GENERAL INTRODUCTION

CANCER

Cancer is a multifaceted disease that represents one of the leading causes of mortality in developed countries. Worldwide, one in eight deaths is due to cancer and it is the second most common cause of death in the US, exceeded only by heart disease, accounting for nearly one of every four deaths (http://www.cancer.org). The World Health Organization (WHO) projects that without immediate action, the global number of deaths from cancer will increase by nearly 80% by 2030, with most occurring in low- and middle-income countries. External factors (such as tobacco, infectious organisms, chemicals, and radiation) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) are mostly responsible for cancer. These causal factors may act together or in sequence to initiate or promote the development of cancer (Khazir et al., 2014).

Cancer results from a multistage and multi-mechanism process that involves mutagenic, cell death and epigenetic mechanisms, during the three distinguishable but closely allied stages: initiation, promotion, and progression. Since reducing the initiation phase to a zero level is impossible, the most effective intervention would be at the promotion phase to eliminate premalignant cells before they become malignant (Karikas, 2011; Bray et al., 2012).

Cancer is generally considered as uncontrolled cell division that results in the aggregation of cells to form tumors. There are many factors which are involved in the pathogenesis of cancer, such as:

1) Individuals that engaged in risk-taking behaviors or lifestyles (e.g. smoking, use of snuff, and lack of proper diet like high in meat and low in fruits and vegetables) (De et al., 2005).
2) Exposure to known carcinogens (e.g. heavy metals of chromium) (Snow, 1992).

3) Genetic mutations to the development of cancer (e.g. familial adenomatous polyposis) (Vander Luijt et al., 2000)

NATURE OF CARCINOGENESIS

On this basis, cancer results from a multistage carcinogenesis process in which distinct molecular and cellular alterations that involves three stages: initiation (normal cell → transformed or initiated cell), promotion (initiated cell → preneoplastic cell), and progression (preneoplastic cell → neoplastic cell) (Jones and Baylin, 2002). It takes many years for the journey of normal cells to develop into complete malignancy (Khan et al., 2007), offering the opportunities to intervene its development (Figure 1) (Beliveau and Gingras, 2007).

Figure 1: Steps in carcinogenesis (Yao et al., 2012)

Initiation

Initiation is a result of rather rapid and irreparable process to the cell, which includes the uptake of a carcinogenic agent and its distribution and transport to organs and tissues by its metabolic activation and the subsequent covalent interaction with target cell DNA, leading to stable genotoxic damage. The transformed cells undergo many changes to form preneoplastic cells (Yao et al., 2012).
**Promotion**

In contrast to initiation, tumor promotion process is not rapid, and oxidative stress and chronic inflammatory are key components in promoting tumor proliferation and angiogenesis which is necessary for solid tumor growth (Coussens and Werb, 2002).

**Progression**

Progression involves the gradual conversion of tumor cells to the invasive cells, leading to increased metastatic potential. Each of these progression processes (angiogenesis/ invasion/ metastasis) involves rate-limiting steps that are influenced by non-malignant cells of the tumour microenvironment (Joyce and Pollard, 2009).

**HEPATOCELLULAR CARCINOMA**

Liver cancer, especially hepatocellular carcinoma (HCC), is the fifth most common cancer and the third foremost cause of cancer associated death globally (Befeler and DiBisceglie, 2002; Bishayee and Dhir, 2009; Viatour et al., 2011). The large number of HCC patients, there is a strong mandate to develop relevant animal models and biomarkers (Dalton and Friend, 2006) that will enable accurate translation from preclinical research to clinical practice when developing new single-agent or combination treatments for HCC. HCC can be secondary to hepatitis B or C, cirrhosis due to alcohol consumption, liver disease due to aflatoxin toxicity, hormonal imbalance and certain metabolic diseases. Hepatocarcinogenesis involves initial genotoxic insult (initiation), clonal expansion from premalignant to malignant lesions (promotion) and finally tumour progression by further clonal expansion (Thorgeirsson and Grisham, 2002; Shire and Roberts, 2012; Shen and Cao, 2012).
Descriptive Epidemiology

HCC has a poor prognosis with the number of deaths almost equal to the number of cases being diagnosed annually (about 600,000) and the 5-year survival rate reported below 9% (Sherman, 2005). The incidence of HCC is on the rise in multiple geographic areas, including Asia Pacific, sub-Saharan Africa, southern Europe as well as North America. The occurrence of HCC in the developing countries has dramatically increased by more than 70% over the last 25 years (El-Serag, 2004). It has been estimated that there will be more than 22,000 new cases and about 18,000 deaths in the United States in 2009 due to liver cancer which represents about 4% of cancer mortality in this country (Jemal et al., 2009). This type of cancer occurs more often in men than women. It is usually seen in people age 50 or older. However, the age varies in different parts of the world.

In most cases, the cause of HCC is usually scarring of the liver (cirrhosis). Cirrhosis may be caused by:

- Alcohol abuse
- Autoimmune disease of the liver
- Hepatitis B or C virus infection.
- Inflammation of the liver that is long-term (chronic)
- Iron overloads in the body (hemochromatosis) as well as carcinogens, such as aflatoxins and nitrosamines are also involved in its etiology.

Patients with hepatitis B or C are at risk for liver cancer, even if they have not developed cirrhosis.

Symptoms

- Abdominal pain or tenderness, especially in the upper-right part
- Easy bruising or bleeding.
- Enlarged abdomen
- Yellow skin or eyes (jaundice)
Signs and tests

Physical examination may show an enlarged, tender liver.
Tests include:

- Abdominal CT scan
- Abdominal Ultrasound
- Liver biopsy
- Liver enzymes (liver function tests)
- Liver MRI
- Serum alpha fetoprotein
- Some high-risk patients may get regular blood tests and ultrasounds to see whether tumors are developing

Complications

- Gastrointestinal bleeding
- Liver failure
- Spread (metastasis) of the cancer (Roberts, 2011)

EXPERIMENTAL MODELS OF HEPATOCELLULAR CARCINOMA

N-Nitrosodiethylamine (NDEA)

N-Nitrosodiethylamine (NDEA), also known as diethylnitrosamine (DEN), is a slightly yellow liquid with a boiling point of 175-177°C and a specific gravity of 0.94. It is soluble in water, ethanol, diethyl ether and organic solvents. Its chemical structure is shown in Figure 2. NDEA has had extensive use as an experimental carcinogen, and although there is evidence of human exposure, no epidemiological studies have specifically investigated NDEA-related human cancer. NDEA has been found in a variety of products that would result in human exposure, including mainstream and side stream tobacco smoke, meat, whiskey etc., (Hoffmann et al., 1980; Bartsch and Montesano, 1984).
Chemical carcinogen $N$-Nitrosodiethylamine (NDEA) is a potent hepatocarcinogenic agent. The rat model of NDEA-induced HCC is considered as one of the most accepted and widely used experimental models to study hepatocarcinogenesis (Ha et al., 2001). Human livers metabolize nitrosamines similar to that of rat liver and also exhibit considerable similarities with regard to morphology, genomic alterations and gene expression, despite their different disease etiologies (Feo et al., 2000). Biotransformation of NDEA produces the promutagenic adducts, O6-ethyl deoxy guanosine and O4- and O6-ethyl deoxy thymidine that may initiate liver carcinogenesis. It has been reported that NDEA metabolism in the liver by cytochrome p-450 (Phase-I) generates reactive oxygen species (ROS) causing oxidative stress and modulating phase II enzymes activity (Mandal et al., 2008). It has been well established that NDEA, being a genotoxic carcinogen, forms alkyl DNA adducts, induces chromosomal aberrations, micronuclei and sister chromatid exchanges in rat liver (Jagadeesh et al., 2009). Mutations induced by NDEA are responsible for the development of hepatocarcinogenesis. Currently, the mechanism of NDEA-induced hepatocarcinogenesis is thought to be as follows: NDEA is hydroxylated by cytochrome P-450 isozymes in the liver, throught an alkylation mechanism, to become bioactive. Subsequently, bioactivated NDEA reacts with DNA, causing ethylation of the bases. The ethyl DNA adducts can interrupt base pairing, resulting in mutations and the activation of proto-oncogenes, for example, ras (Chen et al., 1993; Verna et al., 1996), and inhibition of
tumor-suppressor genes, for example, p53 (Chen et al., 1993; Verna et al., 1996) which often result in HCC.

**MARINE SOURCES**

The bioactive compounds isolated from marine organisms have been shown to exhibit anti cancer, anti microbial, anti fungal, anti inflammatory and other pharmacological activities (Gul and Hamann, 2005; Meyar and Hamann, 2005; Ravichandran et al., 2010; 2011). Now a day, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals (Febles et al., 1995; Lima-Filho et al., 2002). Many bioactive and pharmacologically compounds such as alginate, carrageen, phlorotannins and agar as phycocolloids have been obtained from seaweeds and used as in medicine and pharmacy (Siddhanta et al., 1997).

**SEAWEEDS**

Seaweed is a renewable living resources found in the coastal water bodies. Seaweed is known for their nutrient and chemical composition and it serves as an important source of bioactive natural substances. They have been used as food in the Asian countries for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals (Rajasulochana et al., 2009; Sasidharan et al., 2012). Seaweeds are known as a good resource of biologically active substances. Especially, brown algae contain various bioactive compounds including pigments, steroids, phycocolloids and phlorotannins (Kuda et al., 2005). Previous studies reported that phlorotannins from brown algae have various biological activities such as anti-oxidant, anti-inflammation, anti-allergy, anti-bacterial, and anti-diabetic activities (Nagayama et al., 2002; Kang et al., 2004; Shibata et al., 2008).
DESCRIPTION OF ECKLONIA CAVA (E. CAVA)

Ecklonia cava (class: Phaeophyceae; family: Lessoniaceae; order: Laminariales, species: E. cava) is a brown alga found in the ocean off Japan and Korea. It is seen growing from the low water neap tide mark down to 15–18 m depth (Heo et al., 2005; Kang et al., 2013). E. cava, brown seaweed (Laminariaceae) has long been utilized as a traditional food and traditional folk herb in Korea (Kim et al., 2006; Shim et al., 2009). Recent researches provide the evidence that E. cava has exhibited various biological activities both in vitro and in vivo (Kang et al., 2010; Lee et al., 2010, b; Kang et al., 2012).

An expanding body of evidence has revealed, the biological activities of E. cava including antiplasmin-inhibitory (Fukuyama et al., 1990), anti mutagenic (Lee et al., 1998), bactericidal (Nagayama et al., 2002), antioxidant (Kang et al., 2004), HIV-1 reverse transcriptase (Ahn et al., 2004), radical scavenging (Kang et al., 2005, 2006; Ahn et al., 2007), anticancer (Kim et al., 2006), anti-asthmatic activities (Kim et al., 2006), immunomodulatory (Ahn et al., 2008), anti-allergic (Kim et al., 2008), anti-inflammatory (Jung et al., 2009), tyrosinase inhibitory activity (Heo et al., 2009), type I diabetes (Kang et al., 2010) antiviral activity (Ryu et al., 2011) and Antimetastatic activity (Lee et al., 2011).

DIECKOL

Dieckol (C_{36}H_{22}O_{18} \ (4\-\[4\-\[6\-\[3, \ 5\- \ dihydroxyphenoxy] \ -4, \ 7, \ 9\-\ trihydroxydibenzo \ - p \ - dioxin - 2 \ - yl] \ oxy - 3, \ 5 \ dihydroxyphenoxy] \ dibenzo \ -p- \ dioxin -1, \ 3, \ 6, \ 8 \ - tetrol]) (Figure 3) is a phlorotannin that can be found in Ecklonia cava (Kang et al., 2005). Dieckol have been elucidated to exhibit anti-oxidation (Ahn et al., 2007), anti-cancer (Zhang et al., 2011; Oh et al., 2011; Lee et al., 2012), anti hair loss (Kang et al., 2012), anti-diabetic II (Kang et al., 2013), hepatotoxicity (Kang et al., 2013), and free radical scavenging activities (Yoon et al.,
2013) anti-adipogenic, anti-myeloperoxidase, (Jung et al., 2014), anti-inflammatory (Yayeh et al., 2014).

**Figure 3: Chemical structure of dieckol**
Review of literature