GENERAL INTRODUCTION
CANCER

Cancer is a disease characterized by uncontrollable growth of cells which do not die. Normal cells in the body follow an orderly path of growth, division, and death. Programmed cell death is called apoptosis, and when this process breaks down, cancer begins to form. Unlike regular cells, cancer cells do not experience programmatic death and instead continue to grow and divide, which leads to a mass of abnormal cells that grow out of control. Hence, cancer is considered to be one of the leading causes of morbidity and mortality in economically developed countries and the second leading cause of death in developing countries (WHO, 2011). The burden of cancer in economically developing countries is increasing as a result of population aging and growth as well as, increasingly, an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and “westernized” diets (Jemal et al. 2011).

LIVER AND LIVER CANCER

Liver, the largest organ of the body, is located in the upper right side of the abdomen, behind the rib cage, normally weighs between 1.3 - 3.0 kg and is a soft, pinkish brown, triangular organ. Anatomically, liver consists of four lobes of unequal size and two large vessels carry blood to the liver (Scheme A). The hepatic artery comes from the heart and carries blood rich in oxygen. The portal vein brings blood rich in nutrients absorbed from the small intestine to the liver. These vessels divide into smaller and smaller vessels, ending in capillaries. These capillaries end in the thousands of lobules of the liver. Each lobule is composed of hepatocytes, and as blood passes through, they are able to monitor, add, and remove substances from it. The blood then leaves the liver via the hepatic vein, returns to the heart, and is ready to be pumped to the rest of the body.
Liver is built up of three major liver cell types, the hepatocytes (parenchymal cells), the sinusoidal endothelial cells lining the sinusoids and Kupffer cells. Liver plays an important role in the clearance of compounds from the blood (metabolism, excretion), produces immune proteins to control infections and directly removes germs and bacteria (innate immune system) and synthesizes proteins that regulate blood clotting and various other physiological processes (Scheme B).

Also, liver produces and excretes bile fluid which is required for food digestion and absorption of fats and fat-soluble (Kmiec et al., 2001 and Sherlock et al., 2008). Interestingly, the liver is the only internal organ that actually can regenerate lost tissue: as little as 25% of remaining liver can regenerate into a whole liver (Fausto et al., 2006), predominantly due to the hepatocytes reentering the cell cycle (i.e. the hepatocytes go from the quiescent G0 phase to the G1 phase and undergo mitosis). There is also some evidence of bipotential stem cells, called ovalocytes, which exist in the Canals of Hering that can differentiate into either hepatocytes or cholangiocytes (cells that line the bile ducts). A cancer that starts in the liver is called primary liver cancer. Primary liver cancer, also known as hepatocellular carcinoma (HCC), is one of the most lethal cancers having worldwide prevalence (Thun et al., 2010).
Liver cancer is the fifth most commonly diagnosed cancer and the second most frequent cause of cancer death in men worldwide. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death. An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 (Jemal et al. 2011).

**HEPATOCELLULAR CARCINOMA**

Hepatocellular carcinoma (HCC), a malignant tumour derived from hepatocytes that belong to a primary malignant epithelial tumour of the liver. HCC is the sixth most common cancer worldwide and the third most common cause of cancer-related mortality, with 746,000 deaths each year. The estimated survival of patients with advanced HCC is <10% (Nathan et al., 2009). Almost 80% of HCC cases occur in the developing world; In India there are 19,000 new cases of HCC besides 17,000 deaths, annually (Ferlay et al., 2010). The prevalence of two major risk factors for HCC in India, chronic hepatitis C (HCV) (1-1.9% population) (Sievert et al., 2011) and active hepatitis B (HBV) (~2.4%) (Batham et al., 2007), in combination with an aging population of patients expected to
develop advanced liver disease, hence the incidence of HCC in India can be expected to increase.

**RISK FACTORS OF HCC**

Majority of HCC cases are reported to be infected mainly of hepatitis B and C viruses (Kumar et al., 2007) and the risk factors such as obesity, iron overload, both alcoholic and non-alcoholic cirrhosis, as well as dietary hepatocarcinogens, such as aflatoxins and nitrosoamines have also been implicated as key causes of HCC (Scheme C) (Bartsch et al., 1984, Kensler et al., 2004 and Schütte et al., 2009). The metabolic risk factors for HCC, such as diabetes and obesity, have also resulted in an increased incidence of nonalcoholic fatty liver disease and, in turn, Nonalcoholic Steatohepatitis (NASH) has been associated with the increased incidence of primary liver cancer (McGlynn et al., 2011).

**Scheme C:** Risk factors for HCC development *(Figure adapted from Dig Dis 2009; 27:80-92).*
CHRONIC HEPATITIS AND LIVER CIRRHOSIS

Chronic hepatitis and liver cirrhosis are recognized as important risk factors for the development of HCC. Hepatitis is associated with liver cell necrosis, inflammation, regeneration, and fibrosis, which may proceed to cirrhosis. A common feature in chronic viral hepatitis and liver cirrhosis is long lasting inflammation of the liver associated with chronic regenerative conditions, which enhance the susceptibility of liver cells to genetic changes (Buetow et al., 1989). Following liver cell necrosis, quiescent hepatocytes start to proliferate. Chronic hepatitis is characterized by repetitive cycles of necrosis and regeneration, which facilitate successive acquisition of genomic alterations (Rocken et al., 2001). Chronic HBV infection remains a major risk factor in liver disease such as cirrhosis and HCC. However, approximately 20% of HCV carriers develop HCC, while the incidence of HCC in HBV carrier is about 5% (Colombo et al., 1999).

ALCOHOL AND HCC

Chronic alcohol consumption is one of the major risk factors for developing HCC, which leads to a fatty degeneration of hepatocytes, resulting in 30% in alcoholic liver fibrosis and in 10-20% in liver cirrhosis that is prone to the development of HCC with an annual incidence of 1-2% (Becker et al., 1996 and Seitz et al., 2006). The chronic inflammation leads to stimulation of apoptosis and by the enhanced cell injury to an increased cellular proliferation, hyper-regeneration causing DNA damage due to increased oxidative stress forming acetaldehyde-DNA adducts in presence of toxic metabolites acetaldehyde, which lead to constant genomic alterations (Homann et al., 2006).

DIABETES MELLITUS, OBESITY AND OTHER METABOLIC FACTORS

The interaction of the single components of metabolic syndrome, especially obesity and diabetes mellitus, is complex. The hazardous effect of each single factor is aggravated in case of a coexistence of several factors. A combination of fatty liver, obesity and diabetes is associated with a higher rate of fibrosis
progression (Adams et al., 2005). Recent studies showed that obesity was a genuine promoter of HCC in a mouse model depending on enhanced production of the tumour promoting cytokines IL-6 and TNF, which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3 (Park et al., 2010). The existence of diabetes mellitus further increased the risk of HCC in patients with liver cirrhosis due to alcohol abuse or viral hepatitis (N’Kontchou et al., 2006 and Torisu et al., 2007). A study on metabolic disorders such as hereditary hemochromatosis (HH) is one of the important causes of excess iron accumulation in liver reported to have a 200-fold increased risk of HCC (Kowdley et al., 2004), Moreover, iron overload may interact with HBV, HCV, alcohol and many other known HCC risk factors and act as a cofactor in the pathogenesis of HCC (Kew et al., 2009).

DIAGNOSIS OF HCC

Identification of early HCC which is potentially amenable to aggressive intervention and improved survival is the rationale behind screening for HCC. Since most HCC patients are diagnosed at the end-stage of liver dysfunction, the mortality rate is approximately the same as the incidence rate (Fattovich et al., 2004). Therefore, early detection of HCC is of paramount importance to improve the survival of affected patients (Zhou et al., 2012).

SEROLOGICAL TESTING

The increasing incidence of HCC has improved the prospects for screening the at-risk population, and the basic tools for this evaluation are serological markers. Alpha-fetoprotein (AFP) is the gold standard marker for early prognosis of HCC (Wright et al., 2007). Glypican-3 (GPC-3), a cell surface glycoprotein, is highly specific and is comparable with AFP and its expression is also increased in malignant melanoma patients. Golgi protein 73 (GP73), localized in the membranes of the cis-Golgi complex, is up-regulated in the hepatocytes of patients with cirrhosis or HBV- or HCV related HCC and its specificity for detecting HCC is superior to AFP (Wright et al., 2007).
The other Serological markers of HCC include Des-\(\gamma\)-carboxy prothrombin (DCP), an abnormal form of prothrombin (coagulation factor II) and \(\alpha\)-L-Fucosidase, a ubiquitous lysosomal enzyme are found to be in elevated levels in HCC patients (Giardina et al., 1998 and Wang et al., 2005). Hepatocyte growth factor (HGF), a multi-functional cytokine, affects mitogenesis, cell motility, cell invasion and carcinogenesis and elevated serum levels are observed in HCV-infected patients (Wright et al., 2007).

**DIAGNOSTIC IMAGING**

Recent advances in imaging techniques play a pivotal role in the early detection of HCC and have contributed to the diagnosis of hepatic lesions. Several methods are being used to diagnose hepatic abnormalities in HCC patients. *Ultrasonography* is the oldest technique with high sensitivity and positive predictive value for the early diagnosis of cirrhosis and HCC (Choi et al., 1996 and Wright et al., 2007).

*CT scans* are performed using multi-phase contrast imaging of the liver. CT arteriography is more invasive but also more effective in increasing the rate and accuracy of detection (Wright et al., 2007).

*Magnetic Resonance Imaging* is highly specific and sensitive similar to that of a multiphase CT scan (Yu et al., 2002).

**LIVER BIOPSY**

Liver biopsy has been safely and effectively used as a diagnostic tool than any other emerging technique for hepatic liver lesions. Fine-needle aspiration (FNA) and needle core biopsy are used to obtain cytological and histological samples under ultrasound or CT scan guidance. The combination of these techniques increases the diagnostic power of liver biopsy. The microscopic features of HCC include peripheral endothelial wrapping, atypical naked nuclei and an elevated nuclear to cytoplasmic ratio. The most malignant identifiable histological feature is dysplasia (Inoue et al., 2009). The diagnosis of lesions less than 2 cm in diameter by biopsy has an accuracy of 95.6% (Caturelli et al., 2004).
CURRENT TREATMENT STRATEGIES FOR HCC

The consensus is that, for effective treatment, the tumour must be detected in the early phases of development. Surgical treatments (surgical resection and liver transplantation) and percutaneous treatment (alcoholization and radiofrequency) are considered to be curative or radical.

**Surgical resection** is considered to be the option of choice for the treatment of patients with HCC and its safety has been repeatedly demonstrated over the last few decades. For more advanced HCC tumours, greater than 10 cm in diameter, surgical resection is the best treatment. The resection of large HCC tumours can only be achieved when liver functions are maintained within satisfactory limits. Resection decreases the mortality rate to less than 5%. The survival rate after resection can reach 7% for the 1st year and 74% for the 2nd, depending on residual hepatic reserve and tumour staging (Bruix et al., 2002). Tumour recurrence is observed in about 12, 60 and 70% of patients after 1, 3 and 5 years, respectively (Fuster et al., 1996 and Llovet et al., 1999) and is related to the presence of satellite nodules and tumour differentiation (Michel et al., 1995). Recurrence may be local or may consist of the appearance of metachronic tumours since the cirrhotic liver, especially when involved by extensive inflammatory activity, continues to be a risk factor (Fuster et al., 1996 and Llovet et al., 1999).

**Liver transplantation** is the treatment of choice in cases of HCC limited to the liver with poor hepatic function. Liver transplantation not only eliminates the neoplasia, but can also cure the base liver disease. Choice of liver transplantation is ideal for patients with single tumours smaller than 5 cm, or with up to 3 nodules, none of them larger than 3 cm, without signs of neoplastic invasion of the portal system or of distant metastases. The possibilities of survival reach 84, 74 and 74% in the 1st, 2nd and 5th years after liver transplantation, with a rate of tumour recurrence of only 3.5% (Figueras et al., 1997). Despite its advantages, also involves disadvantages such as the lack of donors, the high cost
and the possibility of tumour recurrence, the frequent postoperative infections, the high rates of perioperative morbidity and the quality of postoperative life.

**Alcoholization and radiofrequency** - Absolute alcohol causes cell dehydration and extensive coagulative cell necrosis in addition to leading to thrombosis of the intra-tumoural vessels. Percutaneous ethanol injection (PEI) is a procedure of easy execution, good tolerability and low cost, which can be applied during repeated sessions (Vilana *et al.*, 1992 and Livraghi *et al.*, 1995a). Using ultrasound, the alcoholization needle is introduced until it reaches the nodule and the amount of alcohol injected per session depends on tumour size. Local recurrence is observed in 17% of cases and the survival of patients reach 85% in the first year, 60% in the third, and 30% in the fifth, rates similar to those obtained with surgical resection (Livraghi *et al.*, 1995b, Yamamoto *et al.*, 2001).

**Chemotherapy** is not routinely administered in the treatment of HCC for a variety of reasons, including the presence of the multi-drug resistant gene (MDR1), which limits systemic chemotherapy efficacy (Chou *et al.*, 1997 and Ng *et al.*, 2000). No consistent positive results have been obtained using hormonotherapy, immunotherapy, or systemic chemotherapy. The lack of response of liver tumours to anticancer drugs is due to complex mechanisms, involving changes in the expression and/or function of the proteins involved in drug uptake/efflux, intracellular processes of signalling, DNA repair and death/survival control (Marin *et al.*, 2008). Thus, current research efforts are focused on gaining a better understanding of the mechanisms of chemotherapy resistance in order to be able to better predict it before starting treatment and to develop novel strategies to overcome it.

**PATHOGENESIS OF HCC**

The development of HCC is a multistep hepatocarcinogenesis process. The environmental, infectious, nutritional, metabolic, and endocrine factors contribute directly or indirectly to hepatocarcinogenesis. The importance of individual factors vary by geographic location, have a direct impact on the
characteristics of these patients, and influence the disease course, making HCC a complex condition associated with a poor prognosis (Venook et al., 2010). Recent studies have revealed insights into the complex role of HBV in malignant transformation of liver cells, chromosomal abnormalities in HCC have been unraveled, new oncogenic pathways and tumour suppressor networks have accumulated (Zimonjic et al., 1999; Levy et al., 2002).

**CHROMOSOMAL AND GENETIC ALTERATIONS**

Chromosomal alterations at different stages of hepatocarcinogenesis were accessed using genome-wide allelotyping, which showed a stepwise increase in allelic losses from cirrhosis, through dysplastic nodules (low grade to high grade), to primary and metastatic HCC (Lee et al., 2008). Loss of heterozygosity (LOH) was uncommon in cirrhotic livers (n = 24; mean fractional allelic loss [FAL] index, 0.09). In contrast, LOH was common in HCC nodules (n = 74; mean FAL index, 0.4). The stepwise increase in frequency of allelic losses provides further evidence for the hypothesis of multistep hepatocarcinogenesis. The close association of high-grade dysplastic nodules with HCC suggests a more aggressive treatment approach that may be beneficial for high-grade dysplastic nodules to prevent further progression to HCC (Lee et al., 2008).

Recurrent allelic losses are common in chromosomes 1p, 4q, 8p, 13q, 16q, and 17p, and allelic gains are most often observed in 1q, 8q, 16p, and 20q (Wong et al., 1999, Guan et al., 2000 and Wilkens et al., 2001). The regions of recurrent chromosomal deletions may harbour the loss or mutation of putative tumour suppressor genes (Chan et al., 2002 and Wong et al., 2002), while regions of recurrent chromosomal gains may be associated with gain of function mutations and oncogenes (Patil et al., 2005). Thus, chromosomal gains and losses may result in deregulation of signaling pathways in HCC leading to tumourigenesis and metastasis.
EPIGENETIC ALTERATIONS

In addition to genetic and chromosomal mechanisms of mutations, epigenetic alterations have been implicated to play an important role in human carcinogenesis. Epigenetic alterations refer to the reversible and heritable changes in gene expression that occur without alteration to the DNA sequence. DNA methylation is an example of such changes and a key epigenetic event in cancer. DNA methylation in the mammalian genome is found at the cytosine residues of CpG dinucleotides, often associated with promoter related CpG islands. Although DNA methylation is essential for normal development and differentiation, aberrant hypomethylation in many human cancers can lead to the expression of oncogenes (Jones et al., 2002), or similarly, hypermethylation can lead to the silencing of tumour suppressor genes (Lehmann et al., 2007).

In HCC, aberrant DNA hypermethylation has been reported in promoter regions of tumour suppressor genes, such as p16INK4A, E-cadherin, RAS-association domain family (RASSF1A), suppressor of cytokine signalling (SOSC-1) and phosphatase and tensin homolog (PTEN). The frequency of aberrant DNA methylation increases from precancerous lesions to dysplastic nodules and finally HCC, signifying their importance in tumour progression (Wong et al., 2008). Demethylation agents such as DNA methyltransferase inhibitors are being developed and utilised as anticancer drugs as they allow re-expression of the aberrantly methylated genes to restore normal tumour-suppressive functions. Azacitidine and decitabine are two DNA methyltransferase inhibitors currently approved by the US Food and Drug Administration (FDA) for chemotherapy against myelodysplastic syndrome (Gore et al., 2011).

DEREGULATED CELL SIGNALING PATHWAYS IN HCC

Genetic and epigenetic changes lead to altered gene expression patterns, resulting in the activation of oncogenes and / or inactivation of tumour suppressor genes and disruption of normal cell signalling pathways. Deregulation of various signalling pathways have been implicated in
pathogenesis of HCC, including proliferation and survival pathways (e.g. epidermal growth factor, insulin-like growth factor [IGF] and hepatocyte growth factor), differentiation pathways (e.g. Wnt and Hedgehog pathways), inflammation pathways (e.g. interleukin-6 [IL-6] and interferon [IFN]), and growth factor-regulated angiogenic signalling (e.g. vascular endothelial growth factor and platelet-derived growth factor) (Whittaker et al., 2010 and Zender et al., 2010).

**Table A:** Molecular targets and therapeutic agents for HCC in clinical trials.

<table>
<thead>
<tr>
<th>Therapeutic Agents</th>
<th>Molecular Targets</th>
<th>Author</th>
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<tbody>
<tr>
<td>Sorafenib SHARP study versus placebo</td>
<td>RAF, VEGFR, PDGFR</td>
<td>Llovet et al., 2008</td>
</tr>
<tr>
<td>Sorafenib Asia-Pacific study versus placebo</td>
<td>RAF, VEGFR, PDGFR</td>
<td>Cheng et al., 2009</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR, PDGFR</td>
<td>Faivre et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zhu et al., 2009</td>
</tr>
<tr>
<td>Brivanib</td>
<td>VEGFR, FGFR</td>
<td>Raoul et al., 2009</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>Philip et al., 2005</td>
</tr>
<tr>
<td>Erlotinib + bevacizumab</td>
<td>EGFR</td>
<td>Thomas et al., 2009</td>
</tr>
<tr>
<td>TSU-68</td>
<td>VEGFR, PDGFR, FGFR</td>
<td>Kanai et al., 2011</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>Siegel et al., 2008</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGF</td>
<td>Zhu et al., 2007</td>
</tr>
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</table>

Recent meta-analysis showed that aggressive HCC subclass was associated with larger tumour size, poor histological differentiation, and specific molecular alterations such as the activation of transforming growth factor (TG F)-alpha and-beta, MYC and AKT, overexpression of AFP and IGF2, and downregulation of IFN-related genes. In contrast, less aggressive tumours were well differentiated, and associated with CTNNB1 mutation and Wnt activation (Hoshida et al., 2009).
and Hoshida et al., 2010). Specific oncogenes required for tumour progression have yet to be identified in HCC.

The improved understanding of the molecular basis of hepatocarcinogenesis has opened up opportunities for targeted therapies in HCC. Agents targeting signaling pathways implicated in the pathogenesis of HCC that are now in preclinical or clinical trials are given in Table A (Llovet et al., 2008 and Hoshida et al., 2010). Sorafenib, a tyrosine kinase inhibitor, is currently the only targeted agent with demonstrable clinical efficacy to be approved by the FDA for HCC treatment (Cheng et al., 2009). Other promising agents in phase II / III clinical trials will likely expand the therapeutic armamentarium for HCC in the future. With further research and identification of new targets / targeted agents, management strategies for HCC will be better defined and personalised to maximise efficacy and cost benefit.

**ALTERNATIVE NOVEL STRATEGIES TO TREAT HCC**

Based on tradition, herbal medicine makes use of herbs and extracts made from plants and plant sources to heal and treat diseases. Disease is an imbalanced state of the body and studies showed herbs can neutralize these imbalances depending on the basis or the nature of a particular disease (Li et al., 2011). Recent biomolecular studies have shown that herbal medicines have pleiotropic effects such as anti-viral, anti-inflammatory and anti-cancer activities. Some herbal compounds are also designed to cure all phases of HCC, including initiation, promotion and progression (Treasure et al., 2005). Three curative strategies have been applied to treat HCC: liver transplantation, surgical resection and local destruction. Despite these strategies, the rate of HCC recurrence is still very high; palliative treatments, such as TACE, anti-viral treatments and immunotherapeutics, are given either before or after treatment. Importantly, the incidence still nearly equals the mortality rate and more than 80% of patients present with advanced disease (Taieb et al., 2006). The overall disappointing results of both curative therapies and palliative treatments in advanced HCC
patients support the research for other more active and specific treatments to be administered alone or in combination with the current therapy. Recent studies on herbal compounds showed curative properties and some have been proven to be effective against HCC by targeting various drivers of HCC. These drugs are called targeted-biological response modifiers. Some of these drugs are listed in Table B.

**Table B:** Targeted-biological response modifier drugs used against HCC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Possible mechanisms of anti-HCC</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Inhibits proliferation; induces apoptosis; inhibits p21(ras), PCNA, cyclin E, factor NF-κ and p34(cdc2); anti-angiogenesis; inhibits MMP-9 secretion; inhibits histone deacetylase; enhances P21(WAF1/CIP1) protein; elevates mitochondrial membrane potential; attenuates ROS</td>
<td>Chuang <em>et al.</em>, 2000, Cao <em>et al.</em>, 2007, López-Lázaro <em>et al.</em>, 2008 and Yoysungnoen <em>et al.</em>, 2008</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Inhibits proliferation; induces apoptosis; downregulates Bcl-2 and upregulates Bax expression; reduces ROS; induces cell-cycle arrest in G1 and G2/M phases; modulates NO/NOS; increases gap-junctional intercellular communication; sharps increment of the mitochondria membrane potential; inhibits TNF-alpha-mediated MMP-9 expression; suppresses the ROS-potentiated invasion.</td>
<td>Hebbar <em>et al.</em>, 2005, Notas <em>et al.</em>, 2006 and Yu <em>et al.</em>, 2008</td>
</tr>
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</table>


Silibinin
Causes G1 arrest; induces Kip1/p27; decreases cyclin D1, cyclin D3, cyclin E, cyclin-dependent kinase (CDK)-2, and CDK4; downregulates metalloproteinase-2; increases acetylation of histone H3 and H4; inhibits cell proliferation; inhibits NO production and of ERK 1/2 cascade.

Tanshinone - IIA
Induces apoptosis; induces cell arrested in G(0)/G(1); downregulates bcl-2 and c-myc; upregulates fas, bax, p53; inhibits DNA synthesis.

Wellington et al., 2001, Varghese et al., 2005, and Momeny et al., 2008
Wu et al., 1991, Yuan et al., 2004 and Zhong et al., 2007

The active development of innovative therapeutic approaches and molecularly targeted agents using herbal medicine could offer an opportunity to study these agents in HCC and gives new hope for the future.

MORIN (3, 5, 7, 2’, 4’-pentahydroxyflavone)

**Occurrence and structure** - Morin (3, 5, 7, 2’, 4’-pentahydroxyflavone), a natural bioflavonoid, belonging to moraceae family, is isolated as a yellow pigment from almond hulls (*P. guajava L.*) and old fustic (*Chlorophora tinctoria*) (Aggarwal et al., 2006 and Wijeratne et al., 2006). Morin is identified in fruits, vegetables, tea, wine, and many oriental medicinal herbs (Das et al., 1994).
PROPERTIES

a). Physical properties

Highly purified morin is an odourless, yellow to brown coloured powder.

b). Chemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Powder</td>
</tr>
<tr>
<td>Synonyms</td>
<td>2, 3, 4, 5, 7-Pentahydroxyflavone; Morin hydrate; Fusic; Morin dihydrate; 2-(3, 5-dihydroxyphenyl)-3, 6, 8-trihydroxy-4H-chromen-4-one; 2-(2, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one</td>
</tr>
<tr>
<td>Empirical Formula</td>
<td>C₁₅H₁₀O₇</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>302.2357 g/mol</td>
</tr>
<tr>
<td>Solubility</td>
<td>DMSO ≥64mg/mL; Water &lt;1mg/mL; Ethanol ≥25mg/mL, freely soluble in alcohol; slightly soluble in ether; acetic acid; soluble in aqueous alkaline solutions with an intense yellow colour, which turns brown on exposure to air.</td>
</tr>
<tr>
<td>Density</td>
<td>1.799g/cm³</td>
</tr>
<tr>
<td>Melting point</td>
<td>300°C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>645.5°C at 760 mmHg</td>
</tr>
<tr>
<td>Viscosity at 25°C</td>
<td>12 centipoises</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.823</td>
</tr>
<tr>
<td>Flash point</td>
<td>249.3°C</td>
</tr>
<tr>
<td>Vapour Pressure</td>
<td>2.94E-17mmHg at 25°C</td>
</tr>
</tbody>
</table>
c). **Physiological properties**

Morin, with its potent antioxidant and metal ion chelating nature, is reported to perform various biochemical effects including antioxidant (Kok *et al.*, 2000), anti-cancer (Bhattacharya *et al.*, 1988), anti-inflammatory (Raso *et al.*, 2001 and Fang *et al.*, 2003) and anti-neoplastic activities.

**BIOMEDICAL SIGNIFICANCE OF MORIN**

Earlier studies in this laboratory elucidated the anti-hepatocarcinogenic efficacy of morin, in attenuating hepatocellular carcinoma in diethylnitrosamine induced rats by regulating the expression of NF-κB-p65, COX-2 and MMPs and by fostering apoptosis (Sivaramakrishnan *et al.*, 2009 and Sivaramakrishnan *et al.*, 2010); the anti-fibrotic potential of morin in experimental liver fibrosis, where morin inhibited stellate cell proliferation by suppressing Wnt/β-catenin signaling and favoring apoptosis both, *in vitro* and *in vivo* (Madankumar *et al.*, 2014 and Madankumar *et al.*, 2015); and anti-proliferative, anti-carcinogenic and anti-inflammatory efficacy of morin in DMBA induced mammary carcinogenesis (Nandhakumar *et al.*, 2012, Ramadass *et al.*, 2012 and Kumar *et al.*, 2014).

Also, studies on morin hydrate revealed that it inhibits aberrant crypt foci in azoxymethane-induced rats (Tanaka *et al.*, 1999), exhibits chemopreventive effects on chemically induced rat tongue carcinogenesis (Kawabata *et al.*, 1999), exerts antioxidant effects (Kok *et al.*, 2000), suppresses inducible nitric oxide synthase and cyclooxygenase-2 expression in macrophages (Raso *et al.*, 2001), exerts anti-inflammatory effects reducing the incidence of lipopolysaccharide induced septic shock (Fang *et al.*, 2003), inhibits the release of inflammatory cytokines, such as IL-6, IL-8, and tumour necrosis factor from mast cells (Kempuraj *et al.*, 2005), inhibits 12-O-tetradecanoylphorbol-13-acetate-induced hepatocellular transformation in human hepatocytes (Hsiang *et al.*, 2005), suppresses the activation of NF-κB and NF-κB regulated gene expression in HeLa, H4, A549 and A293 cells (Manna *et al.*, 2007), inhibits the growth of Human Leukemia HL-60 cells, induces apoptosis through mitochondria
dependent pathway (Kuo et al., 2007), attenuates CD36 expression and oxLDL uptake in U937-derived macrophages (Lian et al., 2008), protects acute liver damage induced by CCl₄ in rats (Lee et al., 2008), prevents 1, 2-dimethylhydrazine induced experimental colon carcinogenesis in rats (Vennila et al., 2009), exerts antifibrogenic effects against DMN-induced hepatic injury (Lee et al., 2009), exerts cellular protection against hydrogen peroxide induced oxidative stress (Zhanga et al., 2009), exerts neuroprotective actions in Parkinson disease models (Zhang et al., 2010), attenuates tau hyperphosphorylation by inhibiting GSK 3β in human neuroblastoma SH-SY5Y cells (Gong et al., 2011), inhibits amyloid formation by islet amyloid polypeptide and disaggregates amyloid fibers (Noor et al., 2012), exerts protective effect on cardiac mitochondrial function during isoproterenol-induced myocardial infarction in male Wistar rats (Al Numair et al., 2012), inhibits interleukin-1β-induced nitric oxide and prostaglandin E2 production in human chondrocytes (Chen et al., 2012), induces heme oxygenase-1 via ERK-Nrf2 signaling pathway (Park et al., 2013), augments phagocytosis mechanism and inhibits LPS induced autophagic signaling in murine macrophage (Jakhar et al., 2014), induces apoptosis by induction of BAD protein in human leukemic cells (Park et al., 2014), promotes Nrf2-regulated cellular defenses against oxidative injury to primary rat hepatocytes (Rizvi et al., 2015a), mitigates acetaminophen-induced liver injury by potentiating Nrf2 regulated survival mechanism through PHLPP2-Akt-Gsk3β axis (Rizvi et al., 2015b), exerts renoprotective mechanism in cisplatin-induced kidney injury (Wei et al., 2015), induces apoptosis by modulation of Bcl-2 family members and Fas receptor in HCT 116 cells (Hyun et al., 2015), inhibits enzymatic activity of N-methylpurine DNA glycosylase, a DNA repair enzyme with various roles in human disease (Dixon et al., 2015), induces apoptosis by modulation of Bcl-2 family members and Fas receptor in HCT 116 cells (Hyun et al., 2015), inhibits enzymatic activity of N-methylpurine DNA glycosylase, a DNA repair enzyme with various roles in human disease (Dixon et al., 2015), exerts renoprotective mechanism in cisplatin-induced kidney injury (Wei et al., 2015), exerts antiarthritis effect with
inhibition of synovial angiogenesis (Zeng et al., 2015), suppresses inflammatory immune response in raw 264.7 macrophages through the inhibition of inflammatory mediators, intracellular ROS levels and NF-κB activation (Dhanasekar et al., 2015), rescues endothelial dysfunction in a diabetic mouse model by activating the Akt/eNOS pathway (Taguchi et al., 2015), promotes Nrf2-regulated cellular defenses against oxidative injury to primary rat hepatocytes (Rizvi et al., 2015a), mitigates acetaminophen-induced liver injury by potentiating Nrf2 regulated survival mechanism through PHLPP2-Akt-Gsk3β axis (Rizvi et al., 2015b) and attenuates Ovalbumin-induced airway inflammation by modulating oxidative Stress-responsive MAPK signaling (Ma et al., 2015).

The above said evidences prove morin could have a benefical effect on several human diseases due to its antioxidant, antidiabetic, anti-inflammatory, anticarcinogenic, antihypertensive, antibacterial, hypouricemic, and neuroprotective activity, by modulating the activity of many enzymes. In addition, in vitro and in vivo studies, demonstrated that morin exhibits very low toxicity levels and its chronic administration is well tolerated. All these findings suggest that Morin is a non-toxic, natural compound and understanding its molecular mechanism against progression of liver carcinogenesis, in vivo and in vitro studies forms the base of the current study.