Chapter 1
INTRODUCTION

1.1 Introduction

Human civilizations have rooted their firm foot in this planet Earth and are now the leaders of this world in every aspect starting from tradition to technology. If we investigate the primitive life that human society used to prevail, they usually used to have raw foods and the environmental condition was susceptible to the proper digestion of such food materials. The human brain is a gift that we have received from Mother Nature and in their whole life a person is known to utilize only 1% of the brain and because of that humans are being able to invent fire and other necessary tools that have changed the whole process of livelihood. Prevalence of proper environmental conditions provides necessary nectar for human health and also provides necessary elements that help human beings to lead a good and healthy life with better immunity. As the scenario of livelihood advances, people started traveling from one place to another for several ongoing factors and can share their culture, tradition, and food. Due to evolutionary changes, the human brain has adopted and advanced in several ways, leading to the evolution of proper science and technology that are harbouring many necessary tools that will change the whole environment of human existence on Earth (Crittenden and Schnorr, 2017) .

With the advent of technologies that were useful in handling many tasks, they created a better form of life in every aspect. The Industrial Revolution came into existence gradually and steadily with that the atmospheric condition of the earth started changing and the water and other food articles that were necessary for the survival of people became more and more affected and more prone to different kinds of ailments that had declined the survival age of the human race. Population bombardment is also one of the factors that has played a significant role in the development of diseases in human society (Ashton, 1997). To mitigate the needs of the people, especially food articles scientists around the world worked together and developed the concept of the Green Revolution. More and more food articles could have been generated by applying the above-said technology. To keep these articles

away from microbes, the concept of pesticides came into play and these pesticides had shown a remarkable solution in solving the problem that the cultivators used to suffer from (Evenson and Gollin, 2003). However, the main problem came into existence regarding health when pesticides used are eventually stored in different parts of the body, creating various health issues in the present era. Many kinds of drugs came out in the market to solve the relevant issues. The literacy rate of India is 77.7% by the year 2022 according to the National Survey of India (Swargiary and Roy, 2022) but still, the people of India lack knowledge regarding the hazardous issues that could develop using drugs without proper prescription. Many of the people used to have medicines without proper consultation with doctors and preferred quack doctors who used to roam in villages and were given medicines without proper knowledge and side effects. Because of this, people become more susceptible to different kinds of diseases and liver disease is among them. The liver is more affected because all the medicines after absorption are used to pass into the circulation through the liver and these medicines damage the liver when taken without proper medical format and without proper consultation (Ramachandran and Kakar, 2009).

Maintaining a healthy liver is essential for general health and well-being. Hepatotoxicity is considered one of the major health problems in modern times for adopting sedentary lifestyles, unhealthy food habits, and increasing obesity. Alcohol and other prescription drugs directly affect liver metabolism, and if this abuse continues for a long period, ultimately results in compromised health (Asrani et al., 2019). Although there are many different types of liver disorders, they only manifest clinically in a few distinct ways and are typically categorised as hepatocellular, cholestatic (obstructive), or mixed diseases. Inflammation and necrosis are the main symptoms of a majority of hepatocellular disorders, including alcoholic liver disease and viral hepatitis. In cholestatic diseases, the main feature is to inhibit the flow of bile as observed in gallstone or malignant obstruction. Mixed pattern has the feature of both hepatocellular and cholestatic disease which can be observed in diseases such as drug-induced liver diseases (Kasper et al., 2018). Cirrhosis of the liver is a primary cause of death and morbidity worldwide.

In 2016, it was the 11^{th} most common cause of death and the 15^{th} most prevalent cause of illness, contributing to 2.2% of all fatalities worldwide. Cirrhosis is liver fibrous tissue produced by viral hepatitis, prolonged alcoholism, hemochromatosis, Wilson's disease, cystic fibrosis, or liver-toxic substances. Cirrhosis is expected to

afflict 26 per 100,000 Europeans and 16.5 per 100,000 Eastern Asians and 23.6 per 100,000 Southeast Asians. However, Cirrhosis-related deaths are reducing in Asia as a result of increased HBV immunisation and medical care for viral hepatitis (Moon et al., 2020). Chronic Liver Disease (CLD) is responsible for the deaths of 1.32 million people as per reported in 2017, however, a major portion of CLD is due to hepatitis C virus (HCV) (Cheemerla and Balakrishnan, 2021). India alone accounted for 18.3% of the two million liver-related fatalities worldwide in 2015 and the record of the past three decades shows that cirrhosis and its complications are rising steadily compared to China and other Asian countries with a large population where it remains controlled comparatively (Mondal et al., 2022). Non-alcoholic fatty liver disease (NAFLD) is increasingly becoming a prominent cause of liver disease, with prevalence in India estimated to range from 9% to 32% of the total population. However, studies suggest that obese or overweight people have been more affected by NAFLD (https://main.mohfw.gov.in/sites/default/files/OG%20print%20ready%20version%20_0.pdf).

Notably, the National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS), an initiative implemented at the district level to promote proactive preventive measures against this peril and expedite advancements towards Universal Health Coverage, incorporated NAFLD in February 2021. This development was highly advantageous for the Indian government (Sarin et al., 2021; Bhadoria et al., 2022). When it comes to clinical phenotyping, uniform reporting, and electronic databases, India's epidemiological data resources on liver disorders are not as strong as those in advanced countries (Mondal et al., 2022). Liver disease is classified into major categories such as acute disorder which occurs over fewer than 3 months, sub-acute disorder which occurs between 3 to 6 months and chronic disorder which lasts for more than 6 months. One of the major signs of liver damage is jaundice, yellow coloration of the eye and skin. Jaundice is the accumulation of bilirubin in the body and is considered a complex disease. Jaundice may be classified into several types: pre-hepatic jaundice, which occurs due to the breakdown of red blood cells; hepatic jaundice, which is caused by a liver dysfunction that impairs the capture, conjugation, and excretion of bilirubin; and post-hepatic jaundice, which results from a blockage in the extra hepatobiliary system (Abbas et al., 2016). Other typical symptoms of liver disease include itching, right-upper-quadrant pain, fatigue, nausea, poor appetite, intestinal bleeding, and abdominal distension (Kasper et al., 2018).

1.2 Liver anatomy

The liver, which weighs around 1500g, is the biggest organ in the human body, constituting almost 2% of the total body weight of an average adult (Juza and Pauli, 2014; Hall and Hall, 2020). The liver is positioned in the upper right region of the abdomen, below the ribcage. It is mostly found in the right upper quadrant of the abdomen, although a part of it extends into the left upper quadrant. The liver is positioned through ligamentous attachment to the diaphragm, great vessels, peritoneum, and upper intestinal organs. It is a dark reddish-brown organ with a soft, spongy texture (Figure 1.1) (Abdel-Misih and Bloomston, 2010; Kasper et al., 2018). It typically extends towards the left hypochondriac region until the left anterior axillary line and takes up the majority of the right hypochondriac and parts of the epigastric sections. The liver rapidly grows in size as the body develops from childhood to maturity. After the growth phase hits a plateau around the age of 18, there is a gradual decline in liver weight starting in middle age. From childhood to maturity, the ratio of liver weight to body weight declines. Infancy to adulthood, the liver weight ranges from 4–5% of body weight to roughly 2% of body weight. Additionally, Females have smaller liver size comparatively and larger body size has larger liver size. The wedge shape of the liver is influenced by the structure of the upper abdominal cavity where it grows. The left hypochondrium is close to the wedge's narrow end, and the anterior edge of the wedge points both anteriorly and inferiorly. The diaphragm of the respiratory system and the anterolateral abdominal and thoracic walls form the superior and right lateral aspects together. The surrounding viscera form an inferior appearance. Current understanding suggests that the fibrous capsule of the liver does not play a major role in preserving its shape. Instead, it enables the liver to expand in size when it undergoes hypertrophy due to disease, surgical removal, or blockage of the hepatic artery or portal vein on the opposite side. The liver appears reddish-brown when it is in a healthy condition. Steatosis, or an increase in fat, causes it to take on a more yellowish tinge and round out the margins. These changes are likely to occur in individuals who are obese and/ or consume an excessive amount of alcohol. Venous outflow blockage (Budd-Ciari syndrome) or congestive heart failure causes the liver to turn blue. The texture of the organ varies from delicate to rigid based on the quantity of blood, adipose, and fibrous connective tissues that are present. Numerous metabolic processes are necessary for immunological defence, nourishment, and homeostasis are regulated by the liver. A few of the functions it is utilized for include bile production, iron and other micronutrient storage, blood sugar and lipid regulation, the synthesis of

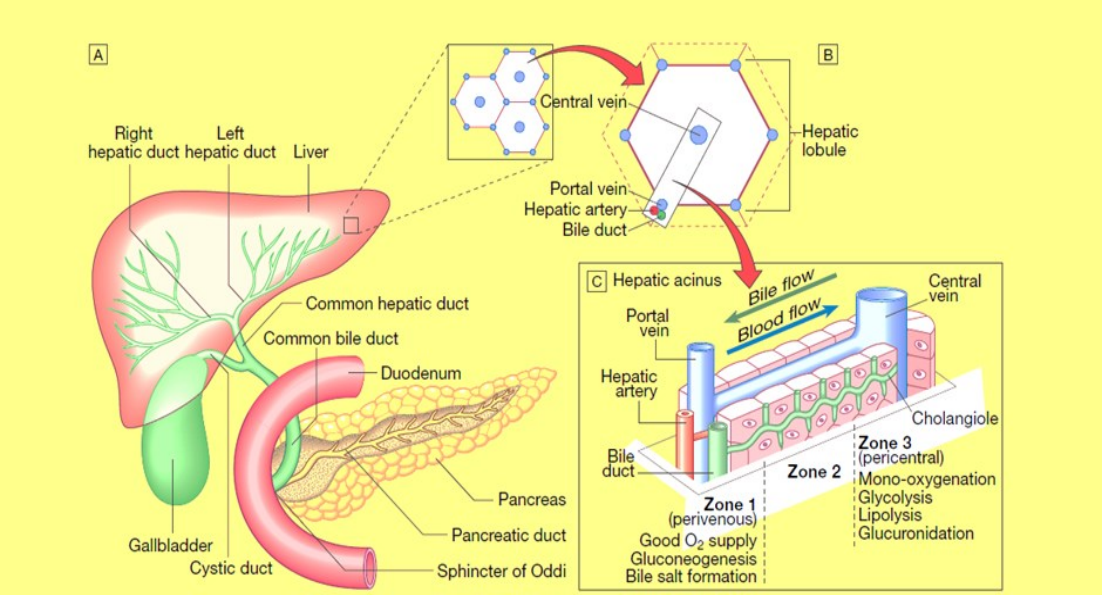

Figure 1.1: Structure and microstructure of Liver. [A] Liver anatomy. [B] Hepatic lobule. [C] Hepatic acinus (adapted from 'Davidsons Principal and Practice of Medicine' 23rd Edition by Ralston et al., 2018)

proteins and clotting factors, the metabolism of amino acids, and the breakdown and elimination of harmful or potentially toxic substances from the blood .

***1.2.1 Surface of the liver*:** The liver exhibits superior, posterior, anterior, inferior, and right surfaces, as well as a well-defined inferior border .

***1.2.2 Superior surface*:** Superior surface is the area that lies directly below the respiratory diaphragm and is the largest, isolated from it by the peritoneum excluding a little triangular zone. The majority of the superior surface is located below the respiratory diaphragm's right superior aspect. Anterior surface is nearly triangular and surrounded by the peritoneum except the attached location of the falciform ligament.

***1.2.3 Anterior surface*:** The anterior attachment of the respiratory diaphragm stays connected with a large portion of it.

***1.2.4 Right surface*:** Right surface is adjacent to the superior side of the respiratory diaphragm on the right and is covered by the peritoneum. It is isolated from the right lung, pleura, and the seventh through eleventh ribs.

***1.2.5 Posterior surface*:** Posterior surface is convex; the right side is wider than the left slide. A triangular bare area develops as a significant amount of posterior surface is joined to the respiratory diaphragm via loose connective tissue. Adjacent to the exposed region, there exists a tunnel or groove that accommodates the inferior vena

cava. The inferior vena cava groove is situated on the left side of the caudate lobe, which forms the back part of the liver. The separation between the left lobe and caudate lobe is achieved by a fissure referred to as the ligamentum venosum. Near the superior end of the ligamentum venosum fissure, the posterior surface of the left lobe has a shallow imprint.

***1.2.6 Inferior surface*:** The lower surface of the liver is bounded by its inferior edge. The strong fissure in the midline, which houses the liver's round ligament and merges with the posterior surface around the origin of the smaller omentum, the porta hepatis, and the inferior layer of the coronary ligament, is what sets it apart. The stomach fundus and the smaller omentum are attached superiorly and inferiorly, respectively, to the left lobe's inferior surface. The quadrate lobe is in close proximity to the pylorus, the inferior portion of the smaller omentum, and the upper segment of the duodenum.

***1.2.7 The Gross anatomical lobes*:** The Gross anatomical lobes based on its external appearance, the liver has historically been divided into the right, caudate, and quadrate lobes; these lobes are characterised in part by peritoneal ligamentous attachments.

***1.2.8 Right lobe*:** Right lobe has the highest volume and adds to the surface of the liver significantly. The ligamentum venosum serves as a dividing structure between the left lobe of the liver, positioned at the front and upper part, and the inferior part separated by the fissure for the liver's round ligament and the falciform ligament. The porta hepatis divides two prominences on the right side, below the grooves formed by the liver's round ligament and the ligamentum venosum.

***1.2.9 Left lobe*:** Left lobe is the smaller in size among the two primary lobes, however in young children, it is almost as big as the right lobe. It lacks subdivisions and is located to the left of the falciform ligament.

***1.2.10 Peritoneal attachment*:** The liver is anatomically linked to the diaphragm, anterior abdominal wall, and other viscera by several peritoneal ligaments.

***1.2.11 Falciform ligament and round ligament of the liver*:** The falciform ligament, derived from the ventral mesogastrium of the embryo, binds the liver to the anterior abdominal wall. The right leaf extends laterally on the outermost surface and is contiguous with the superior layer of the coronary ligament. The spherical ligament of the liver is located at the lower free edge of the falciform ligament and extends into a fissure on the lower side of the liver. It is the residual remains of the umbilical

vein in the foetus that has been damaged or eliminated. Typically, the left umbilical vein disappears shortly after birth and connects to the left branch of the hepatic portal vein during foetal development.

***1.2.12 Coronary ligament*:** The anterior and rear sides of the right lobe of the liver are mirrored by the peritoneum from the diaphragm. This creates the coronary ligament. The "bare area," which is a large triangle-shaped part of the liver that doesn't have any abdominal covering, is in the space between the layers of the coronary ligament.

***1.2.13 Triangular ligaments*:** The left triangular ligament, consisting of a bilayered peritoneum, envelops the upper border of the left hepatic lobe. The anterior leaf's middle part and the left layer of the falciform ligament form a continuous connection, whereas the left layer of the smaller omentum and the posterior layer also make a continuous connection. To expose the respiratory diaphragm and the abdominal segment of the oesophagus, one may shift the left lobe of the liver by separating the triangle ligament. This ligament is situated in front of the stomach and the abdominal portion of the oesophagus. The two layers of the coronary ligament converge laterally at a point designated by a tiny structure called the right triangular ligament. It is situated in close proximity to the right lateral border of the liver's "bare area."

***1.2.14 The porta hepatis*:** The porta hepatis is a profound transverse fissure situated on the lower surface of the liver, positioned between the caudate process at the back and the quadrate lobe at the front. The structure infiltrates the liver's functional tissue and comprises the hepatic artery, hepatic portal vein, right and left hepatic ducts, and hepatic nerve plexuses.

***1.2.15 The perivascular fibrous capsule*:** The perivascular fibrous capsule which is continuous with the fibrous capsule and envelops all of these structures, shields the arteries and bile ducts as they travel through the liver parenchyma. The dense cluster of arteries, supporting connective tissue, and liver parenchyma directly above the porta hepatis is referred to as the "hilar plate" of the liver. Perivascular fibrous capsules are also known as Glisson's sheaths which enclose the divisions of the portal triad structures once they reach the liver and divide into branch segments cause the fibrous capsule of the liver, also known as the "Glisson's capsule," to condense. Consequently, there is a solitary fibrous capsule that envelops each bile duct, hepatic portal vein, and hepatic artery.

***1.2.16 Vascular supply and lymphatic drainage*:** The liver is supplied with blood by the hepatic artery, the portal vein, and the hepatic veins. The portal vein and hepatic artery proceed to the porta hepatis inside the lesser omentum, where they typically divide.

***1.2.17 Lymphatic drainage*:** The lymphatic and common hepatic ducts emerge from the porta hepatis beside the hepatic artery. The hepatic veins exit the liver via its posterior aspect and proceed towards the inferior vena cava. The majority of the hepatic sinusoids are involved in the production of protein-rich lymph that originates from the liver. It passes to nodes superior and inferior to the respiratory diaphragm via both superficial and deep channels. The thoracic duct has an increase in lymph flow when hepatic venous drainage is blocked.

The liver is classically depicted as 2 lobes namely left and right hepatic lobes as per its functional anatomy and morphologic anatomy (Abdel-Misih and Bloomston, 2010; Kasper et al., 2018). The tiny lobules (0.8 to 2 millimeters in diameter) having a hexagonal structure are the functional unit of the liver which can be observed by the unaided eye. There are about 50,000 to 1,00,000 individual lobules in the human liver. The liver is supplied with blood via the hepatic artery and portal vein. These blood vessels transport nutrient-rich and oxygenated blood into the sinusoidal hepatic space. The liver then removes deoxygenated blood through the hepatic venous system (Deheragoda, 2022). Hepatocytes constitute two-thirds of the organ's massare a type of polyhedral cell with a spherical-shaped nucleus (euchromatic and polyploidy) lined on either side by blood-filled sinusoids (Courtney and Townsend, 2022). Kupffer cells are distributed in the lumen of hepatic sinusoids and are capable of phagocytizing bacteria and other foreign matter (Hall and Hall, 2020). The right and left hepatic ducts are responsible for carrying bile from the liver to the gallbladder (Sasse et al., 1992). Underneath the canaliculus remain hepatocytes whose cytoplasm is rich in organelles, most commonly found are endoplasmic reticulum, mitochondria, peroxisomes, and lysosomes (Saxena, 2017)

1.3 Functions of liver

Liver cells (hepatocytes) are functional units of the liver that carry out the liver's metabolic, detoxification, and synthetic functions (Mulaikal and Emond, 2012). Although some of the functions can be recreated through dialysis, however, no manmade organs or instruments are capable of replicating the complete functions of the liver (Carpentier et al., 2009). More than 500 crucial roles of the liver have been

identified, usually in association with different systems or organs. The known functions of hepatocytes can be classified into three categories such as regulation and synthesis, storage and purification, transformation and clearance which help to maintain and regulate the normal physiological homeostasis of the body (Adewusi and Afolayan, 2010; Zhou *et al*., 2016).

A few vital functions of the liver include:

a. It forms and secretes bile, which is held in the gallbladder until it is needed to break down and digest fatty acids.

b. It turns sugar into glycogen, which it stores until the muscles require energy and then secretes glucose into the bloodstream.

c. It produces cholesterol and protein and converts carbohydrates and proteins into fats, which are then stored for later use.

d. It generates prothrombin and fibrinogen, both of which are blood-clotting agents.

e. It produces blood proteins and enzymes that are required for digesting and other biological activities.

f. It yields urea while degrading proteins, which it creates from carbon dioxide and ammonia. The kidneys eventually eliminate it.

g. It is the only solid organ that can renew itself.

h. It receives nutrients that are supplied from the intestine.

i. It has the potential to regulate or increase hormone action and remove circulating hormones.

j. The liver participates in the metabolic processes of carbohydrates, lipids, and amino acids, as well as the degradation of medications and environmental contaminants. The liver is well-known for integrating metabolic pathways and controlling how the body reacts to food and hunger. The primary cause of both liver diseases, such as non-alcoholic fatty liver disease (NAFLD), and lesser-known liver diseases, such as type 2 diabetes, may be attributed to metabolic pathways and associated regulatory mechanisms. Every nutrient that is taken up by the liver through the portal vein from the stomach follows a different route in hepatocytes.

Amino acids from protein-rich foods are used to generate plasma proteins, such as albumin. Albumin has two important functions maintaining oncotic pressure in the vascular area and facilitating the movement of tiny molecules such as hormones,

medicines, and bilirubin throughout the body. To prevent hyperglycemia after a meal, the liver absorbs more than half of the absorbed glucose and transforms it into glycerol and fatty acids for storage or conversion to glycogen. Hypoglycemia is prevented during fasting because glucose is produced by gluconeogenesis or is liberated from glycogen in the liver. Since the liver creates very low-density lipoproteins and further metabolises low and high-density lipoproteins, it is crucial to lipid metabolism. Dysregulation of lipid metabolism is thought to be a major factor in the development of NAFLD. Lipids are now acknowledged to have a substantial impact on the development of hepatitis C by aiding the virus in entering hepatocytes (Harsh Mohan, 2000; Han et al., 2016).

k. The liver is involved in the production of key proteins required in the coagulation process. Coagulation factors such as II, VII, X, and IX are post-transitionally altered by enzymes that rely on vitamin K. An important biomarker of liver function is the reduction of clotting factor synthesis. Therefore, prothrombin time (PT) is one of the important methods available for examining liver function.

l. The synthesis of bile and the metabolism of bilirubin are both controlled by the liver. Every day, the breakdown of haemoglobin produces between 425 and 510 mmol of unconjugated bilirubin. Due to its inability to dissolve in water and move into the urine, the majority of bilirubin in the blood is usually unconjugated and linked to albumin. The sinusoidal membrane is where the unconjugated bilirubin is taken up and turned into mono and diglucuronide by UDP-glucuronyl transferase, improper function of this enzyme causes inherited hyperbilirubinemias. The hepatocyte membrane contains particular carriers that allow the bilirubin conjugates to be discharged into the bile canaliculi.Conjugated bilirubin is transformed into stercobilinogen in the intestines, where it might undergo additional oxidation to become stercobilin, which is then expelled from the stool and gives it a brown appearance. Urobilinogen, a term for the small amount of stercobilinogen that is discharged in the urine after it reaches the liver, is further oxidised to produce urobilin. The liver secretes one or two litters of bile per day, which is made up of cholesterol, phospholipids, bilirubin, and bile acids (Kasper et al., 2018).

m. In the liver, some vitamins (including vitamin K and folate) are kept in smaller concentrations than others, such as vitamins A, D, and B12. The liver can convert certain vitamins into more active forms, such as 25 (OH) vitamin D from 7-dehydrocholesterol. Due to its poor absorption, fat-soluble vitamin K leads to biliary obstruction, which exacerbates coagulopathy. The liver stores other minerals,

which are subsequently eliminated in the bile, including iron, ferritin, haemosiderin, and copper.

n. Approximately 9% of the total healthy liver consists of the hepatic immune system, including both adaptive immune responses such as B and T cells, as well as innate immune system cells including Kupffer cells, macrophages, and natural killer cells. Another atypical lymphocyte is crucial to the host and defence since it shares phenotypic traits with both T cells and NK cells. These cells in the liver have contributed to the prevention of gut microbes from entering the bloodstream. Kupffer cells eliminate bacteria, viruses, old and damaged red blood cells, antigen-antibody complexes, and poisons that have their source inside the body. They make up the biggest mass of tissue-resident macrophages in the body. Numerous inflammatory mediators, which can act locally or be discharged into the systemic circulation, can be produced by these cells. Although the precise mechanism underlying this occurrence is yet unknown, the liver has an immunologically viable environment in which it may withstand the generated immune response, such as liver transplantation and chronic viral infection (Wilson and Waugh, 1996; Harsh Mohan, 2000; Arjun et al., 2022).

o. One crucial liver function is the metabolism of drugs. Drug metabolism is influenced by ageing, nutrition, and heredity. The endoplasmic reticulum of the hepatocyte hosts a complicated process. There are several stages involved in this detoxification:

i. Phase I reaction: Phase I reactions involve oxidation or demethylation, which is carried out through cytochrome P-450. The enzymes that comprise the P-450 system carry out a range of oxidative phase I processes. It is primarily found in the liver but also can be found in other organs such as the kidney, gastrointestinal tract, and brain.

Cytochrome P-450 enzymes consist of an apoprotein and a heme prosthetic group that bind oxygen after electron-transfer events from NADPH. These events result in aliphatic and aromatic hydroxylation, O, N, or S dealkylation, or dehalogenation. This kind of reaction often generates a hydroxyl group, which may then participate in phase II reactions. A family consisting of gene products (isozymes) that may have similar functions is composed of genes with a 40% similarity in amino acid composition. For example, the CYP3 family consists of many genes labelled with numerical designations such as 1, 2, and so on, along with an additional A subfamily. The primary enzyme responsible for the metabolism of erythromycin in humans is CYP3A4.

ii. Phase II reaction: The majority of chemicals still need extra metabolism after a phase I reaction since they are not highly water soluble. In phase II processes, a large water-soluble polar group is often attached to a hydroxyl oxygen by glucuronidation or sulfation, resulting in the formation of ether or ester linkages. For some substances, the hepatic metabolism only requires these single stages. However, phase I oxidation occurs either before or after the phase II reaction for the majority. Bilirubin, morphine, furosemide, and acetaminophen are among the substances that need to be glucuronidated. Sulfation is just as crucial as glucuronidation, especially when it comes to the metabolism of bile acids and steroid molecules. Multiple sulfotransferase species with comparable specificities exist, using 3-phosphoadenosine-5-phosphosulfate, a compound derived from ATP and sulphate ions. Phase 2 reactions generally have no adverse effects, however they may infrequently generate dangerous or carcinogenic byproducts (Tso and McGill, 2003; Hodges and Minich, 2015).

1.4 Different kinds of liver diseases

1.4.1 Hepatitis

Hepatitis is a condition characterised by liver inflammation, mostly caused by infectious viruses like hepatitis A, B, and C, as well as other factors such as infections, toxins (such as alcohol and drugs), autoimmune diseases, or genetic abnormalities. There are five separate strains of the hepatitis virus, specifically referred to as Hepatitis A, B, C, D, and E. The predominant strains among the five that lead to liver cirrhosis, liver cancer, and mortality resulting from viral hepatitis are types B and C. The death toll around the world due to hepatitis B and C was estimated to be 354 million. 71 million persons worldwide were afflicted with HCV alone, with five percent of those cases occurring in six nations: Pakistan, China, Egypt, Russia, India, and the United States. It is possible to avoid some hepatitis variations with immunisation. According to a WHO report, immunisation, diagnostic procedures, medications, and awareness campaigns could help avert over 4.5 million premature deaths in middle-class nations by 2030 (Waheed et al., 2018; Cheemerla and Balakrishnan, 2021; https://www.who.int/health-topics/hepatitis#tab=tab_1 accessed on 19/12/2022). Symptoms: Mild to severe fever, uneasiness, diarrhoea, nausea, abdominal pain, loss of appetite, dark coloured urine, yellowish skin and eyes, depression, fatigue, sleep disturbance, grey-colouredfaeces, and joint pain (Evon et al., 2019; Available at https://www.cdc.gov/hepatitis/abc/index.htm#:~:text=Symptoms%20of%20hepatitis%20can%

20include,%2C%20joint%20pain%2C%20and%20jaundice).

1.4.2 Liver cancer

The liver is susceptible to cancer, just like other bodily components. It usually develops within cirrhosis and inflammation and is the sixth most prevalent location for early-stage cancer in humans. The lining of the bile duct is where adenocarcinomas, which include bile duct cancer, originate. Itching skin, pale faeces, dark yellow urine, and yellow skin and eyes are some of its symptoms. Hepatocellular Adenoma are uncommon, benign tumours of epithelial origin that affect less than 0.004% of the population (Bioulac-Sage et. al., 2013). The symptoms include pain in the epigastric region, tachycardia, hypotension, and orthostasis. About 90% of primary liver malignancies are due to hepatocellular carcinoma (HCC), whereas the remaining 10% are due to cholangiocarcinoma (CCA). With its unique metabolic and immunosuppressive condition, in addition to its anatomical position and organisation, the colon is often the site of primary HCC or CCA and metastatic cancer that spreads from other organs. HCC can be prevented by vaccination against HBV and careful management of HCV infection and effective treatment of HBV (Kasper et al., 2018; Li et al., 2021). Symptom: Reduction of weight (without trying), decrease in appetite, eating a little amount of meal yet feeling really full, vomiting or nausea, liver enlargement, a sensation of fullness beneath the right and left side fullness under the ribcage, spleen enlargement, Pain in the vicinity of the right shoulder blade or the abdomen (belly) abdominal swelling or fluid accumulation, itchiness, yellow coloration of the skin and eyes (Jaundice). (https://www.cancer.org/cancer/liver-cancer/detection-diagnosis-staging/signs-symptoms.html)

1.4.3 Immune system abnormality

1.4.3.1 Autoimmune hepatitis:

It refers to chronic and progressive inflammation of the liver where the cause of the disease is not known. It can manifest either as acute or chronic hepatitis that can lead to cirrhosis. Symptoms: Edema, myalgia, anorexia, upper abdomen pain, mild pruritus, and fatigue.

1.4.3.2 Primary biliary cirrhosis

The bile ducts are slowly destroyed in this disease. Irreversible scarring caused by the build-up of harmful substances leads to the disease. Symptoms: Fatigue, Itchy skin, dry eyes, and mouth.

1.4.3.3 Primary sclerosing cholangitis

The disease is distinguished by cholestasis with inflammation and fibrosis of the bile duct. The condition later may lead to liver cirrhosis with portal hypertension. Symptoms: Cholangitis, deficiency of fat-soluble vitamins, osteoporosis, weight loss, portal hypertension, variceal bleeding, ascites (Khurana, n.d.)

1.4.4 Abnormal genetics

1.4.4.1 Wilson's disease

Wilson's disease (WD) is a hereditary condition caused by a mutation in the ATP7B gene on chromosome 13. This mutation leads to an abnormal accumulation of copper in the body. The estimate of one case per 30,000 individuals globally in non-isolated populations for Wilson's disease (WD) in 1984 continues to be relevant in the present day. The incidence of WD is higher in China and other Asian countries as compared to Western countries, with 58.7 instances per 1,000 inhabitants (Członkowska et al., 2018). Symptoms: Chronic hepatitis, cirrhosis, ascites, and jaundice.

1.4.4.2 Hemochromatosis

Hemochromatosis is a metabolic condition characterised by excessive iron accumulation in the body. The accumulation of excessive iron in the heart, liver, pancreas, joints, and pituitary gland leads to a pathological condition that might result in death. Symptoms: Persistent exhaustion, stomach aches, joint pain, and abnormal heartbeat (Crownover and Covey, 2013).

1.4.5 Others

1.4.5.1 Fatty liver disease

Fatty liver is diagnosed when lipids accumulate in an amount above 5 percent of its total weight. The worldwide prevalence of non-alcoholic fatty liver disease (NAFLD), formerly referred to as metabolic dysfunction-associated fatty liver disease (MAFLD), was estimated to be 24%. The primary disease subtype that can progress beyond fibrotic response to cirrhosis is the non-alcoholic steatohepatitis (NASH) phenotype, which accounts for more than half of MAFLD cases. Obesity and type-2 diabetes are associated with an increased probability of fibrotic development; However, since 1975, obesity rates have risen globally, with the United States leading in this respect. The possible management of steatosis is through weight loss, controlled medications, and a healthy lifestyle (Eslam et al., 2020; Cheemerla and Balakrishnan, 2021). Symptoms: Fatigue, upper right

abdomen discomfort, ascites, splenomegaly, erythema of the hands, and jaundice (Clark et. al., 2002).

1.4.5.2 Alcoholic liver disease

Chronic and frequent alcohol use is ranked as the third most significant contributor to the overall burden of illness worldwide and is a major component in the development of liver disease. Globally about 3.5 million deaths occur due to the negative use of alcohol. Most of the alcohol-related mortality is secondary to cirrhosis. A 2016 survey found that, China and India were classified as moderate consumers (4-6 L/year) of alcohol, while Russia was one of the countries with the highest consumption (12 L/year). The pathology of alcoholic liver ailment involves three lesions namely fatty liver, alcoholic hepatitis, and cirrhosis however progressive injury rarely exists in a pure form. Nevertheless, giving up alcohol causes liver damage and fat deposition to return to normal. (Kasper et al., 2018; Cheemerla and Balakrishnan, 2021). Symptoms: Tiredness and low energy, sickness, decreased appetite, and weight loss, edema and ascites, jaundice, impotence in the male, shrinking of testicles in males and breast swelling in females, easy bruising and abnormal bleeding, pale-coloured stools (Robert et. al., n.d.).

1.4.5.3 Diabetes

Diabetes Mellitus and liver disease are linked, and this could lead to fatal outcomes. Diabetes is a result of peripheral glucose metabolism and hepatic insulin metabolism; however, liver illness can also be a cause of diabetes. There could be a direct hepatogenic effect of the Hepatitis C virus. Diabetes, in conjunction with obesity, dyslipidemia, and hypertension, results in a metabolic liver disease. Symptoms: slight soreness in the right upper quadrant, increased ALT and AST levels, and generally no symptoms (Simona et. al., 2006).

1.4.5.4 Obesity

Non-alcoholic fatty liver and obesity are significantly correlated, particularly with visceral fat. Hepatic steatosis is caused by changes in the liver's metabolism brought on by an increased concentration of free fatty acids. Inflammation is consequently caused by this process. Additionally, because insulin is essential for controlling the metabolism of localised fatty acids, insulin resistance, and hyperinsulinemia are frequently observed in obese individuals. Because visceral lipolysis in obesity is resistant to insulin, free fatty acids build up in the liver, causing fatty liver and hepatic steatosis. Symptoms: Ascites, fatigue, erythematous palms, splenomegaly, right upper quadrant abdominal discomfort, and jaundice (Andre, 2002).

1.5 Pathophysiology of liver diseases:

1.5.1 Degeneration and intracellular accumulation of substances

Damage to the hepatocytes can result in considerable cell expansion and irregularly clumped cytoplasm with huge clear gaps. Several substances, such as fat, iron, and residual biliary debris, may accumulate in hepatocytes. Accumulation of excessive fat drop in the liver causes a common condition known as steatosis. Alcoholic liver disease and Raye syndrome are two disorders that cause an abundance of small fat vesicles that do not displace the nucleus microvesicular steatosis. Macrovesicular liver disease is characterised by a single fat vacuole that fills up the hepatocyte and pushes the nucleus to the exterior of the cell (Quentin and Christopher, 2018).

1.5.2 Necrosis

Cell death, or necrosis, is a frequently observed sign in both acute and chronic liver disorders. All types of liver damage including microbiological toxic, circulatory, and traumatic often lead to necrosis of liver cells. The amount of involvement of hepatic lobule in necrosis varies. Consequently, necrosis of liver cells is divided into three types namely focal, zone, and Confluent. Small hepatocyte clusters known as focal necrosis are typically accompanied by lymphocytes. On the other hand, zonal necrosis denotes damage to a specific area of the brain brought on by an ischemia or drug-related event. Confluent necrosis involves multiple lobules (Krishna, 2017).

1.5.3 Apoptosis

Apoptosis is a type of programmed cell death that differs from necrotizing necrosis. Oncotic necrosis often results from severe metabolic disruption and is represented by cellular swelling leading to cellular membrane burst with the release of intracellular contents. While necrosis refers to the death of a large group of adjacent cells, apoptosis involves the separation of a cell from its neighbouring cells and its subsequent shrinking rather than swelling. Additionally, a phenomenon known as piecemeal necrosis can occur. Other processes involved in apoptosis include internucleosomal DNA degradation, nuclear condensation, lobulation, and fragmentation.

1.5.4 Fibrosis

Hepatic fibrosis, a common feature of most chronic liver diseases, is mostly attributed to the excessive accumulation of extracellular matrix proteins, particularly collagen. Liver fibrosis typically necessitates liver transplantation and is associated with cirrhosis, liver failure, and portal hypertension. Fibrosis can form immediately

within the sinusoids surrounding one or more hepatocytes, or it can first develop around the portal tracts or the terminal hepatic vein. As fibrosis progresses, scar tissue surrounds the nodules of regenerating hepatocytes which is the site for the formation of cirrhosis (Ramón and David, 2005; Hernandez-Gea and Friedman, 2011; Tae et al., 2022).

1.5.5 Cirrhosis:

Fibrosis is an initial stage of cirrhosis, characterised by the production of regenerating nodules surrounded by fibrous bands in response to long-term liver injury. This condition ultimately leads to portal hypertension and end-stage liver disease. Circulatory disorders such as loss of kidney functioning, bleeding, and liver cancer are the result of complicated consequences of liver failure. Any injury to the liver or to its functions whether minor or major if persists for a long time might turn into severe cases of cirrhosis, which is known to be permanent. In that stage of liver damage, the main choice of treatment is liver transplantation rather than trying to defeat the cirrhosis using the medicine and its counterparts (Schuppan and Afdhal, 2008; Tsochatzis et al., 2014).

1.6 Different kinds of synthetic drugs responsible for liver diseases

A large number of synthetic drugs have been produced and used round the world to get rid of various ailments. However, the consumption of such drugs may lead to various liver disease as listed in Table 1.1.

1.7 Clinical features of liver disease:

Depending on the underlying aetiology, severity, and stage of the disease, liver disease can present with a wide range of clinical characteristics. The following list includes some typical clinical features:

1.7.1 Jaundice: Jaundice is identified by turning the skin, mucous membrane and eyes into yellow colour. It happens when the liver is unable to adequately process and remove bilirubin, a yellow pigment, which builds up in the body (Elhaj and Hamad, 2020).

***1.7.2 Abdominal pain and swelling*:** Hepatic disorders may lead to abdominal discomfort or pain, specifically localised in the right upper quadrant of the abdomen. As the condition advances, the buildup of fluid in the abdominal cavity may cause abdominal distension, which is medically referred to as ascites (Sivakrishnan and Pharm, 2019).

Table 1.1: Drugs responsible for liver disease

Type of drug	Examples	References
NSAID	Aspirin, Ibuprofen	Tripathi, 2018
Preferential COX2 inhibitors	Nimesulide, Nabumetone	Kwon et al., 2019; LiverTox (accessed on 30-07-2023)
Selective COX2 inhibitors:	Celcoxib, Rofecoxib	Hajj et al., 2009; LiverTox (accessed on 30-07-2023)
Analgesic-antipyretics with poor anti-inflammatory action	Acetaminophen, Nefopam	Rotundo et al., 2020; Tracqui et al., 2002
Disease-modifying antirheumatic drugs (DMARD)	Gold, Leflumonide	Tripathi, 2018
Drugs Used for Gout	Probenecid, Sulfinpyrazone	LiverTox (accessed on 30-07-2023)
Synthesis inhibitor	Allopurinol	LiverTox (accessed on 30-07-2023)
Drugs for cough	Carbocisteine, Sodium and potassium citrate	Akimoto et al., 2021; Takaki et al., 2022; Francis et al., 1984
Antitussives (cough center suppressants)	Codeine, Noscapine	Akhigbe et al., 2020; Winter and Flataker, 1961
Drugs for bronchial asthma	Aminophylline, Montelukast	Tripathi, 2018; LiverTox (accessed on 30-07-2023)
Hormone and related drugs	Follicle-stimulating hormone, Luteinizing hormone	Wang et al., 2016; Geisthövel, 1979
Thyroid hormones	Methimazole, Perchlorates	Tripathi, 2018; Minicozzi et al., 2019
Oral antidiabetic drugs	Glipizide, Pioglitazone	Kamal and Bhabhra, 2019; Tripathi, 2018
Corticosteroids	Hydrocortisone, Prednisolonine	LiverTox (accessed on 26-07-2023);
Skeletal muscle relaxants	Chlormezanone, Baclofen	Sheu et al., 1995; Tripathi, 2018
Sedative Hypnotic Drugs	Nitrazepam, Alprazolam	Jochemsen et al., 1983; Tripathi, 2018
Antiepileptic drugs	Phenobarbitone, Carbamazepine	LiverTox (accessed on 28-07-2023);
Angiotensin-converting enzyme inhibitors	Lisinopril, Benazepril	Al-Rifaie et al., 2020; LiverTox (Accessed on 26-07-2023)
Angiotensin antagonist	Telmisartan	Tripathi, 2018
Anti-hypertensive	Hydrochloride thiazide, Chlorothiozide	Arizon et al., 2004; LiverTox (Accessed on 26-07-2023)
Beta-adrenergic blockers	Atenolol	Schwartz et al., 1989;
Alpha + beta adrenergic blockers	Labetalol, Carvedilol	LiverTox (accessed on 30-07-2023)
Hypolipidaemic drugs	Lovastatin, Simvastatin	Tolman, 2002; Kiortsis, 2007
Drugs for peptic ulcer	Cimetidine, Ranitidine	
Anti emetics	Ondansetron, Granisetron	Lewandowski and Chapman, 2008; Tripathi, 2018
Anti-microbial drugs	Sulfonamides, Nitroimidazoles	LiverTox (02-08-2023); Tripathi, 2018
Antitubercular drugs	Rifampin, Isoniazid	LiverTox (accessed on 30-07-2023)
Antifungal drugs	Clotrimazole, Amphotericin	LiverTox (accessed on 30-07-2023)
Anti-malarial drugs	Chloroquine, Mefloquine	Tripathi, 2018
Amoebic and other anti-protozoal drugs	Mebendazole, Albendazole	Tripathi, 2018

***1.7.3 Fatigue and weakness*:** Chronic liver illness often results in weariness, weakness, and a pervasive sense of malaise. These symptoms may be attributed to the liver's diminished capacity to metabolise foods and eliminate poisons from the body (Hartleb et al., 2022).

***1.7.4 Loss of appetite and weight loss*:** Liver disease can cause a decreased appetite, leading to unintentional weight loss. This can result from the altered metabolism of nutrients and a reduction in the production of bile, which aids in fat digestion (Hartleb et al., 2022).

***1.7.5 Nausea and vomiting*:** Liver dysfunction can contribute to feelings of nausea and episodes of vomiting. These symptoms may be associated with the accumulation of toxins in the body or impaired digestion and absorption of nutrients (Hartleb et al., 2022).

***1.7.6. Changes in stool and urine*:** Liver disease can affect the colour and consistency of stool and urine. Stool may become pale or clay-coloured due to decreased bile pigment excretion, while urine may become dark or tea-coloured due to the presence of bilirubin.

***1.7.7 Itching*:** Pruritus, or itching, is a common symptom of liver disease. It arises as a result of bile salts storing up in the skin, which causes irritation and itching (Hegade et al., 2015).

***1.7.8 Easy bruising and bleeding*:** The liver produces clotting factors necessary for normal blood clotting. Liver disease can lead to a decrease in these clotting factors, resulting in easy bruising and prolonged bleeding (Lisman and Porte, 2017).

***ix. Mental confusion and cognitive changes*:** Advanced liver disease, such as cirrhosis, can cause hepatic encephalopathy, a condition characterized by mental confusion, forgetfulness, personality changes, and in severe cases, coma. This is due to the accumulation of toxins in the bloodstream that would normally be metabolized by the liver (Blei and Córdoba, 2001).

1.8 Diagnosis of liver disease:

A mix of laboratory testing, imaging tests, physical examinations, medical history review, and occasionally liver biopsy are used to diagnose liver disease. These are a few typical diagnostic techniques for assessing liver disease:

1.8.1. Medical history and physical examination

The healthcare provider has to investigate about indications, risk factors, alcohol consumption, medication use, and family history of liver disease. Further, they will

conduct a physical examination to assess signs such as jaundice, abdominal tenderness, and fluid accumulation.

***1.8.2 Blood tests*:** Blood tests can provide valuable information about liver function, liver damage, and the presence of specific liver diseases. Commonly performed blood tests include:

1.8.2.1 Liver function tests: These tests evaluate the concentrations of albumin, bilirubin, prothrombin time, and liver enzymes including alanine aminotransferase [ALT] and aspartate aminotransferase [AST].Deviant levels may suggest impaired liver function or injury (Thapa and Walia, 2007).

1.8.2.2 Viral hepatitis serology: Blood tests can detect the presence of antibodies or viral genetic material (RNA or DNA) for hepatitis viruses (including hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E) (Easterbrook et al., 2017).

1.8.2.3 Autoimmune and metabolic liver disease markers: Blood tests can help identify markers associated with autoimmune liver diseases (e.g., antinuclear antibodies, anti-smooth muscle antibodies) or metabolic liver diseases (e.g., iron studies, alpha-1 antitrypsin levels).

1.8.2.4 Tumour markers: In certain cases, tumour markers like alpha-fetoprotein (AFP) may be measured to screen for liver cancer.

1.8.3 Imaging studies

Various imaging techniques can provide detailed images of the liver to assess its size, structure, and the presence of abnormalities. They might consist of:

1.8.3.1 Ultrasound

A non-invasive imaging method that visualises the liver by using sound waves. It employs to measure the size of the liver, find lumps or tumours, and determine whether liver stiffness or cirrhosis is present (Gerstenmaier and Gibson, 2014).

1.8.3.2 Computed Tomography (CT) scan

CT scans produce finely resolved cross-sectional images of the liver using X-rays. It also can provide information about liver anatomy, detect masses, and evaluate liver structure (Zeb et al., 2012).

1.8.3.3 Magnetic Resonance Imaging (MRI)

MRI creates finely detailed images of the liver by using radio waves and magnetic fields. It also can provide information like CT scans but without exposure to ionizing radiation (Schwimmer et al., 2015).

1.8.3.4 Fibroscan or transient elastography

This method quantifies liver stiffness, which serves as an indicator for the occurrence of liver fibrosis or cirrhosis. This procedure is non-invasive and used to assess liver function (Eddowes et al., 2019)

1.8.4 Liver biopsy

On a certain occasion, a liver biopsy may be conducted to get a tiny portion of liver tissue for microscopic analysis. This can assist in identifying the source and intensity of liver illness. Typically, a needle passes through the skin into the liver for a biopsy while the patient is under regional anaesthesia (Ratziu et al., 2005).

1.8.5 Additional tests

Additional tests are likely necessary based on the suspected underlying cause of liver illness. These can include genetic testing, specific viral load quantification, imaging of the bile ducts (such as endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography), or specialized functional liver tests (Rubin et al., 2013).

1.9 Herbal drugs used for the treatment of liver diseases:

Presently, many governmental, non-governmental, and other groups in India are endeavouring to carry out scientific investigations on medicinal flora. The notable organisations are the National Botanical Research Institute (NBRI), the Tropical Botanical Garden and Research Institute (TBGRI), the Central Council for Research in Ayurvedic Sciences (CCRAS), the Directorate of Medicinal and Aromatic Plants Research (DMAPR), the Central Drug Research Institute (CDRI), and the Central Institute of Medicinal and Aromatic Plants (CIMAP). Significant effort and progress have been made in the standardization of raw materials and finished goods. Recent years have seen a substantial amount of study on ethnobotanical research worldwide, not just in India. The main interest of modern medicine has been in its social consequences and the acknowledgment of preventative medicine, on the one hand, and the creation of several effective therapeutic methods, on the other (Agrawal and Paridhavi, 2012).

Medicinal plants are considered a rich source of phytochemical compounds that help develop modern drugs. Plants have been used for centuries as a medicine to treat diseases without scientific knowledge and proper guidance. All parts of the plant including stems, leaves, flowers, roots, and seeds are scientifically established to have medicinal properties (Khan and Ahmad, 2019). The collection of herbs that

work together to heal illnesses is known as a herb-herb combination. Even while a single plant may be curative, its potency is diminished when compared to formulations made from a combination of many plants, such as polyherbal medicines. There is a shift from a one-to-one target model due to an increase in interest and based on knowledge of empirical evidence on the herb-herb combination (Xie 2000; Che et al., 2013).

The World Health Organisation (WHO) defines herbal medicines as complete, branded pharmaceuticals that contain aerial, subterranean, or other plant material, or mixtures of these components, as well as an active ingredient. According to the pharmacodynamics scale, herbal medicines may be divided into three categories: (1) those with active components that are known and whose dosages have been demonstrated to be effective; (2) those whose active ingredients need to be standardized; and (3) those whose efficacy is unknown but which have a history of traditional use (Khan and Ahmad, 2019). Herbal medicines are mostly utilized for the treatment of minor or persistent illnesses and typically comprise a few biologically active substances. It has become increasingly popular as the efficacy of pharmacological effects of medicinal plants increases in the 21st century. Egyptian papyrus writings and ancient Chinese documented the use of plants for healing even earlier than 3000 BC. Herbs were employed in traditional medical practises by Native American and African indigenous tribes. Ayurveda and other sophisticated medicinal systems, like Siddha, Unani, and traditional Chinese medicine, use herbal products with great efficacy to treat a wide range of ailments. Ayurveda came to exist in about 900 BC in India. The word Ayurveda, a Sanskrit word is interpreted as knowledge or science (Veda) of life (Ayur). Identifying disorders and recommending herbs for treatment vary between Naturopathic Medicine, Traditional Chinese Medicine, and Ayurvedic medicine. Of these, Chinese herbal medicine, or Traditional Chinese Medicine (TCM) has prospective applications comparable to the Indian medical system. However, it is proven that some plants induce toxicity and are not safe for consumption (Goyal et al., 2012; Khan and Ahmad, 2019).

1.9.1 Silymarin as an example of single herb

Silybum marianum L. seeds are used to make silymarin and belong to the family Asteraceae or Compositae. It is commonly known as milk thistle, and has been utilised as a natural cure for illnesses of the liver and biliary tract. Most liver illnesses, including cirrhosis, jaundice, and hepatitis can be treated with milk thistle

to protect and regenerate the hepatic cells. Silymarin functions as a scavenger of free radicals and has shown strong protection in many experimental types of hepatic illness. Clinical trials have not reported any serious adverse events or deaths related to silymarin therapy; it is often well tolerated with a low frequency of side effects. Silymarin therapy should be initiated as soon as possible for maximal benefit in patients with fatty liver disease and other particular liver disease presentations, such as acute liver failure. At this point, the liver still has a high capacity for regeneration, and the best outcomes can be obtained by removing oxidative stress, which is the root cause of cytotoxicity. A novel formulation Eurosil 85® was created to increase the oral bioavailability of silymarin. This formulation has been utilised most frequently in clinical studies on silymarin. (Vargas-Mendoza et al., 2014; Gillessen and Schmidt, 2020)

1.9.2 Liv 52 as an example of polyherbal formulation

Liv 52 is a formulation that contains seven specific herbs, which are *Cichorium intybus*, *Capparis spinosa*, *Terminalia arjuna*, *Solanum nigrum*, *Cassia occidentalis*, *Tamarix gallica*, *Achillea millefolium*, and Mandur Bhasma. It is available in both tablet and syrup forms. Produced by The Himalaya Drug Company, this medicine is used for the treatment of many hepatic ailments, such as liver damage caused by radiation therapy, anorexia, reduced appetite, viral hepatitis, ALD, pre-cirrhotic diseases, and early cirrhosis of the liver. The presence of several hepatoprotective chemicals in Liv-52 likely mitigated the detrimental impact of carbon tetrachloride and other toxic substances on the liver. Liv.52 syrup dosage recommendations range from 2-3 tablespoons two to three times per day to 2-3 tablets twice each day. It is stated that liv.52's constituents have a variety of hepatoprotective qualities. Molecules like esculetin and p-methoxybenzoic acid, found in *Capparis spinosa* and *Cichorium intybus*, are known to exhibit hepatoprotective and antioxidant properties in laboratory animals. *Solanum nigram* effectively defended liver cells from the DNA damage brought on by free radicals, whereas flavonoids and arjunolic acid that were extracted from *Terminalia arjuna* were shown to raise glutathione levels. Nevertheless, *Achillea millefolium* and *Cassia occidentalis* were also discovered to possess hepatoprotective and antioxidant qualities. These herbs are arranged and explained following Ayurvedic principles to improve adequacy and keep a strategic distance from lethality. Despite being used largely to treat ALD, Liv-52 was also effective in treating advanced cirrhosis brought on by alcohol, according to a European RCT. The herbs included in liv-52 possess defensive qualities and exhibit

diuretic, anti-inflammatory, anti-oxidative, and immunomodulating effects (Huseini et al., 2005; Ganesh et al., 2022).

1.10 Oxidative stress and free radicals

A high quantity of free radicals or a drop in antioxidant content are associated with oxidative stress. Since they have several unpaired electrons, free radicals are unstable and extremely reactive to other living things. Previously, it was thought that free radicals only included radicals with an oxygen core, which are called reactive oxygen species (ROS). However, they also include a subset of reactive nitrogen species (RNS). Free radicals, or ROS, are produced by the body's ongoing metabolic processes and primarily target proteins, nucleic acids, carbohydrates, and lipids. Other sources of ROS which is endogenous origin include mitochondria, peroxisomes, xanthine oxidase, inflammation, arachidonic acid pathways, free metal ions, exercise, cigarette smoke, respiratory burst, and exogenous origin include UV irradiation, and environmental pollutants. The lack of reactivity of dioxygen during partial reduction leads to the formation of reactive oxygen species (ROS). Superoxide anion (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), nitric oxide (NO), and several other chemicals are instances of reactive oxygen species (ROS) that harm cells and spread, ultimately impacting DNA. ROS performs a dual role, when present in higher concentrations poses a harmful effect on the biological system whereas beneficial at moderate concentrations like guarding against infection. ROS levels fluctuate within the spectrum of redox biology and cytotoxic/ cytostatic levels as part of normal activity. For instance, H_2O_2 facilitates the process of differentiation, migration, and proliferation. Cellular homeostasis, signalling, and many biological activities all need it. However, excess ROS accumulation is damaging to biological components including DNA, lipids, and proteins. Ageing, carcinogenesis, cardiovascular disease, autoimmune illness, and many other conditions are caused by changes in DNA. 8-OH-G is a good indicator of carcinogenesis and can show DNA modification through oxidative stress (Neha et al., 2019).

1.10.1 Antioxidant and its defense system

Although the oxidation reaction also has harmful aspects, it is recognised to preserve the composite structure of our bodies, making it an essential component of life. In general, oxidation is a chemical transformation process of reactive free radicals, which can trigger several chain events that can kill cells. Antioxidants through countering the effect of ROS can reverse oxidative stress-related disease. Antioxidants play a crucial role in preserving the best possible functioning of cells

by counteracting free radicals. Numerous antioxidants, both man-made and natural, have been identified. Endogenous antioxidants may be classified into two categories: enzymatic and non-enzymatic. Glutathione peroxidase, superoxide dismutase, and catalase are types of natural enzymatic antioxidants. Non-enzymatic antioxidants include melatonin, uric acid, lipoic acid, bilirubin, and glutathione. Exogenous antioxidants include carotenoids, natural flavonoids, vitamins E, A, and C, as well as other compounds. The synthetic antioxidants derived from petroleum include tert-butyl hydroquinone (TBHQ), butylated hydroxytoluene (BHT), propyl gallate (PG), octyl gallate (OG), and butylated hydroxyanisole (BHA). While synthetic antioxidants are often regarded as potent agents that prevent food spoilage, there are assertions that they may also have adverse effects on human DNA and enzyme systems. A dietary antioxidant known as NDGA (Nordihydroguaiaretic acid) has been linked to renal cystic disease, especially in rodents (Krishnaiah et al., 2011; Neha et al., 2019).

1.10.2 Antioxidants assist defense mechanism of the body by

a) Preventing the formation of new radicals, it is important to consider the role of Catalase (CAT), Selenium (Se), Zinc (Zn), Copper (Cu), and Superoxide Dismutase (SOD).

b) Capturing free radicals to prevent or avoid a vicious cycle (Carotenoids, Vitamins E and C).

c) Repairing the harm created by free radicals (lipases, protease).

***Thus, the objectives of the current study is as follows*:**

1. To the standardized each ingredients of the traditionally used polyherbal formulation for hepatoprotective activity.
2. To screen the phytochemical constituent of the polyherbal formulation.
3. To evaluate the *in-vitro* antioxidant activity of standardized polyherbal formulation.
4. To evaluate the *in-vivo* hepatoprotective activity of standardized polyherbal formulation.