in the liver. Liver regulates blood sugar levels in response to nervous and endocrine signalling. It is a major site for gluconeogenesis, lipid metabolism- cholesterol, triglyceride and lipoprotein production. Synthesis of bile acids and secretion of these materials into the bile takes
place in the liver, thus facilitating the absorption of dietary fat and fat soluble vitamins. It also metabolizes exogenous compounds, such as drugs and toxins. It is involved in catabolism of T\textsubscript{3} and T\textsubscript{4}, steroidal and other hormones. It also synthesizes hormones such as IGF-I, angiotensin and erythropoietin (Zakim and Boyer, 1996).

**Hepatotoxicity**

Hepatotoxicity refers to liver damage, that includes hepatitis (inflammation of the liver), hepatic necrosis (death of liver cells) and hepatic steatosis (fat accumulation in liver).

The first indication of liver damage is rise in levels of liver marker enzyme in blood. In liver damage, these enzymes- ALT, AST, ALP and GGT are released into circulation and are usually assessed for liver function.

**Alcohol induced liver disease**

Alcoholic liver disease (ALD), which is due to excessive alcohol intake, has become a global health problem. Liver is the major organ for synthesis of vitals, metabolizing ingesta, and detoxifying noxious substances. People who drink a lot are more likely to suffer from clinical manifestations like steatohepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma (Pritchard et al., 2007; Le et al., 2004). The toxic byproducts of alcohol breakdown like acetaldehyde, can react with cellular proteins to generate adducts and cause severe tissue damage and can also induce steatosis (accumulation of fat). The development of steatohepatitis is identified through leukocytes infiltration that can occur at any stage of ALD. It's a risk factor for mortality (Diehl, 1997). In the beginning, ALD is asymptomatic and the person can recover if he ceases to consume alcohol (Mandrekar et al., 2011). However, repeated intake of alcohol may lead to the progression of ALD.

The development of ALD involves multi-factorial risk factors like amount and pattern of alcohol consumption, ethnicity, age, obesity, smoking, gender, co-existing chronic viral hepatitis, iron overload and host genetic factors (Torrueillas et al., 2014). In alcoholic liver disease, hepatic injury can occur through multiple ways, like ethanol metabolism or endoplasmic reticulum stress (ER stress) (Ji, 2014). Chronic consumption of alcohol leads to lipid peroxidation. It also provokes inflammatory cytokines, cause acetaldehyde toxicity, membrane damage and finally apoptosis.
Alcohol is soluble in water and fat; hence it can penetrate all the tissues, affecting almost every organ of the body (Patere et al., 2011). Alcohol metabolism via alcohol dehydrogenase in liver generates high NADH/NAD^+ ratio, which alters the oxidation–reduction potential of the hepatic cell. NADH induces fatty acid synthesis, and reduced NAD^+ results in decreased fatty acid oxidation. Elevated fatty acids levels lead to increased TG synthesis and accumulation, leading to fatty liver.

Thus, alcohol is one of the causes of accumulation of excessive hepatic fat, leading to fibrosis and cirrhosis (Yang et al., 2012; Purohit et al., 2004). Hepatic fibrosis involves the accumulation of matrix materials (Collagen, proteoglycans) and some macromolecules within the ECM (extracellular matrix). In advanced fibrosis, collagen content increases to 3-10 folds. Hepatic fibrosis is a healing process to chronic insults and is the final stage of CLD, (Smith, 2012; Yang et al., 2012; Friedman, 2008). Progressive fibrosis leads to damage of hepatic cells and ultimately results in cirrhosis and is linked to high risk of hepatic malignancies (Schuppan and Afdhal, 2008). Pharmacotherapy options are limited for controlling ALD. Complete abstinence from alcohol is the only recommended way of heptato-protection against the problem of ALD (Vuittonet et al., 2014). Currently, the only definitive treatment for advanced level fibrosis and cirrhosis is liver transplantation. The demand for organ grafts is the major hindrance for a successful transplantation (Crespo et al., 2012) stressing the need for effective anti-fibrotic treatments (Beyer et al., 2012; Inagaki et al., 2012).

**Pathogenesis of ethanol toxicity**

It was first (in 1940s) believed that pathological condition in the alcoholics was due to malnutrition, but now it is accepted that hepatotoxicity is due to alcohol. In the presence of biomolecules and mineral enriched diets, ethanol favours accumulation of fat, with striking ultra-structural lesions both in rats and human leading to severe liver damage (Lieber, 2000). Thus, the pathogenesis of alcohol related liver disease is multifactorial.

**Malnutrition**

Alcoholics commonly suffer from malnutrition. Alcohol, unlike other drugs, gives a high calorific value of 7 kcal/g. The calories from alcohol are “empty calories”
in the absence of vital biomolecules and this decreases the appetite. Thus, ethanol changes many normal nutrient consumption in the diet leading to malnutrition and related symptoms. Furthermore, continued alcohol use can alter the function of digestive system which may disturb the body’s ability to use vitamins. Alcohol also changes the processing of nutrients resulting in secondary malnutrition because of maldigestion/malabsorption caused by GI complications associated with ALD (Lieber, 2000).

In animals, malnutrition leads to different forms of ALD (Bergheim et al., 2011). The reason why malnutrition is involved in progression of liver disease in the alcoholics remains unclear. Lieber (Lieber, 1997) reports that nutritional deficiencies and direct toxic effects related to ALD are now linked with metabolism of alcohol.

**Metabolism of ethanol**

Liver is the main organ involved in alcohol metabolism. Hepatocytes are the main players in ethanol metabolism (Maher, 1997). Alcohol dehydrogenase and cytochrome P450E1 (CYP2E1) metabolizes ethanol to acetaldehyde and subsequently to acetate by acetaldehyde dehydrogenase. CYP2E1 expression is elevated in response to increased ethanol entry into hepatic cells and Kupffer cells (Thakur et al., 2007).

![Figure 2. Oxidative metabolism of ethanol](Cederbaum, 2012)
Acetaldehyde is a highly reactive metabolic product that results in oxidation of lipids, nucleic acids (NA) and formation of protein adducts. In addition, ethanol consumption leads to the generation of ROS (Lieber, 2000). The resulting oxidative stress can have deleterious effects on hepatocytes, which include dysregulation of fatty acid metabolism, changes in macromolecules turnover, mitochondrial dysfunction and cellular stress, ultimately leading to death of hepatocytes (Wu and Cederbaum, 2003).

Generation of ethanol derived free radicals

Ethanol can non-enzymatically react with hydroxyl radicals (OH\(^\bullet\)) generated via iron-catalysed degradation of H\(_2\)O\(_2\) (hydrogen peroxide) called Fenton's reaction (Cohn et al., 2006). From this reaction several free radicals can be formed from methanol and water (\('CH\(_2\)CH\(_2\)OH, CH\(_3\)CH\(_2\)O\(^\bullet\), or CH\(_3\)C'OH). \(\alpha\)-Hydroxyethyl radical (CH\(_3\)C'HOH, HER) comprises a major portion when alcohol reacts with OH\(^\bullet\) (Valko et al., 2005).

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \longrightarrow \text{OH}^\bullet + \text{OH}^- + \text{Fe}^{3+} \]

\[ \text{CH}_3\text{CH}_2\text{OH} + \text{OH}^\bullet \longrightarrow \text{CH}_3\text{C'}\text{OH} + \text{H}_2\text{O} \]

OH\(^\bullet\) attack on ethanol results in HER formation. Other iron-oxygen complexes raised from Fenton's reaction (Cohn et al., 2006) and ferrous iron autooxidation also participate in this reaction (Reinke et al., 2002).

Chemically generated HERs react with glutathione, vitamin C and E (El-Beltagi and Mohamed, 2013), thereby reducing the levels of antioxidants observed in chronic ethanol consumption. HERs react with oxygen, forms peroxo radical intermediate leading to formation of acetaldehyde (Setshedli et al., 2010).

\[ \text{CH}_3\text{C'}\text{OH} + \text{O}_2 \rightleftharpoons \text{CH}_3\text{C}(\text{OO}^\bullet)\text{OH} \rightleftharpoons \text{CH}_3\text{CHO} + \text{O}_2^\bullet^- + \text{H}^+ \]

Toxicity associated with ethanol oxidation by ADH

The oxidation of ethanol to acetaldehyde through alcohol dehydrogenase contributes to the reduction of nicotinamide adenine dinucleotide (NAD) to NADH. The high amount of reduced equivalents formed overwhelms the liver cells capacity.
maintain redox homeostasis and results in raise in the NADH/NAD ratio (Kono et al., 2000).

**Hyperlipidemia and fat accumulation**

The high ratio of NADH/NAD⁺ generated due to ethanol consumption and the concentration of α-glycerophosphate leads to hepatic triacylglycerol accumulation. Fatty acid oxidation therefore decreases in the mitochondria, resulting in changes in mitochondrial structure and function (Cazanave and Gores, 2010) causing several metabolic changes such as, induced lipogenesis in liver, high mobilization of peripheral fat, enhanced uptake of lipids from blood and decreased activity of tricarboxylic cycle.

In addition to the raised NADH, microsomal induction also increase the activity of lipogenic enzymes, enhances the synthesis of low and very low density lipoproteins (LDL and VLDL). LDL and VLDL changes and cholesterol turnover are involved in ethanol-induced hyperlipidemia.

**Free radical generation by xanthine oxidase**

Increased cytosolic NADH/NAD⁺ ratio also influence the activity of xanthine dehydrogenase and xanthine oxidase (XO), facilitating generation of free radicals (Kundu et al., 2012) and purine breakdown.

\[
\text{Hypoxanthine} \xrightarrow{\text{XO}} \text{Xanthine} \xrightarrow{\text{XO}} \text{Uric acid}
\]

\[
\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{H}_2\text{O}_2
\]

**Free radical generation by ferrous ion**

Increase in the cytosolic reducing equivalents in cytosol also can cause reduction of ferric (Fe³⁺) to ferrous (Fe²⁺) ions, which are involved in the generation of hydroxyl radical from hydrogen peroxide (Pessayre et al., 2012).

\[
\text{Fe}^{3+} + \text{NADH} \rightarrow \text{Fe}^{2+} + \text{NAD}^+ + \text{H}^+
\]

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{OH}^* + \text{OH}^- + \text{Fe}^{2+}
\]
Free radical production by the mitochondria

Alcohol induced hypermetabolic state in liver is characterised by enhanced mitochondrial respiration, demanding for the reoxidation of NADH (Adachi and Ishii, 2002). In mitochondrial matrix, reducing equivalents will act as substrate for the electron transport chain (ETC). This leads to increased instability of electrons. Electrons are transferred to molecular oxygen to create superoxide anion. Due to changes in NADH levels and modifications formed in complexes of mitochondria, chronic alcoholic consumption results in elevation of ROS (Bailey and Cunningham, 2002). Thus, NADH changes via Alcohol dehydrogenase plays an important role in ALD (Lieber, 1997) including steatosis.

PUFA and repeatedly heated PUFA

Vegetable oils rich in polyunsaturated fatty acid (PUFA) and its dietary intake is increasing due to its beneficial effects. PUFA is considered to be good for health, due to its hypocholesterolemic effect, and hence PUFA is highly consumed over saturated fatty acid (Patere et al., 2011). However, many studies have shown that increased consumption of PUFA is detrimental to health. Processing make oils unstable to oxidation. Studies show that maternal n-6 PUFA status during pregnancy influence fat accumulation in adipose in childhood (Moon et al., 2013) and the omega-6 fatty acids could speed up the augmentation of prostate tumor cells (Isabelle et al., 2007).

PUFA potentiates the severity of the liver diseases, by increasing the oxidative stress of the hepatic cells (Purohit et al., 2004). High ethanol consumption increases the calorie intake and hence alcoholics normally like to take fried foods and now they are generally made in PUFA (Leibowitz, 2007). Vegetable oil with high concentration of PUFA affects oxidative status of the cells. When taken in large quantities the cells become susceptible to oxidation. PUFAs have more unsaturated double bonds in their structure and have risk of being altered, denatured and react easily resulting in numerous damage to the cells. Excess consumption of PUFA is problematic and promotes chronic inflammation.

Food items prepared by repeatedly heated vegetable oils rich with PUFA are highly detrimental to health. Repeated heating destroys the beneficial property of PUFA and cause various changes in properties of the oil. Repeated heating of oil at
high temperatures produce more peroxidative indices (Liu et al., 2014; Ng et al., 2014; Adam et al., 2008), causes microsteatosis, inflammation and necrosis of the liver tissues (Jaarin et al., 2010). It also leads to genotoxic and proneoplastic changes in liver (Srivastava et al., 2010). Thus, the intake of alcohol along with fried food exacerbates the alcohol related liver diseases (Leibowitz, 2007). Alcohol induced liver fibrosis is accelerated by supplementing PUFA in diet (Aruna et al., 2004). When alcohol fed rodents were given foods rich in PUFA, it leads to inflammation, accumulation of fat and fibrosis (Varma et al., 2004; Latha et al., 2010; Aruna et al., 2006). Products of lipid peroxidation generated from the Alcohol + ΔPUFA are assumed to stimulate stellate cell propagation and their deposition in matrix, leading to fibrosis due to overproduction of aldehydes.

**Reactive oxygen species and their relationship in Alcohol + ΔPUFA induced liver damage**

The generation of ROS is a naturally occurring intracellular metabolic process. But the concentration of ROS in the cell is kept fairly constant by enzymic (e.g. SOD, CAT and GPx) and non-enzymic (e.g. reduced glutathione, vitamin C & E) antioxidants that are able to dispose the unwanted reactive oxygen species and generate nontoxic by-products. However, the production of ROS in the cells impairs antioxidant mechanism (Dey and Cederbaum, 2006). Many drugs and chemicals induce oxidative stress in the body, including ethanol and heated PUFA.

Ethanol or its metabolites change the delicate equilibrium in the liver by either acting as prooxidants or decreasing the antioxidant status or both (Seitz and Becker, 2007). Numerous evidences support the theory that acute and chronic ethanol toxicity is mediated through the production of harmful free radical species in a variety of tissues (Das and Vasudevan, 2007). PUFAs are most likely liable target to a ROS attack because of their low bond dissociation energy. The reaction of these radicals with these membrane machinery leads to lipid peroxidation. The enhanced generation of free radicals has been shown to cause DNA cleavage (Rakonczay et al., 2003).
Lipid peroxidation

Lipid peroxidation (LP) is explained as the oxidative deterioration of PUFA to form free radical intermediates and peroxides, which damage cellular constituents. LP is initiated by the construction of hydrogen atom from PUFA in the membrane lipids. It is a cascaded reaction providing free radicals that promotes peroxidation (Gutteridge, 1995). In addition, one of the lipid peroxidation products, malondialdehyde produces DNA adducts, that mediate DNA damage (De Bont and van Larebeke, 2004).

Lipid peroxidation is commonly used as an indicator to assess the cell membrane damage as a consequence of free radical production. The oxidative injury induced by alcohol in patients with alcoholic liver disease and in experimental animals exposed to Alcohol + ΔPUFA can be monitored by the detection of LP products, such as conjugated diene, lipid hydroperoxides and malondialdehyde (Cederbaum, 2001).

Protein damage

ROS plays a significant role in oxidative process. ROS alters the structure of proteins by attacking arginine, lysine and other residues to produce carbonyl protein (Chevion et al., 2000). Oxidation of cystine residues may produce mixed disulphides (Dalle-Donne et al., 2005). A random attack of reactive oxygen species on proteins is unlikely to be very damaging. ROS formed by activated macrophages might be a major cause of ALD (Kaviarasan et al., 2008).

Alterations in the lipid metabolism

The liver coordinates synthesis of fatty acids, esterification of triacylglycerols, and their packaging into very low density lipoproteins for export during fed conditions. In fasting it controls the rates of β-oxidation and ketogenesis. By balancing these processes, the liver handles large amount of fat without accumulating triacylglycerol. However, in certain forms of liver disease, this fine balance is disrupted and elevated levels of hepatocellular free fatty acid and triacylglycerol occur. The most common liver disease in which fatty acid metabolism is deranged is ALD.
Alcohol metabolism changes the intramitochondrial redox potential through production of NADH by alcohol dehydrogenase. This impairs β-oxidation and TCA cycle pathways ensuing increased production of FFA, triacylglycerol and VLDL and their secretion (Galli et al., 2001). A “redirection” of the incorporation of acetate from fatty acids to cholesterol synthesis, as a consequence of reduced formation of the acceptor glycerolipids, results in the increased cholesterol synthesis. Ethanol administration also stimulates the synthesis of phospholipids from exogenous fatty acids in rat hepatocytes; these alterations affect lipid levels in various tissues (Visioli et al., 1998).
The accumulation of lipids and the consequent steatosis are the most prominent alterations taking place in the liver of alcoholics, resulting from impaired lipid metabolism. Prolonged administration of ethanol also results in changes in the composition of fatty acids in an array of tissues including liver (Bataller and Brenner, 2005). Thus, chronic administration of ethanol produce marked alterations in lipid metabolism.

**DNA damage**

DNA damage is one of the most sensitive biological markers for evaluating the oxidative stress. The importance of ROS in inducing genetic toxicity was widely accepted and extensively studied (Milder and Patel, 2012). Direct breakage of DNA strands occurs when ROS interacts with DNA. Superoxide radicals can directly or indirectly damage DNA whereas hydrogen peroxide mediates DNA damage by the generation of hydroxyl radical (Al-Assaf and Abdullah, 2011).

![Diagram of DNA damage](image)

**Figure 4. Mechanism of oxidative damage to DNA** (Yu and Anderson, 1997)

The cumulative and inevitable effect of free radical attack on mitochondrial DNA is an increase in the frequency of mutations, which result in the production of proteins with impaired function. An accumulation of errors or damage of primary genetic material has already been proposed as initiators during cellular senescence.
and death of cell (Yu and Anderson, 1997). Ethanol exerts its cell toxicity via DNA damage (Saravanan and Pugalendi, 2005).

**Extracellular matrix**

Extracellular matrix (ECM) occupies very less percentage of the liver, but it influences liver function in a tremendous way. In fibrotic condition, changes in the protein level, specially the collagens, result in modification of the architecture of liver and affect various cell mechanisms. ECM proteins are multifunctional. They are signalling molecules, structural elements, maintain morphology of the liver and regulate growth factors. They perform individually as well as synergistically. Liver synthesizes various types of matrix proteins in normal and pathological conditions.

**Liver-normal condition**

The central vein, capsule and bile duct and surrounding areas of ECM are very much similar to all other epithelial organs. Basement membrane consists of collagen IV, glycoproteins, laminin and other proteins. Collagens I, III, V, VI and fibronectin are the important components in interstitium of the portal space. The lobular areas and the adjacent portal tracts are connected by collagen I network. Discontinuous deposits of collagen IV is also present in normal liver. In the microvilli of hepatocytes, fibronectin is dominant. In the space of Disse, type III and VI collagens are found. Collagen III is discontinuously arranged as deposits and distribution of type IV is in increasing order from the portal to the central region (Purohit et al., 2004).

**Liver-fibrosis condition**

Fibrosis is a chronic disease condition. Initially, disturbance in the extracellular matrix leads to qualitative and quantitative modifications in liver. It causes changes in morphology and physiology of liver. During advanced stage of fibrosis, the collagen content is increased 3 to 10 fold in ECM and it results in transport problems between the sinusoid to hepatocytes. Activation of HSC (perisinusoidal cells, lipocytes) is potentially involved in this modification. Morphological differences due to this ECM in the periportal and central regions influence the function of these areas (Bataller and Brenner, 2005).

Activation of stellate cell is an important event in liver damage; it is changed from quiescent state to a fibrogenic cell. These transitional cells undergo proliferation.
in region with maximum injury subsequently. Accumulation of ECM proteins causes changes throughout the dissection with basement membrane and affects the space in the sinusoidal epithelium. It increases synthesis of collagens and laminin for new matrix (Bortolotti et al., 2006) and as a result normal liver cell around Disse changes. The increased ECM affects the normal exchange process between sinusoidal blood and liver cells. Severe fibrosis leads to cirrhosis, forms fibrous with matrix protein (collagen) and becomes protease resistant, impeding the resolution of cirrhosis.

**Matrix metalloproteinases**

A number of proteases have been involved in the proteolytic deprivation of collagen and other ECM components, and the well-known are the members of the matrix metalloproteinase (MMP) family. MMPs come under metzincin super family of proteases that comprisemany metallo-endopeptidases family (Verma et al., 2007). The metzincins have a conserved structural topology that includes a harmony design within catalytic domain of 3histidines that give a zinc binding place. A sealed ‘Met-turn’ motif resides beneath the active site of zinc ion. Based on the structure,metzincins are divided into 4 subfamilies, namely, serralysins, adamalysins, astracins and matrixins (Gomis-Rüth, 2003).

MMPs are structurally and functionally related to zinc endopeptidases that are capable of destroying ECM proteins such as interstitial basement membrane, fibronectin, laminin, collagen, proteoglycansand thus are involved in connective tissue remodeling events involved in different pathological conditions (Egeblad and Werb, 2002). MMPs have been classified into collagenases, stromelysins, gelatinases and matrilysins, based on ECM proteins present in tissues (McCawley and Matrisian, 2001).

All MMPs are secreted as zymogens, having a secretory signal sequence and a propeptide sequence whose proteolytic cleavage is needed for activation. Next to the propeptide is the catalytic domain that has the consensus zinc binding moiety. MMP activity is broadly regulated at 3 levels, i.e., transcription, cleavage of zymogen and by regulation of enzyme activity by a number of normal inhibitors. Generally, MMPs are expressed in small quantities during normal-state. Cytokines, growth factors and cellular interactions trigger rapid induction of MMP expression (Sternlicht and Werb, 2001).
Table 1. The matrix metalloproteinase (MMP) family (Verma et al., 2007)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>E.C. No.</th>
<th>Pseudonyms</th>
<th>Mol. Wt. (Latent/Active)</th>
<th>Collagen substrates</th>
<th>Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>3.4.24.7</td>
<td>Collagenase-1, Fibroblast Collagenase, Tissue Collagenase Interstitial Collagenase</td>
<td>55,000/45,000</td>
<td>I, II, III, VII, VIII, X</td>
<td>Aggrecan, Gelatin</td>
</tr>
<tr>
<td>MMP-2</td>
<td>3.4.24.24</td>
<td>72kDa Gelatinase, 72 kDa TBE-1 Gelatinase/Type IV Collagenase, Gelatinase</td>
<td>72,000/66,000</td>
<td>I, II, III, IV, V, VII, X, XI</td>
<td>Aggrecan, Laminin Elastin, Gelatin,</td>
</tr>
<tr>
<td>MMP-3</td>
<td>3.4.24.17</td>
<td>Procollagenase, PTR1 protein, Transin-1 SL-1, Stromelysin-1</td>
<td>57,000/45,000</td>
<td>II, III, IV, IX, X, XI</td>
<td>Aggrecan, Laminin MMP Elastin, Gelatin</td>
</tr>
<tr>
<td>MMP-7</td>
<td>3.4.24.33</td>
<td>Matrilysin, Uterine Metalloproteinase, Matrin, PUMP-1 Protease</td>
<td>28,000/19,000</td>
<td>IV, X</td>
<td>Aggrecan, Laminin Elastin, Gelatin</td>
</tr>
<tr>
<td>MMP-8</td>
<td>3.4.24.34</td>
<td>Collagenase2, PMNL Collagenase, Neutrophil Collagenase</td>
<td>75,000/58,000</td>
<td>I, II, III, V, VII, VIII, X</td>
<td>Aggrecan, Gelatin Elastin,</td>
</tr>
<tr>
<td>MMP-9</td>
<td>3.4.24.25</td>
<td>92 kDa Gelatinase, Gelatinase B, 92 kDa Gelatinase/Type IV Collagenase</td>
<td>92,000/86,000</td>
<td>IV, V, VII, X, XIV</td>
<td>Aggrecan, Elastin</td>
</tr>
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<td>MMP-10</td>
<td>3.4.24.22</td>
<td>SL-2, Stromelysin-2, Transin-2</td>
<td>57,000/44,000</td>
<td>III, IV, V</td>
<td>Aggrecan, Elastin, Laminin, Gelatin,</td>
</tr>
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<td>MMP-11</td>
<td>SL-3, Stromelysin-3, ST-3</td>
<td>51,000/44,000</td>
<td>Aggrecan, Fibronectin</td>
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<td>MMP-12</td>
<td>3.4.24.65</td>
<td>HME, Macrophage Metalloelastase MME</td>
<td>54,000/45,000 and 22,000</td>
<td>IV</td>
<td>Elastin, Fibronectin</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Collagenase-3</td>
<td>60,000/48,000</td>
<td>I, II, III, IV</td>
<td>Aggrecan</td>
<td></td>
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<td>MMP-18</td>
<td>3.4.24.65</td>
<td>Xenopus Collagenase-4, xCol4</td>
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<td>Aggrecan,</td>
<td></td>
</tr>
<tr>
<td>MMP-19</td>
<td>RASI-1</td>
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<td>IV</td>
<td>Fibroneectin, Lamiin</td>
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<td>MMP-20</td>
<td>Enamelysin</td>
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<td>IV</td>
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<td>MMP-28</td>
<td>Epilysin</td>
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<td></td>
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<td>MT1-MMP</td>
<td>MMP-14, Membrane-Type Metalloproteinase-14</td>
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<td>I, II, III</td>
<td>Aggrecan, Elastin, Gelatin, Laminin</td>
<td></td>
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<td>MT2-MMP</td>
<td>MMP-15, Membrane-Type Metalloproteinase-15</td>
<td>72,000/60,000</td>
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<td>Fibronectin, Gelatin</td>
<td></td>
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<tr>
<td>MT3-MMP</td>
<td>MMP-16, Membrane-Type Metalloproteinase-16</td>
<td>64,000/52,000</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MT4-MMP</td>
<td>MMP-17, Membrane-Type Metalloproteinase-17</td>
<td>57,000/53,000</td>
<td></td>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>MT5-MMP</td>
<td>MMP-24</td>
<td>62,000</td>
<td></td>
<td></td>
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<tr>
<td>MT6-MMP</td>
<td>MMP-25 Leukolysin</td>
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<td>Gelatin, Fibronectin</td>
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</tr>
</tbody>
</table>

**Biomedical importance of MMPs**

MMPs participate in a number of proteolytic events, both in physiological and pathological conditions. Functions of the MMPs are cell relocation, bone elongation, neurite growth, lesion healing-angiogenesis, uterine involution, ovulation, menstruation, enamel formation, mammary gland development, hair follicle development, antigen processing and presentation, sperm maturation, and embryo implantation. During pathological conditions MMPs are actively involved in tumor growth and migration, arthritis, glaucoma, lupus scleroderma, fibrosis, cirrhosis, multiple sclerosis, infertility, aortic aneurysms and many more diseases (Webster and Crowe, 2006).

MMPs are primarily meant to destroy ECM proteins. Cell migration is facilitated principally by degradation of ECM by MMPs. However, effects of ECM degradation are considerably more complex. In normal liver, low expression of interstitial collagenase (MMP-1) gelatinase A (MMP-2), stromelysin (MMP-3), gelatinase B (MMP-9) is expected (Sbardella et al., 2012). In case of pathological conditions, especially in early stages, an elevated expression of metalloproteinases,
especially, gelatinase A has been reported, which is majorly because of activation of stellate cells (Shiomi et al., 2010). During initiation of fibrosis, along with an abnormal production of collagen III and I, the degradation of collagen V by gelatinase A takes place, significantly contributing to the capillarization of sinusoid. Therefore, there exists a homeostasis between pro-fibrogenic process and pro-fibrolytic process in liver fibrosis (Latella et al., 2015). The balance between these processes is modulated through competitive, additive and synergistic action of different stimuli via additional regulatory mechanisms. As a matter of fact, homeostasis of metalloproteinases are the result of a highly complex regulation of gene expression, extracellular activation of proenzyme forms and highly specific inhibition involving tissue inhibitors of metalloproteinases (TIMPs) (Mannello and Medda, 2012). MMP expression increases with advancing fibrosis thus decreasing the connective tissue deposited. Due to their critical role in ECM regulation, MMPs are known to be potential biochemical markers for studying liver toxicity (Poli, 2000).

**Tissue inhibitors of matrix metalloproteinases**

One of the most important regulatory mechanisms in metalloproteinase activity is controlled by a family of specific molecules called Tissue inhibitors of matrix metalloproteinases (TIMPs). TIMPs bind at the active site of MMPs and hence inhibit their degradative ability. TIMPs are small molecules ranging between 21-28 KDa, they bind to MMPs in a stoichiometric ratio of 1:1 reversibly inhibiting MMP activity. TIMPs work at two levels: first, they bind to the carboxy terminus of the enzymes to retard their activation. Second, they bind at the active site of the enzymes, reversibly blocking the enzyme activity (Brew et al., 2000).

TIMPs show varied expression and specificity for different type of MMPs. TIMP-1 and TIMP-2 participate in inhibition of a broad range of MMPs. TIMP-1 forms a reversible complex with pro MMP-9. TIMP-1/pro MMP-9 complex is believed to recruit MMP-3, thus, forming a next complex that results in MMP-3 inactivation. TIMP-3 is involved in MMP-1, 3, 7 and 13 inactivation. Unlike other TIMPs, it is involved in inactivation of more distantly related metalloproteinase called ADAM-17. TIMP-4 is known to have an extra restricted inhibitory activity that primarily inhibits MMP-2 and MMP-7 and to some extent MMP-1, 3 and 9.
<table>
<thead>
<tr>
<th></th>
<th>TIMP-1</th>
<th>TIMP-2</th>
<th>TIMP-3</th>
<th>TIMP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (kDa)</td>
<td>28.5</td>
<td>21</td>
<td>24-27</td>
<td>24</td>
</tr>
<tr>
<td>Tissue localization</td>
<td>Wide distribution, but lower levels. Uterus, decidua, ovary, heart, muscle, placenta, kidney, thymus, spleen, low in liver, testes, brain</td>
<td>Wide distribution, Brain (meninges, choroid plexus), heart, uterus, ovary, thymus, decidua, muscle, spleen, kidney, testes, placenta, low in liver</td>
<td>Thymus, heart, kidney, ovary, placenta, brain, muscle, uterus, decidua, spleen, in lung, low in liver</td>
<td>Most restricted. Brain (highest of all TIMPs) (cerebellar purkinje cells), heart, testes, thymus, ovary, muscle, placenta. Also in blood vessels, platelets, breast. Absent in liver and spleen</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Yes</td>
<td>No</td>
<td>High</td>
<td>No (unresolved)</td>
</tr>
<tr>
<td>Soluble</td>
<td>Yes</td>
<td>Yes</td>
<td>ECM associated</td>
<td>Yes</td>
</tr>
<tr>
<td>Regulation</td>
<td>Principally transcriptionally</td>
<td>Constitutive</td>
<td>Principally transcriptional</td>
<td>Highly regulated</td>
</tr>
<tr>
<td>Number of disulfide bonds</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>C-terminal tail</td>
<td>None</td>
<td>QEFLDIEDP</td>
<td>KSIINATDP</td>
<td>KEFVDIVQP</td>
</tr>
<tr>
<td>MT-MMP inhibition</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ADAM’s inhibition</td>
<td>No</td>
<td>No</td>
<td>Yes-ADAM-17</td>
<td>No</td>
</tr>
<tr>
<td>Hemopexin C-domain binding</td>
<td>MMP-9</td>
<td>MMP-2 outer rim of domain at junction of hemopexin modules III &amp; IV. Displaces TIMP-4</td>
<td>MMP-2</td>
<td>MMP-2 (competed off by TIMP-2)</td>
</tr>
<tr>
<td>Trimolecular complex formation</td>
<td>MMP-9/TIMP-1/ MMP-3 (inhibition)</td>
<td>MT-1-MMP/TIMP-2/ proMMP-2</td>
<td>Does not form with MT1-MMP and MMP-2</td>
<td>Does not form with MT1-MMP and MMP-2</td>
</tr>
</tbody>
</table>
Apart from its role in checking MMPs, TIMPs are also believed to play a significant role in the maintenance of ECM. However, few more functions of TIMPs have been reported, such as cell growth-promoting activity, erythroid potentiating activity, embryogenesis-stimulating activity, and steroidogenesis stimulating activity. Since, TIMPs are believed to have a dual function; it is a preferred target to study tissue fibrosis (Giannandrea and Parks, 2014).

**Fatty liver (hepatic steatosis)**

Accumulation of fat in the liver cells is the initial and recurrent response to alcohol (Lieber, 2001). In patients with steatosis, lipid accumulates in bulk (macrovesicular) or small (microvesicular) droplets in liver cells. However, this condition infrequently causes sickness (Clark and Diehl, 2002).

**Alcoholic fatty liver**

Frequent alcohol consumption in large amount increases the risk of rising various illness of the liver (Lieber, 2001). Conventionally, they have been considered serially linked, making progress from fatty liver to perivenular fibrosis/ hepatitis to cirrhosis.

---

**Figure 5. Progression of alcoholic liver disease** (Lieber, 2001)
**Perivenular fibrosis**

Fibrosis involves deposition of ECM, specifically collagen. It can happen at any place in the hepatic acinus. Initially, the deposition is around the central veins and venules. Prolonged intake will develop into more harsh condition, such as cirrhosis (Sadana Addagudi et al., 2013).

**Alcoholic hepatitis (steatohepatitis)**

Steatohepatitis is associated with hepatocellular injury and acute/chronic inflammation. Accumulation of fat in the liver leads to progression of hepatitis. Fatty liver is more susceptible to many factors that activate inflammation. The development of steatohepatitis seems to be an important regulating step in the pathogenesis of cirrhosis (Clark and Diehl, 2002).

**Cirrhosis**

The most significant form of alcoholic liver damage is cirrhosis. Heavy alcohol consumption leads to damage of hepatocytes. Fibroblasts emerge at the site of injury and induce collagen development. One of the characteristic features of cirrhosis is the development of scar tissue that blocks the blood vessels and changes the normal structure (McClain et al., 2002), worsening the hepatic function. Upon continued hepatocyte damage and collagen accumulation, the liver reduces in size and appears nodular, with hardened texture leading to “end stage cirrhosis” (McClain et al., 2002). It takes 10-20 years of heavy drinking for cirrhosis to occur. Most liver-related morbidity and mortality occur in the subset of individuals, who have become cirrhotic. Various transcription factors e.g., PPAR-α, carnitine palmitoyl transferase I (CPT-I), acyl-CoA oxidase (ACO), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), PPAR-γ, sterol regulatory element-binding protein-1c (SREBP-1c) induce the expression of gene products integral to the fatty liver.

**PPAR-α in fatty liver**

PPAR-α is a master factor and plays a crucial role in fatty acids metabolism and mediates fatty acid oxidative gene expression and is involved in inflammatory processes. PPAR-α participate in metabolism of amino acids, lipoproteins, glucose/glycerol, cholesterol, bile acids and xenobiotics. The molecular mechanisms and specific target genes of PPAR-α and their functional consequences has been
studied (Rakhshandehroo et al., 2010). PPAR-α is a major regulatory transcription factor of lipid metabolism predominantly present in the liver. During energy deprivation, fatty acids and fatty acid-derived compounds (ligands) bind to PPAR-α and trigger uptake and utilization by peroxisomal and mitochondrial fatty acid β-oxidation (Rakhshandehroo et al., 2010).

Oxidation of alcohol produces NADH and it favors the anabolic pathways of fatty acid and TG synthesis and reduces β-oxidation of fatty acids. It increases influx of fatty acids to liver. It leads to fatty acid accumulation in liver. Further, alcohol inhibits adenosine monophosphate activated kinase (AMPK) activity (You and Crabb, 2004). This leads to increased lipogenesis and decreased lipolysis in hepatic cells by preventing the expression of peroxisome proliferating-activated receptor α (PPAR-α) (Nakajima et al., 2004). So, PPAR α expression is an important factor to identify the pathological condition in the formation fatty liver during Alcohol +ΔPUFA induced liver toxicity.

**Cytokines in liver**

Liver is one of the most active organs engaged in inflammatory response. Cytokines are major factors involved in acute and chronic inflammation. Endotoxins that are produced in intestine, travel via blood stream to the liver and induce the production of cytokines by activating kupffer cells. Various detoxifying enzymes are induced in chronic alcoholic response in liver, thus, generating ROS that leads to the production of cytokines. Compounds that can control the cytokine levels in ALD are highly helpful in the therapy of ALD (Singh et al., 2014).

Cytokines are the key markers in inflammatory diseases. In a normal cell, it is minimally secreted. Gradual lipid accumulation in hepatocytes provokes hepatic stellate cells and Kupffer cells to synthesize various types of cytokines that would lead to portal inflammation, slow necrosis or apoptosis, and eventually end up in fibrosis (Braunersreuther et al., 2012).

TNF-α is an important cytokine secreted by most body cells, particularly in HSCs, Kupffer cells, and adipocytes. TNF-α expression and insulin resistant in steatohepatitis are closely linked. TNF-α act on hepatic mitochondria, causes swelling and rupturing of the mitochondrial membrane, thus disrupting the respiratory chain complexes. Such, reduced functioning of mitochondrial complexes have been
reported in experimental animals that were challenged with hepatotoxicity (Wei et al., 2008; Sudheesh et al., 2012; Sudheesh et al., 2013). In cases of hepatic steatosis, IL-6 protects the liver by preventing mitochondrial dysfunction (Braunersreuther et al., 2012). IL-6 blocks the hepatic cytokine signalling (Pearce et al., 2013).

A number of cytokines are abnormally regulated in the liver in response to alcohol consumption. TNF-α, a vital mediator of ALD (Barnes et al., 2014), initiates the injury when challenged with ethanol exposure. In addition to LPS–TLR-4 signalling, TNF-α is important for the prognosis of ALD (Kawaratani et al., 2013). Ethanol also generates an affinity for monocytes. In ALD, monocytes produce elevated levels of TNF-α compared to monocytes from non-alcoholics (Elmore, 2007).

IL-1, a known pro-inflammatory mediator cytokine is elevated in ALD patients, after chronic ethanol exposure. IL-1 receptor antagonists (Anakinra) are one of the promising leads in alcoholic hepatitis treatment (NIAAA, 2013). Similarly, IL-6 is an important inflammatory cytokine and is involved in activation of antibodies secreting cells and provokes inflammatory reaction (Hill et al., 1992). It activates various signalling pathways via STAT-3 and prevents hepatocellular damage and promotes liver regeneration (Wang et al., 2011).

TGF-β is also an important cytokine marker in ALD (Zhang et al., 2014). TGF-β level is generally elevated during hepatic injury. It is secreted by various liver cells like hepatocytes, HSCs and Kupffer cells during necrotic condition. TGF-β activates HSCs, further increasing the severity of liver damage. In severe conditions, it leads to liver fibrosis and cirrhosis (Jeong, 2008). An elevated level of TGF-β also leads to steatosis (Ciuclan et al., 2010).

**NFκB and STAT-3 Pathways**

NFκB is one of the important cell survival pathways that are activated during liver injury and inflammation. STAT-3 is another important pathway triggered through immune responses and inflammation. These pathways act separately as well as in collaboration with various regenerative processes. Stress and cytokines rapidly activate both NFκB and STAT-3. Induction of NFκB and STAT-3 control the expression of immune response and anti-apoptotic genes. The activation of and cooperation between STAT-3 and NFκB plays a key role in regulating inflammatory
responses. These pathways promote progression of various cancers in colon, gastric and liver. NFκB and STAT-3 control expression of various genes responsible for cell proliferation, survival and repairing tissues (Karin, 2008; Karin and Lin, 2002; Haura et al., 2005).

**Figure 6. Inflammatory cytokines in alcoholic liver disease** (Kawaratani et al., 2013)

NFκB and STAT-3 act collaboratively in alcohol induced liver damage. Injured hepatocytes release some cytokines and Kupffer cells produce TNF-α. These cytokines activate NFκB and STAT-3 molecular mechanisms, leading to progression of ALD (Yue and Turkson, 2009). Inhibition of these pathways by specific targeting would reduce the ALD (Kawaratani et al., 2013).

**The pathways of cell death in liver**

During progression of ALD, continuous ethanol metabolism leads to cell injury, leading to cell death. It is mainly through three major pathways, namely, apoptosis, necrosis and necroptosis (Elmore, 2007). Necrosis, a non-apoptotic pathway where the cell undergoes random rupturing and release cell contents into
extra-cellular space, causing damage to neighbouring cells because of accumulation of inflammatory response. Necroptosis, a phenomenon where the cell seems to undergo necrosis morphologically, though in reality, it undergoes a highly regulated programmed cell death, similar to apoptosis.

**Apoptosis**

Every cell has an in-built mechanism of programmed cell death called apoptosis. Older and unhealthy cells trigger a series of mechanism that causes death and clearance from the organism in a systematic way, thus, not affecting the neighboring healthy cells. Apoptosis plays an important role in maintenance of liver. Apoptosis is characterized by specific morphological changes, blebbing of cell membrane, cellular protein degradation, condensation of chromatin, characteristic fragmentation in DNA, ultimately leading to the formation of small fragments of closed bodies called “apoptotic bodies” (Nishawar et al., 2008). These apoptotic bodies are finally scavenged by kupffer cells.

**Apoptotic Pathways**

Apoptosis occurs via two major pathways i.e. the extrinsic pathway and the intrinsic pathway. Extrinsic pathway is cell membrane receptor mediated pathway, while, the intrinsic pathway is mitochondrial regulated. However, both of the pathways have a common end pathway regulated by the caspases. The extrinsic pathway involves plasma membrane receptors such as TNF related apoptosis stimulating ligand receptors (TRAIL-R1 and TRAIL-R2), Fas and tumor necrosis factor receptor (TNF-R1) (Yin, 2000). This condition triggers the activation of key proteins such as procaspase-8 and -10 resulting in the formation of a complex known as death-inducing signalling complex (DISC), which in turn activate the effector caspases (caspase 3 and 7) (MacFarlane and Williams, 2004). Activation of caspases triggers irreversible mechanisms that result in cell death.

The intrinsic pathway involves the activation of proapoptotic markers, namely, Bax and Bak. Several factors such as oxidative stress, accumulation of ROS, endoplasmic reticulum stress, chemical and radiation exposure may trigger intrinsic pathway. It is principally known to be a mitochondrial mediated pathway. The pro-apoptotic markers are regulated by an anti-apoptotic marker known as Bcl-2. Mitochondrial dysfunction leads to the release of inter mitochondrial membrane
proteins such as cytochrome c and SMACs (second mitochondria derived activator of caspases) into the cytosol. The release of cytochrome c and SMACs triggers apoptosome formation. Apoptosome is a complex formed by the association of cytochrome c, procaspase-9 and adaptor apoptosis associated factor 1 (Apaf-1). This apoptosome catalyzes the activation of down-stream effector proteins like caspases 3, 6 and 7 which trigger the degradation of the cellular organelles (Martinon and Tschopp, 2004).

**Figure 7. Mechanism of apoptosis** (Martinon and Tschopp, 2004)

**Drugs used in the management of liver disease**

**Corticosteroids**

Corticosteroids used in therapies of alcoholic hepatitis have received a great deal of interest. Guidelines sustain the use of corticosteroids in alcoholic hepatitis. However, steroids seem to have a beneficial effect on short term survival but not on long-term survival.

**Adverse effects**

Corticosteroids are very strong medicines. High doses of corticosteroids can actually cause liver disease. The main side effects reported are appetite, obesity, gastric problems, headache, fluctuation in blood glucose and BP. Side effects are arrested when therapy is stopped. Prolonged usage leads to reduced immunity.
Colchicine

Colchicine has been used in the therapy of fibrosis and colchicine may progress liver activities and reduce the consequence of hepatic fibrosis.

Adverse effects

The therapeutic option for colchicine is fairly narrow because of toxic effects with even little overdoses. Colchicine intake causes stomach disturbances. Prolonged usage leads to suppression of bone marrow muscle and neuronal complications. Thrombophlebitis and decreased spermatogenesis have also been reported.

Antioxidant therapy

The deleterious effects of alcohol, at least partly, involve alcohol induced oxidative stress (McDonough, 2003). Intake of antioxidants could trigger the detoxification mechanism (Zhang et al., 2013) and represent a potential group of therapeutic agents for ALD.

Silymarin, vitamin E, selenium S-adenosyl methionine and polyenoylphosphatidyl choline are some nutrients and antioxidants which are under assessment or accepted for the prevention and/or treatment of ALD (Frazier et al., 2011).

Ethno medical importance of natural remedies

Herbal products are extensively used in traditional medicines. Therapeutic plants have a beneficial role in maintenance of human health. About 80% of the world population relies on the habitual medicine which is mostly based on plant materials (World Health Organization, 2002). The customary medicine refers to a wide range of very old, natural health care practices including folk/ethnic practices as well as Ayurveda, Siddha, Amchi and Unani. They attract increased attention because of their natural origin and accumulated evidence during years of application (Guo et al., 2001).

One of the problems faced in crude plant drugs is alterations in efficacies due to preparative protocol differences. Such differences could happen due to genetic changes, seasonal difference, soil variation, modified climates, dietary status of the
medicinal plants. So, phytochemical approach concentrates in isolating pureactive principles as drugs.

Herbal drugs have gained significance in modern years because of their efficacy and cost effectiveness. There are more than 100 drugs with all structural information that are prepared from higher plants and used in modern medicines. A proper ethnopharmacological search and follow-up research can lead to variety of drugs (Pandey Govind, 2011).

Wheatgrass

Medicinal plants or herbs have received much attention as dietary supplements. Their isolated bioactive constituents serve as a major source of drug development for preventive and curative applications. Plants are good source of phenolics and flavonoids, which have been reported to show superior antioxidant activity. Due to the presence of potent antioxidant agents in plants, there are number of herbal medicines in Ayurveda which are being increasingly used to treat liver diseases (Faremi et al., 2008; Sathaye et al., 2011; Lu et al., 2011; Ashok et al., 2007).

Wheat (Triticum aestivum) germinated over a period of 6 to 10 days is known as wheatgrass. It is a sub species of the family Poaceae (Padalia et al., 2010). It is widely grown in temperate regions; however, India too has been cultivating WG because of wheat being one of the staple foods of India. The first experimental trial on WG was reported way back in 1930s by Charles F Schnabel (Murphy, 2002). The extract of WG has traditionally been used as a healer in several illnesses like sore throat, liver diseases and constipation (Ellen Coleman, 1994). WG is also known as “complete food” as it contains vitamin C and E, β carotene, ferulic acid, vanilic acid, and phenols especially flavonoids. Further WG extract has good antioxidant properties. It is known to be a rich source of chlorophyll, which accounts for prohibiting the metabolic activation of carcinogens. Dry powder of WG is available in market as nutritional supplements and it is mostly marketed as ayurvedic product. It is non-toxic and approved by Food and Drug Administration authority.

Wheatgrass in folk medicine

WG is used as a herbal medicine in a number of disorders like thalassemia and myelodysplastic syndrome (Marawaha et al., 2004; Mukhopadhyay et al., 2009). It is believed to strengthen the immune system and regress the spread of cancer cells, thus,
increasing the life span of cancer patients (Moller et al., 1999). WG juice is found to have healing properties in various degenerative diseases and it benefits the blood cell, bone, glands, kidney and other parts of the body (Padalia et al., 2010; Kulkarni et al., 2006; Shakya et al., 2012). There is a major concern about contaminations due to solvent usage in the preparation of plant extracts. To minimize this, traditional extracts are always been prepared as decoctions of aqueous extracts (Rahim and Khan, 2006). WG fresh juice has long been used as a nutritional supplement (Shakya et al., 2012; Kothari et al., 2008; Alitheen et al., 2011) in several parts of the world.

**Wheatgrass (Triticumaestivum)**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Poales</td>
</tr>
<tr>
<td>Family</td>
<td>Poaceae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Pooideae</td>
</tr>
<tr>
<td>Tribe</td>
<td>Triticeae</td>
</tr>
<tr>
<td>Genus</td>
<td>Triticum</td>
</tr>
<tr>
<td>Species</td>
<td>T. aestivum</td>
</tr>
<tr>
<td>Binomial name</td>
<td>Triticumaestivum</td>
</tr>
</tbody>
</table>

Figure 8. Structure of Wheatgrass

**Pharmacological properties**