CHAPTER 1

Introduction

1.0.1. KEYWORDS: - HCC, apoptosis, mistletoe, liver cancer


1.1. CANCER

Cancer can be defined as a disease in which a group of abnormal cells grow relentlessly disregarding the rules of normal cell division. Normal cells are continually regulated by signals that decide whether the cell should divide, differentiate into another cell or die. Due to the innumerable mutations they acquire, cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. The basis of modern cancer biology rests on a simple principle – virtually all mammalian cells share similar molecular network that control cell proliferation, differentiation and cell death. The prevailing theory, which underpins research into the genesis and treatment of cancer is that normal cells are transformed into cancer cells as a result of changes in these network at the molecular, biochemical and cellular level, and for each cell there are a finite number of ways this disruption can occur (Hejmadi et al., 2009).

According to the International Agency for Research on Cancer GLOBOCAN project, it is predicted that India’s cancer burden will be nearly two-fold more in the next 20 years, from slightly over a million new cases in 2012 to approximately 1.7 million by 2035 (Mallath et al., 2014). More
than six lakhs deaths due to cancer were reported in 2012. Among the Indian population, the most commonly diagnosed cancers are those of the lungs, breast, colon, rectum, stomach and liver (Murthy & Mathew, 2004).

### 1.2. LIVER CANCER

Cancer of the liver is among the foremost causes of death due to cancer in developing countries. The incidence of liver cancer is two to threefold higher in developing countries when compared to the developed countries (Torre et al., 2012). According to a recent survey, mortality rate due to liver cancer increased by 3% from 2010 to 2014; higher death rate was reported in men compared to women (Siegel et al., 2017).

There are mainly 4 types of primary liver cancer:

- **Hepatocellular carcinoma (HCC)** - Also known as hepatoma, this is the most prevalent type, accounting for over 75% of liver cancer cases. Men are more prone to HCC than women. Cirrhosis and hepatitis of the liver are the major risk factors for the development and progression of HCC.

- **Fibrolamellar carcinoma** - This is a rare subtype of HCC, wherein the cancer cells have a plate-like structure. This cancer develops in younger people. Cirrhosis/hepatitis is not a major risk factor for the development of fibrolamellar carcinoma. High alpha-fetoprotein (AFP) levels are not detected in this cancer type; hence AFP cannot be used as a biomarker for the detection of this cancer.

- **Cholangiocarcinoma** - Cancer occurring along the bile duct is called cholangiocarcinoma. Bile ducts essentially transport the bile produced in the liver to the gall bladder. If the cancer develops in the portion of the duct inside the liver, it is called intrahepatic cholangiocarcinoma. If the cancer develops in the duct region outside the liver, it is known as extra hepatic cholangiocarcinoma.

- **Angiosarcoma** - Also known as hemangiosarcoma, this rare type of liver cancer develops in the blood vessels of the liver.

- **Hepatoblastoma** - This is a rare case of liver cancer that usually develops in younger children and can be treated surgically.
1.3. HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is one among the five most common cancers worldwide (Pang and Poon, 2007) and develops primarily due to oncogenic mutations in the genome; resulting in increased cellular proliferation and abnormal cellular growth (Swamy et al., 2016). It is the most common and fatal type of primary liver cancer, having a 5-year survival rate of less than 10% (He and Karin, 2011). The incidence of HCC is reportedly higher in the Asian countries, due to the prevalence of Hepatitis B (HBV), whereas Hepatitis C (HCV) infection is the cause of HCC in Europe and other western countries (El Serag et al., 2003). Mortality rate due to HCC has been increasing in India with a mortality rate of 6.8 per 100,000 in men and 5.1 per 100,000 in women (Acharya, 2014).

1.3.1. SYMPTOMS AND RISK FACTORS OF HCC

Initial stages of HCC are usually asymptomatic and there is rapid tumour formation (Lin and Kao, 2011). Sometimes, it may present with unspecific symptoms such as weight loss, fever, bloating and anorexia (El Serag et al., 2008).

The primary risk factors for HCC include hepatitis [Chronic Hepatitis B (HBV) or hepatitis C (HCV) infection], liver cirrhosis, fibrosis, aflatoxin exposure, alcohol consumption, tobacco usage and metabolic syndrome (obesity, diabetes, fatty liver disease), non-alcoholic steatohepatitis (Swamy et al., 2016, Ingle et al., 2016). A study in a tertiary care hospital in India reported HBV related cirrhosis and cryptogenic cirrhosis as the leading causes for HCC development (Pal et al., 2013).

1.3.2. MOLECULAR PATHOGENESIS OF HCC

The liver consists of cells called hepatocytes (Fausto et al., 2006). It is the primary organ involved in the breakdown, metabolism and storage of nutrients and aids in the absorption of nutrients by secreting bile into the intestine (Carriage and Henson, 1995). Once infected by HBV/HCV (Hepatitis virus B/C) virus, the hepatocytes are targeted by the immune cells for destruction (Herzer et al., 2007); this may lead to a severe case of fibrosis, a major risk factor for HCC (Block et al., 2003). The HBx gene present in HBV encodes a viral protein known as HBx protein, which plays a critical role in causing HCC (Bouchard and Schneider, 2004).
Hepatocarcinogenesis is an exceedingly complex, multi-step process in which the external stimuli induce changes in the hepatocyte leading it to proliferate relentlessly. The chief site of origin of most HCC include the dysplastic nodules (Theise et al., 2002) and liver stem cells (Lee et al., 2006). One of the chief hurdles in treating HCC stems from the fact that most HCC patients often suffer from cirrhosis and have impaired liver function (Pang and Poon, 2007).

Tumor protein, TP53 is commonly mutated in several cases of HCC (Totoki et al., 2014; Ahn et al., 2014); specifically point mutation (G to T transition) at third position of 249 codon (Woo et al., 2011) is related to the poor prognosis of HCC. Mutation in the following genes are observed in HCC cases: proto-oncogenes (c-myc, K-ras, H-ras), tumor growth suppressors (Rb1, p73, p16), growth hormone receptors (AR, IGF, EGFR, Her2), signal transducer (AXIN1), cell cycle regulators (CDKN2A, cyclin A2, cyclin D1), PTEN, SOCS, E-cadherin (Kan et al., 2013; Lee, 2015; Swamy et al., 2016).

1.3.3. SIGNALING PATHWAYS ASSOCIATED WITH HCC DEVELOPMENT

- MAPK (Mitogen activated protein kinase) pathway - This pathway is a focal point for cell proliferation and differentiation. The prolonged activation of proteins in this pathway often leads to excessive cellular proliferation (Avila et al., 2006). Upregulation of MAPK has been observed in HCC cell lines in vitro and documented in several cases of human HCC (Schmidt et al., 1997; Ito et al., 1998). MAPK pathway (involving proteins Ras-Raf-MEK-ERK) forms a signaling cascade that communicates extracellular signals on the receptor surface to the DNA in the nucleus, culminating in cellular division. Signaling begins when the ligand (usually EGF, VEGF, PDGF, TGF etc.) bind to their cognate receptors on the cell surface and stops when the DNA in the nucleus undergoes transcription, followed by translation, resulting in protein expression. This might produce a significant change such as cellular division. Mutational changes might cause one or more proteins in the signaling cascade to remain infinitely in the “switched on” state, causing relentless cellular proliferation (Pang and Poon, 2007).

ERK activation leads to cellular division and eventually protects the cell from chemotherapy-induced toxicity. On activation, ERK affects apoptosis by downregulating the members of BH3 (Bcl-2 homology domain-3) family, the pro-apoptotic proteins Bad and Bim at the
protein level. ERK specifically inactivates Bad by triggering phosphorylation at Serine 112 (Howie et al., 2008).

- **PI3K/Akt/mTOR** – This pathway regulates cellular proliferation, growth and metabolism in most cancer cells including HCC (Zhou et al., 2011). Growth factors (EGF, IGF) on binding to their cognate receptors, activate PI3K (Phosphoinosititide-3-kinase). Activation of PI3K starts off a signaling cascade: PIP3 (phosphatidyl-inositol 3, 4, 5- triphosphate) activates AKT, which in turn activates mTOR (mammalian target of rapamycin) and Bcl-2 associated death promoter. Activation of mTOR enhances cellular proliferation; in contrast inactivation of bad increases cellular proliferation and decreases apoptosis (Swamy et al., 2016). Overexpression of IGF (Insulin-like growth factor) and IGF receptors is observed in most HCC cases (Alexia, 2004).

- **Apoptotic pathways** – Apoptosis (the cell suicide program) is activated when the DNA has undergone severe damage. Subsequently, DNA repair enzymes are recruited to repair the damaged DNA. However, if the damage is severe, then the cell undergoes suicide (Elmore, 2012). Cancer cells dodge this process and hence survives despite the mutation. Therapies targeting the extrinsic or intrinsic apoptotic pathway are in development (Shanmugham et al., 2011; Shikha et al., 2015).

- **Other pathways** – Dysregulation of Wnt β Catenin pathway, JAK/STAT pathway, Hedgehog pathway, IGF pathway are also commonly observed in HCC.

### 1.3.4. DIAGNOSIS OF HCC

Tumor biopsy is generally carried out to identify abnormal cancerous growth. Analysis of body fluids for specific molecular markers has aided in the faster detection of tumors and has therefore increased the survival rate in certain cancers (Sidransky, 2002). Since liver cirrhosis is a chief risk factor for HCC development, HCC cases can be detected by ultrasonography (USG), which has helped to reduce the increased mortality rate due to HCC (Zang et al., 2004). EASL (European Association for the Study of the Liver) recommends USG screening once every six months for the detection of HCC in patients with liver cirrhosis (EASL, 2012). Additionally, spiral computed tomography and Magnetic Resonance Imaging (MRI) may also aid in the earlier detection of HCC (Llovet et al., 2004).
The common molecular biomarkers for the detection of HCC include Serum alpha-fetoprotein [AFP], Glypican-3, Serum Fas/FasL, TGF-β and VEGF/VEGF receptors

Other diagnostic tests for HCC: Abnormally high levels of serum alanine amino transferase (ALT), increased prothrombin time and low serum albumin level are key diagnostic functional tests indicating the occurrence of liver cirrhosis, a risk factor for the development of HCC (Lau et al., 2008).

1.3.5. TREATMENT OF HCC

Surgery, involving transplantation resection, is presently the most effective treatment for HCC. However, the rate of HCC recurrence is high and the rate of long-term survival is poor. Additionally, surgical resection can be performed if HCC is detected early in the patient and has not metastasised to other organs (Lok et al., 2010). However, HCC often turns unresectable in most patients at the time of presentation (Raza and Sood, 2014).

Current treatment modalities:
Sorafenib or Nexavar (Bayer Healthcare Pharmaceuticals, Monteville, USA) is a multitargeted MAP (Mitogen activated protein) kinase inhibitor approved by the United States Food and Drug Administration (USFDA) for the treatment of advanced cases of HCC (Bruix and Sherman, 2005). It is a tyrosine kinase inhibitor and it acts by competing with ATP (Adenosine Triphosphate) to bind at the tyrosine kinase domain of the (tyrosine kinase) receptor. Sorafenib exerts anti-tumor effects on the liver cancer cells by binding to VEGFR-2/3 (Vascular Endothelial Growth Factor Receptor) and PDGFR-β (Platelet derived growth factor receptor) thus inhibiting angiogenesis. Additionally, it specifically blocks MAP kinase signaling by inhibiting Raf, MAPK and ERK, thus bringing the overall tumor signaling to a standstill (Samant and Shevde, 2011). Phase II and III studies of the SHARP (Sorafenib HCC Assessment Randomized Protocol) trial in sorafenib showed an increase in survival time (10.7 months) in patients receiving sorafenib treatments compared to placebo group (7.9 months). Sorafenib treatment delayed time to progression of HCC with a median of 5.5 months in sorafenib group compared to 2.8 months in the placebo group (Zhu et al., 2013). However, since sorafenib targets the MAPK signalling pathway in normal cells as well, adverse effects such as diarrhoea, hand-foot skin syndrome, hypertension and fatigue have been reported in majority of patients (Granito et al., 2016). This was corroborated in the SHARP
trial wherein 80% of the sorafenib treated group reported adverse effects compared to 20% in the placebo group (Zhu et al., 2013).

Other drugs used in the treatment of HCC include:

- **Bevacizumab** – Also known as avastin (Genentech Inc., Roche, California, USA), it is a recombinant humanized monoclonal antibody that blocks angiogenesis by targeting the VEGF signaling pathway (VEGF, VEGFR) (Hsu et al., 2010). Major side effects associated with bevacizumab treatment include hypertension, thrombosis and bleeding (Siegel et al., 2008).

- **Sunitinib** – Marketed as Sutent (Pfizer Inc, New York, USA), it targets VEGFR2, PDGFR, c-KIT (proto-oncogene c-Kit/CD117), FLT-3 (fms like tyrosine kinase 3/CD135) and RET kinase (Iyer et al., 2010). A randomized control trial in Phase III compared sunitib with sorafenib. Overall survival was better in sorafenib treated group whereas progression-free survival was superior in the sunitinib treated group. However, due to several major side effects in sunitinib treatment such as fatigue, diarrhea and nausea, the use of sunitinib was discontinued (Cheng et al., 2003).

- **Brivanib** – Developed by Bristol Myers Squibb (New Jersey, USA), brivanib alaninate or BMS-582664, is a dual inhibitor of PDGFR and FGFR (fibroblast growth factor receptor). A phase II study evaluated the efficacy of brivanib in advanced HCC and overall survival was observed to be 10 months. Major side effects of brivanib therapy include hypertension, proteinuria and hemorrhage (Park et al., 2011).

- **Linifanib** – Also identified as ABT-869 (Abbot Laboratories, Illinois, USA), linifanib is a strong inhibitor of VEGFR and PDGFR. Major side effects of linifanib therapy include hypertension and fatigue (Toh et al., 2013).

- **Ramucirumab** – Known by the brand name cymarga (ImClone Systems, New York, USA), is a recombinant human monoclonal antibody which binds to VEGFR2 and thus blocks the binding of VEGF to VEGFR2. By this blockage, downstream effects of VEGF in angiogenesis are inhibited. Major side effects of ramucirumab therapy include hypertension and gastrointestinal hemorrhage (Zhu et al., 2013)

- **Cediranib** – marketed as Recentin (AstraZeneca, Cambrige, UK), it is a potent inhibitor of PDGFR, c-kit and VEGF.
• **Regorafenib** – Known as Stivarga (Bayer Healthcare Pharmaceuticals, Monteville, USA), it targets VEGF signaling. Major side effects of regorafenib therapy are hand foot syndrome, diarrhea, weight loss, hypertension, dysphonia.

• **Cetuximab** – Marketed as Erbitux (Bristol Myers Squibb, New Jersey, USA), it is a EGFR inhibitor which binds and inhibits EGFR, which is overexpressed in HCC.

• **Panitumumab** – Marketed as Vectibix (Amgen Inc., California, USA), it is a IgG2 human monoclonal antibody which binds to the extracellular domain of EGFR, thus preventing its activation. Side effects related to its therapy include dermatological toxicities, fatigue, diarrhea and decreased magnesium levels (Lacouture et al., 2010).

• **Everolimus** – Marketed by Novartis (Basel, Switzerland) under the trade name Zortress/Certican and Evertor by Biocon (Bangalore, India), it selectively inhibits mTORC1 complex.

• **Doxorubicin** – Discovered at Farmitalia (now acquired by Pfizer) and marketed under the trade name Adriamycin, it is a naturally occurring anthracycline employed as the standard cytostatic agent in the therapy of liver carcinoma. It has been used systemically and locally in the treatment of liver carcinoma as a single agent or in combination with other agents such as cisplatin (Asghar and Meyer, 2012). It intercalates with the base pairs of the DNA double helix and stabilizes topoisomerase II complex after it has broken the DNA chain for replication; hence it prevents the resealing of the DNA double helix and inhibits the process of replication (Tacar et al., 2013). Additionally, it produces cytotoxic effects by generating quinone free radicals (Rossi, 2013). Major side effects include myelosuppression and dose-dependent cardiac toxicity (Patel et al., 2013).

• **Cisplatin and mitomycin** are also occasionally used in HCC therapy

### 1.4. Phytochemicals and HCC

Traditional plants are a rich source of pharmacologically active phytochemicals. Several phytochemicals have been analytically isolated from plants and evaluated for their potential benefits in combating tumorigenesis. Increasing evidence suggests that phytochemicals such as curcumin, resveratrol, capsaicin, green tea catechins etc. are beneficial in the treatment of hepatocarcinogenesis (Mann et al., 2009). A ‘renegade’ cell would be able to turn cancerous and
proliferative relentlessly, once it has acquired the following features (Hanahan and Weinberg, 2000):

- Evade apoptosis, the orchestrated cell suicide program
- Sustain proliferative signaling by producing growth factors
- Inhibit the production of growth suppressors
- Enable replicative immortality
- Evade immune destruction
- Induce the production of new blood vessels

A tumour generally consists of a population of many such genetically diverse renegade cells that have evolved due to inherent genomic instability. Each core hallmark that a cancer cell exhibits is regulated by partially redundant signaling pathways. A primary reason for the ineffectiveness of chemotherapy (which targets specific genes) in killing tumour cells is because the tumour develops resistance. When a specific pathway is challenged by chemotherapeutic insult, the cancer cells in the tumour veers more towards other hallmarks, as an evasive course of action (Efferth et al., 2017). Hence, therapies targeting numerous redundant pathways at the same time is of primary importance. Molecular studies show that natural products are capable of targeting multiple cellular signalling pathways (Efferth and Koch, 2011). Plant extracts (with a complex mixture of phytochemicals) that have more than one gene target are excellent candidates for cancer therapy.

1.5. PARASITIC PLANTS

The ancient Greek used the word ‘parasitos’ to imply a creature living on another organism. It was essentially derived from the Greek words ‘para’ (beside) and ‘sitos’ (food), to refer to an entity that sponges food from another organism (Taylor, 2017). Similarly, the parasitic plants rely on their flowering host plant for nutrition; they obtain water and nutrients from their host via a complex system known as ‘haustoria’ (Tennakoon and Weerasuriya, 1998). In general, parasitic plants are thought to have a negative effect on the growth of their host plants as they compete with their host for resources (Glatzel and Geils, 2009). Based on their dependence on hosts, they are classified as either holoparasite/obligate parasites or hemiparasites/facultative parasites. Holoparasitic plants are unable to photosynthesize and therefore depend wholly on their host for nutrients, carbon and water. Hemiparasites, on the other hand can photosynthesize by themselves for their carbon needs and rely less on their hosts for nutrition. Of the known parasitic plants, approximately 390 species
are holoparasitic and 4100 species are hemiparasitic in nature (Rubiales and Jorgensen, 2011). Depending on which part of the hosts the parasites attach to, the parasitic plants may be classified as root parasites (they attach to the roots of the host) or aerial parasitic (they attach to the shoots of the host). Of the known parasitic plants, 40% are known to be root parasites and 60% are aerial parasites (Bell et al., 2011). Approximately 1400 hemiparasitic plants belong to the mistletoe family (Mathiasen and Nickrent, 2008). Unlike other parasites, mistletoes do not have extremely negative effects on their hosts (Press, 2006; Watson, 2009; Bell et al., 2011).

1.6. MISTLETOE AND CANCER THERAPY

With regard to taxonomy, mistletoes belong to the order Santalales; hence they are commonly found in the families Loranthaceae, Santalaceae and Misodendraceae (Nickrent, 2011). The word “mistletoe” originated from the Celtic name which means “all heal” (Vega, 2002) as it was used to treating a wide range of diseases (Ameer et al., 2015). The ancients Celts and the Vikings considered the mistletoes to have exceptional healing powers and they were used by the Celtic druids in rituals and ceremonies more than two thousand years ago (Sherman, 2015). Accumulating evidence over the years have shown that mistletoes possess powerful anti-oxidant, anti-cancer, anti-hypertensive and anti-microbial properties (Lim et al., 2016). Specifically, mistletoes have a strong ethnopharmacological track record in cancer therapy (Anderson 1982, Klein., 2015). Rudolf Steiner, in 1920 used fermented mistletoe extract for cancer therapy as an anthroposophic medicine (Wagner, 2012). Viscum Album or the European mistletoe is widely used in complementary cancer treatment as it contains numerous chemopreventive and cytotoxic compounds such as lectins, viscotoxins and flavonoids (Kienle et al., 2009). Due to its powerful immune-modulating properties (Bussing, 2006), various marketed formulations of European mistletoe extracts such as Iscador, Helixor, Eurixor, Isorel have been used in oncology for several years (Melzer et al., 2009).

1.7. Elytranthe parasitica (L.) Danser

The genus Elytranthe comprises of several plants which demonstrate remarkable anti-tumour potential (Devehat et al., 2002). Elytranthe parasitica (L.) Danser is a perennial climbing woody parasitic shrub that belongs to the genus Elytranthe and family Loranthaceae. Known as parasite honeysuckle, it grows extensively in the Western Ghats of India on peepal, mango, neem and jackfruit trees (Bhat, 2003).

It has been used traditionally as a veterinary medicine and as a leaf paste to eradicate ticks (Quattrocchi, 2008). Plants of the same genus growing in Indonesia, *Elytranthe maingayi* and *Elytranthe tubaeflora*, have been traditionally used in treating cancer (Devehat, 2002). Earlier studies on phytochemical investigation of *Elytranthe parasitica* revealed the presence of phytochemicals such as flavonoids, tannins, saponins, phytosterols and carbohydrates (Sodde et al., 2011). The aqueous and methanolic extracts from the stem of *Elytranthe parasitica* showed powerful anticancer activity in Ehrlich’s Ascites Carcinoma *in vivo* (Sodde et al., 2011). It also showed moderate free radical scavenging and antioxidant activity (Sodde et al., 2011). A related specie, *Macrosolen cochin chinensis* (Common Chinese mistletoe) was able to inhibit MCF-7 breast cancer cells at an IC$_{50}$ 0.63 ppm, L1210 leukemia cancer cells at an IC$_{50}$ 41.0 ppm, HCT-116 colon cancer cells at an IC$_{50}$ 20 ppm, and A-431 epidermoid cancer cells at an IC$_{50}$ 20 ppm (Artanti et al., 2004).

1.8. CONCLUSION

Most mistletoes species, with the exception of *Viscum album* are understudied. Despite its potent anticancer properties, no phytocompound(s) has been separated and characterized analytically.
from the plant. Hence, exploring the phytochemistry and biological activity of unidentified mistletoes such as *Elytranthe parasitica* (L.) Danser would prove useful for cancer therapy.

1.9. REFERENCE


