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While the heart resounds and attracts the music of the mandolin, 
there, inside, you filter and apportion, you separate and divide, 
you multiply and lubricate, you raise and gather the threads and the grams of life, 
the final distillate, the intimate essences....

Ode to the Liver; Pablo Neruda

1. LIVER

The adult human liver normally weighs between 1.3 - 3.0 kilograms, and is a soft, pinkish-brown 
organ. It is the largest glandular organ of the human body. Liver has a wide range of functions, 
including detoxification, protein synthesis, combating infections in the body, producing quick 
energy, storing iron, vitamins and other essential nutrients. Liver plays a vital role in the 
clearance of compounds from the blood, producing immune proteins to control infections and 
directly removing germs and bacteria (innate immune system) and synthesizing proteins that 
regulate blood clotting and various other physiological processes. Further, liver produces and 
excretes bile fluid which is required for food digestion and absorption of fats and fat-soluble 
vitamins. Liver is thus essential for survival and proper functioning of body.

1.1 Anatomy: Traditionally liver was divided into four lobes, right, left, caudate and quadrate 
lobe. However, now the Couinaud nomenclature is frequently used in which liver is divided in to 
eight segments. It is divided into right and left lobe by the line between the gallbladder and 
inferior vena cava. Further each lobe is partitioned into 2 sub-lobes, and each sub-lobe into 2 
segments (Figure 1) (Misdraji et al., 2010). Portal vein and hepatic artery supply blood and 
oxxygen to the liver. Portal vein supplies up to 70% blood and 40% oxygen while the hepatic 
artery is responsible for 30% blood and 40% oxygen (Misdraji et al., 2010; Sherif et al., 2010). 
Bile is secreted from the right and the left hepatic duct which meets to form a common hepatic 
duct. The hepatic duct further joins with the cystic duct coming out of the gall bladder to form 
the bile duct (Figure 1). Each segment of liver has a single portal triad (Figure 2) also called the 
pedicle. A portal triad is formed by the terminating portal vein branches, hepatic artery branches, 
and a bile duct.
1.2 Structural organization of liver: The hexagonal liver lobule is the basic structural unit of the liver (Figure 2). The central vein and portal tract are located at the center, and three angular points of the hepatic lobule, respectively (Kang, 2013). The endothelium-lined sinusoids of the hepatic lobule represent the functional unit of the liver, where afferent blood flow is exposed to functional hepatic parenchyma prior to being drained into hepatic venules (Townsend et al., 2012). The hepatic sinusoids are 7 to 15 μm wide but have the ability to increase in size by up to 10-fold (Townsend et al., 2012). Sinusoids are unique capillaries that differ from other capillaries of the body, because of the presence of open pores or fenestrae lacking a diaphragm and a basal lamina underneath the endothelium (Braet and Wisse, 2002). Endothelial fenestrae usually measure 150–175 nm in diameter. The different cell types of hepatic lobule are divided into different groups and perform certain set of functions. Hepatocytes and bile duct cells are the major parenchymal cells. The second groups of cells are sinusoidal cells that include the hepatic sinusoidal endothelial cells and Kupffer cells (hepatic macrophages). The final group is perisinusoidal cells, which consist of hepatic stellate cells and pit cells (Kang, 2013). Sinusoidal endothelial cells are separated from hepatocytes by the space of Disse (perisinusoidal space) (Braet and Wisse, 2002; Townsend et al., 2012). This forms an extravascular fluid compartment.
wherein the hepatocytes project microvilli, that allows proteins and other plasma components from the sinusoids to be taken up by the hepatocytes. The fenestrations of the endothelial cells also restrict the movement of molecules between the sinusoids and hepatocytes (Braet and Wisse, 2002; Townsend et al., 2012).

1.2.1 Hepatocytes: Hepatocytes, in the hepatic parenchyma, represent around 70% of the liver cell population (Vera-Ramirez et al., 2013). These cells are arranged in arrays of one or two cells thick and are connected by sinusoids (blood vessels) which further connect the portal venous system to the systemic venous system (Kang, 2013; Vera-Ramirez et al., 2013). Main functions of hepatocytes include secretion of proteins and bile, detoxification of xenobiotics, acute phase response and metabolism of glucose, glycogen and cholesterol (Malhi et al., 2010). Hepatocytes are generally quiescent cells with low turnover and a long life span however; these cells can grow in response to stimulus such as damage to other cells (Preet, 2012).

1.2.2 Cholangiocyte: Cholangiocytes also known as bile duct cells are present in the bile duct epithelium and represent around 3% of the liver cell population. These cells are involved in the transportation and control the rate of bile flow, secretion of water and bicarbonate to control the pH of bile (Vera-Ramirez et al., 2013).
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1.2.3 *Endothelial cells*: The sinusoidal endothelial cells constitute around 2.5% of the lobular parenchyma which form the lining of the sinusoids and thus forms the sinusoidal plexus which aids in blood circulation (Vera-Ramirez et al., 2013). The endothelial cells also enable the transfer of molecules and proteins between serum and hepatocytes (Preet, 2012). They further function in antigen presentation, cytokine secretion and blood clotting (Preet, 2012). These cells contain fenestrations with a diameter of 150 to 175 nm. They do not, therefore, form a tight basement membrane barrier between themselves and the hepatocytes. Their fenestrations or pores further promote the free passage of blood components through this membrane into the liver parenchymal cells (Townsend et al., 2012).

1.2.4 *Kupffer cells*: Kupffer cells are located within the sinusoidal lining. They form the 2% of the liver cells (Vera-Ramirez et al., 2013). These cells are the resident macrophages of liver. These cells protect the liver from the antigens and have both the endocytotic and phagocytic ability (Preet, 2012). They form almost one-quarter of all the lysosomes of the liver. They also protect the liver from gut-derived particulate materials and bacterial products. These cells play an important role in the defense system of liver by releasing cytokines. They also remove damaged erythrocytes from circulation (Preet, 2012; Townsend et al., 2012).

1.2.5 *Hepatic stellate cells*: Hepatic stellate cells (HSCs) also known as perisinusoidal or Ito cells are lipid filled cells that are found in the space of Disse. They represent around 1.4% of the liver cells (Vera-Ramirez et al., 2013). These lipid-filled cells are the primary storage site for vitamin A. They are also involved in the synthesis of extracellular matrix and regulation of contractility of the sinusoids. HSCs are activated to a myofibroblastic state in case of acute and chronic hepatic liver injuries. Activated HSCs lead to decreased intracellular vitamin A, and increased production of extracellular matrix. Thus, HSCs play a vital role in the development and progression of hepatic fibrosis to cirrhosis (Preet, 2012; Townsend et al., 2012).

1.2.6 *Pit cells*: Pit cells, also known as liver-associated lymphocytes are natural killer cells involved in defense mechanism against tumor cells or viruses. These cells are rarely found and are only involved in cytotoxic activity (Vera-Ramirez et al., 2013).
1.3 Important functions of liver: The liver plays a crucial role in important physiological processes ranging from regulation of the amount of energy, storage and distribution, synthesis of factors, proteins and detoxification. Some of the important functions are listed here:

- The liver manufactures many substances which serve other organs or tissues in response to multiple metabolic signals. It is the only organ producing acetoacetate which is used by muscle, brain, and kidney, but not itself (Jeschke, 2009).
- The liver receives blood from the arterial and portal circulation, processes nutrients, metabolizes toxins and wastes, and stores, transforms, and distributes them to the vascular, biliary, or lymphatic circulations (Jeschke, 2009).
- The liver acts as a physiologic reservoir of blood with 25% to 30% of its volume composed of blood (Greenway and Lautt, 1989). Liver can release up to 60 percent of its blood volume in case of acute failure without loss of its own function (Eipel et al., 2010).
- Bile secretion is another important function of liver. Hepatocytes secrete 80% of the total daily production of bile (approximately 1500 mL) while the rest of the 20% is secreted by the bile duct epithelial cells (Jeschke, 2009). Bile plays an important role in digestion of fats.
- Excretion of bilirubin: Bilirubin is a breakdown product of heme and is almost completely excreted in the bile (Jeschke, 2009).
- Liver is the secondary lymphoid organ of the reticuloendothelial system (RES). In spite of being a secondary lymphoid organ, 60 % of the cellular system of RES are present in the liver (Jeschke, 2009). The cellular components include phagocytes, Kupffer cells and endothelial cells. The main function of these cells is to recognize antigens and initiate immune response (Townsend et al., 2012).
- Liver is responsible for the synthesis of acute phase proteins. The acute phase response is series of events which is initiated to prevent tissue damage and to activate repair processes (Moshage, 1997). The acute phase response is initiated by activated phagocytic cells, fibroblasts, and endothelial cells, which release proinflammatory cytokines leading to the systemic phase of the acute phase response (Moshage, 1997).
- Liver plays a central role in drug metabolism. The smooth endoplasmic reticulum of the hepatocyte is the principal site of metabolism in the liver. Hepatic-based reactions are broadly classified into phase I and II reactions. Phase I reactions involve oxidation, reduction and hydrolysis reactions that increase the polarity and water solubility of compounds. The
improved polarity makes the excretion of the compounds much easier (Townsend et al., 2012). Phase I reactions however do not necessarily detoxify chemicals and in fact, may produce toxic metabolites. Phase I reactions occur in the cytochrome P450 (CYP) system (Townsend et al., 2012). Phase II reactions generally involve transferase reactions wherein compound is coupled to a conjugate which makes the compound less toxic (Townsend et al., 2012).

- Carbohydrate Metabolism: The liver has an essential role in energy metabolism as it provides readily available source of energy to the central nervous system, red blood cells, and adrenal medulla in the form of glucose (Jeschke, 2009). Also the glucose absorbed by the hepatocyte is converted directly into glycogen for storage. Glycogen acts as the primary source of glucose during the fasting phase (Jeschke, 2009). Glycogenesis, glycogenolysis, and the conversion of galactose into glucose all represent hepatic functions that ensure sufficient glucose synthesis.

- Lipid Metabolism: Liver has three main sources of free fatty acids: fatty acids synthesized from carbohydrates and amino acids, fat absorbed from the gut, fat liberated from adipocytes in response to lipolysis. These fatty acids are etherified with glycerol to form triglyceride (TGs). The export of TGs is dependent on the synthesis of very low density lipoproteins. In cases of an excess supply of fatty acid, there is lipid accumulation in the liver which leads to fatty liver. Phospholipid and cholesterol synthesis takes place in the liver. Cholesterol is a standard for the determination of lipid metabolism (Jeschke, 2009).

- Protein Metabolism: Liver is involved in synthesis and secretion of 17 of the major plasma proteins. Hepatic cells are responsible for the synthesis of albumin, fibrinogen, prothrombin, and other factors involved in blood clotting (Kang, 2013).

- Vitamin Metabolism: The liver plays an important role in uptake, storage, and mobilization of vitamins, especially fat soluble vitamins (A, D, E and K). The absorption of these fat soluble vitamins is dependent on bile salts. Vitamin A is exclusively stored in the Ito cells (fat-storing cells) of the liver (Chen, 2013; Jeschke, 2009). The initial step in vitamin D activation occurs in the liver where vitamin D$_3$ is converted to 25-hydroxycholecalciferol (Wills and Savory, 1984). Vitamin K is essential for the $\gamma$-carboxylation of the vitamin K-dependent coagulation factors II, VII, IX, and X. These factors are inactive without $\gamma$-carboxylation (Jeschke, 2009).
• The liver plays a vital role in synthesizing all the clotting factors (except Von Willebrands factor), coagulation inhibitors and also most of the fibrinolytic proteins (Kujovich, 2005).
• Liver is also responsible for the synthesis or secretion of many hormones. Angiotensinogen is made and secreted into the bloodstream by the liver. It also synthesizes and secretes insulin-like growth factor-I (IGF-I), insulin-like growth factor binding proteins (IGFBPs), and hepatocytes growth factor (HGF) (Jeschke, 2009).

1.4 Liver disease: Liver disease is a common term for a number of diseases and disorders that disrupt the function of liver (Pubmed, 2013). Some of the commonly known liver diseases include non alcoholic fatty liver disease, viral hepatitis (Hepatitis A, B, C, D and E), cirrhosis and liver cancer (Table 1). Liver diseases remain a global health problem and have been found to be a major cause of worldwide mortality and morbidity (Lozano et al., 2012; Murray et al., 2012). The total number of global deaths due to hepatitis B and hepatitis C in 2010 were estimated to be 7,86,000 and 4,99,000 respectively (Lozano et al., 2012). More than one million deaths and 31,027,000 Disability Adjusted Life Years (DALY) were due to liver cirrhosis (Lozano et al., 2012; Murray et al., 2012). DALY denotes a summary measure for burden of disease, which is composed of the addition of years of life lost to premature mortality and years of life lost to disability (Lozano et al., 2012). Another 752,100 deaths were due to liver cancer (Lozano et al., 2012). An estimated 57% of cases of liver cirrhosis and 78% of cases of primary liver cancer result from hepatitis B or C virus infection (WHO, 2013).

Liver diseases may be arbitrarily classified as acute and chronic based on the duration or the persistence of the injury (Malhi and Gores, 2008). Acute injury resolves upon removal of the injury causing agent and liver function is restored completely. Chronic liver injury results due to continuous acute injury over a long period (Kanel and Korula, 2011; Malhi and Gores, 2008). The main features of acute and chronic hepatic injury are as follows (Cross, 2013; Kanel and Korula, 2011):

1.4.1 Acute hepatic injury involves
• Variation in bile flow,
• Elevations of serum transaminases [alanine aminotransferase (ALT), aspartate aminotransferase (AST)],
• Cholestasis (condition where bile cannot flow from liver to duodenum) along with elevation of serum bilirubin levels,
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- Fatty changes (rare in case of drug or toxin induced acute injury),
- Necrosis and inflammation (mild).

1.4.2 Chronic hepatic injury involves

- Continuous injurious process resulting in repetitive phases of injury and repair,
- Elevation of serum transaminases (depending on the etiology of the disease process),
- Elevations in alkaline phosphatase (ALP) (suggesting biliary tract involvement or cholestasis),
- Elevations in serum bilirubin (suggesting more serious disease),
- Hypoalbuminemia,
- Severe fatty changes in liver,
- Severe inflammation,
- Collagen deposit leading to distortion of hepatic architecture, resulting in hepatic fibrosis,
- Fibrosis progression to cirrhosis.

Table 1: List of various causes of liver diseases

<table>
<thead>
<tr>
<th>Classification</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>Hepatitis A, B, C, D and E, Epstein–Barr virus, Cytomegalovirus, Herpes simplex, Exotic viruses</td>
</tr>
<tr>
<td>Genetic/metabolic</td>
<td>Haemochromatosis, Wilson disease, Hereditary hyperbilirubinaemias, α1-Antitrypsin deficiency, Cystic fibrosis, Hepatic porphyria, Amyloid</td>
</tr>
<tr>
<td>Toxic/drug induced</td>
<td>Alcohol, Drugs, Poisons</td>
</tr>
<tr>
<td>Autoimmune</td>
<td>Autoimmune hepatitis, Primary biliary cirrhosis, Primary sclerosing cholangitis,</td>
</tr>
<tr>
<td>Neoplastic</td>
<td>Primary, Malignant, Benign, Secondary</td>
</tr>
<tr>
<td>Bacterial/spirochaetal</td>
<td>Leptospirosis, Tuberculosis, Pyogenic liver abscess</td>
</tr>
<tr>
<td>Protozoal</td>
<td>Kala-azar (visceral leishmaniasis), Amoebiasis, Malaria</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Polycystic liver disease, Congenital hepatic fibrosis, Sarcoid, Liver disease in pregnancy</td>
</tr>
</tbody>
</table>
1.5 Mechanism involved in liver disease

1.5.1 Oxidative Stress: Numerous in vitro and in vivo studies have implicated oxidative stress and associated protein, lipid and DNA damage, as important pathophysiological force which lead to chronic liver injury, in terms of hepatic inflammation and fibrosis (Cubero and Trautwein, 2011; Morisco et al., 2008). Oxidative stress is a result of imbalance between the production of free radicals i.e. reactive oxygen species (ROS) and the cellular antioxidant system which scavenges these free radicals (Cubero and Trautwein, 2011). ROS not only damages all components of the cell, including proteins, lipids, and DNA, but can even act as a messengers through a phenomenon called redox signaling (Vera-Ramirez et al., 2013). Polyunsaturated lipids are indispensable for supporting the cell as they are the major structural components of cell membranes, endoplasmic reticulum and mitochondria. Free radicals can disrupt the structural component of the cells by the peroxidation reaction of lipids (Muriel, 2009). Chronic oxidative stress leads to persistent injury which causes parenchymal damage and that in turn induces an immune response at the injury site (Vera-Ramirez et al., 2013). This immune response leads to massive infiltration of activated Kupffer cells, monocytes/macrophages and lymphocytes which are characteristics of chronic inflammation (Sommer, 2005).

Oxidative stress induced inflammation is mediated by the activation of transcription factor NF-κB (nuclear factor-kappa beta). NF-κB binds to specific DNA regions known as κB sequences and activates the genes involved in inflammation, cell survival, proliferation, and differentiation in response to free radicals (Elsharkawy and Mann, 2007; Pahl, 1999; Sun and Karin, 2008; Yuan et al., 2006). In unstimulated cells, NF-κB is present in an inactive form as a heterodimeric complex which is inhibited by its interaction with IκB family of inhibitors. In response to an external stimulus such as by ROS, IκB is phosphorylated which leads to subsequent release of NF-κB that gets translocated from the cytoplasm to the nucleus wherein activation of target genes takes place (Gilmore, 1999; Yamamoto and Gaynor, 2001). NF-κB promotes the production and secretion of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) in Kupffer cells (Dienes and Drebber, 2010; Papa et al., 2009) and also inducible NO synthase (iNOS) and cyclooxygenase-2 in monocytes (Taylor et al., 1998; Yuan et al., 2006).

The inflammatory cells are known to release growth factors, cytokines, chemokines, and also ROS, which activate HSCs into becoming myofibroblast-like cells (MFs) (Muriel, 2009; Vera-Ramirez et al., 2013). This state of HSCs also involves induction of β platelet-derived growth
factor (β-PDGF) receptor, and the PDGF signaling is sustained through a positive feedback mechanism that involves ROS (Parsons and Green, 2011). This is achieved by PDGF binding to its receptor which activates phosphatidylinositol 3-kinase (PI3K), and extracellular regulated kinase (ERK) that in turn activates ROS production. Two other mitogens that play an important role in the activation of HSCs are TNF-α and IL-6. These two cytokines are activated by the redox sensitive transcription factor NF-κB as discussed earlier (Friedman, 2008).

1.5.2 Apoptosis: Persistent injury in the liver parenchyma leads to activation of inflammatory cells that release various inflammatory cytokines and growth factors. TNF-α is a major cytokine which based on the redox state of the cells activates pathways, that act as either prosurvival or proapoptotic and necrotic signals. TNF-α interacts with type 1 receptor (TNFR1) that further forms an association of the TNF-receptor-associated death domain (TRADD), TNF-receptor-associated factors 2 and 5 (TRAF-2 and TRAF-5) and the Rieske iron-sulfur protein of mitochondrial ubiquinol-cytochrome c reductase (RIP-1) (Vera-Ramirez et al., 2013). These interactions of TNF-α lead to activation of NF-κB and AP-1 transcription factors, which in turn are responsible for the activation of genes that are essential for the survival of hepatocytes (Chaisson et al., 2002). Glutathione (GSH) depletion may alter the vulnerability of hepatocytes to TNF-α and instead initiate TNF-α induced apoptosis axis, which will ultimately lead to mitochondrial dysfunction, thus enhancing the production of ROS and cell death (Diogo et al., 2011; Han et al., 2006; Yuan and Kaplowitz, 2009). During this condition TRADD/RIP-1/TRAF-2 complex may dissociate from TNRF1 and may bind Fas ligand-associated death domain (FADD), which leads to caspase 8/10 recruiting that will cleave the proapoptotic protein BH3 interacting domain death agonist (Bid). Cleaved Bid protein translocates to the mitochondria, to enhance its permeability which in turn release cytochrome c, and also activates intrinsic apoptosis pathway and ROS production (Micheau and Tschopp, 2003; Parsons and Green, 2011). TNF-α stimulation of RIP-1 may also lead to its translocation to the mitochondria and increased permeability of the mitochondrial membrane may yet again elicit ROS release, without any contribution of cytochrome c, aggravating necrotic and caspase independent cell death (Vandenabeele et al., 2010). All these events may eventually lead to ROS-mediated and persistent activation of c-Jun amino-terminal kinases (JNKs), through the oxidative inhibition of JNK phosphatases, which may further cause hepatocyte apoptosis (Malhi and Gores, 2008; Yuan and Kaplowitz, 2009) (Figure 3). Thus chronic oxidative stress in liver may then aggravate a
molecular shift or transition from survival signals to apoptotic signals in injured hepatocytes. This further contributes to induction of ROS in the damaged region, again inducing the inflammatory response and activating HSCs in a continuous cycle.

1.5.3 Steatosis: High level of hepatic and circulating free fatty acids (FFA) indicates liver steatosis and steatohepatitis (Guicciardi and Gores, 2005). Metabolic disorder leading to obesity or over weight patient that present metabolic syndrome such as diabetes, insulin and leptin

Figure 3: Mechanism of liver injury. The central role of hepatocyte apoptosis in liver injury: vulnerable hepatocytes undergo apoptosis when stressed. Apoptosis can be initiated via Kupffer cell release of TNF-α, leading to activation of JNK. Activated NK and NK T cells release Fas or TRAIL, and interferon gamma, which up-regulates Fas or TRAIL release, leading to death receptor–mediated hepatocyte apoptosis. Hepatocyte apoptosis can also occur via activation of the intrinsic pathway (not shown here). Apoptotic hepatocytes are engulfed Kupffer cells, leading in turn to their activation. Activated Kupffer cells secrete TNF-α, interleukins, and interferon to promote the inflammatory response. They also secrete transforming growth factor β, leading to activation of stellate cells. Stellate cells can also be directly activated by apoptotic bodies. Activated stellate cells secrete collagen type I, leading to liver fibrosis. Attenuation of hepatocyte apoptosis, or forced apoptosis of activated stellate cells, such as with proteasomal inhibitors or TRAIL, can lead to resolution of fibrosis. [Reprinted from Gastroenterology, 134, Malhi & Gores, Cellular and Molecular Mechanisms of Liver Injury, 1641-1654, 2008 with permission from Elsevier Annexure I(ii)].
resistance causes a chronic build up of triglyceride in the hepatocytes, along with ROS production, due to mitochondrial impairment (Guicciardi and Gores, 2005; Malhi and Gores, 2008). Mitochondrial dysfunction results in the impairment of mitochondrial $\beta$-oxidation and FFA accumulation, which in turn induce microsomal FFA oxidation (\(\alpha\)-oxidation) involving cytochrome P450 isoforms CYP2E1 and CYP4A that compensates for FFA overload (Vera-Ramirez et al., 2013). CYP2E1 and CYP4A activity again induces the production of ROS, which further leads to lipid peroxidation (Leclercq et al., 2001). ROS generated by CYP2E1 also actively promotes insulin resistance, which decreases insulin signaling in the liver, and thus leads to fat accumulation in hepatocytes. Activation of nuclear peroxisome proliferator-activated receptor-$\gamma$ (PPAR-$\gamma$) is responsible for these changes as it activates the expression of enzymes involved in lipid catabolism in mitochondria and peroxisomes (Westerbacka et al., 2007). This imbalance in lipid homeostasis and enhanced production of ROS, leads to lipid accumulation and oxidative stress which further induces hepatocyte apoptosis that provokes an acute inflammatory response which progresses to liver necrosis, chronic inflammation and finally liver fibrosis.

1.5.4 Fibrogenesis: As discussed earlier regardless of the causative etiology oxidative stress plays a key role in HSC activation which contributes to liver injury. Activated HSC transdifferentiate into MF cells characterized by an increase in cell proliferation, loss of vitamin A-storing capability, expression of $\alpha$-smooth muscle actin ($\alpha$-SMA), and overproduction of extracellular matrix (ECM) (Friedman, 2000). The latter leads to fibrogenesis, which will eventually result in cirrhosis, if not treated effectively. Activated MF cells produce and deposit large amounts of collagen type I and type III fibers at the liver injury site.

The end products of oxidative stress such as 4-hydroxy-2, 3-nonenal may play a role in the expression of transforming growth factor (TGF-$\beta$1) in Kupffer cells and macrophages (Chiarpotto et al., 2005). Fibrogenic activity is essentially induced by TGF-$\beta$1, which is activated by binding to its TGF-$\beta$1 type II receptors. Activated TGF-$\beta$1 associates with Smad protein and its phosphorylation forms a heterodimeric complex which gets translocated to the nucleus wherein it binds to specific motifs that lie on the promoter region of the collagen, type I, alpha 1 (COL1A1) gene (Parsons et al., 2007). TGF-$\beta$1 is also activated by connective tissue growth factor (CTGF), which is normally expressed by hepatocytes, endothelial cells, ductular epithelial cells and MF cells (Gressner et al., 2007). CTGF expression in hepatocytes is also upregulated by factors such as products of lipid peroxidation (hydroxynonenal and
The contraction of active MFs actively contributes to lobular distortion and portal hypertension as fibrosis advances and cirrhosis develops (Vera-Ramirez et al., 2013). Thus oxidative stress induces various signals that promote both the inflammatory and fibrogenic processes in liver (Figure 3). All these changes in cells that affect MF and inflammatory cells, as well as hepatocytes, in a continuous process reinforce ROS mediated signaling (Vera-Ramirez et al., 2013).

1.6 Current treatment for liver disease: Liver disease like viral hepatitis, nonalcoholic steatohepatitis (NASH), and alcoholic liver disease can lead to the development of cirrhosis which can further develop into hepatocellular carcinoma. The management of liver diseases remains a challenge to the modern medicine as there is now a general agreement among hepatologists that the number of useful drugs currently available for treatment is far from sufficient, and that there is a need for a wider range of safe and efficient therapeutic agents (Muriel and Rivera-Espinoza, 2008). The available treatment options and the problems associated with these treatments are discussed here.

1.6.1 Hepatitis B: The FDA approved drugs for treatment of hepatitis B are baraclude, epivir-HBV, hepsera, intron A, pegasys, tyzeka, viread (FDA, 2013). Interferon therapy that has been used against hepatitis B has a long list of side-effects ranging from influenza-like illness, alopecia, leucopenia to emotional distress, thrombocytopenia etc (Uhl et al., 2014).

1.6.2 Hepatitis C: Treatment of chronic hepatitis C virus (HCV) infection is based on the combination of pegylated interferon-α and ribavirin. However, development of resistant viruses is a major concern with this line of therapy (Pawlotsky, 2011). Some of the FDA approved drugs for the treatment of hepatitis C include sovaldi, lysio, pegasys, incivek (FDA, 2013). Longer duration of treatment with these drugs are not preferred as leads to wide range of side effects (Hep Magazine, 2014).

1.6.3 Hepatitis D: There is no effective therapy for hepatitis D infection as interferon therapy is only associated with therapeutic success in about 30% of the treatments (Uhl et al., 2014).

1.6.4 Non-Alcoholic Fatty Liver Disease (NAFLD): Lifestyle interventions aimed at weight loss is essential for all patients with NAFLD and is considered suitable treatment for NAFLD. If lifestyle intervention fails, liver-directed pharmacotherapy with pioglitazone or vitamin E can be
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considered for those with advanced, but pre-cirrhotic, NASH (Dyson et al., 2014). Treatment with pioglitazone causes side effects ranging from weight gain (Sanyal et al., 2010), congestive cardiac failure (Lago et al., 2007), bladder cancer (Piccinni et al., 2011) to reduced bone density (Lecka-Czernik, 2010).

1.6.5 Alcoholic liver disease (ALD): There is no US Food and Drug Administration–approved therapy for any form of ALD (Crittenden and McClain, 2013). Glucocorticosteroids represent the most widely accepted therapy in patients with severe alcoholic steatohepatitis (ASH). However, some patients do not respond to the treatment and there are chances of developing life-threatening infections while taking corticosteroids like prednisone (Frazier et al., 2011; Jaurigue and Cappell, 2014). Pentoxifylline is used as treatment for alcoholic hepatitis and is associated with side effects such as nausea and vomiting (Frazier et al., 2011). Combination therapy of corticosteroids and pentoxifylline has yielded no positive results (Jaurigue and Cappell, 2014).

1.7 Alternative therapy: As there are few effective drugs for liver disease there is need to develop new effective and safer drugs for their treatment. Table 2 lists a number of drugs that have been tested for their hepatoprotective activities including their clinical benefits and toxicity (Muriel and Rivera-Espinoza, 2008). As indicated in the table plant derived compounds have gained interest in providing alternative treatment options for liver disease.

2. PHYTOCHEMICALS

Natural products have been known for their medicinal properties since ancient times and have played an important role in traditional medicine systems such as Ayurvedic, Chinese and Egyptian (Sarker and Nahar, 2007). Plant-based foods that contain significant amounts of bioactive phytochemicals are considered desirable to provide health benefits against various chronic diseases (Liu, 2003). Various phytochemicals have been isolated and characterized from sources such as fruits, vegetables, spices and beverages (Doughari et al., 2009). Phytochemicals are naturally occurring chemical compounds in plants that serve various functions and are responsible for their flavor, color, smell and texture (Son et al., 2008). The non-nutrient plant chemical compounds or bioactive components are referred to as phytochemicals (’phyto-‘from Greek - meaning ‘plant’) or phytoconstituents (Doughari, 2012). Phytochemicals include compounds that exhibit biological properties such as antioxidant, anti-inflammatory, antiproliferative, or DNA repair, have evolved essentially for plants to act as a defensive system.
and also to cope with environmental changes (Doughari et al., 2009; Huffman, 2003; Nweze et al., 2004; Tuteja et al., 2001).

Table 2: List of agents useful in the treatment of liver diseases

<table>
<thead>
<tr>
<th>Drug</th>
<th>Origin</th>
<th>Main beneficial effects</th>
<th>Clinical relevance</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>Herbal</td>
<td>Antifibrotic</td>
<td>No beneficial properties were demonstrated</td>
<td>Very toxic at high doses</td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>Synthetic</td>
<td>Reduce cytokine production, antifibrotic</td>
<td>Most studies show no important effects</td>
<td>Medium</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Herbal ‘Curcuma longa’</td>
<td>Reduce harmful cytokines, antifibrotic</td>
<td>No studies available in human hepatic disorders</td>
<td>Safe (consumed in diet)</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>‘Glycyrrhizina glabra’ Herbal</td>
<td>Anti-inflammatory, antioxidant</td>
<td>Improves mortality and liver function in some patients with subacute hepatic failure</td>
<td>Not reported</td>
</tr>
<tr>
<td>Interferons</td>
<td>Endogenous Produced by recombinant DNA</td>
<td>Antiviral, antifibrotic</td>
<td>Effective in hepatitis B and C. Not tested directly as antifibrotic in humans</td>
<td>Several side effects at therapeutic doses</td>
</tr>
<tr>
<td>Liv 52</td>
<td>Combination of various plants</td>
<td>Diuretic, anti-inflammatory, immunomodulation</td>
<td>Antiviral, Prevention of cirrhosis. Anti-hepatotoxic</td>
<td>Not reported</td>
</tr>
<tr>
<td>Nitric oxide (NO)</td>
<td>Endogenous NO donors</td>
<td>Vasodilator, antifibrotic in some circumstances</td>
<td>Not studied in human liver diseases</td>
<td>Hypotension Oxidative stress</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Grapes, peanuts, synthetic</td>
<td>Antioxidant, antifibrotic, Immunomodulation</td>
<td>Not studied in human hepatic diseases</td>
<td>Not reported (consumed in diet)</td>
</tr>
<tr>
<td>Silymarin</td>
<td>Herbal ‘Silybum marianum’</td>
<td>Antioxidant, antifibrotic, Immunomodulation</td>
<td>Anticholestatic Antifibrotic, Antiviral</td>
<td>Very low</td>
</tr>
<tr>
<td>S-adenosyl-L-methionine</td>
<td>Endogenous Synthetic</td>
<td>Methyl donor antifibrotic, anti-cancer</td>
<td>Anticholestatic Antifibrotic, increases survival of hepatic liver diseases</td>
<td>Very low</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Synthetic</td>
<td>Antioxidant, antifibrotic, Immunomodulation</td>
<td>Not tested in human liver diseases</td>
<td>Teratogenic</td>
</tr>
</tbody>
</table>

[Reprinted from J. Appl. Toxicol, 28, Muriel P and Espinoza YR, Beneficial drugs for liver diseases, 93–103, 2008 with permission from John Wiley and Sons: Annexure I(iii)]
Review of Literature

2.1 Classification of Phytochemicals: The exact classification of phytochemicals is not known due to their vast and varied numbers. The various classes of phytochemicals as reported in literature are:

2.1.1 Alkaloids: Few well known alkaloids include morphine, codeine, berberine, vinblastine atropine, morphine, ergotamine, cocaine, nicotine and ephedrine (Doughari, 2012; Kennedy and Wightman, 2011).

2.1.2 Terpenes: Examples of some important terpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol which fall under the category of monoterpenes (De Martino et al., 2010). Taxol, the well known anticancer agent, is a diterpene (Williams et al., 2000). Examples of triterpenes include amyrins, ursolic acid and oleanic acid (Doughari, 2012).

2.1.3 Glycosides: Few very important examples include digoxin, digitoxin, and ouabain which are steroid-like compounds, designated as cardiac glycosides, used for treatment of congestive heart failure, anti-arrhythmic agents and also have potential for anti-cancer treatment (Kirch, 2001; Newman et al., 2008).

2.1.4 Saponin: Diosgenin and hecogenin are other well known steroidal saponins that are used in the commercial production of hormones. Progesterone is derived from diosgenin (Kumar et al., 2014; Sarker and Nahar, 2007). Other steroidal hormones, e.g. cortisone and hydrocortisone, can be prepared from the starting material hecogenin (Sarker and Nahar, 2007).

2.1.5 Tannins: Some examples of tannins include theaflavins, daidezein, genistein and glycitein (Doughari, 2012). Condensed tannins formed by condensation of catechin units are also known as phalbatannins (Saroya, 2011).

2.1.6 Anthraquinones: Examples include chrysophanol, aloe-emodin, rhein, salinos poramide, luteolin and emodin (Doughari, 2012). Luteolin is known to possess antioxidant, anti-inflammatory and anti cancer activity (López, 2009).

2.1.7 Polyphenols: Polyphenols are secondary metabolites of plants which are responsible for defense against ultraviolet radiation and pathogens (Beckman, 2000). Polyphenols are now known for their protective effects against cancers, cardiovascular diseases, neurodegenerative diseases, diabetes and osteoporosis (Arts and Hollman, 2005; Graf et al., 2005; Pandey and Rizvi, 2009). Polyphenols are classified on the basis of number of phenol rings and the structural
elements that bind these rings to one another. The main groups include phenolic acids, flavonoids, stilbenes and lignans (Spencer et al., 2008).

2.1.7.1 Phenolic acids: Phenolic acids are mainly divided into two classes: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxybenzoic acid is usually low in concentration except in certain red fruits, black radish and onions it is found in higher concentrations (Shahidi and Naczk, 1995). Examples of hydroxycinnamic acids include caffeic, ferulic and sinapic acids (Manach et al., 2004; Pandey and Rizvi, 2009).

2.1.7.2 Flavonoids: These form an important group of polyphenols widely distributed among the plant flora. They can be divided into several classes according to the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and flavanones (Pandey and Rizvi, 2009). Different groups arise from the variation in number and arrangement of the hydroxyl groups and their extent of alkylation and/or glycosylation. Citrus fruits contain large quantities of flavones: tangeretin, nobiletin, and sinensetin (Shahidi and Naczk, 1995). Quercetin and kaempferol are the most common flavonols present in nearly 70% of plants (Pandey and Rizvi, 2009). Genistein and Daidzein are two isoflavones known for their protective role in breast cancer and osteoporosis (Adjakly et al., 2013). Anthocyanins are found in red wine, certain varieties of cereals, and certain leafy and root vegetables but they occur most abundantly in fruits. Cyanidin is the most common anthocyanidin in foods (Manach et al., 2004). Flavanols exist in both the monomer form (catechins) and the polymer form (proanthocyanidins) (Manach et al., 2004). Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found mainly in grapes, tea and in seeds of leguminous plants (Arts et al., 2000).

2.1.7.3 Lignans: is another class of polyphenols that are characterized by their 1,4-diarylbutane structure. Lignans are found in high concentrations in (lariciresinol, matairesinol, secoisolariciresinol) flax and sesame seeds (Tangney and Rasmussen, 2013). Cereals, grains, fruit, and certain vegetables also contain lignans, but their concentration is limited in these sources in comparison to seeds (Heinonen et al., 2001). Secoisolariciresinol which is found in linseed is considered to be a phytoestrogen (Pandey and Rizvi, 2009).

2.1.8 Stilbenes: One of the best studied, naturally occurring polyphenol stilbene is resveratrol which is found largely in grapes. It is also found in low quantities in wine (0.3–7 mg aglycones/L and 15 mg glycosides/L in red wine) (Smoliga et al., 2011; Tomé-Cameiro et al., 2013).
2.2 **Health benefits of phytochemicals:** Cells in humans and other organisms are constantly exposed to oxidative stress. The term "oxidative stress" was first introduced in the eighties by Helmut Sies (1985), and is defined now as “A situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents” (Santillán et al., 2013). The free radicals generated have beneficial role, however, a physiological balance must be maintained between ROS and antioxidant defense system that scavenges them (Frei, 2004; Pham-Huy et al., 2008; Poljsak et al., 2013) (Figure 4). For the last two to three decades beneficial role of phytochemicals in the prevention of chronic diseases such as liver disease, cancer, neurodegenerative disease and cardiovascular disease have been extensively studied (Girish and Pradhan, 2012; Lobo et al., 2010; Mattson and Cheng, 2006; Patil et al., 2009; Son et al., 2008). The fact that as oxidative stress is implicated in the etiology of several chronic diseases, suggests, the use of phytochemicals as an antioxidant therapy could be a promising avenue for treatment of these diseases. Therapeutic strategy wherein the antioxidant capacity of cells is increased may come a long way in treatment of these diseases (Lobo et al., 2010).

![Figure 4: Oxidative stress due to excessive reactive oxygen species is reduced by the antioxidative defenses (the physiological balance is represented by the dashed line) (Poljsak et al., 2013).](image)

2.3 **Phytochemicals as hepatoprotective agents:** Even though liver diseases are a global health problem, yet the modern medicine is limited not only in terms of the available treatment and cure options but also in terms of the fact that the available choice of drugs show serious adverse effects (Kshirsagar et al., 2011; Wolf et al., 2008). As the current available choice of useful drug treatments is limited, thus the scientific community looks forth to any suggested safe, efficient and alternative therapeutic options (Muriel and Rivera-Espinoza, 2008). As oxidative stress plays a key role in pathogenesis of liver diseases, most of the hepatoprotective drugs belong to the group of free-radical scavengers, and their mechanism of action involves membrane...
stabilization, neutralization of free radicals and immunomodulation (Morisco et al., 2008). Thus, a phytochemical with significant antioxidant property would be an ideal candidate for preventing liver diseases.

Plant drugs are known to play a major role in the management of liver diseases worldwide including India. There are numerous plants and polyherbal formulations with claims of hepatoprotective activities. About 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to possess liver protective activity (Girish and Pradhan, 2012) Globally more than 600 commercial herbal formulations and in India more than 93 medicinal plants are used in different combinations as hepatoprotectants. Authors also described a comprehensive list of hepatoprotective plants and their active principles or phytochemical constituents studied in various models of liver injury along with the dosage used. Herbal medicines are easily available, inexpensive and considered to be safe. This has increased their demand in primary health care (Sheetal and Singh, 2008).

2.4 Mechanism of action: Oxidative stress is the common pathway of chronic liver diseases of different etiology. Phytochemicals possess strong antioxidant activity and so their use in standard therapies for liver disease is common (Prete et al., 2012). The mechanism of hepatoprotection by these compounds generally involves a series of multiple effects. The antioxidant effects is confirmed by reduced levels of malondialdehyde a marker of lipid peroxidation (LPO), enhances the reduced glutathione level and the activities of antioxidant enzymes, glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in numerous studies (Gao and Zhou, 2005; Prete et al., 2012). NF-kB mediated inhibition of inflammatory cytokines and chemokines has been shown with different phytochemicals such as silymarin, curcumin, ellagic acid and picroliv (Anand et al., 2008a; Devipriya et al., 2007; Schubert and Muller-Goyman, 2003; Shapiro et al., 2006). They can also decrease formation of leukotrienes, prostaglandins,and TNF-α by Kupffer cells (Negi et al., 2008). Phytochemicals can also protect normal structure of mitochondrial membrane and enhance the activity of ATPase in mitochondria, thereby modulating the balance of liver energy metabolism (Gopi and Setty, 2010; Reddy et al., 2009). Immune dysfunction is a component of liver disease, and thus, immunomodulation by herbal therapy prevents oxidative stress and inflammation and in turn
strengthens the detoxifying power of liver cells (Jiang et al., 1997). All these effects contribute to the protection of liver and tilt the balance of injury towards liver regeneration.

2.5 Problems associated with plant based drugs: In spite of the potential shown by herbal products as hepatoprotectants in various in vitro and in vivo studies there is still a lack of new drugs that are clinically proven. This is due to certain problems associated with these plant based drugs as discussed below.

2.5.1 Phytochemicals and Hormesis: The term ‘hormesis’ has been used to describe a biphasic dose response, with a low dose, prompting a favorable effect and a high dose, exhibiting a toxic effect (Calabrese, 2005). It can be further explained as a process in which exposure to a low dose of a chemical agent induces an adaptive beneficial effect on the cell or organism that is damaging at higher doses (Mattson, 2008). Plants develop various biosynthetic pathways for the production of toxins that help in protecting them against attack by microorganisms and insects and many of these toxins involved in plant defense have been identified as phytochemicals. Several phytochemicals show a favorable anticarcenogenesis effects at low doses while they may themselves act as carcinogens at high doses (Calabrese, 2005) (Figure 5). Vitamin E (µM concentrations) and several other polyphenols have shown beneficial effects against oxidative stress induced in cell culture models of atherosclerosis, cancer, and neurodegenerative disorders (Barbaste et al., 2002; Butterfield et al., 2002; Kline et al., 2007). However when tested clinically (probably at higher doses) these antioxidant phytochemicals have remained ineffective in the primary prevention studies (Riccioni et al., 2007). In other words, it is a specific dose range in which a phytochemical can produce a beneficial effect. The relatively low amount of phytochemicals i.e. typically consumed in daily diet is unable to produce antioxidant effects which are reported to be maintained when their concentrations in cells reach µmolar levels (Mattson, 2008). High doses of phytochemicals may on the other hand be toxic, owing to their prooxidative effects at high concentrations or their potential to react with beneficial concentrations of ROS normally present at physiological conditions and required for optimal cellular functioning (Bouayed and Bohn, 2010; Decker, 1997) (Figure 5). Fukumoto and Mazza (Fukumoto & Mazza, 2000) reported dual antioxidant and pro-oxidant activities for a variety of plant-derived polyphenols including gallic acid, protocatechuic acid, syringic acid, vanillic acid,
ellagic acid, caffeic acid, coumaric acid, chlorogenic acid, ferulic acid, myricetin, quercetin, rutin, kaempferol, (+)-catechin, (−)-epicatechin, delphinidin, and malvidin. However, there are limited in vitro studies on the pro-oxidant nature of natural photochemicals and even fewer studies being carried out in laboratory animal model systems (Babich et al., 2011). The volume of research on the antioxidant properties of polyphenols as related to their biological effects greatly overshadows the lesser number of studies on the biological consequences of the pro-oxidant nature of polyphenols (Bouayed and Bohn, 2010). While the latter studies are important for determining the dosage of these phytochemicals which will yield beneficial instead of the deleterious effects.

2.5.2 Bioavailability (BA): BA of phytochemicals may be defined as the proportion of the administered compound, that is digested, absorbed, and utilized in normal metabolism (Scheepens et al., 2010). Since several of these phytochemicals are poorly absorbed, hence their BA relies heavily upon estimates of amount absorbed (Carlos et al., 2011; Manach et al., 2004). Health benefits of phytochemical will depend on their BA however, only limited information is available on these aspects and hence this is a major hurdle in the development of these plant based drugs (Rein et al., 2013). The transport mechanisms i.e. delivery of phytochemicals to the target sites, their metabolisms in the human body, and the biomarkers exerting the health benefits are also poorly understood (Epriliati and Ginjom, 2012). The metabolites resulting from digestive or hepatic enzymes may differ from the native substances in terms of biological activities. Thus, the knowledge on the BA of polyphenols is essential if their health effects are to be clearly elucidated and pharmacologically enhanced (Manach et al., 2004; Rein et al., 2013). It is usually observed that at the doses found effective in the laboratory set up including pre-clinical studies, for these phytochemicals is high (Girish and Pradhan, 2012) and are not practical for their clinical translation. Low of BA leads to low plasma concentrations and or significant metabolites which makes it difficult to correlate the in vitro and in vivo mechanisms of action. The low potency due to low BA and lack of exclusive patent protection, thus, are some challenges associated with developing natural phytochemicals into “conventional pharmaceuticals” (Manach et al., 2004; Rein et al., 2013).
2.6.3 Safety/Toxicity: There is no Universal (or individual for different countries) regulatory system that can ensure the safety and activity of phytopharmaceuticals (Bent, 2008; Silva et al., 2010; Teschke and Schulze, 2013). Evidence-based proof of the efficacy of many phytochemical agents is frequently lacking, however, in recent years, data on evaluation of the therapeutic and toxic activity of some herbal medicinal products is becoming available. A few reports of acute/or chronic liver damage after ingestion of some herbals that contain pyrrolizidine alkaloids, germander, greater celandine, kava, atractylis gummifera, callilepis laureola, senna alkaloids, chaparral, indicate that the clinical safety should be a must (Bunchomtavakul and Reddy, 2013; Javaid and Bonkovsky, 2006; Seeff, 2007). Even at the
laboratory level the toxicity/safety profile of the phytochemicals must be evaluated (Stickel and Schuppan, 2007).

Thus, it is essential that the active molecules must be isolated and tested in suitable culture and animal experiments and finally in randomized, placebo controlled studies to enable rational clinical use of these agents (Dhiman and Chawla, 2005; Prete et al., 2012). Further the use of a suitable delivery/carrier system is necessary to improve the BA, achieve controlled release of the compound and overcome toxicity issues if any.

3. NANOTECHNOLOGY

Nanotechnology is defined as 'the design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel superior characteristic or property' (Bawa, 2013). The last quarter of the century has witnessed a boom in the field of nanotechnology. This is essentially owing to the nanocarriers based medicines which hold a lot of promise as they can circumvent different obstacles faced in the development of therapeutics (Shi et al., 2010). Some nanomedicines have been successfully developed and approved for clinical use while some are undergoing clinical trials (Bawa, 2013).

As discussed earlier, the development of plant-based drugs including hepatoprotective drugs require a carrier/delivery system which is necessary to improve the BA, stability, control release and overcome toxicity issues of the encapsulated compound or material. This will help improve and establish efficacy of plant based drugs in clinical trials and thus fasten their journey from lab through clinics to the market. It is assumed that the phytopharmaceutical research involving incorporation of phytochemicals into novel formulations such as nanoparticles, microemulsions, matrix systems, solid dispersions, liposomes, and solid lipid nanoparticles would help these herbal medicine to see the light of the day (Mishra et al., 2013).

The major hindrance to the absorption of most of the phytochemicals is their inherent skewed hydrophilic or lipophilic nature, which also contributes to their low BA. Moreover these phytochemicals get rapidly metabolized and cleared from the physiological system and do not remain in systemic circulation for a sufficient period elicits beneficial effects. Incorporation of
phytochemicals into a nano system will help improve their uptake and biodistribution and the nanosize and the colloidal nature imparted especially to the lipophilic molecules tends to overcome their limited wettability/solubility (Anthony et al., 2012).

3.1 Nanocarriers for hepatic delivery of drugs: Nanocarriers for efficient hepatic delivery of drugs have been investigated and these include organic nanoparticles (bionanocapsules and human serum albumin (HSA)), inorganic nanoparticles (metal-based), polymer based nanoparticle and lipid based nanoparticles ((Li et al., 2010) (Figure 6). Limited nanocarriers based hepatoprotectants that have been approved by FDA (PEG-Intron for hepatitis C and pegasy for hepatitis C). Bionanocapsules (hollow virion-free nanoparticles) that were derived from the hepatitis B virus envelope L particles containing the pre-S1 peptide as its native hepatocyte infectivity mechanism, both for gene and drug delivery have been reported (Yamada et al., 2003). HSA has also been used for its effective protein and drug carrier abilities (Greupink et al., 2006; Moreno et al., 2010). Inorganic iron (III) oxide-based paramagnetic nanoparticles have also been used as targeted therapeutic carriers (Purushotham et al., 2009; Yang et al., 2009) . Although, the first two classes of nanocarriers have showed potential, the development of polymeric and lipid based nanoparticles have proved to be superior options for delivery of therapeutics (Li et al., 2010). The latter two have been widely used as they provide synthetic versatility which allows manipulation of these carriers in terms of size, charge and surface properties.

Nanoparticles present in systemic circulation face significant physiological barriers that hamper their delivery and uptake by the liver cells (Li et al., 2010) (Figure 7). Nanoparticles may react with serum proteins (non-specific interactions) and their surface can be deposited with antibodies and/or complement proteins (opsonization). Latter can be avoided by the presence of a hydrophilic coat of surfactants incliding tween 80 and polyethylene glycol (PEG).

The following two interactions decline the overall circulation time and amount of the nanoparticles:

1) mechanical entrapment of aggregates in the alveoli capillaries (aggregates larger than 7 μm) (Azarmi et al., 2008).
2) clearance by resident macrophages of the reticuloendothelial system (RES) in the liver, spleen and bone marrow, especially if the size exceeds 200 nm as endothelial cells lining the liver sinusoids (part of the RES) have scavenger receptors which internalize hard solid particles up to 0.23 μm in vivo (Jacobs et al., 2010).

Thus, nanoparticles have to overcome these physiological and anatomical constraints to hepatic delivery. This is achieved mainly due to modulation of the size and charge of the nanoparticles. Integration of PEG moieties help to minimize protein binding and in effect reduce non-specific scavenging of the nanoparticle therapeutics by the RES. Therapeutic delivery strategies are typically divided into passive and active targeting (Li et al., 2010; Yuan et al., 2013).
3.1.1 Passive targeting: Passive targeting may be defined as amassing nanoparticles at a specific body site owing to certain anatomic or pathophysiological features (Santos-Magalhaes and Mosqueira, 2010) resulting in an increase in concentration of these nanoparticles in the diseased cells, while minimizing non-specific undesirable effects to other organs. This is achieved by modulating size properties of nanocarriers and their surface-modification, leading to their long term circulation and/or administration at a specific site (Li et al., 2010) (Figure 8). Sinusoids are specialized capillaries characterized by the presence of 100—200 nm fenestrations along their endothelial wall and absence of basal lamina (Jacobs et al., 2010). Thus, following systemic administration, the defining size properties of typically < 200 nm in diameter of nanoparticles will significantly facilitate passive liver targeting in the absence of significant aggregation with serum proteins or self-aggregation (Li et al., 2010; Romero et al., 1999). Thus, nanoparticles will pass through the sinusoidal fenestrations and effectively build up a high concentration of the nanoparticle carried therapeutics in the space of Disse, where diffusion to the various liver cell types can occur. However, deformable nanocarriers of up to 400 nm may extravasate through the sinusoid endothelial fenestrations via forced extrusion probably due to transient interactions with the sinusoidal endothelial cells (Romero et al., 1999).
In hepatocellular carcinoma (HC) passive accumulation of nanoparticle in the liver cells can also be achieved by enhanced permeability and retention (EPR) effect (Matsumura and Maeda, 1986). The EPR effect occurs due to the characteristic features of the tumor microenvironment which include (a) leaky tumor vasculature, as a result of rapid and incomplete tumor angiogenesis to meet the elevated demands for oxygen and nutrients, leading to increased permeability and extravasation of macromolecules, and (b) impaired lymphatic drainage, which again aids in the retention of nanoparticles in the tumor tissues (Gu et al., 2007). Since, the size of the gap junction between endothelial cells is reported to vary between 400 and 600 nm, nanoparticles are therefore expected to be extremely efficient at extravasating from the tumor microvasculature which leads to their high local concentration in tumor (Iyer et al., 2006; Maeda et al., 2000).

Figure 8: Passive and active liver targeting strategies of nanoparticles (Li et al., 2010). (a) Nanocarriers (<200 nm) aid in the evasion of early clearance by the Kupffer cells. (b) The size of the nanoparticles aids in extravasation into the space of Disse through sinusoidal fenestrations in the absence of basal lamina, which (c) results in the increase of a high local concentration of nanoparticles, hence facilitating their diffusion across the loosely organized extracellular matrix in the space of Disse. This then leads to either (d) non-specific endocytic uptake or (e) receptor-mediated uptake by the hepatic stellate cell (i.e. cellular target for liver fibrosis) or hepatocyte (i.e. cellular target for HBV infection and HCC) [Reprinted from Nano Today, 5, Li et al., Polymer- and lipid-based nanoparticle therapeutics for the treatment of liver diseases 296-312, 2010 with permission from Elsevier: Annexure I(iv)]
3.1.2 Active targeting: The specific delivery of the nanoparticles to the diseased cells aids in the capitalization of the therapeutic effects of the drugs while minimizing unwanted side effects on normal liver cells (Li et al., 2010). As discussed in earlier sections human liver constitutes various cell types, including parenchymal hepatocytes and the non-parenchymal sinusoidal endothelial cells (SECs), Kupffer cells, and HSCs. Hepatocytes are essentially implicated in the development of HBV infections and HC and thus used for the treatment of these diseases. HSCs are considered to be the main target for therapeutic interventions in liver fibrosis due to their major roles in the secretion and maintenance of ECM. Thus, both hepatocytes (Kren et al., 2009; Mao et al., 2005; Suriano et al., 2010) and HSC (Adrian et al., 2007; Li et al., 2008; Li et al., 2010; Moreno et al., 2010) based ligand approach provide diverse targeting opportunities for nanoparticles to deliver therapeutics (Mishra et al., 2013).

3.2 Nanocarriers for hepatic delivery of phytochemicals based drugs

As discussed in an earlier section phytochemicals are now globally accepted as an option for treating liver diseases. The inherent nano size property of nanocarriers aids in circumventing several limitations of phytochemical based drugs, offering advantages like (Bonifacio et al., 2014; Saraf, 2010; Thapa et al., 2013):

a) Increasing solubility
b) Enhancing BA
c) Enhancing stability
d) Sustained delivery
e) Lowered toxicity
f) Reducing the dose
g) Protection against physical and chemical degradation of the entrapped phytochemicals.

There are only few literature reports on the use of nanocarriers for encapsulating phytochemicals for delivery to the liver cells. The same are discussed here:

3.2.1 Liposomes based drugs: Authors prepared small unilamellar liposomal vesicles (266-466 nm) were prepared to encapsulate silibinin (silymarin, CAS22888-70-6) and target it to liver (Maheshwari et al., 2003). The prepared formulation was evaluated for its hepatoprotective activity in mice against carbon tetrachloride (CCL4) induced hepatotoxicity. Authors showed
improved hepatoprotective performance of silymarin in liposomes (55.6%) in comparison to the plain drug (33.08%) at similar dose of 50 mg/kg.

El-Samaligy and co-workers (El-Samaligy et al., 2006) encapsulated silymarin (a well-known hepatoprotective agent obtained from seeds of *Silybum marianum* (Compositae), into hybrid liposomes for buccal administration to improve its low oral BA and poor absorption from GIT. Hybrid liposomes (660 nm) were prepared using reverse evaporation technique and were composed of lecithin, cholesterol, stearylamine, and Tween 20 in the molar ratio 9:1:1:0.5. Hepatoprotective activity was assessed in acute model of carbon tetrachloride (CCl₄) induced liver damage in rats. Rats received 0.25 mL of CCl₄ in liquid paraffin (1:1, v/v) per 100 g body weight, i.p, while silymarin hybrid liposome (12.5 mg/kg) was administered 3 days prior to CCl₄ treatment and continued till end of experiment. Animals were sacrificed 48 h after the CCl₄ treatment. Hepatoprotective effects were measured using biochemical parameters, serum AST and serum ALT, and histologically. Authors observed that silymarin hybrid liposome produced a significant decrease in both transaminase levels, as well as improved histoarchitecture in CCl₄ model of hepatic injury in comparison to the orally administered free silymarin suspension (El-Samaligy et al., 2006).

### 3.2.2 Polymer based drugs:

Only a few polymer based drug carriers are reported for delivery of phytochemicals to liver. Chen and co-workers (Chen et al., 2005) prepared oleanolic acid nanosuspension (ON) by nanoprecipitation method and evaluated ON for its hepatoprotective effects. Oleanolic acid is a naturally derived triterpene used for the treatment of hepatitis in Chinese medicine. However, its poor solubility often leads to poor BA. Prepared ON was 284.9 nm in size and was compared with corresponding oleanolic acid suspension (p.o 100 mg/kg) on pretreatment against CCl₄ induced liver injury in mice. Hepatoprotective activity was evaluated in terms of ALT and LPO. ON produced significantly better effects than oleanic acid suspension as amelioration in ALT and MDA levels was 80 and 66% with ON while oleic acid suspension showed 61 and 43% reduction, respectively.

In another study, authors used the nanosuspension method to prepare nanoparticles encapsulating extract of *Cuscuta chinensis* (CN), and compared the hepatoprotective and antioxidant effects of the same with ethanolic extract from seeds of *Cuscuta chinensis* (CE). The authors prepared CN by dissolving lyophilized ethanolic extract of CE (CE) (2 g) and Pluronic F68 (PF68) (1 g) in 120 ml of ethanol. The solution was then quickly injected into 280 ml aqueous solution containing...
PF68, which was then homogenized at 22,000 rpm for 30 min, resulting in the formation of nanospheres (267.6 nm). Rats were pretreated with CN (267.6 nm) and CE (p.o) for 7 days before the administration of single dose of acetaminophen to induce hepatotoxicity. Authors measured the levels of AST, (ALT), and ALP; and also evaluated hepatoprotective effects, in terms of histopathology. Authors also evaluated antioxidant parameters (SOD, catalase, glutathione peroxidase (GPx) and MDA). The authors reported that CN at a dose of 50 mg/kg showed better hepatoprotective effects than 125 mg/kg free CE and attributed the effects to the improved solubility of nanosized CE (Yen et al., 2008).

The same group of authors reported the use of nanoprecipitation method to prepare novel naringenin-loaded nanoparticulate system (NARN) to improve restricted BA of naringenin (NAR). Authors reported particle size of lyophilized NARN as 66.2±0.38 nm, and when reconstituted in buffered solution of pH 1.2 and 4.5 the mean particle was less than 65 nm while at pH 7.4 or in pure water the size was 457.10±18.49 and 369.57±18.11, nm respectively. The hepatoprotective effects were evaluated in a model of acute liver failure induced by CCI₄ in rats. NARN and NAR were given for three consecutive days prior to CCI₄ treatment at a dose of 100 mg/kg. NARN showed a significantly higher release rate of NAR attributed to its improved solubility when encapsulated as NARN. Authors reported that NARN presented significantly (p<0.05) better effects as compared to NAR with amelioration in serum liver injury markers (ALT, AST), and antioxidant parameters (LPO, SOD, CAT). Further the authors reported that NARN suppressed all the three hepatic caspases (caspase 3, 8, 9) evaluated by them while only caspase 3 and 9 were suppressed by NAR (Yen et al., 2009).

In another study, authors prepared curcumin nanoparticles (CURN; 142.90 nm) by using the same method (nanoprecipitation) and evaluated their free radical scavenging ability, antilipid peroxidation effect, and cytotoxicity against human hepatoma cell lines HepG2, PLC/PRF/5, and Hep3B in comparison with the corresponding free drug. Authors concluded that CURN showed significantly better (p<0.05) antioxidant and antihepatoma effects (Yen et al., 2010).

In yet another study, Bisht and co-workers prepared polymeric nanoparticles of curcumin (NanoCurc™; Size < 100 nm) comprising N-isopropylacrylamide (NIPAAM), vinylpyrrolidone (VP) and acrylic acid (AA) via free radical mechanism (Bisht et al., 2011). Authors evaluated hepatoprotective effects in CCI₄ induced model of hepatotoxicity in mice. NanoCurc™ and FC were administered via i.p route at a dose of 25 mg/kg on days 1-4, 6, 8, 9, 11 and 13 while CCI₄
was injected at a dose of 1 μg/g of body weight on days 5, 7, 10 and 12 and animals were sacrificed on day 14. Authors observed that NanoCurc™ showed sustained intrahepatic curcumin levels in hepatocytes and non-parenchymal cells. Further NanoCurc™ significantly (p<0.001) inhibited CCl₄ induced liver injury, production of pro-inflammatory cytokines and fibrosis compared to void nanoparticles. Authors demonstrated enhanced antioxidant levels in the liver and inhibition of pro-fibrogenic transcripts associated with activated myofibroblasts. Authors also showed that NanoCurc™ directly induced stellate cell apoptosis in vitro (Bisht et al., 2011). Interestingly authors did not compare the effects of NanoCurc™ with free curcumin.

In a recent study curcumin nanoparticles (CUR-NP) by solid-in-oil-in-water (s/o/w) emulsion method, using PLGA and polyvinyl alcohol (PVA) as the carrier and the stabilizer, respectively (Sankar et al., 2013). Authors reported mean particle size of CUR-NP as 130.8 nm and studied their therapeutic effects against sodium arsenite-induced hepatic oxidative damage in rats in comparison with free curcumin at a dose of 100 mg.kg p.o. Rats were given sodium arsenite (25 ppm) daily through drinking water for 42 days while treatment was given during the last 14 days of the toxin exposure. Authors report that CUR-NP ameliorated the hepatotoxicity induced in terms of altered histoarchitecture, ALT, AST, LPO, SOD, CAT, GSH, glutathione peroxidase (GPx) and glutathione reductase (GR). The authors reported that although the effects were statistically comparable to that of free curcumin, but in term of % amelioration CUR-NP showed better effects.

Considering the success of polymeric and liposome based nanoparticles to deliver drugs to liver and their limitation(s) especially toxicity, stability, difficulty in scalability, another appropriate alternative for drug delivery into the liver would be the solid lipid nanoparticulate systems.

The encapsulation of poorly absorbed molecules into lipid based materials promises enhancement of the biopharmaceutical performance (Anthony et al., 2012; Desai et al., 2012). These lipidic nanosystems have the ability to protect the enclosed drug molecules from oxidation, photodecomposition and hydrolytic or enzymatic degradation. This is accomplished in storage as well as upon in vivo administration. These nanocarrier systems are inclined to be transported (a) across the gut via lymphatics (Paliwal et al., 2009) overcoming first-pass metabolism encountered by the corresponding free drug; (b) across biological membranes, including those of GIT, blood vessels, organs, and blood–brain barrier (Tabatt et al., 2004;
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Trevaskis et al., 2008) via pinocytosis and transcytosis and also an active or passive uptake; and (c) by inhibition of various efflux transporters. Lipid-based nanosystems (LNs) constitute liposomes, SLNs, nanostructured lipid carriers, and self-nanoemulsifying drug delivery systems (SNEDDS). Thus the use of lipids for improving the bioavailability and solubility of drugs and in addition advantages like biocompatibility makes them an ideal choice for delivering therapeutics.

3.2.3 SLNs improved oral bioavailability of herbal drugs for hepatic delivery: There are reports on utilizing SLNs as carrier for improving the oral bioavailability of plant based drugs. However, only few reports the use of SLNs for hepatic delivery of plant based drugs. An early study reported the use of SLNs for incorporating silymarin (He et al., 2007). Authors prepared silymarin-loaded solid lipid nanoparticles (SM-SLNs) via both cold and hot homogenization technique (cold-SM-SLNs and Hot-SM-SLNs). Authors formulated SM-SLNs using Compritol® 888 ATO, soybean lecithin and poloxamer 188. The size, PDI and zeta potential of cold-SM-SLNs and hot-SM-SLNs reported were 155.0 nm, 0.24 and -34.2 mV and 170.7 nm, 0.34 and -39.1 mV respectively. The entrapment efficiency achieved with cold homogenization method was much better (87.3 %) than that of hot homogenization method (42.6 %). Based on the entrapment efficiency the authors further carried out pharmacokinetic studies with cold-SM-SLNs and demonstrated the improved targeting efficiency (calculated on the basis of area under the concentration curve) of cold-SL SLNs versus silymarin suspension (32.19 vs 10.15) at a dose of 32 mg/kg body weight in mice. The authors thus proposed SLNs based drugs for targeting liver via oral route (He et al., 2007).

In another study authors prepared taspine solid lipid nanoparticles (Ta-SLN; 173 nm) and Ta-SLN modified by galactoside (Ta-G2SLN; 192.3) using the film evaporation-extrusion method (Lu et al., 2008). The nanoparticles were spherical or near-spherical with smooth surface and high encapsulation efficiency (Ta-SLN: 90.67 %; Ta-G2SLN: 81.03 %). The authors reported that i.v injection of Ta-SLN or Ta-G2SLN resulted in a higher plasma and liver concentration and a longer retention time in mice compared with the administration of free Ta. The authors concluded that SLN tended to be preferentially delivered to the liver and Ta-G2SLN may further enhance liver targeting.

In another study baicalin SLNs (BSLN) were prepared by emulsification ultrasonic dispersion method (Yan et al., 2012). The SLNs prepared with the composition of baicalin-soybean lecithin-
glyceryl monostearate-poloxamer 188 in ratio 1:5:15:30 had mean particle size of 68.6 nm. Zeta potential and encapsulation efficiency were -22.13 mV and 84.7% respectively. In vivo biodistribution studies (30 mg of BSLNs and equivalent dose of baicalin suspension was injected via tail vein in Sprague Dawley rats) and reported a high targeting rate of BSLNs for liver (6.931) which was more than that observed for other organs (heart, lung, kidney).

In a recent study authors prepared cucurbitacin B loaded SLNs (Cu B-SLNs; 124.8 nm) and found that the concentration of poloxamer 188 and soybean lecithin had effects on the mean particle size and size distribution (Hu et al., 2013). The authors reported zeta potentials values around -33 mV and in vitro release demonstrated the sustained release after a burst release. On administration of free and encapsulated Cu B (at a dose of 2 mg/kg/BW in mice via tail vein), targeting efficiency of Cu B-SLNs was 1.94 times higher in liver as compared to that of Cu B solution in the liver.

### 3.3 Solid Lipid Nanoparticles (SLNs):

In the year 1992 the development of first lipidic nanoparticles (LNs) was reported. These systems continued to receive interest till date due to their ability to surpass the limitations of vesicular colloidal systems i.e. liposomes and polymeric nanoparticles (Kaur et al., 2008; Lee et al., 2007; Lucks et al., 1992). They are identical to oil/water emulsion for parenteral nutrition, with the liquid lipid of the emulsion being replaced with the solid lipid (Cavalli et al., 2000). SLNs can be prepared from fatty acids; mono-, di-, and triglycerides; and phospholipids, which are normal constituents of the human body and are therefore considered biocompatible (Rawat et al., 2008; Westesen et al., 1997). SLNs are reported to efficiently incorporate lipophilic drugs (Hu et al., 2004) because the latter can be incorporated easily within the lipidic core. They are also reported to be suitable for hydrophilic drug molecules (Bhandari and Kaur, 2013).

#### 3.3.1 Advantages of SLNs:

SLNs have many advantages including the ones claimed for polymeric nanoparticles, liposomes and fat emulsions, while they can overcome the disadvantages associated with each of these systems (Ghadiri et al., 2012; Kakkar and Kaur, 2011; Kaur et al., 2008; Lim et al., 2012). These advantages are listed below:

1. The nanoparticles and the SLNs particularly those in the range of 120–200 nm are not taken up readily by the cells of the RES and thus bypass liver and spleen filtration and removal without the release of encapsulated drug.
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2. SLN formulations have been found to be stable for period ≥ one year which is major advantage with respect to other colloidal carrier systems.

3. High drug payload achieved with SLNs versus a limited drug loading for polymeric nanoparticles is another highlight of SLNs.

4. Lipids used for their production are relatively cheaper than synthetic polymer used in polymeric NPs, for example, PLGA.

5. Excellent scalability and reproducibility of significant properties, when prepared in large batches, using a cost-effective high-pressure homogenization technique as the preparation procedure, is again an exclusive advantage with SLNs.

6. Controlled release of the incorporated drug can be achieved for up to several weeks. Further, by coating with or attaching ligands to SLNs, there is an increased scope of drug targeting.

7. The feasibility of incorporating both hydrophilic and hydrophobic drugs.

8. The carrier lipids are biodegradable and hence safe for human consumption. This is again important in reference to polymeric nanoparticles wherein the safety of the monomers formed after degradation (even if the polymer is biodegradable) may remain a concern.

9. In addition avoidance of organic solvents during preparation of SLNs by most methods, which is necessary for polymeric nanoparticles and in most instances of vesicular systems, is also an added advantage.

3.3.2 Methods of SLNs preparation: SLNs are produced by using several methods viz,

a) High pressure homogenization including cold and hot homogenization methods ((Müller and Runge, 1998).

b) Microemulsification method (Gasco, 1993).

c) Spray drying method (Freitas and Muller, 1998).

d) Preparation using supercritical fluid (Kaiser et al., 2001).

e) Solvent emulsification-evaporation (Sjostrom and Bergenstahl, 1992).

f) Solvent emulsification-diffusion (Quintanar-Guerrero et al., 2005).

g) Solvent injection (Schubert and Muller-Goyman, 2003).

h) Preparation via water-in-oil-in-water double emulsion (w/o/w) (Cortesi et al., 2002).

i) High shear homogenization (Kržič et al., 2001) and/or ultrasound dispersion (Song and Liu, 2005).

j) Preparations using membrane contactor (Charcosset et al., 2005).
For the purpose of present study SLN preparation by microemulsification is discussed and this method was used for the preparation of SLNs.

3.3.3 **Microemulsion based SLNs preparation**: Technique of microemulsion based SLN preparation was developed by Gasco which was based on the dilution of a hot microemulsion using cold water (Gasco, 1993). Microemulsions typically are formed by stirring an optically transparent mixture of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, and sodium taurodeoxycholate), co-emulsifiers (sodium monooyctylphosphatate) and water at 65-700 rpm. The hot microemulsion consequently formed is dispersed in cold water (2-8°C) under continuous stirring. Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. The droplet structure in microemulsion is already present and thus, no energy is required to achieve submicron particle sizes (Boltri et al., 1993; Gasco, 1997). Additionally, temperature gradient and the pH value are key parameters for the quality of the final lipid nanosuspension.

4. **DRUG AGENTS SELECTED**

4.1 **Sesamol**: Sesamol (1, 3-benzodioxol-5-ol) is an established antioxidant which is extracted from roasted seed of sesame (Sesamum indicum Linn.) family Pedaliaciae. Sesame is hugely prized oil crop since ancient times with India and China being its largest producers. Sesame seeds are considered to possess not only nutritional, but medicinal value too. They were employed in the ancient Chinese medicine as energy boosters and to prevent ageing (Budowski, 1950; Fukuda et al., 1994). On ripening, sesame splits and releases the seeds (Figure 9), and hence the famous phrase, “OPEN SESAME”. However, we interpret the phrase as ‘open research on sesame’ so as to develop and establish sesamol (an important antioxidant molecule obtained from this plant) for its therapeutic use in various disorders whose pathogenesis involve oxidative stress.

Sesame seed is an important source of oil (44-58%) which contains sesamolin, sesamin, and sesamol (392, 238, and 11.5-16.1 mg/100 g of oil, respectively) (Mohamed and Awatif, 1998). Sesamol is a phenolic compound and is formed by hydrolysis of sesamolin during thermal oxidation (Mohamed and Awatif, 1998). The long shelf life of sesame oil has been attributed to sesamol thus, sesame seeds though prone to rancidity the oil obtained from these seeds remains preserved.
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Sesame oil has been found to be effective against various diseases, including atherosclerosis, hypertension, and the effects of aging (Fukuda et al., 1985; Namiki, 1995). Sesame oil contains phenol, sesamin, sesamol, sesamolin, and a relatively small amount of tocopherol, which contributes to its superior oxidative stability (White, 1992). Sesame oil has been reported to ameliorate hydroxyl-radical generated lipid peroxidation and hepatic injury by inhibiting superoxide anion generation in iron-intoxicated mice. However, according to the authors the doses of sesame oil used in the study were pharmacological rather than physiological doses, and may be practically too large for human consumption (Hsu et al., 2007). Thus, sesamol one of the important antioxidant component derived from sesame oil is now being studied for its multiple biological effects.

4.1.1 Structural and physiochemical properties: Sesamol, 5-hydroxy-1, 3-benzodioxole or 3,4-methylenedioxyphenol is an established antioxidant molecule (Chennuru and Saleem, 2013; Geetha et al., 2009; Suja et al., 2004) (Figure 10) and its antioxidant activity is attributed to the presence of the benzodioxole group in its ring, which scavenges hydroxyl radical to produce another antioxidant molecule 1,2-dihydroxybenzene (Kumagai et al., 1991).

![Figure 10: Chemical structure of sesamol](image-url)

While the phenolic groups of molecules are generally responsible for the antioxidant activity of many natural molecules (McPhail et al., 2003; Wright et al., 2001), the benzodioxole derivatives activities (Hartley et al., 2012; Parise-Filho et al., 2011; Tagashira and Ohtake, 1998; Tseng et al., 2001). These activities have been attributed to their effect on various enzymes as well as
scavenging of ROS. Sesamol has so far been reported to exhibit numerous beneficial properties such as neuroprotective (Narasimhan et al., 2011), cardioprotective (Ying et al., 2011), hepatoprotective (Jnaneshwari et al., 2014), anti-mutagenic (Geetha et al., 2009; Kato et al., 1996), renoprotective (Gupta et al., 2009), radioprotective (Misra et al., 2011), anti-ageing (Kapadia et al., 2002; Ramachandran et al., 2010; Sharma and Kaur, 2006) and anti-inflammatory effects in various animal models (Chu et al., 2010; Hsu et al., 2009).

Area of drug research is divided into two stages: drug discovery/design and development. Drug development depends on the biopharmaceutical and pharmacodynamic properties of the drug since these properties control the rate and extent of drug reaching its site of action. Of the various physicochemical properties, solubility and ionization constant (pKa) are two important factors which monitor drug liberation from the dosage form and absorption. Various physiochemical properties of sesamol can be found in PubChem database (Pubchem, 2013a). It is a small molecule with a molecular weight of 138.12. The organoleptic properties of sesamol are listed in Table 3. The solubility of sesamol has earlier been determined in our laboratory and reported as $38.8 \pm 1.2$ mg/mL at 25°C in water (Geetha et al., 2009). Partition coefficient is another important factor which monitors the ability of a molecule to cross the biological membranes. Partition coefficients (log P) measured at a given pH are known as distribution coefficients (log D). Both log P and log D values for various drugs, measured using n-octanol as organic phase are reported to correlate well with their permeability, distribution and other in vivo pharmacokinetic parameters of the respective drug molecules (Malkia et al., 2004). Partition coefficient of sesamol is reported to be 1.29 (Geetha et al., 2009) and distribution coefficient as being $>1$ in a separate study (unpublished work from our laboratory). The number of H bond donors and H bond acceptors in the sesamol molecule are 1 and 3 respectively (Pubchem, 2013a). Lipinski’s rule of five states that, in general, an orally active drug should not violate more than one of the following criteria a) molecular mass less than 500 daltons, b) octanol-water partition coefficient i.e. log P not greater than 5, c) not more than 10 hydrogen bond acceptors, and d) not more than 5 hydrogen bond donors (Lipinski et al., 1997). It may thus be concluded that sesamol fulfils all the criteria defined under the Lipinski’s rule. The digestibility of sesamol in in vitro gastrointestinal digestion models with a sequential use of digestive enzymes in physiological concentrations and the stomach/duodenal environment are also reported (Jan et al., 2009). Sesamol was found to be stable in the gastrointestinal digestion
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models for 540 min. Thus, physicochemical characterisation of sesamol which is an important tool required for designing and developing dosage form of a drug indicates that it is an ideal candidate for drug development.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sesamol</th>
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<tbody>
<tr>
<td>Nature</td>
<td>Crystalline needles</td>
</tr>
<tr>
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</tr>
<tr>
<td>Odour</td>
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</tr>
<tr>
<td>Melting Point</td>
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<tr>
<td>Hygroscopicity</td>
<td>Nil</td>
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</tbody>
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4.1.2 Pharmacology of sesamol: Sesamol has been studied for its anti-oxidant and anti-inflammatory effects in numerous in vitro and in vivo studies. We discuss here in detail the hepatoprotective effects of sesamol.

4.1.2.1 Hepatoprotective effects

4.1.2.1.1 In vitro studies: The authors studied the effect of sesamol and 20 other related compounds on the, on the LPO of rat liver microsomes induced by CCl₄ or NADPH and on the LPO of mitochondria induced by ascorbate/Fe²⁺. Sesamol was found to be very effective inhibitor of LPO of rat liver microsomes (Uchida et al., 1996).

4.1.2.1.2 In vivo studies: Protective effect of sesamol and its related compounds on CCl₄ induced liver injury in rats was reported almost two decades back (Ohta et al., 1994). Significant effect by alleviation of ALT, AST, LDH, ALP, direct bilirubin and total bilirubin parameters was observed upon intraperitoneal, subcutaneous and oral administration of sesamol.

Effect of sesamol on mortality and ROS-associated liver injury in Wistar rats with cecal-ligation-and-puncture-induced sepsis (septic rats) has also been examined (Hsu et al., 2006). Sesamol was administered every 6 h after the induction of sepsis and the survival rate was determined during the ensuing 48 h. The authors reported that hepatic lipid peroxidation; hydroxyl radical, and superoxide anion levels were significantly lowered in sesamol-treated septic rats. Furthermore, sesamol inhibited the production of nitrite and the expression of iNOS in the liver of septic rats.
The authors concluded that sesamol delayed mortality and attenuated oxidative stress-associated liver injury by inhibiting the production of nitric oxide, at least partially, in septic rats.

In another study, the effect of sesamol on systemic oxidative stress and hepatic function in acutely iron-intoxicated mice was investigated (Hsu et al., 2007). Sesamol reduced the levels of lipid peroxidation, hydroxyl radical, iron production, superoxide anion generation, and xanthine oxidase activity in iron-intoxicated mice. Further, sesamol also decreased the serum levels of AST and ALT, and ameliorated iron-intoxication-induced histological changes in the liver. The prophylactic effect of sesamol on mitochondrial oxidative stress, hydroxyl-radical-generated lipid peroxidation, and hepatic injury in acetaminophen (APAP)-overdosed rats was also investigated (Chandrasekaran et al., 2009). APAP (1,000 mg/kg) was given to induce mitochondrial oxidative-stress-associated hepatic injury in rats, and then, immediately after the APAP administration animals were injected with sesmaol (10 mg/kg, i.p.). Sesamol prevented significant rise in the levels of AST, ALT, centrilobular necrosis, ferrous ions, hydrogen peroxide, hydroxyl radicals, lipid peroxidation, and a significant decrease in mitochondrial aconitase activity in the liver tissue of rats, 24 h later.

In another study by same group of authors sesamol was also found effective against acetaminophen-induced liver injury in rats (Chandrasekaran et al., 2011). Equimolar doses (1 mmol/kg) of sesamol and N-acetylcysteine significantly inhibited acetaminophen (300 mg/kg)-increased serum aspartate transaminase and alanine transaminase levels, 6 h post-administration. Sesamol and N-acetylcysteine maintained hepatic glutathione levels and inhibited lipid peroxidation. The authors concluded that the protective effect of sesamol against acetaminophen-induced liver damage is comparable to that of N-acetylcysteine by maintaining glutathione levels and inhibiting lipid peroxidation in mice.

The therapeutic effect of sesamol against monocrotaline-induced sinusoidal obstruction syndrome (SOS) in rats was examined in a separate study (Periasamy et al., 2011), where in a single dose of monocrotaline (90 mg/kg) induced SOS in rats. Sesamol (5, 10, 20, and 40 mg/kg) was subcutaneously injected 24 h after monocrotaline treatment. Liver pathology revealed that sesamol offered significant protection against SOS. A single dose of sesamol therapeutically attenuated SOS by decreasing the recruitment of inflammatory cells, downregulating MMP-9, and upregulating TIMP-1 expression.
In a recent study, cyclophosphamide (CP) induced hepatotoxicity was ameliorated by sesamol (Jnaneshwari et al., 2014). CP (150 mg/kg) was injected intraperitonially to experimental rats and from day 2 rats were orally treated with sesamol. Elevated levels of endogenous reactive oxygen species, lipid peroxidation, and decreased levels of glutathione, total thiols, along with reduction in antioxidant enzymes including SOD, CAT, glutathione-s-transferase, and glutathione peroxidase, were evident in CP-intoxicated animals. Pro-inflammatory mediators like tumor necrosis factor -α, interleukin (IL)-1β, IL-6 and cyclooxygenase-2 were also elevated. Moreover, the levels of liver function markers like serum ALT and AST were also altered. The altered parameters were significantly restored to normal by oral administration of sesamol (50 mg/kg) suggesting its anti-oxidative, anti-inflammatory and hepatoprotective abilities.

4.1.3 Pharmacokinetics: Drugs are administered by various routes and in most cases reach the site of action via systemic circulation. Oral drug administration represents the most convenient and common route of drug delivery. Further to this it is observed that in addition to other reasons like short gastric residence time and, drug instability on metabolism in the gastrointestinal tract, or lack of intestinal permeation of a drug may limit the bioavailability of several orally administered drugs. If a drug is injected intravenously, the total amount is immediately available to exert a therapeutic effect. BA is 100% for IV injection and it varies for other routes, depending on the extent of absorption and the extent of first pass hepatic metabolism in addition to other factors. For example, in case of oral route, the swallowed drug reaches the stomach, where it dissolves and a part of it may be absorbed. Absorption is completed in the small intestine. From here, the drug travels to the hepatic portal vein, where it may be metabolized at least partially before reaching the systemic circulation via the hepatic vein. Several factors can prevent the drug from reaching the systemic circulation (which may at times be desirable). Bioavailability is a term used to define the rate and extent of drug released from the dosage form that reaches the site of action/systemic circulation. In literature, there are couples of reports on the pharmacokinetics, bioavailability and elimination profile of sesamol (Jan et al., 2008; Jan et al., 2009). Authors have reported that the maximum concentrations of sesamol achieved after sesamol (po at a dose of 50mg/kg and iv at a dose of 5 mg/kg BW) in rats was 1.4 ±0.7 and 2.3 ±0.6 μg/mL respectively. Further to it, the half-lives were 563.7 ± 36.9 and 29.2 ±6.1 min and AUCs were 501.3 ± 200.8 and 141.4 ±9.0 min · μg/mL, respectively (Jan et al., 2008). These results showed that inspite of 10 times high oral dose, the Cmax was higher for i.v administration.
The authors report an oral bioavailability of 35.5 ± 8.5%, for sesamol in rats. After sesamol administration (po), the maximum concentrations of sesamol sulfate and glucuronide (two most common metabolites of sesamol were) 17.5 ± 6.8 and 34.0 ± 13.0 µg/mL; the half lives were 257.3 ±72.6 and 122.9 ± 15.5 min; and AUCs were 2034.4 ± 717.7 and 1266.7 ± 438.9 min µg/mL, respectively. The authors further carried out the tissue distribution of sesamol in rats by measuring the concentrations of sesamol and sesamol metabolites in various tissues using HPLC. It was reported that sesamol metabolites (glucuronide/sulfate) were widely distributed in rat tissues, with the highest concentrations in plasma and lungs and the lowest in brain. It was assumed by that sesamol is at first transported to the liver followed by its distribution to other tissues (lung, kidney, and brain). Authors further analysed the area under curve (AUC), which represents the total drug exposure integrated over time and estimated AUCs for plasma and whole tissue after oral administration of sesamol (300mg/kg bodyweight) and indicated that the AUC followed the order; intestines > lung > plasma > brain > kidney > liver. Further the authors also report that the AUCs of sesamol metabolites (glucuronide/sulfate) were remarkably highest in the kidneys, lungs, and plasma (Jan et al., 2008).

The same group also studied the excretion of sesamol in Sprague-Dawley rats (Jan et al., 2009). After oral administration of sesamol (100 mg/kg), the change in its concentration was determined in various excreta within 24 h period. They reported that sesamol conjugated metabolites were rapidly eliminated from urine and feces in 0-4 h. The majority of intact sesamol glucuronide was excreted in the urine. The authors suggested that sesamol conjugated metabolites are primarily eliminated from the plasma via the kidney by active tubular secretion. Sesamol was excreted in much smaller quantities in the two subsequent 4–8 and 16–24 h periods. Urinary amounts of sesamol and its conjugated metabolites increased in response to sesamol ingestion, reaching a peak at 4–8 h. The measured amounts of sesamol were 109.4 ± 4.8 nmol/mL for the free form, 8426.2 ± 108.5 nmol/mL for sesamol glucuronide, and 4853.8 ± 62.5 nmol/mL for sesamol sulfate. The unchanged sesamol was present in very low concentrations and represented only 0.4 ± 0.1% up to 24 h after administration(Jan et al., 2009).

To investigate the elimination of sesamol in rats, the authors also determined the concentration of sesamol and its conjugated metabolites in rat feces within 24 h after administration of sesamol. In the feces the concentrations of sesamol and conjugated metabolites reached a maximum at 4–8 h after administration. However, the concentration of free sesamol was
significantly greater than its conjugated metabolites in the feces. The percentage of sesamol and sesamol glucuronide excreted in feces in rats was highest in the first 24 h, accounting for $1.170 \pm 0.304$ and $0.806 \pm 0.266\%$ of the total excretion for sesamol and sesamol glucuronide, respectively (Jan et al., 2009).

4.1.4 Toxicity studies: There is no human or animal data for the chemical disposition of sesamol. However, there are few acute, sub-chronic, and chronic toxicity studies in animals and humans. We present here all such studies that have been conducted and reported in literature, to the best of our knowledge.

4.1.4.1 Acute studies

4.1.4.1.1 Animal studies: In a study conducted with rats of unspecified strain and sex which were injected intradermally with 0.1 ml of an aqueous solution containing 5 mg of sesamol ($n=10$). It was observed that 6 rats developed necrosis at the site of injection within 4 days of administration (Ambrose et al., 1958). The authors also conducted studies in rabbits (unspecified strain and sex) which were again injected intradermally with 0.16-5 mg of sesamol dissolved in 0.1 ml of water while the control rabbits were given water and 0.05 ml of a 4% solution of formaldehyde. They observed irritation in the formaldehyde dosed rabbits however rabbits dosed with $\geq 0.5$ mg of sesamol showed a more striking irritation within 30 min of injection. However, no such effects were observed when the authors used a 5% aqueous solution of sesamol, equivalent to 50 mg/kg, for application on the shaved right flank of 3 rabbits (unspecified sex and strain) for 4 days (Ambrose et al., 1958).

The same authors also studied effects of sesamol in eyes of rabbits (unspecified strain and sex) by administering 1.2 mg, 2.3 mg, and 4.6 mg of sesamol in the conjunctival sac of one eye with the other eye serving as the control as it was administered only water (Ambrose et al., 1958). Authors reported that 4 h after the application of sesamol, all the rabbits in each group experienced the same symptomatology including edema of the nictitating membrane, swelling of the palpebral folds, and conjunctivitis. Further to it, after 24 h, slight chemosis of the eye was seen in rabbits treated with 2.3 and 4.6 mg sesamol. After 24 h eyes of the rabbits treated with 1.2 mg of sesamol appeared normal while the eyes of rabbits treated with 2.3 mg appeared normal after 48 h. 3 of the 6 rabbits treated with 4.6 mg sesamol experienced a slight edema in nictitating membrane at 48 hours, but the eyes of these rabbits were otherwise normal after 48 hours (Ambrose et al., 1958)
4.1.4.1.2 Human studies: In a study conducted in humans, 13 patients with contact allergy to sesame oil were patch tested using 5% sesamol and 8 patients tested positive to sesamol (Neering et al., 1975). However, in another study a 35-year-old women who suffered from a deep burn, was treated with a Chinese ointment ("Shiunkoh") which composed of 60% sesame oil. After 10 days, the woman experienced edema, erythema, and vesicles around the burn. The women tested positive to skin patch tests with 1% sesamin or 1% sesamolin in petrolatum and negative to 1% sesamol in petrolatum. These results led the authors to conclude that sesamin and sesamolin are the primary allergens in sesame oil (Hayakawa et al., 1987; Kubo et al., 1986).

In yet another study it was reported that a 25-year-old woman developed cheilitis on her lips following the use of a new lipstick and also showed a strong positive reaction in skin patch tests with the new lipstick and sesame oil. However, no reaction was seen with sesamol at concentrations of 0.1%-5.0% in petrolatum. Further, patch tests revealed that sesamin (0.1% pet) and sesamolin (0.3% pet) showed positive reactions. This was confirmed as sesamol was not detectable in the sesame oil used as an ingredient in the lipstick upon high performance liquid chromatographic (HPLC) analysis (Hayakawa et al., 1987).

4.1.4.2 Sub-chronic studies

4.1.4.2.1 Animal studies: In an early report the depilated skin of rats (unspecified strain, sex, and number) was topically applied with 50 mg/kg of sesamol in cotton seed oil or ethyl alcohol daily for 30 days and no local or systemic effects were observed (Ambrose et al., 1958). In another study conducted, rats, (6 week old male F344 rats; n=5) were fed 1% or 2% sesamol in a basal diet for 4 weeks while control rats were fed only the basal diet (n=5). After 4 weeks the body weights of the sesamol treated rats were 10% to 15% less than control rats. Further, the rats fed on 2% sesamol diet developed large ulcers with thickened epithelium in the central region of the forestomach (Hirose et al., 1987). In another study conducted by Ito and co workers (Ito et al., 1988) authors reported that sesamol showed no hepatocarcinogenic potential in male Fischer rats.

4.1.4.2.2 Human studies: 5 subjects were given topical dermal application of 9 sensitizing doses of 1.25 mg of sesamol dissolved in alcohol to the anterior cubital surface of the right arm, 24 hours apart and a challenge dose was applied 12 days after the last sensitizing dose. No signs of hyperemia or irritation were observed after application of either the sensitizing or the challenging doses (Ambrose et al., 1958).
4.1.4.3 Chronic studies

4.1.4.3.1 Animal studies: Hirose and co-workers studied the effect of naturally occurring antioxidants including sesamol on rat forestomach epithelium in comparison to the synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), of which the former is a known forestomach carcinogen. Groups of five F344 male rats were given diet containing BHA, BHT, gallic acid, sesamol, caffeic acid, chlorogenic acid, ferulic acid, eugenol or esculin for 4 weeks at a level of 0.7% for BHT or 2% for other compounds. The histological examination indicated that the forestomach of BHA treated group showed hyperplasia mainly in the prefundic region near the esophageal orifice while sesamol induced large ulcers and hyperplasia in the central region (Hirose et al., 1987).

The same group of researchers further studied the carcinogenic potential of sesamol in male and female F344 rats and B6C3F1 mice. They treated a group of 30 rats with 2% sesamol diet for 104 weeks and a group of mice (30) for 96 weeks. The histological examination of forestomach revealed that sesamol was associated with squamous cell carcinoma at incidences of 31% (p<0.001) in male rats, and 38% (p<0.001) and 17% (p<0.05) in male and female mice, respectively (Hirose et al., 1990).

Authors observed the effects of sesamol administration at a dietary level of 2% in groups of 30 male and female F344/DuCrj rats and B6C3F1 mice for 104 and 96 weeks, respectively. They reported the induction of squamous cell carcinomas in the forestomach in nine of 29 (31%) male rats, 3 of 30 (10%) female rats, 11 of 29 (38%) male mice and 5 of 30 (17%) female mice treated with sesamol. Further to it, they reported that papillomas developed in 10 of 29 (34%) male rats and 14 of 30 (47%) female rats, but not in any of the treated mice. They further observed that hyperplasias developed in almost all rats and mice of both sexes. They also found significant differences from control values for all three lesions in rats and for carcinoma and hyperplasia categories in mice. The incidence of other tumors in the 2% sesamol group was comparable with control values. The authors thus concluded that sesamol induces squamous cell carcinomas in the forestomach of rats and mice, males being more susceptible than females (Tamano et al., 1992).

4.1.4.3.2 Human studies: There are no reports on the chronic toxicity effects of sesamol in humans.

4.1.5 Mutagenicity studies: The mutagenicity study for sesamol was evaluated in the Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538. In the presence of metabolic activation, sesamol at a concentration of 33,333 µg/plate, dissolved in
dimethylsulfoxide, was non-mutagenic. In addition, sesamol was non-mutagenic without metabolic activation at a concentration of 100-5000 μg/plate (Zaika, 2009).

Mutagenicity study was also carried out in mouse lymphoma system using strains L5178Y (TK+/TK-). Sesamol was mutagenic at doses of 8-260 μg/ml, dissolved in dimethylsulfoxide. Further, in the presence of metabolic activation also sesamol was mutagenic at doses ranging from 8-260 μg/ml (Seifried et al., 2006).

4.2 Curcumin: In past few years there has been a growing interest in phytochemicals for their therapeutic potential in various diseases. Curcumin is one of these compounds, and this polyphenol has been studied vigorously and extensively reported to possess various biological properties such as anti-oxidant, anti-inflammatory, anti-fibrogenic, and anti-cancer to name a few (Asher and Spelman, 2013; Beevers and Huang, 2011; Noorafshan and Ashkani-Esfahani, 2013). Due to its numerous biological properties, curcumin is being marketed in many countries including India, Japan, Korea, China, Thailand, South Africa, Turkey, Nepal and The United States of America in the form of tablets, soaps, ointments and cosmetics (Prasad et al., 2014) (Figure 11).

Curcumin has gathered much attention due to its pleiotropic effects with more than 90 clinical studies (ongoing and recruiting), investigating the effects of curcumin in various human disorders such as Alzheimer’s disease, asthma, irritable bowel syndrome, cancers, osteoarthritis, rheumatoid arthritis, depression, ulcerative colitis, osteosarcoma, proteinuric chronic kidney disease, and cystic fibrosis to name a few (Nabavi et al., 2014).

Curcumin is a bright yellow-colored phenolic compound that is isolated from rhizomes (Figure 12) of Curcuma longa L. (turmeric) belonging to a member of Zingiberaceae family, which is grown in India, Southeast Asia, and other tropical areas (Gupta et al., 2013a). Diferuloylmethane, demethoxycurcumin and bisdemethoxycurcumin are the different natural analogs of curcumin (Nabavi et al., 2014) (Figure 13).
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Figure 11: Curcumin is marketed in many forms such as capsules, ointment, tablets, soap, cosmetics and energy drinks (Prasad et al., 2014).

Figure 12: Curcumin is obtained from the rhizome of the herb *Curcuma longa* L.
4.2.1 Chemistry of curcumin: Curcumin has a molecular formula of $C_{21}H_{20}O_6$ with a molecular weight of 368.37 g and melting point of 183°C (Pubchem, 2013b). It gives brilliant yellow and red color at pH 2.5–7 and >7, respectively (Tonnesen and Karlsen, 1985a). Chemically, curcumin is a 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) and exhibits keto-enol tautomerism with keto form being predominant in acidic and neutral solutions while a stable enol form in alkaline medium (Balasubramanian, 2006). The fact that curcumin in solution exists primarily in its enolic form plays an important role in the free radical scavenging capability of curcumin (Tomren et al., 2007). Curcumin is lipophilic in nature as its structure constitutes two aromatic rings connected by two unsaturated carbonyl groups which consequently lead to its poor solubility in water (Tonnesen, 2006). Authors have reported that the stabilization of the structure of curcumin by hydrogen-bonding associated with the central -OH group, accounts for its functional site which results in its molecular biological activities (Priyadarsini et al., 2003). The yellow-orange colored powder although insoluble in water and ether, is found to be soluble in ethanol, methanol, dimethyl sulfoxide and acetone. It is stable at acidic pH but unstable at neutral and basic pH (Tomren et al., 2004), however the stability

Figure 13: The three common analogs of curcumin are diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin (Nabavi et al., 2014).
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improves at pH greater than 11.7. More than >90% curcumin gets rapidly degraded within 30 minutes in phosphate buffer systems of pH 7.2 (Tonnesen and Karlsen, 1985a, b).

4.2.2 Safety: Curcumin is a natural product which is consumed in the human diet, and has so far been reported to be safe in animals and humans, even at at high doses (Nabavi et al., 2014; Rivera-Espinoza and Muriel, 2009; Vera-Ramirez et al., 2013). The FAO and WHO Expert Committee on Food Additives reported that 3mg/kg body weight daily intake of curcumin is acceptable which designates its safety (Nabavi et al., 2014). The same was confirmed by the European Food Safety Authority (EFSA) in 2010 which also advocated a dose of 3 mg/kg/day of curcumin as safe (EFSA., 2010). The risk of hepatotoxicity due to curcumin has been closely evaluated, and the conduct of various liver function tests revealed that curcumin at even dose as high as 2-4 g per day did not affect functioning of liver (Goel et al., 2008).

4.2.3 Curcumin in liver disease: Oxidative stress is widely implicated in liver disease irrespective of etiology and thus the antioxidative properties of curcumin complement with the fact of it being a safe, inexpensive and easily available compound makes it a potential candidate for exploring its therapeutic-curative potential for several liver diseases (Vera-Ramirez et al., 2013). As discussed earlier current therapies available for various hepatic diseases is limited. Curcumin can be considered an alternative curative-therapeutic agent for hepatic diseases as it possesses antioxidant, anti-inflammatory, antifibrotic and thus hepatoprotective effect (Rivera-Espinoza and Muriel, 2009) (Figure 14).

4.2.3.1 Antioxidant effect: Curcumin has well known antioxidant activity, which is comparable to another potent antioxidant vitamin C and is reported to have more than 10 times higher antioxidant activity than vitamin E (Motterlini et al., 2000). The antioxidant properties of curcumin reside in its chemical structure which has both a phenolic group and one diketonic ring in the same molecule of both these features that contribute to its antioxidant activity (Anand et al., 2008b).

4.2.3.2 Anti-inflammatory effect: The hydroxyphenyl unit in curcumin has been reported to manifest anti-inflammatory activity while acylation and alkylation of the phenolic hydroxy reduces its anti-inflammatory activity (Dulbecco and Savarino, 2013; Mukhopadhyay et al., 1982). It is also reported that the phenolic hydroxyl groups of curcumin are required for inhibition of COX-1 activity (Hong et al., 2004). The authors further reported that addition of alkyl or alkoxy groups at the 3- and 5-positions on the phenyl ring could enhance the anti-inflammatory effect.
Curcumin has been widely reported to inhibit the expression NF-κB dependent inflammatory chemokines, cytokines, and inflammation-promoting enzymes in Kupffer cells and hepatic tissue homogenates (Bassiouny et al., 2011; Charoensuk et al., 2011; Nanji et al., 2003).

4.2.3.3 Antifibrotic effect: Curcumin is known to possess antifibrotic activity due to its inhibitory effect on TGF-β. Latter is known for its profibrinogenic effects as it plays an important role in promoting the activation of stellate cells to myofibroblasts and thus the production of extracellular matrix (Branton and Kopp, 1999; Shen et al., 2003). TGF-β is one of the molecular target of curcumin which acts upon it via NF-κB (Hanai et al., 2006). Curcumin has been reported to inhibit the upregulation of TGF-β mRNA and TGF-β protein expressions in hepatic tissues of bile duct-ligated rats (Reyes-Gordillo et al., 2008). Authors have attributed the preventive role of curcumin against fibrosis to its antioxidant action which aids in the down regulation of NF-κB and also TGF-β (Dohmen et al., 2004; Leask and Abraham, 2004). Antifibrotic effect of curcumin is also mediated by its effect on metalloproteinases, which are involved in remodeling the extracellular matrix (Kang et al., 2002; Miquel et al., 2002). Curcumin has also been reported to have significantly reduce liver fibrosis and injury induced by CCl₄ and Concaavalin A through the negative modulation of the expression of Toll-like receptor TLR 2, TLR4, and TLR 9 (Tu et al., 2012).

4.2.4 Hepatoprotective effect in various diseases of the liver

4.2.4.1 Hepatitis B: Hepatitis B virus (HBV) infection causes hepatitis which may progress to cirrhosis, and hepatocellular carcinoma (HC) (Jazayeri et al., 2009). During hepatitis B viral infection, the metabolic regulator peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) coactivates the transcription of HBV via the forkhead transcription factor FOXO1 and hepatocytes nuclear factor-4α (HNF4α) (Quasdorff et al., 2008). A report has shown that curcumin downregulates PGC-1α and significantly suppresses HBV gene expression (Rechtman et al., 2010) which was earlier reported to be a good strategy for anti-HBV therapy (Ganem and Prince, 2004).

4.2.4.2 Hepatitis C: The hepatitis C virus (HCV) can cause chronic hepatitis which may also progress to fibrosis cirrhosis, and hepatic carcinoma (Degos et al., 2000). A report demonstrated that curcumin suppressed PI3K/Akt-SREBP-1 pathway which led to inhibition of hepatitis C virus replication (Kim et al., 2010).
4.2.4.3 *NAFLD*: Different studies have revealed that nonalcoholic fatty liver diseases showed pathologies similar to alcoholic liver injury. Studies have reported that i.p administrations of curcumin alleviates fibrosis by modulating intrahepatic gene expression of monocyte chemoattractantprotein-1, CD11b, procollagen type I, NF-xB, ICAM-1, COX-2, TNF-α, and protein levels of α-smooth muscle-actin (Leclercq et al., 2001). Curcumin is also reported to inhibit oxidative stress via modification of mitochondrial ROS and it was reported that curcumin can also alleviates the abnormal increase in the level of aminotransferases (Ramirez-Tortosa et al., 2009). In another report curcumin decreased hepatic triglycerides in obese mice by downregulating the gene expression of sterol regulatory element-binding protein-1c in the liver (Kuo et al., 2012). It further decreased the expression genes of mitochondrial DNA (mtDNA), nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (Tfam), which are responsible for the lower mitochondrial respiratory chain (MRC) complex I activity and adenosine triphosphate production. The authors concluded that curcumin can prevent fatty liver disease (Kuo et al., 2012).

4.2.4.4 *ALD*: The protective action of curcumin against experimental model of alcoholic liver diseases was evaluated in rats and the authors reported that treatment with dietary curcumin (75 mg/kg/ day) reduced fatty liver, necrosis, and inflammation (Nanji et al., 2003). The authors thus
proposed that curcumin could inhibit lipid peroxidation, activation of NF-κβ, and the expression of TNF-α, IL-12, MCP-1, MIP-2, COX-2, and iNOS. It has been reported that chronic exposure to ethanol increases, Ca21- dependent phospholipase 2 (PLA2) activity (Hugund et al., 1994) which in turn, increases the formation of arachidonic acid release from phospholipids, which is then converted to physiologically relevant eicosanoids (Holtzmann, 1991). Curcumin (80 mg/kg/day) has been reported to inhibit not only the production of arachidonic acid (in the liver) (Rajakrishnan et al., 2000) but also the PLA2 activity (Aggarwal et al., 2004).

Authors have also reported significant increase in the levels of prostaglandins (PGE1, PGE2, PGF2a and PGD2) in the liver of alcohol treated rats, which was decreased by co-treatment with curcumin at a dose of 80 mg/kg body weight daily for sixty days (Rajakrishnan et al., 2000). The decreased production of PGs mediated via curcumin (3, 10, 30, or 100 μM) is probably through the inhibition of PLA2, lipoxygenase and COX activities as reported in an in vitro study (Huang et al., 1991). The decrease in PGs also suggested that increased arachidonic acid may be utilized for the synthesis of phospholipids, which can be useful for plasma membrane synthesis (Rajakrishnan et al., 2000). In vitro and in vivo studies have further shown that curcumin ameliorated the ethanol-induced histopathological changes of the liver and also attenuated cellular released ALT, AST, and LDH (Rong et al., 2012; Samuhasaneeto et al., 2009). Further to it the ethanol exposure resulted in ROS generation, MDA elevation, GSH depletion and antioxidant defense system impairment, which were significantly attenuated by curcumin treatment. Authors reported a very high dose of 400 mg and 1200 mg/kg/day of curcumin for protective role in ethanol induced injury in rats which was mediated by NF-κβ activation modulation (Samuhasaneeto et al., 2009). Authors also concluded that a higher dose of 1200 mg/kg did not produce better effects than 400 mg/kg dose. In another report a dose of 75 mg/kg/day of curcumin for attenuation of ethanol induced changes in mice (Rong et al., 2012). The reported evidence strongly suggests that curcumin helps in maintaining the membrane structure, integrity and function, protecting the liver from alcohol toxicity (Nabavi et al., 2014; Rivera-Espinoza and Muriel, 2009).

4.2.4.5 Hepatotoxicity: Hepatic toxicity is known to be induced by many drugs and toxins as liver is the main site of metabolizing the drug/toxins(Vera-Ramirez et al., 2013). Hepatic toxicity can lead to oxidative stress which in turn can cause steatosis, acute cell death, and finally culminate in cirrhosis (Vera-Ramirez et al., 2013). Common type of hepatic toxicity studied in
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laboratory is induced by iron, CCl₄, thioacetamide, paracetamol, endotoxin, and antitubercular drugs (Rivera-Espinoza and Muriel, 2009; Vera-Ramirez et al., 2013).

Authors have reported the ameliorative effects of curcumin against hepatic toxicity induced by iron (Messner et al., 2009), thioacetamide (TAA) (Shapiro et al., 2006), paracetamol (Girish et al., 2009), chloroquine (Dattani et al., 2010), methotrexate (Hemeida and Mohafez, 2008), erythromycin estolate (Pari and Murugan, 2004), endotoxin (Kaur et al., 2006) isoniazid, rifampicin, and pyrazinamide (Adhvaryu et al., 2007). The antioxidant property of curcumin protects against drug-induced liver injury (Negi et al., 2008).

Authors reported that pretreatment with curcumin at a dose of 60 mg/kg/day for 7 days decreased the levels of LPO measured as thiobarbituric acid reactive substances (TBARS) and increased GSH and SOD levels in the liver homogenates from a single dose of LPS given on 7th day in rats (Kaur et al., 2006). In another study mice were pretreated with a single dose of 100 mg/kg of curcumin 3 h prior the GalN/LPS challenge which resulted in ameliorative effects on liver injury induced (Yun et al., 2010).

Curcumin (5 to 10 μM) significantly reduced iron-dependent oxidative stress and iron toxicity in rat epithelial cell without blocking the iron uptake (Messner et al., 2009). In a study conducted authors demonstrated the hepatoprotective effects in male B6C3F1 mice pretreated with curcumin (17 mg/kg/day, p.o.) for 12 days followed by a single APAP exposure (400 mg/kg, ip) (Bulku et al., 2012). Authors further reported that Curcumin ameliorated APAP-induced liver damage through normalization of proapoptotic (Bax, caspase-3) and antiapoptotic signaling pathways. Authors have shown that inhibition of COX-2 and iNOS expression through NF-κβ pathways by curcumin aids in the amelioration of drug-induced liver injury (Puri and Sanyal, 2012). In another study authors (Somanawat et al., 2013) evaluated the protective effects of curcumin in paracetamol induced hepatic injury in mice. Authors demonstrated that paracetamol treated rats showed siginificant improvement in altered levels of ALT, AST, MDA, GSH, IL-12, and IL-18 on cotreatment with curcumin at a dose of 200 and 600 mg/kg.

The antioxidant properties of curcumin and its ability to inactivate NF-κβ (Maheswari et al., 2006; Singh and Aggarwal, 1995) and thus pro-inflammatory cytokine production are the most important mechanisms of action of curcumin to prevent acute CCl₄- induced liver injury. In another study authors reported the protective effect of curcumin in acute and sub-chronic rat liver injury induced by CCl₄ (Park et al., 2000). In acute study, pretreatment with curcumin (100 or
200 mg/kg/day for four days) significantly prevented the increase of ALT and AST levels in rats after treatment with a single dose of CCl₄ (0.2 ml/kg, i.p.). In the sub-chronic liver damage, CCl₄ was administered by gavage (1 ml/kg, mixed with an equal volume of corn oil) twice a week for 4 weeks while co-treatment with curcumin (50 or 100 mg/kg) was done daily. Authors reported that curcumin (100 mg/kg) prevented partially, but significantly, alterations in the level of ALT and AST. However, authors also noted that a 50 mg/kg dose of curcumin did not produce any significant effects on these parameters. Furthermore normalised hydroxyproline and MDA levels were observed after treatment with curcumin at a dose of 100 mg/kg/day (Park et al., 2000).

Curcumin treatment (200 mg/kg, p.o.) given before and 2 h after CCl₄ administration in rats provided hepatoprotection by attenuating oxidative stress and inhibiting NF-κB mediated inflammation (Reyes-Gordillo et al., 2007). In another study authors showed that co-treatment of curcumin (200 and 400 mg/kg/day body weight for 8 weeks) with CCl₄ reduced the levels of inflammatory cytokines, including IF-γ, TNF-α, and IL-6. Further to it, curcumin also inhibited HSC activation by elevating the level of PPARγ and reduced the abundance of PDGF, TGF-β, their receptors, and type I collagen (Fu et al., 2008).

In a study conducted abuthors observed the antifibrotic effects of curcumin (100mg/kg/day for two months) in a model of CCl₄ induced cirrhosis in rats (three months induction period)(Reyes-Gordillo et al., 2008). It has been reported that curcumin upregulated the expression and activity of matrix pro-MMP-2 and proMMP-9 in human bronchial epithelial cells, and during the prevention and healing of indomethacin-induced gastric ulcers (Swarnakar et al., 2005). Recovery from liver fibrosis may be explained through modulation of MMPs; in addition, to the downregulation of NF-κB, TGF-β, and other cytokines. Another report showed that curcumin inhibited collagen synthesis and HSC activation in vivo and in vitro (Kang et al., 2002) which further supported the ability of curcumin to reverse CCl₄ fibrosis (Reyes-Gordillo et al., 2008).

4.2.4.6 Liver cancer: Curcumin’s NF-κB inhibitory activity (Darvesh et al., 2012) and its interaction with other transcription factors (Notarbartolo et al., 2005) influences apoptotic mechanisms in HC. It has been revealed that curcumin has an apoptosis inducing effect which is mediated by p38 activation-induced FasL expression signaling in human HC Huh7 cells (Wang et al., 2013). MicroRNAs (miRNAs) regulation is another mechanism involved in the pathogenesis of human HC (Ladeiro et al., 2008) and authors have showed that curcumin administration could control the expression of miR-199 and miR-200 families (Hassan and Al-
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Olayan, 2012). Curcumin also modulated the expression of miR-21 and miR-34a and upregulated tumor suppressor let-7a miRNA (Subramaniam et al., 2012). Liver cancer promotes neovascularization and angiogenesis in HC tumors that are regulated by different molecular signaling pathways including extracellular signal-regulated kinase 1/2 and serine/threonine kinase AKT (Schmitz et al., 2008). Curcumin administration significantly repressed different angiogenic biomarkers such as VEGF, and COX-2 expressions (Yoysungnoen et al., 2006). Another report confirmed that the repressive effect of curcumin on STAT3 and hypoxia-inducible factor-1α expression in human HC led to reduced tumor angiogenesis and progression (Bae et al., 2006) other possible antiproliferative mechanism of curcumin against HC is through induction of endoplasmic reticulum stress and mitochondrial dysfunction (Cheng et al., 2010).

4.2.5 Pharmacokinetics and metabolism of curcumin

4.2.5.1 Serum concentration: Curcumin in many studies have shown very low serum concentrations. The first reported study to examine the uptake, distribution, and excretion of curcumin was carried out by Wahlstrom and Blennow in Sprague-Dawley rats (Wahlstrom and Blennow, 1978) . The authors reported that only negligible amounts of curcumin was present in the blood plasma of rats after oral administration of 1 g/kg of curcumin indicating the poor absorption from gut. In another study authors reported that at a dose of 400 mg p.o administration of curcumin in rats, no curcumin was found in the heart blood. This was in contrast to the ≤5 μg/mL concentration of curcumin in the portal blood from 15 min to 24 h (Ravindranath and Chandrasekara, 1980). In another report maximum serum concentration was 1.35 ± 0.23 μg/mL after 0.83 h when rats were administered curcumin at a dose of 2 g/kg (p.o), while, in humans, curcumin was not detectable at the same dose (Shoba et al., 1998). In a later study authors (Pan et al., 1999) investigated the pharmacokinetic properties of curcumin administered via oral or intraperitoneal (i.p.) routes in mice. Oral administration at a dose of 1.0 g/kg of curcumin showed low plasma levels of 0.13 μg/mL after 15 min, while a maximum plasma level of 0.22 μg/mL was obtained at 1 h. Plasma concentrations then declined below the detection limit by 6 h. On i.p. administration of 0.1 g/kg plasma curcumin levels peaked (2.25 μg/mL) within 15 min of administration and declined rapidly within 1 h (Pan et al., 1999). In yet another study authors showed that 10 mg/kg of curcumin given via (intravenous) i.v. route in rats yielded a maximum serum curcumin level of 0.36 ± 0.05 μg/mL, while at a 50 times higher dose administered orally serum curcumin concentration was 0.06 ± 0.01 μg/mL (Yang et al., 2007).
yet another study authors observed that upon oral administration of curcumin to rats at a dose of 500 mg/kg, peak concentration of 64.29 ± 1.69 µg/ml was observed in serum at 24 h and declined to 8.04 ± 1.09 µg/ml in the next 24 h (Suresh and Srinivasan, 2010). Phase I clinical trial at three different oral dose of 4, 6 and 8 g of curcumin administered daily for three months was conducted among 25 patients who had precancerous lesions (Cheng et al., 2001). The study revealed that serum curcumin concentrations levels were 0.51 ± 0.11, 0.63 ± 0.06, and 1.77 ± 1.87 µM for 4, 6 and 8 mg dose, respectively. In another phase trial conducted with 15 patients having advanced colorectal cancer, curcumin was administered at doses between 0.45 and 3.6 g daily for four months (Sharma et al., 2004). Authors reported that in 3 of the 6 patients administered 3.6 g dose of curcumin, had the mean plasma curcumin concentration of 11.1 ± 0.6 nmol/L after one hour on day 1, while, curcumin concentration remained undetectable in the plasma of patients administered doses lower than 3.6 g.

4.2.5.2 Tissue distribution: There are limited number of studies addressing the uptake and distribution of curcumin in body tissues. It has been reported in a study that on administrating 400 mg curcumin (p.o) to rats, only traces of unchanged curcumin was found in the liver and kidney. It was also observed that although 90% of the administered dose was detectable in the stomach and small intestine at 30 min, only 1% of it was present at 24 h. The same authors in another report studied the tissue distribution of a tritium-labeled (3H) curcumin (Ravindranath and Chandrasekhara, 1981). The authors reported that, on administering 10, 80 and 400 mg of (3H) curcumin to rats, and measuring substantial amounts of radioactivity was observed in the blood, liver and kidney, 12 days after dosing (at a dose of 400 mg). However, the proportion of curcumin absorbed at different doses (10, 80 and 400 mg) remained constant (60% to 66% of the administered dose), indicating dose independent kinetics exhibited by curcumin. (Pan et al., 1999) reported that following i.p. administration at a dose of 0.1 g/kg to mice, 177.0, 26.9, and 7.51 µg/g, levels of curcumin were found in the intestines, liver, and kidneys respectively was produced while only traces (0.41 µg/g) were observed in the brain tissue at 1 h. In another study by Garcea et al. (2004) administration of 3600 mg of curcumin to patients with colorectal cancer, curcumin concentration was found to be 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g, in normal and malignant colorectal tissue, respectively. Further to it the authors also reported that curcumin showed pharmacological activity in the colorectum as indicated by the effects on measured levels of M(1)G and cyclooxygenase-2 (COX-2) protein (Garcea et al., 2005). In yet
another study, curcumin was not detectable in the liver tissue of patients, with hepatic metastases from colorectal cancer, who had received 450-3600 mg of curcumin daily for 1 week prior to surgery (Garcea et al., 2004).

Upon oral administration of curcumin to rats at a dose of 500 mg/kg, peak concentration of the same was observed in the intestine at 1 h while in liver and kidney the peak concentrations were observed at 6 h (Suresh and Srinivasan, 2010). The authors reported that 3.88 to 48.3 per cent of the administered curcumin was detected in blood, liver, kidney and intestine from 1 to 24 h. Curcumin concentration in the intestinal tissue gradually decreased from 36.2 mg at 1 h to 2.21 mg by 24 h. Further, curcumin was not detectable in the intestine after 4 days. Curcumin reached peak concentration of 9.03 ± 1.11 and 135.2 ± 5.26 μg/whole tissue in kidney and liver, respectively after 6 h and on day 4, only 0.027 ± 0.006 and 4.77 ± 0.69 μg/whole tissues was detectable respectively. This study is in contrast to earlier report by Ravindranath & Chandrasekhara (Ravindranath and Chandrasekhara, 1980) where after oral administration of curcumin at a dose of 400 mg in rats, no amount was detectable in liver and kidney at 24 h. The authors suggested that the variation in result could be due to use of more sensitive HPLC method for the detection and analysis of curcumin (Suresh and Srinivasan, 2010).

4.2.5.3 Metabolites: Studies have been carried out to evaluate the metabolism of curcumin in rodents and in humans. In an early study, authors reported that in suspensions of isolated hepatocytes or liver microsomes, 90% of the added curcumin was metabolized within 30 min (Wahlstrom and Blennow, 1978). Liver is the major organ accountable for the metabolism of curcumin (Hoehle et al., 2006; Wahlstrom and Blennow, 1978). In an early study authors have reported that glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin are the major biliary metabolites of curcumin in rats (Holder et al., 1978). In another study authors have demonstrated that on oral administration of curcumin major metabolites were glucuronides/sulfates (Asai and Miyazawa, 2000) (Figure 15). It was thus indicated that on oral administration curcumin is first absorbed from the gut and then gets metabolized in liver, where the metabolized forms (glucuronide/sulfate conjugates) remain in the systemic blood circulation (Anand et al., 2007; Basnet and Skalko-Basnet, 2011) while on i.p administration hexahydrocurcuminol, hexahydrocurcumin, and tetrahydrocurcumin metabolites are formed (Marczylo et al., 2009) (Figure 16).

Whether the curcumin metabolites are biologically active remains an unanswered question.
Although most studies indicated that metabolites are less active than curcumin, few studies however suggest that they may actually be more active than curcumin (Anand et al., 2007; Sandur et al., 2007).

![Curcumin sulfate](image1)

![Curcumin glucuronide](image2)

**Figure 15:** Chemical structures of metabolites of curcumin following oral administration (Nabavi et al., 2014).

![Hexahydrocurcuminol](image3)

![Hexahydrocurcuman](image4)

![Tetrahydrocurcumin](image5)

**Figure 16:** Chemical structures of metabolites of curcumin following intraperitoneal administration (Nabavi et al., 2014).

### 4.2.5.4 Half-life
Systemic elimination or clearance of a compound from the body is an important factor in determining its relative pharmacological activity. Authors reported that when
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1 g/kg curcumin was administered orally to rats, % of it was excreted in the feces, and negligible amounts were found in the urine. (Wahlstrom and Blennow, 1978). In another report, i.v. and i.p administration of curcumin resulted in biliary excretion of the molecule from cannulated rats (Holder et al., 1978). Ravindranath and Chandrasekhara (1980) showed that when radiolabelled curcumin was administered orally to rats at a dose of 400 mg/rat, nearly 40% of curcumin was found in the feces in unchanged form. Curcumin was not detectable in urine, however, some of the derivatives like curcumin glucuronide and sulfates were observed in urine. At lower doses of 80 mg and 10 mg of [3H]curcumin, most of the label were excreted within 72 h, while with 400 mg, considerable amounts of the label was present in tissues 12 days after dosing (Ravindranath and Chandrasekhara, 1980). In a clinical study conducted with 15 patients oral curcumin doses between 36 and 180 mg were administered daily for up to 4 months. Neither curcumin nor its metabolites were found in urine, but curcumin was recovered from feces (Sharma et al., 2001).

In another study authors reported that on administering curcumin (2g/kg p.o) 0.31 ± 0.07 and 1.7 ± 0.5 h, were the values for absorption and elimination half-lives (Shoba et al., 1998). The elimination half-life values for i.v. (10 mg/kg) and oral (500 mg/kg) curcumin in rats were reported to be 28.1±5.6 and 44.5±7.5 min, respectively (Yang et al., 2007). There is not enough evidence in the literature which can further help in deducing reasons for controlling the in vivo elimination half-life of curcumin (Anand et al., 2007; Nabavi et al., 2014).

These studies show that curcumin is hydrolytically unstable at intestinal pH, rapidly metabolized, conjugated in the liver, and excreted in the feces, all contributing to its limited systemic bioavailability.

4.2.6 Strategies to overcome low bioavailability of curcumin: The poor bioavailability of curcumin limits its therapeutic usefulness and thus, many attempts as described below have been made to improve the low BA of this pluripotent compound (Bisht and Maitra, 2009; Prasad et al., 2014) (Figure 17). Adjuvants, nanoparticles, liposomes, micelles, and phospholipid complexes based formulations of curcumin have been reported to provide longer circulation times, better permeability, and resistance to metabolic processes (Anand et al., 2007).

4.2.6.1 Adjuvants: The synergistic inhibitory effect of genistein and curcumin against pesticide-induced cell growth of oestrogen-dependent breast carcinoma cell lines (MCF-7) has been
reported (Verma et al., 1998). It has been indicated that epigallocatechin-3-gallate, a component of green tea, could counteract certain undesirable activities assigned to curcumin suggesting the limited BA of curcumin (Balasubramanian and Eckert, 2004). In yet another study curcumin uptake by rat skin after topical application of a curcumin hydrogel demonstrated that eugenol and terpineol could enhance curcumin absorption by 2.2- and 2.5-fold, respectively, at 8 hours (Fang et al., 2003).

Adjuvants like pipeline have been used to overcome low BA of curcumin as the former is inhibitor of hepatic and intestinal glucuronidation (Antony et al., 2008). In humans co-administering curcumin orally (2g) with pipeline (20 mg), increased its BA by 2,000% at 45 minutes while administration of pipeline at 20 mg/kg with curcumin 2 g/kg in rats increased the bioavailability by only 154% (Shoba et al., 1998). A later study however, showed that pipeline (20 mg/kg orally) when co-administered with curcumin (2 g/kg orally) enhanced the bioavailability of curcumin up to 20 times in epileptic rats (Sakaida et al., 2003; Sharma et al., 2010). Similar conclusions were drawn by a study where intestinal absorption of curcumin was relatively higher when co-administered with pipeline, and further to it the elevated levels were maintained for a considerably longer period in the body tissues (Suresh and Srinivasan, 2010).

4.2.6.2 Self-microemulsifying drug delivery system: Self-microemulsifying drug delivery system (SMEDDS) is used to improve the solubility, dissolution and oral absorption of poorly water-soluble drugs (Zhang et al., 2008). The technology involves the use of an isotropic mixture of oil, surfactant, cosurfactant and drug substance that forms a microemulsion under the conditions of gastrointestinal fluid and gastrointestinal motility after oral administration. The microemulsion formed is less than 100 nm in size and leads to improved solubility of hydrophobic drugs which in turn enhances their absorption across the gastrointestinal tract (Gupta et al., 2013b).

A study reported an improvement of 1213% in the oral bioavailability of curcumin via SMEDDS as compared with curcumin suspension (Wu et al., 2011). Another similar study showed that rats dosed with liquid and pelleted SMEDDS showed 14 and 10 fold increased absorption of
curcumin in plasma as compared to the aqueous suspension of curcumin (Setthacheewakul et al., 2010).

Figure 17 Various strategies applied for enhancing bioavailability of curcumin. GMO, glyceryl monoleate; PLGA, poly(lactide-co-glycolide acid); PHEMA, poly(2-hydroxyethyl methacrylate); PEG-PEI, polyethylene glycol-poly(ethylene imine). (Prasad et al., 2014)

4.2.6.3 Liposomes, micelles and phospholipid complexes: Liposomes can carry both hydrophilic and hydrophobic molecules and have been used as a carrier for curcumin in various models of carcinogenesis (Li et al., 2007; Pandelidou et al., 2011). In a study, authors reported that liposomal vehicle helped in loading more curcumin into cells than human serum albumin or aqueous DMSO (Kunwar et al., 2006). In a pharmacokinetic study carried out with liposomal encapsulated curcumin (LEC) preparation, it was observed that LEC showed a much higher bioavailability and better absorption as compared to the free curcumin in rats (Takahashi et al., 2009). Authors designed silica-coated flexible liposomes loaded with curcumin (CUR-SLs) and...
curcumin-loaded flexible liposomes (CUR-FLs) and reported improvement in bioavailability of CUR-SLs and CUR-FLs (7.76 and 2.35 times) respectively as compared to suspension. The authors concluded that silica coating enhanced the stability of flexible liposomes, and thus CUR-SLs exhibited a 3.31 times improved bioavailability in comparison with CUR-FLs (Li et al., 2012). In yet another study, authors demonstrated that N-trimethyl chitosan chloride (TMC)-coated liposomes encapsulated with curcumin showed higher bioavailability in comparison with uncoated liposomes encapsulating curcumin and curcumin suspension (Chen et al., 2012). Propylene glycol liposomes (PGL) have been designed by authors and in vitro reports suggested that, PGL showed higher uptake of curcumin in comparison with conventional liposomes and free curcumin suspension (Zhang et al., 2012). These studies have indicated that liposome as a carrier can enhance the bioavailability of curcumin.

Micelles and phospholipid complexes are known to improve the gastrointestinal absorption of curcumin, thereby giving higher plasma levels and lower kinetic elimination which leads to enhanced bioavailability (Anand et al., 2007). In an in vitro study the intestinal absorption of curcumin and micellar curcumin formulation with phospholipid and bile salt was evaluated and intestinal absorption of curcumin was found to increase from 47% to 56% in the latter (Suresh and Srinivasan, 2007).

In a pharmacokinetic study, authors reported a 60-fold higher biological half-life for curcumin with polymeric micelle curcumin in rats as compared to curcumin solubilised in a mixture of dimethylacetamide (DMA), polyethylene glycol (PEG) and dextrose (Ma et al., 2007). Letchford and co-workers have showed a $13 \times 10^5$-fold increase in curcumin solubility in a polymeric micellar formulation containing methoxy poly(ethylene glycol)-block-polycaprolactone diblock copolymers (Letchford et al., 2008). A study reported the use of curcumin-loaded MPEG-PCL (Cur/MPEG-PCL) micelles improved the half life and area under curve (AUC) of curcumin in vivo. Further to it authors also reported inhibition of angiogenesis by Cur/MPEG-PCL micelles on transgenic zebrafish model (Gou et al., 2011).

In another study, authors demonstrated a significant improvement in curcumin bioavailability after oral administration of curcumin-phospholipid complex. The authors showed that curcumin-phospholipid complexes (100 mg/kg of curcumin) achieved a plasma curcumin level of 600 ng/ml at 2.33 hours after oral administration while free curcumin on oral administration at a corresponding dose showed a plasma concentration of only 267 ng/ml after 1.62 hours.
Furthermore, authors showed a 1.5 times more increase in the curcumin half-life in rats (Liu et al., 2006). In another study authors reported a 3 times increase in the aqueous solubility and a better hepatoprotective effect of a curcumin-phospholipid complex compared to free curcumin (Maiti et al., 2007). In another report authors evaluated that phosphatidylcholine increased the oral BA of curcumin in vivo. Authors reported peak plasma levels of curcumin were 5-fold higher than when an equivalent amount of unformulated curcumin was administered. Similarly, liver levels of curcumin were higher after administration of formulated curcumin (Marczylo et al., 2007).

4.2.6.4 Nanoparticulate delivery system(s): Nanoparticle-based delivery systems are useful for enhancing the bioavailability of curcumin (Anand et al., 2007). A study by Bisht et al. (2007) reported the synthesis of a polymer-based nanoparticle of curcumin called 'nanocurcumin’ (≤ 100 nm size) (Bisht et al., 2007). Solid lipid nanoparticles (SLNs) loaded with curcuminoids have also been developed and characterised for topical application wherein the light and oxygen sensitivity of curcuminoids was markedly reduced by incorporating curcuminoids into this carrier system. Authors further reported that in an in vivo study with healthy volunteers, a cream incorporating curcuminoid-loaded SLN showed improved efficiency over the one containing free curcuminoids (Tiyaboonchai et al., 2007).

In another report transferrin-mediated solid lipid nanoparticles of curcumin (Tf-C-SLN) were formulated that increased the photostability, and enhanced the anticancer activity of curcumin against MCF-7 breast cancer cells. The authors conducted flow cytometric studies which revealed an enhanced anticancer activity with Tf-C-SLN as compared to curcumin solubilized surfactant solution (CSSS) and curcumin-loaded SLN (C-SLN) indicating the potential of Tf-C-SLN in enhancing the anticancer effect of curcumin in breast cancer cells in vitro (Mulik et al., 2010).

Authors have prepared curcumin-loaded apotransferrin nanoparticles via sol-oil chemistry, and demonstrated that considerable quantities of drug was released steadily over a fairly long period (up to 50% of curcumin was still present at 6 h). This was in contrast to the intracellular soluble curcumin (sol-curcumin) which reached peak value at 2 h and was found to be completely eliminated at 4 h (Gandapu et al., 2011). Authors prepared colloidal nanoparticles of curcumin (theracurmin) and showed that a 40 times higher AUC was obtained with the latter in
comparison to free curcumin powder on oral administration in rats. The authors observed that oral administration of theracurmin (30 mg) when administered in healthy human volunteers orally resulted in 27-fold higher AUC than that of curcumin powder (Sasaki et al., 2011). The polymeric nanoparticles of curcumin prepared by Cheng et al. (2013) showed significantly higher concentration of curcumin in plasma of Tg2576 mice, on oral administration. Further to it, a 6 times higher AUC and mean residence time in comparison with free curcumin was observed in brain of Tg2576 mice (Cheng et al., 2013). In another study polylactic-co-glycolic acid curcumin (PLGA-curcumin) nanoparticles were prepared by emulsion-diffusion-evaporation method and the authors found a significant increase (p ≤ 0.001) in the AUC value of the nanoparticulate formulation in comparison to free curcumin suspension and curcumin with piperine, indicating the improved bioavailability with the nanoparticulate systems. The authors also reported T_{max} to be 2 hours with a sustained release attained up to 48 hours in plasma in comparison to free curcumin, where there were no detectable levels beyond 6 h (Shaikh et al., 2009).

In an in vitro study, PLGA-curcumin nanoparticles showed fast and more improved cellular uptake than free curcumin and i.v administration of free curcumin or PLGA-curcumin (2.5 mg/kg), 2 time higher serum concentration was observed for latter in comparison to former (Anand et al., 2010). In another study, PLGA-curcumin nanoparticles enhanced the cellular uptake performed in cisplatin resistant A2780CP ovarian and metastatic MDAMB-231 breast cancer cells by two and six fold, respectively in comparison with free curcumin (Yallapu et al., 2010).

In another study with curcumin-PLGA-nanoparticles, authors reported that the relative bioavailability was increased 5.6-fold and also exhibited longer half-life compared with that of native curcumin on oral administration. The authors stated that the enhanced oral bioavailability exhibited by curcumin was due to enhanced water solubility, improved absorption by means of enhanced permeability, higher release rate in the intestinal juice, inhibition of P-glycoprotein-mediated efflux and also increased residence time in the intestinal cavity (Xie et al., 2011). In yet another study, authors reported that curcumin encapsulated in high molecular weight PLGA showed 1.67 and 40 fold higher bioavailability than that of low molecular weight PLGA conjugated curcumin and conventional curcumin, respectively (Tsai et al., 2012). In a recent study formulations of PLGA and PLGA-polyethylene glycol (PEG) (PLGA-PEG) blend
nanoparticles containing curcumin were prepared and they were reported to increase the bioavailability of curcumin by 15.6 and 5.4 times, respectively. The authors concluded that encapsulating curcumin in these PLGA based nanoparticles was suitable for oral delivery of curcumin (Khalil et al., 2013).

Nanoglobules based nanoemulsion formulation of curcumin was evaluated for the prospective bioavailability enhancement. The authors demonstrated that nanoemulsion encapsulated curcumin showed increased release of curcumin in comparison with curcumin suspension during ex vivo study which indicated enhanced solubility of curcumin by nanoemulsion (Kumar et al., 2012). In another study, authors revealed that curcumin encapsulated into hydrogel nanoparticles formed a homogenous curcumin dispersion in aqueous solution in comparison with free curcumin and which led to a 95% release of curcumin in an in vitro release profile for the former (Guzman-Villanueva et al., 2013). In another study, authors prepared nanoemulsion curcumin (NEC) formulation which enclosed up to 20% curcumin (w/w) and demonstrated a 10 times higher (AUC) and 40 times higher C(max) in NEC in comparison with free curcumin in mice (Zhongfa et al., 2012).

Cyclodextrins (CD) are cyclic oligosaccharides which have been used to improve delivery and bioavailability of curcumin. Curcumin complexed with CD (CDC) showed longer half-life and thus superior cellular uptake in the cancer cells in comparison with free curcumin (Yadav et al., 2010). In a more recent report, authors have formed molecular inclusion complex of curcumin-beta-cyclodextrin and incorporated it into nanoparticles (CD). An improved curcumin permeability (1.8 times) across animal skin tissue was observed for the latter systems (Rachmawati et al., 2013).

Curcumin has multiple pharmacological activities that have the potential for many hepatic diseases but its poor bioavailability has hindered its widespread clinical use. There is need to improve pharmacokinetic and pharmacodynamic properties and thereby significantly reduce the required dosages of curcumin and to increase the patient compliance for the product (Dulbecco and Savarino, 2013).