CHAPTER -1
GENERAL INTRODUCTION

Liver is a complex and important organ in the body about 1.5 kg of weight. It is a main metabolic organ responsible for clearing the blood from undesired endo- and exogenous compounds. The liver is generally divided into four lobes: right, left, quadrate and caudate lobes. It supplies blood via the portal vein and hepatic artery. It occupies approximately 4% of body weight and blood flow is 1.5 l/mins. Its main functions are: (i) To store carbohydrates, proteins, fats, certain vitamins and iron (ii) To control the production and removal of cholesterol (iii) To produce bile and blood clotting factors (iv) To remove and detoxify the waste products, drugs and other noxious substances (V) To produce substances which are responsible for immune system, and to remove bacteria from the blood stream to combat infections. Two - third of the liver is the parenchyma, which contains the hepatocytes, and the remaining is the biliary tract. It receives its blood supply via the hepatic artery and portal vein.

1.1.1. Hepatocytes

About 60 - 80% of the cytoplasmic mass of the liver is made up of hepatocytes. They are organized into plates of one or two cell thickness and have an average lifespan of 150 days. To achieve their role as the chief functional cells of the liver, they are highly differentiated and produce a plethora of enzymes and receptors and are specialized in metabolizing and excreting different classes of molecules. Up to 500,000 receptors were present in hepatocytes among them asialoglycoprotein is the most abundant receptor (Spiess, 1990).

1.1.2. Kupffer cells

Kupffer cells are the resident macrophages of the liver and are located along with sinusoids which accounts for 80 % of resident macrophages in the body and they constitute about 15% of the liver cells. The principle role of Kupffer cell is immune response mediation from soluble components of blood and phagocytosis which are responsible for protecting the liver.
1.1.3. Stellate cells

A type of cell in the liver, hepatic stellate cell is otherwise called as Ito cells, is found in the perisinusoidal space. The stellate cell is the major cell type involved in liver fibrosis, which is the formation of scar tissue in response to liver damage. In normal liver, stellate cells are in a quiescent state and represent 5 -8% of the total number of liver cells.

![Diagram of liver structure and cells](image)

Fig.1.1. Structure and cells in liver

1.1.4. Functions

The liver has many functions including the storage of essential Nutrients, breakdown of erythrocytes, bile Secretion, synthesis of plasma Proteins and cholesterol. Hepatocytes absorb and store excess nutrients in the blood such as glucose (glycogen), Iron, Retinol (Vitamin A), Calciferol (Vitamin D). These nutrients are released when their levels are too low.

1.1.4.1 Breakdown of Erythrocytes

Red blood cells have a life span of 120 days. RBC’s weakened and rupture, releasing hemoglobin into the blood plasma. Hemoglobin is absorbed by phagocytosis by Kupffer cells in the liver. Hemoglobin is split into two group heme and globins. In heme groups, Iron is removed from heme leaving a substance called bilirubin (bile pigment) and the removed iron is carried to bone marrow, where it is used for the formation of new hemoglobin in RBC’s. Bilirubin becomes a component of bile and globins-Hydrolysed to amino acids and returned to the blood.
1.1.4.2. Bile Secretion

Bile contains HCO₃ (Bicarbonate), bile salts, bile pigment, cholesterol and stored in gall bladder, these are concentrated and acidified and it discharged into small intestine via bile duct.

1.1.4.3. Synthesis

Liver involved in protein metabolism by the way of amino acids synthesis. In carbohydrate metabolism various pathways are carried out in liver such as Gluconeogenesis, Glycogenolysis, and Glycogenesis for production of energy for the cell development, maturation and cell division. In lipid metabolism, liver involves in cholesterol synthesis and metabolism. The main blood products like coagulation factors I, II, V, VII, IX, X, XI and protein C, protein S and antithrombin are produced in liver. Liver also act as a main site for red blood cell production and it produces insulin-like growth factor 1 (IGF-1), a polypeptide protein of trombopoetin such as albumin globulin and fibrinogen.

1.1.4.4. Breakdown

Liver breaks down hemoglobin, insulin and other hormone. It breaks down or modifies toxic substances (methylation) which sometimes may result in intoxication. Apart from that liver converts ammonia to urea.

1.1.4.5. Other functions

Albumin is one of the major osmolar components of blood serum. Hepatic blood stream contains globulins that are group of proteins which helps to regulate the function of the circulatory system. Bilirubin is produced in the liver and bone marrow cells as the end product of red-blood-cell breakdown.

1.1.5. Liver Diseases

The liver plays a major role in many functions from production of protein and blood clotting factors to cholesterol, glucose and iron metabolism. A number of illnesses affect the liver. Cirrhosis is a scar tissue which occurs when the normal liver cells is injured by various factors and leads to chronic liver disease. The following symptoms of liver diseases include fatigue, weight loss, nausea, vomiting, and jaundice. The treatment of a particular liver disease depends on its specific cause. There
responsible for causing liver diseases like cancer they are Hepatitis viral infection, alcohol consumption, environmental pollution, chemical carcinogens, excess of iron loaded in the body and auto immune responses. The liver is the center of metabolism can be affected by genetic disorders, intoxication and tumor growth. Chronic liver disease damages the hepatocytes, which are responsible for involving the critical metabolic functions performed by the liver. Due to the damage of these cells leads to inflammation in the liver called hepatitis. Inflammation is further exacerbate damage to the liver and initiates a process of wound healing to cope with the ongoing damage. This process involves the production of a number of extracellular matrix proteins, which maintain the structural integrity of the liver. Inflammation of liver by virus is called as viral hepatitis caused by different types of viruses such as hepatitis A, B, C, D, and E viruses. Among these A and E cause acute viral hepatitis. The hepatitis B, C, and D viruses can cause chronic hepatitis, in which the infection is prolonged, sometimes lifelong. Chronic hepatitis can lead to cirrhosis, liver failure, and liver cancer. High-frequency ionizing radiation can damage the DNA or genes inside the body and high exposures to pesticides can also cause liver cancer.

1.1.5.1. Hepatocellular carcinoma (HCC)

Cancer is a leading lethal disease, among various diseases attributed to mortality in humans all over the world. Liver cancer is a major health problem worldwide. It is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide with an estimated 50000 to 1 million new cases per year (El-Serag, 2012). It is also known as primary liver cancer or hepatoma. The initial symptoms of liver cancer are variable. In developing countries where the access to health care is much limited, it is very common that the cancer is diagnosed at the later stages. In addition patients from these regions may actually have more aggressive liver cancer diseases. In contrast patients in areas of low liver cancer frequency tend to have liver tumors that progress more slowly and therefore remains without any symptoms for a longer time. (Cho et al., 2009).

Hepatocellular Carcinoma (HCC) is a threatening problem worldwide. HCC is the fifth most common malignant neoplasm in India and remains an important cause of deaths in Asian and western countries. Around one million people
worldwide (Siegel et al., 2014). Internal malignancy is the most common form of this
disease in some parts of the world (Andy et al., 2006). It is the third most common form
of cancer in India. Risk factors behind HCC include hepatitis B virus (HBV), hepatitis C
virus (HCV), aflatoxins, alcohol and oral contraceptives (Altekruse, K.A. McGlynn
2009). Smoking, androgenic steroids and diabetes mellitus are also suspected risk factors
for HCC (Aggarwal and Shishodia, 2006).

The prognosis of HCC is dismal with 5-year survival being 1– 4%. The global
distribution of HCC is very variable. According to the age adjusted HCC incidence per
100000 populations per annum, different geographic regions can be divided into three
incidence zones: low (<5), intermediate (between 5 and 15) and high (>15) zones. Most
Asian countries are in intermediate or high incidence zones of HCC (Forner et al., 2010).
In India, the mean incidence of HCC in four population-based registries is 2.77% for
males and 1.38% for females. The prevalence of HCC in India varies from 0.2% to 1.6%.
The geographic model of HCC occurrence is frequently correlated with the etiologic
factors. Hepatitis B virus (HBV) infection is the most common etiologic factor in high
incidence areas, while hepatitis C (HCV) infection is more prevalent in the low incidence
areas. Unlike other low incidence zone, in India HBV is the main etiological factor
associated with HCC (Khan et al., 2010).

HCC is one of the few cancers with well-defined major risk factors. In 80% of
HCC develops in cirrhotic livers, and cirrhosis is the strongest predisposing factor.
Cirrhotic patients have a higher risk; their annual HCC incidence is 2–6.6%, whereas it is
0.4% in non-cirrhotic patients (Lok et al., 2010). Environmental carcinogens such as
aflatoxin B1 increase the neoplastic risk three-fold, which correlates with a specific
mutation on codon 249 of the p53 tumor suppressor gene. Characteristically, in
developing countries HCC related to hepatitis B virus infection results from acquired
infection at birth or early in life (Sherman, 2010).

There are no specific symptoms of liver cancer, and in fact, the earliest signs are
usually subtle and can be mistaken for simple worsening of cirrhosis and liver function.
Abdominal pain is uncommon with liver cancer and usually signifies a very large tumor
or widespread involvement of the liver. Additionally, unexplained weight loss or
unexplained fevers are warning signs of liver cancer in patients wit
whenever the overall health of a patient with cirrhosis deteriorates, every effort should be made to look for liver cancer. HCC is a highly malignant tumor with a very high morbidity and mortality and a poor prognosis (Yao et al., 2008). HCC is the most frequent form of primary liver cancer, it is one of the most common life threatening solid tumors with global annual diagnosis exceeding one million new cases and remains the third leading cause of cancer death (Jemal et al., 2007).

Many kinds of chemical hepatocarcinogens trigger malignance in different places of the liver, which lead to varied patterns of cellular proliferation preceding development of HCC (Femke et al., 2009). HCC development is based on the activation of different carcinogens in different places of liver lobules. DEN is a common environmental chemical carcinogen (Ziech et al., 2014). It is found in tobacco, smoke, meat, whiskey cosmetics, and gasoline and also in many processed foods like milk, meat products, steamed and fried fish, and alcoholic beverages (Brown, 1999). It has extensive use as a carcinogen in experimental animal model. DEN is metabolized in the pericentral zone of the liver lobule, but more actively in the periportal zone, and oxidative stress caused by DEN can contribute to hepatocarcinogenesis (Qi et al., 2008).

HCC is associated with pronounced symptoms of weight loss and tissue wasting (Livraghi et al., 2008). N-nitrosodiethyamine (DEN) is a representative chemical of a family of carcinogenic N-nitroso compounds. Administration of DEN to animals has been shown to cause cancer in liver and at low incidence in other organs also. N-nitroso compounds in particular DEN are well-known hepatic carcinogen and causes liver necrosis (Liu et al., 2009). N-nitrosamines cause a wide range of tumors in all animal species, and these compounds are considered to be effective health hazards to human beings. These nitroso compounds and their precursors have been found in the environment, in certain occupational settings, in food stuffs such as meat products, milk products, tobacco products, cosmetics and pharmaceutical products as well as an endogenous formation in the human body from dietary components (Bartch and Montesano, 1984). DEN causes oxidative stress and cellular injury due to the enhanced generation of reactive oxygen species (ROS) (Bartsch et al., 1989).
Today, a large number of researchers involved in drug development and management of liver cancer and trying to develop new drugs from natural sources to fight againsts HCC (David and Gordon, 012). Some researchers trying to synthesize novel drugs for liver cancer as combinational organic synthesis is still promising and holds great interest to accelerate drug discoveries and development of new catalysts (Kuntz et al., 1999). Treatment of HCC has dramatically changed in the last few years. Better knowledge of the molecular mechanisms responsible for tumor initiation and progression has allowed the development of molecular targeted therapies that specifically block the disrupted pathways (Boucher et al., 2009). The galangin mediates apoptosis through a mitochondrial pathway, and could be a potential chemotherapeutic drug for the treatment of HCC (Hai-Tao et al., 2010). 2'-fluoro-6, 7-methylenedioxy-2-phenyl-4-quinolone (CHM-1), a synthetic 6, 7-substituted 2-phenyl-4-quinolone, was identified as a potent and selective antitumor agent in human HCC (Wang et al., 2008).

![Fig.1.2.Causes of HCC](image-url)
Reactive oxygen species (ROS) are highly dangerous byproducts of cellular metabolism that have direct effect on development and growth of the cell and its survival on the development of cancer. As liver is the main site for metabolic biotransformation of DEN, the production of ROS in liver may be responsible for oxidative stress which causes liver damage. The cellular damage caused by ROS is measured in terms of lipid peroxidation (LPO) (Spiteller, 1996). Liver possesses an efficient antioxidant defense system to inactivate ROS, which are overwhelmed under conditions of oxidative stress and cause damage on critical cellular biomolecules such as lipids, proteins and deoxyribonucleic acid. DEN has been suggested to cause an uncompromised generation of free radicals in the liver, which in turn increases the demand of antioxidant enzymes. Subsequently, it leads to oxidative stress and initiation of carcinogenesis (Perez et al., 2005). One of the focuses in current cancer chemoprevention studies is the search for nontoxic chemopreventive agents that inhibit the initiation of malignant transformation (Verna et al., 1996).
Fig.1.4. Molecular changes in HCC

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. (Shindo et al., 1994). The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids (Crane et al., 1957). Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets.
1.1.6. Production of free radicals in the human body

Radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and in the cytochrome P-450 system. Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions. Some internally generated sources of free radicals are Mitochondria, Xanthine oxidase, Peroxisomes, Inflammation, Phagocytosis, Arachidonate pathways, Exercise, Ischemia/reperfusion injury. Some externally generated sources of free radicals are Cigarette smoke, Environmental pollutants, Radiation, Certain drugs, pesticides, Industrial solvents (Mellors and Tappel, 1966).

Treatment of liver cancer is primarily based on the stage at which the disease was detected and few other factors like the specialist who treats the patient, evolution of the disease etc.. Many herbs and nutrients have an influence on the liver cancer prevention and treatment. The treatment of liver cancer may include surgery, the use of radiotherapy, chemotherapy etc...

1.1.7. Treatments used for Liver Cancer are:

1.1.7.1. Liver transplant for Cancer Treatment.

If the cancer affected areas of the liver is small it is possible to receive a transplant from normal tissues. For larger tumors a liver transplant will not prevent the recurrence of cancer.

1.1.7.2. Radiofrequency Ablation Treatment for Liver Cancer.

At an early stage in cancer where the tumors are less than 2cm in diameter, Radiofrequency ablation can be used to kill the cancer cells. Small electrodes are inserted into the liver through skin. Once the needles are in place, electricity is passed through the needle to generate heat and kill the cancer cells.
1.1.7.3. Chemotherapy Treatment for Liver Cancer.

Chemotherapy or Chemo utilizes are potent medicines to kill cancer cells. Unlike radiofrequency ablation, chemotherapy affects healthy cells in other organs of the body. Chemotherapy can be administered intravenously and in several other ways. Once the medicine reaches the cancerous area, it blocks certain pathways in the cell cycle to kill cancer cells. The new therapeutic radio pharmaceutical has been developed and tested in several centres around the world, including India.

The available anticancer drugs have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. A single "cure" for cancer has proved elusive since there is not a single type of cancer but as many as 100 different types of cancer. In addition, there are very few demonstrable biochemical differences between cancerous cells and normal cells. For this reason the effectiveness of many anticancer drugs is limited by their toxicity to normal rapidly growing cells in the intestinal and bone marrow areas. A final problem is that cancerous cells which are initially suppressed by a specific drug may develop a resistance to that drug. For this reason cancer chemotherapy may consist of using several drugs in combination for varying lengths of time.

Role of chemotherapy:
1. Damage the DNA of the affected cancer cells.
2. Inhibit the synthesis of new DNA strands to stop the cell from replicating, because the replication of the cell is that allows the tumor to grow.
3. Stop mitosis or the actual splitting of the original cell into two new cells. Stopping mitosis stops cell division (replication) of the cancer and may ultimately halt the progression of the cancer.

1.1.7.4. Antibiotics:

A number of antibiotics such as anthracyclines, dactinomycin, bleomycin, adriamycin, mithramycin, bind to DNA and inactivate it. Thus the synthesis of RNA is prevented. General properties of these drugs include: interaction with DNA in a variety of different ways including intercalation (squeezing between the base pairs), DNA strand breakage and inhibition with the enzyme topoisomerase II. Most of these compounds have been isolated from natural sources and antibiotics. How
specificity of the antimicrobial antibiotics and thus produce significant toxicity. The anthracyclines are among the most important antitumor drugs available. Doxorubicin is widely used for the treatment of several solid tumors while daunorubicin and idarubicin are used exclusively for the treatment of leukemia.

1.1.7.4.1. Dactinomycin (Actinomycin D):

At low concentrations dactinomycin inhibits DNA directed RNA synthesis and at higher concentrations DNA synthesis is also inhibited. All types of RNA are affected, but ribosomal RNA is more sensitive. Dactinomycin binds to double stranded DNA, permitting RNA chain initiation but blocking chain elongation. Binding to the DNA depends on the presence of guanine.

1.1.8. Mannich Bases

Mannich bases are an important class of compounds in medicinal chemistry with a wide range of biological properties including antimicrobial (Mete et al., 2010), anticancer (Reddy et al., 2008, Dommicok et al., 1997), anti-inflammatory, analgesic, and anticonvulsant activities. Some of these compounds are also known for their applications as antituberculosis (Sriram et al., 2005), antimalaria, antifungal agents. The biological activity of such molecules has been attributed to the α-β unsaturated ketones liberated from Mannich bases by deamination process under physiological conditions. (Erciyas et al., 1994). These β-unsaturated ketones alkylate certain cellular constituents especially thiol groups. Among these cellular thiols, glutathione (GSH) is the most abundant, and mannich bases are reported to inhibit one or more of the following enzymes in the GSH metabolism namely GSH S-transferases, GSH reductase, gamma-glutamyl transpeptidase, and GSH peroxidase. It is important to note that the disadvantages of traditional alkylating agents where carcinogenic and mutagenic properties have been noted can be prevented by using mannich bases, since they have little or no affinity for amkino groups. Hence interactions with nucleic acids may be avoided (Farmer, 1982). Despite the wide spectrum of biological activities associated to Mannich bases, knowledge on their toxicity is limited. The inhibitory effect of the mannich bases on the peroxidation of linoleic acid at concentrations within the specific range. All the Mannich bases were capable of scavenging DPPH radicals in a concentration-dependent manner (Tamilvendan et al., 2012).
1, 3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl]-urea

Urea (6 g, 0.1 M), 2-naphthol (14 g, 0.1 M), and benzaldehyde (10 ml, 0.1 M) were taken in an equimolar ratio. A concentrated aqueous solution of urea and 2-naphthol was prepared. Benzaldehyde was added in drops with continuous stirring of the solution. The mixture first becomes oily and then slowly turned into a white crystalline mass, which was separated by suction filtration and washed several time with diethyl ether, dried and recrystallized using ethanol by slow evaporation method. Hence, the newly synthesized mannich derived organic compound namely 1, 3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl]-urea (1, 3BPMU) was considered to investigate the therapeutic effect against DEN-induced liver cancer in rats (Tamilvendan et al., 2012).

![Chemical structure of 1,3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl]-urea (1,3,BPMU)](image)

Fig.1.5.Chemical structure of 1,3-Bis-[(3-hydroxy-naphthalen-2-yl)-phenyl-methyl]-urea (1,3,BPMU)
1.2. OBJECTIVES

✓ To study of *In-vitro* effect of 1, 3 BPMU by cytotoxicity studies.

✓ To investigate the *In vitro* effect of 1, 3 BPMU by growth inhibition studies.

✓ To investigate antioxidant effect of 1, 3 BPMU on Diethyl nitrosamine (DEN) induced hepato cellular carcinoma in *wistar albino* rats.

✓ Proteomic analysis of Liver tissue of *wistar albino* rats for differential gene expression.