HCC is the fifth most prevalent cancer in the world and the third leading cause of cancer related death with a 5% survival rate over 5 years and more than 500,000 deaths annually (Iakova et al., 2011). HCC develops due to hepatitis B and hepatitis C virus infections, alcohol abuse, or other metabolic disorders that lead to liver cirrhosis. The common denominator in HCC of different etiology is the induction of oxidative stress by inflammatory cells, resulting in chronic hepatic injury and cell death, followed by oncogenic transformation of surviving hepatocytes and compensatory proliferation that lead to tumorigenesis (Maeda et al., 2005; Vucur, 2010).

N-Nitrosodiethylamine (NDEA) is a commonly used carcinogen that induces multifocal liver tumors resembling human HCC (Koen et al., 1984; Peto et al., 1991; Ishii et al., 2010). Most previously reported HCC models were generated through “initiation and promotion” steps. For example, HCCs were initiated by NDEA, and then promoted by nodularin (Song et al., 1999), phenobarbital and thioacetamide (Park et al., 2001), carbon tetrachloride and 70% partial hepatectomy (Solt et al., 1980), or by hepatitis B viral antigen (Zheng et al., 2007). Although they are technically feasible, these models require a long induction period, ranging from 12 to 18 months and produce HCC (Peto et al., 1991).

NDEA is an experimental hepatocarcinogen found in a variety of products to which humans may be exposed, for example, tobacco smoke, meat, and whiskey (Brown, 1999; Mesallamy et al., 2011). Currently, the mechanism of DEN-induced hepatocarcinogenesis is thought to be as follows (Verna et al., 1996); NDEA is hydroxylated by cytochrome P-450 isozymes in the liver, through 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 (Corcos et al., 1984; Beer et al., 1986) and inhibition of tumor-suppressor genes, for example, p53 (Smith et al., 1991), which often result in HCC.

HCC tumor spread is partly dependent on neo angiogenesis (Spinzi and Paggi, 2008; Faivre et al., 2009). VEGF is considered one of the most important factors involved in neoangiogenesis, development and/or progression of HCC, being associated with a poor survival (Claudio et al., 2004; Mas et al., 2007; Zhu and Raymond, 2009). HCC patients with serum and tissue VEGF overexpression have a lower survival rate (Zhou et al., 2006). Oral multikinase inhibitor (e.g. Sorafenib, sunitinib) of the VEGF, PDGFR of downstream intracellular serine/threonine, is used with success in HCC therapy.

Anwar et al., (2008) reported HCC tumorigenesis includes alteration in cellular proliferation markers, cell cycle regulators, suppressor genes, oncogenes and their receptors, apoptosis related factors as well as modification in cellular proliferation markers, cell cycle regulators, suppressor genes, oncogenes and their receptors, apoptosis related factors as well as modification of genes involved in angiogenesis and immune response (Blum, 2007; Calvisi et al., 2007).

Tischoff and Tannapfe, (2008) clearly demonstrated that both DNA hypomethylation and CpG hypermethylation are the dominant events during HCC development and progression. Therefore, epigenetic changes may serve as indicator or biomarker for screening of patients with an increased risk for HCC. Therapeutic strategies being able to modify the methylation status or multikinase inhibitors of liver cancer cells and to target tumor suppressor genes may be highly beneficial in the treatment of human HCC.

Tumor suppressor genes represent genes that are likely to play a role negatively regulating cell growth. Loss or inactivations of these genes are associated with malignancy and carcinogenesis process.
Apart from deletions and mutations, growing evidence has indicated that epigenetic alterations are implicated in inactivation of tumor suppressor genes (Munakata et al., 2007).

Zhou et al., (2006) reported that mutations in the p53 gene abrogated its normal functions, leading to genomic instability and loss of growth control, p53 over expression may be involved in determining the differentiation and the proliferative activity in many cancers including HCC.

Disruption of the G1/S and G2/M check points leads to uncontrolled cell growth, resulting in the development and progression of cancers (Masaki et al., 2000).

Qin and Tang, (2002) investigate that the p27 protein is a member of cyclin/cyclin-dependent kinase inhibitors involved in cell-cycle progression. p27 also promotes cell migration in metastatic HCC cells through the regulation of RhoA activity. Reduced p27 expression correlates with poor prognosis meanwhile high p27 expression, correlated with prolonged survival, used as prognostic parameter for HCC.

Buhlmann et al., (2008) recently demonstrated the p73 accumulation in HCC, suggesting that p73 plays a role in the malignant phenotype. P 73 expression status is related to prognosis of HCC patients.

Proto-oncogenes encode a wide range of proteins products involved in the control of cell proliferation and differentiation, including growth factors, growth factor receptors, components of signal transduction pathways and transcription factors. Aberrations of many oncogens were identified in HCC being associated with poor prognosis (Cui et al., 2001; Anwar et al., 2008). The overexpression of epidermal growth factor (EGF) and epidermal growth factor receptors (EGFR) were observed in HCC, being associated with late-stage
disease, increased cell proliferation and degree of tumor
differentiation. EGFR can be considered as a marker for predicting the
metastasis and recurrence of HCC. Because of high prevalence of
EGFR overexpression in HCC, inhibitors of EGF-EGFR pathways are
potential therapeutic agents. Studies of EGFR inhibitors \textit{in vitro},
(phase I or phase II) studies were being carried out in HCC therapy
(Huether \textit{et al.}, 2005; Philip \textit{et al.}, 2005).

Deregulation of c-myc gene expression was frequently observed
in experimentally induced HCC in rodents, as well as in primary
human liver tumors. Disease-free survival in patients with c-myc
amplification is significantly shorter than in those without
amplification. Other nuclear oncogenes overexpressed are c-Ki-ras, c-
Ha-ras, c-fos, c-fms and b-catenin with important role of HCC
malignant phenotype (Li \textit{et al.}, 1990; Walzer and Kulik, 2008).

Angiogenesis is a multistep process, physiological angiogenesis
occurs during liver regeneration, leading to the formation of new blood
vessel from pre-existing vasculature, meanwhile pathological
angiogenesis occurs in HCC (Blum and Spangenberg, 2007; Zhu and
Raymond, 2009). Angiogenesis makes significant contribution to
tumor growth, invasiveness, and metastatic potential of HCC.
Differentially expressed angiogenesis genes and proteins were
identified including, vascular endothelial) platelet-derived growth
factor receptor (PDGFR) growth factor (VEGF), basic fibroblast growth
factor (bFGF), matrix metalloproteinases (MMPs) and its inhibitors
(TIMPs), angiopoitin-1 (Ang-1) angiopoitin-2 (Ang-1) have been
evaluated and found to be related to HCC tumorigenesis and
prognosis.

MMPs are proteins possessing key roles in the growth and
infiltration of cancer cells. Expression of MMPs and TIMPs were
investigated in vitro and surgically resected HCC tissues. A high
expression was observed for MMP-2, MMP-9, MT1-MMP and TIMP-2.
Expression of MMP-7, MT2-MMP and TIMP-1 was found at a low frequency and a low amount in both cells and tissues. MMPs and TIMPs are involved in the progression of HCC (Sugimachi et al., 2003).

Mounting evidence indicates the involvement of cytokines in hepatocarcinogenesis (Budhu and Wang, 2006; Liu et al., 2009). Interleukin-6 (IL-6) is a pleiotropic cytokine that plays a critical role in normal hepatic growth and liver regeneration following a reduction in hepatic mass. Concentrations of IL-6 in serum are increased in situations of chronic liver inflammation including alcoholic hepatitis, HBV and HCV infections, and steatohepatitis, conditions that may lead to development of HCC (Naugler et al., 2007).

Serum levels of IL-6 and IL-10 are frequently elevated in patients with HCC but not in benign liver disease or non-HCC tumors (Gaosawara et al., 2005; Zekri et al., 2005). IL-6 and IL-10 may help to identify a subset of HCC patients and may serve as complementary tumor markers in these patients and (Shin et al., 2003; Hsia et al., 2007). These studies suggest that increases in IL-10 and perhaps other Th2 cytokines correlate with progression of HCC (Hsia et al., 2007). Proinflammatory IL-1β was elevated in HCC patients compared with healthy individuals (Wang et al., 2008). Serum IL-15 was higher in HCC indicating the degree of liver inflammation (Beckebaum et al., 2004), TNF-α expression was elevated in HCC patients, especially those with recurrence. In addition, the levels of the TNF-α Rs (TNFαRI and TNFα RII) were higher in HCC patients a (Kakumu et al., 1997). In other studies, TNF-α level was lower in HCC tumor tissue versus the tissue surrounding the tumor and in HCC patients versus healthy individuals (Budhu and Wang, 2006) IFN-α was not detected in HCC, and was concluded that IFN-α may not play a large role in liver inflammation. The proinflammatory cytokines IL-12 and IL-2 are also increased in HCC. Th1 cytokines are mainly up-regulated in HCC (Budhu and Wang, 2006).
Cox-2 is an isoform of cyclooxygenase, which is the key enzyme converting arachidonic acid to prostaglandins. Overexpression of Cox-2 affects many mechanisms involved in carcinogenesis, such as angiogenesis, inhibition of apoptosis, invasion and metastasis (Rahman et al., 2001). It has been shown that Cox-2 induces angiogenesis, which in turn aids tumor growth, invasion and metastasis. It was found positive correlations between Cox-2 and iNOS expression in HCV-positive HCC and this could be partially attributable to modulation of angiogenesis by Cox-2 (Cervello and Montalto 2006). Overexpression of Cox-2 is generally higher in well-differentiated HCC compared with less-differentiated HCC or histological normal liver, with implication in the early stages of hepatocarcinogenesis (Cervello and Montalto, 2006), and increased expression of Cox-2 being significantly associated with shorter disease-free survival in patients with HCC. Cox-2 inhibitors might be effective in prevention of both cancer development and disease progression of HCC (Rahman et al., 2001; Cervello and Montalto, 2006). Experimental studies on animal models with liver cancer have shown that both selective and non-selective Cox-2 inhibitors exert chemopreventive as well as therapeutic effects.

Apoptosis is a key mechanism causing cell death and organ diseases, failure of apoptosis is now understood to contribute to the development of human malignancies. Apoptosis rarely occurs in normal livers but increases in HCC, indicating that bcl-2 and bcl-xL expression play important role in regulating the apoptosis of normal liver and HCC. The relationship between bcl-2 related genes and HCC is still unclear (Guo et al., 2002).

**BIOACTIVE POTENTIAL OF ECKLONIA CAVA**

The phlorotannins (*E.cava*) of brown seaweeds have pointed out a variety of biological effects, including anti-plasmin inhibitor (Nakayama *et al.*, 1989), anti-allergic (Sugiura *et al.*, 2006) anti-
oxidant (Ahn et al., 2007), anti-diabetes 1 (Kang et al., 2010a), anti-hypertensive (Wijesinghe et al., 2011), and hepatoprotective (Kang et al., 2012). The biological properties of the brown seaweed *E. cava* are attributed to the biologically active secondary metabolites such as phlorotannins including eckol, phloroglucinol, phlorofucofuroeckol and dieckol (Heo et al., 2009). Especially, dieckol isolated from *E. cava* has been reported to show excellent antioxidant and protective effects against oxidative stresses caused by hydrogen peroxide (Ahn et al., 2007).

Protective effect of polyphenol extracted from *E. cava* against ethanol induced oxidative damage *in-vitro* and in zebrafish model. Chang liver cell line was used, EPE extract on protective effect against ethanol-induced apoptosis and oxidative stress through the inhibition of ROS generation. Furthermore, additions of EPE extract to zebrafish model improved ethanol induced survival rate, oxidative stress and cell death, dieckol indicated that intake of EPE could be beneficial to the human health (Kang et al., 2014).

Lee et al., (2014) demonstrated that the CPC system is a useful process for the isolation and purification of phlorotannins from *E. Cava*, preparative isolation and purification of phlorotannins from *E. cava* using centrifugal partition chromatography by one-step. Crude sample from *E. cava* dried, four phlorotannins dieckol, phlorofucofuroeckol-A, 2, 7-phloroglucinol-6,6-bieckol and pyrogallol-phloroglucinol- 6,6-bieckol were isolated in high yields by a one-step CPC operation.

Kang et al., (2012) neuroprotective effects of phlorotannins isolated from a brown alga, *E. cava*, against H$_2$O$_2$-induced oxidative stress in murine hippocampal HT22 cells. HT22 murine hippocampus cell line was used, phlorotannins isolated from *E. cava* including PG, EK, TA, ES, and DK can protect neuronal cells against H$_2$O$_2$-induced neurotoxicity. Specifically, suppressing the overproduction of
intracellular ROS, increasing in intracellular Ca2+ levels, and apoptosis may contribute to the protective effects of phlorotannins against H2O2-induced oxidative stress and cell death. H2O2 is a freely diffusible form of ROS that poses an established risk to human health, particularly to neurons. *E. Cava* highlighted the potential of phlorotannins to protect against H2O2-induced neuronal toxicity.

Takahashi *et al.*, (2012) *E. cava* polyphenol (ECP) protects the liver against ethanol-induced injury in rats. Male wistar rats was used, ECP treatment suppressed the ethanol-induced increase in hepatocyte cell death by maintaining intracellular GSH levels. ECP treatment also suppressed the ethanol-induced increases in type I collagen and α-SMA, both of which are markers of HSC activation, by maintaining intracellular ROS and GSH levels. ECP protection against ethanol-induced liver injury was confirmed in *in-vivo* experiments. *E. Cava* increased serum AST and ALT activities were attenuated by administering suggest that ECP could prevent ethanol-induced liver injury.

Effect of anticoagulative sulfated polysaccharide purified from enzyme assistant extract of a brown seaweed *E. cava* (EC) on Wistar rats. Male rats (~300 g) was used, EC exhibited good anticoagulant properties comparable with commercially available fucoidan *in vitro*. In addition, in vivo studies demonstrated the successful use of EC as an anticoagulative agent (Wijesinghe *et al.*, 2011).

Brown alga *E. cava* attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. Male Sprague Dawley rats (180-220 g BW, 6 weeks old used), ECM, a polyphenol-rich extract of *E. cava*, has a potent radical scavenging activity and its administration showed a significant improvement from all the diabetic indications in experimental animals. As its mode of action, ECM activates both AMPK/ACC and PI3K/Akt signaling in C2C12 skeletal muscle cells, which is consistent with the *in-vivo*. Although the therapeutic
potential of *E. cava* is demonstrated in type 1 diabetes mellitus model (Kang *et al.*, 2010).

**PHARMACOLOGICAL ACTIVITIES OF DIECKOL**

Hemeoxygenase 1 partly mediates the anti-inflammatory effect of dieckol in lipopolysaccharide stimulated murine macrophages, RAW 264.7 and BV-2 cells were used. Dieckol at relatively lower and safer concentrations attenuated NO release and iNOS expression in murine macrophages. This suppressive effect of dieckol on NO and iNOS could be emanated from the diminished activity of PI-3 K/Akt and NF-κB signaling cascades modulated partly by HO-1. Thus, dieckol derived from brown algae appears to be a potentially indispensable natural compound to fight against the rampant cellular oxidation and inflammation through HO-1 up-regulation (Yayeh *et al.*, 2014).

Kang *et al.*, (2013) described that protective effect of a marine polyphenol, dieckol against carbon tetrachloride-induced acute liver damage in mouse. Male ICR mice were used, weighing 25–30 g, dieckol isolated from *E. cava* can act as an effective hepato-protective agent via apoptosis pathway by up-regulating the expression of Bcl-xL and down-regulation of Bax in the liver. Thus, dieckol could be used as an effective agent for preventing CCl₄ related liver diseases.

Protective effect of dieckol isolated from *E. cava* against ethanol caused damage in vitro and in zebrafish model. Chang liver cell line was used, dieckol can protect cells against damage and apoptosis by activating the expression of Bcl-xL and PARP and down-regulating Bax and cleaved caspase-3 in Chang liver cells. Dieckol isolated from *E. cava* could be useful for preventing ethanol related liver diseases. However, extensive clinical trials are suggested to be performed on patients with alcoholic liver diseases (Kang *et al.*, 2013).

Dieckol, inhibits adipogenesis through AMP-activated protein kinase (AMPK) activation in 3T3-L1 preadipocytes. 3T3-L1
preadipocytes cells used, dieckol, a marine seaweed polyphenol exhibited prominent anti-adipogenic effect and inhibits adipogenesis through down-regulation of PPARγ, C/EBPa, FABP4 and SREBP-1 in 3T3-L1 cells. Moreover, the inhibitory effect of dieckol on adipogenesis was associated AMPK activation. Therefore, dieckol may be an effective candidate for preventing obesity or obesity-related diseases (Ko et al., 2013).

Thermostability of a marine polyphenolic antioxidant dieckol, derived from the brown seaweed *E. cava*. African green monkey kidney (Vero) were used, dieckol derived from *E. cava* showed a higher thermostability than ascorbic acid which is widely used as a food additive or an antioxidant agent in the food and pharmaceutical fields. Results, suggested that dieckol, a phlorotannin derived from *E. cava*, is a useful functional ingredient for application as a natural antioxidant in food, and is more applicable than ascorbic acid to food processes where higher temperatures are used (Kang et al., 2012).

Hepatoprotective effects of dieckol-rich phlorotannins from *E. cava*, a brown seaweed, against ethanol induced liver damage in BALB/c mice. Male BALB/c mice, weighing 20–25 g were used; the liver injury induced by intake of ethanol is associated with oxidative stress. Our results indicated that DRP could reduce the ethanol induced liver injury in vivo through reducing the total cholesterol, inhibition of ROS generation and reduction of MDA formation. This hepatoprotective effect should due to the presence of bioactive compounds in the DRP (Kang et al., 2012).

Dieckol inhibits 12-O-tetradecanoylphorbol-13-acetate-induced SK-Hep1 Human Hepatoma Cell Motility through Suppression of Matrix Metalloproteinase-9 Activity. Human hepatocellular carcinoma SK-Hep1 cells were used. Dieckol inhibits TPA-induced SK-Hep1 cancer cell motility via suppression of MMP-9 activity. Also, dieckol negatively regulates MAPK signaling and AP-1 activity in response to
TPA, leading to blockade of MMP-9 transcriptional activity and MMP-9 enzyme activity, dieckol might be developed to a promising therapeutics that blocks metastasis by inhibiting motility or invasion of hepatocellular carcinoma cells, particularly of SK-Hep1 cancer cells (Oh et al., 2011).

With this literature as a background resource, clearly shows no detailed studies on an inhibitory effects of dieckol on $N$-Nitrosodiethylamine induced hepatocarcinogenesis in rats.