CHAPTER I
INTRODUCTION

Liver cancer (hepatocellular carcinoma) is a cancer arising from the liver. It is also known as primary liver cancer or hepatoma. The liver is made up of different cell types (for example, bile ducts, blood vessels and fat-storing cells). However, liver cells (hepatocytes) make up 80% of the liver tissue. Thus, the majority of primary liver cancers (over 90% to 95%) arises from liver cells and is called hepatocellular cancer or carcinoma. The initial symptoms (the clinical presentations) of liver cancer are variable. It is becoming much more common for patients to be identified by screening people at high risk for the cancer and finding the cancer before there are any symptoms at all. In countries where liver cancer is very common, the cancer generally is discovered at a very advanced stage of disease for several reasons. For one thing, areas where there is a high frequency of liver cancer are generally developing countries, where access to health care is limited. For another, screening examinations for patients at risk for developing liver cancer are not available in these areas. In addition, patients from these regions may actually have more aggressive liver cancer disease. In other words, the tumor usually reaches an advanced stage and causes symptoms more rapidly. In contrast, patients in areas of low liver cancer frequency tend to have liver cancer tumors that progress more slowly and therefore remain without symptoms longer. 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 with cirrhosis. However, whenever the overall health of a patient with cirrhosis deteriorates, every effort should be made to look for liver cancer. Hepatocellular carcinoma (HCC) is a highly malignant tumor with a very high morbidity and mortality and a poor prognosis (Yu and Keeffe, 2003). 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 (Ahmedin Jemal et al., 2007). HCC is associated with pronounced symptoms of weight loss and tissue wasting (Kwe, 1996).
N-nitrosodiethylamine (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 N-nitrosodiethylamine (DEN) are well-known hepatic carcinogen and causes liver necrosis (Tricker et al., 1991). Recent findings have suggested that 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 (Piot and Sirica, 1980). 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). The free radicals generated by the enzymes of mixed function cytochrome P450-dependent monoxidase system may augment an oxidative stress by the formation of H2O2 and superoxide anions (Farber and Gerson, 1984). ROS are highly dangerous by products 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 (Gey, 1993). 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 (Gey, 1993). One of the focuses in current cancer chemoprevention studies is the search for nontoxic chemopreventive agents that inhibit the initiation of malignant transformation.

It has been noticed that flavonoids may be a cancer preventive (Neuhouser, 2004). Flavonoids may block several points in the progression of carcinogenesis, including cell transformation, invasion, metastasis and angiogenesis, through inhibiting kinases, reducing
transcription factors, regulating cell cycle and inducing apoptotic cell death (Birt et al., 2001). Belonging to the flavone group of flavonoids, luteolin has a C₆-C₃-C₆ structure and possesses two benzene rings (A, B), a third, oxygen-containing (C) ring and 2 to 3 carbon double bonds. Luteolin also possesses hydroxyl groups at 3',5',7' and 4' carbon positions (Ross and Kasum, 2002). The hydroxyl moieties and 2 to 3 double bonds are important structure features in luteolin that are associated with its biochemical and biological activities (Chan et al., 2003). As in other flavonoids, luteolin is often glycosylated in plants, and the glycoside is hydrolyzed to free luteolin during absorption (Hempel et al., 1999). Some portion of luteolin is converted to glucuronides when passing through the intestinal mucosa (Shimoi et al., 1998). Luteolin is heat stable and loss due to cooking are relatively low (Le Marchand., 2002). Based on the observations that luteolin is able to interfere with almost all the aspects of carcinogenesis and it is relatively safe for animals and humans, it is assumed to be a potential chemopreventive agent against cancer through blocking cell transformation, suppressing tumor growth and killing tumor cells. Using luteolin to suppress chronic inflammation can potentially prevent inflammation-associated carcinogenesis.

A tumour marker is a substance produced by a tumour or by the host, detectable in biological fluids or tissues and useful to differentiate neoplastic from non-neoplastic disease. These markers are commonly used in diagnosis, staging and prognosis of cancer and can be useful to localize the tumour burden, as well as to monitor therapeutic effectiveness, detect recurrence or localization of the tumour and screen the general population or groups at risk. Tumour markers, also called biomarkers, have been classified as follows: enzymes, isoenzymes, hormones, oncofoetal antigens, carbohydrate epitopes, oncogene products and genetic alterations. The most widely used tumor marker for diagnosis of HCC is α-feto protein (AFP), which is a unique immunomodulatory glycoprotein (65 kDa) normally made by the immature liver cells in the fetus. Its detection during monitoring of HCC treatment is well accepted in patients with increased AFP levels prior to therapy and is recommended by the European Association for the Study of the Liver (EASL). It was reported that elevated serum level of AFP is observed in the adult animals which are exposed to hepatocarcinogens (Sell and Becker, 1978). CEA (Carcinoembryonic antigen) is a 180 kDa D-glycoprotein and is one of the members of the immunoglobulin supergene family and their level is elevated in the
serum of patients with variety of cancers. Elevated level of CEA in the serum is widely used as
tumor marker. Their level increases with an increase in the size of tumor and metastasis
(Toth et al., 1982). The use of a biomarker is of particular relevance for HCC diagnosis because
it commonly occurs in patients with Liver Cirrhosis (LC), considered as the most important risk
factor and as pre-malignant disease (Fattovich et al., 2004). Cancer biomarkers are quantifiable
molecules involved in the physiologic or pathologic events occurring between exposure to
carcinogens and the development, progression of cancer. Biomarkers may be the consequence of
a continuous process, such as increased cell mass, or a discrete event, such as genetic mutation.
Analysis of tumor markers can be used as an indicator of tumor response to therapy.

Hepatospecific enzymes were activated when hepatocellular damage give rise to
abnormalities of liver function and these enzymes are remarkably increased in HCC
(Ramakrishnan et al., 2007). One of the most sensitive and dramatic indicators of hepatocyte
injury is the release of intracellular enzymes, such as transaminases, phosphatases and LDH in
the circulation after DEN administration. The measurement of phosphatase activity is useful as
an indicator of liver function (Padmakumaran et al., 1998). In the liver, it is closely connected
with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether
extra-hepatic or intra-hepatic leads to increased serum levels of ACP and ALP activities.
Aminotransferases (aspartate transaminases (AST) and alanine transaminases (ALT)) are reliable
marker enzymes of liver and they are the first enzymes to be used in diagnostic enzymology
when liver damage has occurred (Whittby et al., 1984). Among the macromolecules that leak
from the damaged tissues, enzymes are released into the body fluids due to their tissue specificity
and catalytic activity. Hence, the study of these enzymes in serum and tissues has been found to
be more important in the assessment of respective tissue damage. The activities of serum
enzymes such as Aspartate and Alanine transaminases (AST and ALT), Lactate dehydrogenase
(LDH), Alkaline phosphatase (ALP) and γ-glutamate transminase (GGT) normally denote the
function of liver. The elevations in the transaminases are considered as the most sensitive
markers in the diagnosis of hepatocellular damage and loss of functional integrity of the
membrane (Plaa and Hewitt, 1989). ALP is another key hepatic marker enzymes. Elevated
activity of this enzyme in serum indicates pathological alterations in bile flow.
Mast cells (MCs) play an important role in the inflammatory component of a developing neoplasm. Recent research indicates that mast cells are a novel target for therapeutic intervention in the treatment of cancer (Gounaris et al., 2007).

A qualitative and quantitative analysis of silver-stained Argyrophilic nuclear organizer regions (AgNOR) proteins was performed during hepatocarcinogenesis induced in rats initiated by diethylnitrosamine (DEN) using the resistant-hepatocyte model. The quantitative distribution of total AgNOR proteins has been investigated extensively in cytohistological preparations of cancer lesions (Crocker, 1990). A frequent finding is that cancer cells have a greater amount of AgNOR protein than the corresponding normal and hyperplastic cells (Derenzini and Ploton, 1994).

Histopathological studies using Light microscope showed that in the rats induced with DEN, there was a loss of liver architecture and the lobules of neoplastic hepatocytes exhibited focal area of fatty change. Neoplastic cells have vesicular to hyper chromatic nuclei with a typical mitotic figure. It also showed sinusoidal dilatation with cords of neoplastic hepatocytes (Janani et al., 2010)

Chemical composition of extracellular matrix (ECM) plays a pivotal role in cellular and tissue development, regeneration and differentiation. It also plays a key role in pathogenesis of hepatocellular carcinoma (HCC). This study explored premalignant changes in the liver tissue content of collagen (as hydroxyproline, HP), total glycosaminoglycans (TGAGs), free glucosamine (FGA), total sialic acid (TSA), lysosomal membrane integrity variations (calculated as total and free cathepsin D activities) and liver histology. Serum alfa-fetoprotein (AFP) level was used as an early marker for HCC in two groups of Wistar rats. Literature that describes glucosamine (GA) levels in liver cancer is sparse. SA is a monosaccharide found typically attached to cell surface glycoconjugates (glycolipids, glycoproteins, and proteoglycans). It plays an important role in many physiologic and pathologic processes, including progression and spread of human malignancies (Varki and Varki, 2007).

Computer-aided drug designs have gained popularity and have become an integral part of the industrial and academic research for drug development (Kalyanaraman et al., 2005). Transforming ligands into active compounds with non-promiscuous-binding behaviour, known
as hits and then refining them into a structure or series of structures with relevant biological and drug-like activity known as leads, are the key starting points for drug discovery programs (Kenakin, 2003). The Protein-Ligand interaction plays a significant role in structural based drug designing. Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect is a receptor (Alberto and Diego, 2006). Docking is the process by which two molecules fit together in three-dimensional space.

The present investigation has been carried out with the following objectives:

Ø To evaluate the anti-tumor property and mechanism of the luteolin against experimentally induced HCC

Ø To investigate the anti-inflammatory activity of luteolin against DEN induced HCC on Mast cells of albino rats

Ø To evaluate the efficacy of luteolin on in vivo tumour markers and enzymatic as well as non-enzymatic antioxidant status in DEN Induced HCC in albino rats

Ø To evaluate the histoarchitecture of liver cells by Electron and Light microscopy of luteolin on DEN Induced HCC albino rats

Ø To understand the molecular mechanism of the recepting activity of the luteolin with HCC receptors by docking using Patch dock tool