Introduction
CANCER is a complex disease associated with complex signaling pathways and is an outcome of dysregulated multiple molecular and cellular events (Pietras and Ostman, 2010). Moreover, it is a major socio-medical problem posing as world’s top “economic killer”. Recently, the International Agency for Research on Cancer (IARC) has released the latest cancer database providing information of the most recent estimates for 28 types of cancer (Ferlay et al., 2013). According to GLOBOCAN 2012, global cancer burden has risen to 14.1 million new cases and 8.2 million cancer related deaths worldwide (Bray et al., 2013; Siegel et al., 2013). As per the predictions made by IARC, a substantive increase of 19.3 million new cancer cases per year is estimated by 2025 (Ferlay et al., 2013). Furthermore, incidence rates of cancer remain highest in more developed countries, however the mortality (or cancer related deaths) is still higher in less developed regions due to inadequate detection systems and treatment facilities.

 Nearly, two-third of all cancer cases are linked to inadequate components in diet, environmental exposure to pollutants and toxicants present in occupational areas (Anand et al., 2008; Theodoratou et al., 2014). Accumulation of various hazardous xenobiotics in the environment has been contributing to the rising trend of cancer incidence (Jain et al., 2013). Exposures to chemical carcinogens lead to various biochemical and genetic alterations in the cell contributing to the onset of carcinogenesis. Malignancy of liver is one of the most common cancers worldwide. According to the latest statistics (GLOBOCAN 2012) liver cancer ranked second in cancer related deaths. Liver malignancy poses a major problem in less developed countries where 83% of the total estimated i.e. 782,000 new cases occurred in 2012. It is the fifth most common cancer in men and the ninth in women. Moreover,
estimated incidence and mortality in India was found to be doubled in the last five years (Ferlay et al., 2013).

Hepatocellular carcinoma (HCC) is the major histological type of liver cancer and accounts for 85-90% of total liver malignancies (Gomez and Lobo, 2011; Siegel et al., 2013). More than half a million cases of HCC are reported per year worldwide. Geographic area of Southeast Asia and sub-Sahara Africa has been reported with more prevalence of HCC cases (Altekruse et al., 2014). The incidence is expected to continue to increase tremendously for the next two decades. Unfortunately, HCC is considered as the lethal disease as its incidence rate mirrors the mortality rate. The mean age of HCC patients has been recorded between 20 and 50 years (Ferlay et al., 2013). HCC aggressiveness is accompanied by its multifactorial etiology, asymptomatic behaviour, poor prognosis and late diagnosis. Various well studied major clinical risk factors for its development include cirrhosis, chronic infections with hepatitis virus and heavy alcohol consumption (Malek et al., 2014). Moreover, environmental and acquired factors contributed to the increased likelihood of developing HCC (Herbst and Reddy, 2012). Non-viral factors associated with the HCC incidence include hereditary hemochromatosis, obesity, diabetes, inadequate diet, N-nitroso compounds, aflatoxins, pesticides, etc (Hamed and Ali, 2013). Inspite of the advances in surgical and nonsurgical therapies, a number of controversial issues regarding proper screening, diagnosis, staging, and management continue to persist and evolve.

Magee and Baren's (1956) were first to report the landmark discovery revealing hepatic carcinogenic effect of nitrosamines. Since then number of nitrosamines and N-nitroso compounds were investigated for their carcinogenic effect. Approximately 90% of nitrosamines were found to be carcinogenic in wide variety of experimental animals and exhibited organ specific effect. Nitrosamines have been linked to carcinomas of lung, liver, kidney, mammary gland, stomach, pancreas, bladder, or oesophagus (Tyagi et al., 2014). N-Nitrosodimethyamine (NDMA) and N-Nitrosodiethylamine (NDEA) are the known environmental potent hepatic carcinogens and have been used as an initiator in several hepatic cancer models (Inami et al., 2009; Bharati et al., 2012). Tobacco, cosmetics, pharmaceutical products, agricultural chemicals, colour fixatives and flavouring preservatives are the major sources of NDEA exposure (Sadik et al., 2008). Nitrosation of amines in foods has the direct correlation with the presence of nitrosamines in the body. The average human intake of NDEA through food is around 1mg/day (Glory and Thiruvengadam, 2012). Exogenous occurrence and endogenous formation of nitrosamines exaggerate its potency for
carcinogenesis (Wang et al., 2011). In addition to above contributing factors, genetic susceptibility to nitroso carcinogens, infection of certain viruses and micronutrient deficiency increases the intake levels of pro-carcinogens in the body sufficient to cause cancer. NDEA like any other nitrosamines is stable under physiological conditions.

NDEA requires activation by microsomal mono-oxygenase such as cytochrome-P450 (CYP) dependent hydroxylation to yield α-hydroxynitrosamines. Finally, NDEA is metabolized into its active ethyl radical metabolite (CH$_3$CH$_2^+$) and various reactive metabolites, which are highly reactive towards DNA, proteins and lipids, thus exhibiting carcinogenic effect (Inami et al., 2009). Human liver expresses cytochrome 2A3 and cytochrome 2E1, enzymes primarily involved in NDEA activation, thus rendering liver a target organ for its metabolism and carcinogenesis (Aiub et al., 2011a and b). NDEA in various experimental studies has been used as an inducer of HCC by modulating hepatic biochemical and molecular events (Heindryckx et al., 2009; Bharati et al., 2012). Free radicals produced during bio-activation of NDEA and reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion etc. produced during CYP metabolism might be responsible in oxidative stress. Oxidative damage to DNA, protein and lipid during NDEA exposure act as crucial factor for HCC development (Valko et al., 2006; Qi et al., 2008; Heindryckx et al., 2009).

Therapeutic approaches have been classified into three categories such as curative, palliative and symptomatic. Despite the advancement in therapeutic modalities, attainment of optimal liver cancer management is still considered as an unfinished project. Moreover, conventional therapeutic and radical attempts to get rid of the tumor through the cut, burn, and poison technique of surgery, radiation and chemotherapy have not been successful in managing HCC. An important goal of cancer research is to elucidate the processes involved in the induction of human cancer so that possible therapeutic interventions effectively combating this dreadful disease could be developed. An equally significant objective is to recognize points along the carcinogenesis pathway which may be targets for mechanism based prevention strategies. Different stages of carcinogenesis offer multiple possible targets for preventive agents to interfere the development of tumor. Carcinogenesis could be prevented by any of the following ways: modulating the xenobiotic metabolizing enzymes, scavenging the free radical product formed after activation, enhancing the carcinogen detoxification system, modulating DNA repair processes, altering gene expression involved in cell proliferation, apoptosis, differentiation, invasion, cell signaling etc (Anand et al., 2008).
Exploring and targeting specific checkpoints along the carcinogenesis pathway might serve as a beneficial attempt in preventing HCC.

Mammalian cells are equipped with highly efficient xenobiotic metabolizing system and antioxidant defense system contributing in ameliorating any sudden changes in the cellular micro-environment. Liver is the prime target organ for the metabolism of xenobiotics and drug detoxification. Phase I and Phase II xenobiotic metabolizing enzymes aid in the metabolism of the xenobiotics (or carcinogens) into the excreted form. Phase I enzymatic reaction includes oxidation, reduction or hydroxylation of carcinogen to convert to a more polar form suitable for excretion. However, in case of pro-carcinogen such as NDEA, phase I reaction mediated by cytochrome P450 isozymes resulted in the activation to its carcinogenic form (Aiub et al., 2011a and b). Phase II enzymes include transferases (e.g. methyl transferases, glutathione-s-transferases, UDP-glucuronosyltransferases) and mediate conjugation reactions converting the active product of phase I reaction to less active or inactive species. Active species so formed during these reactions if not eliminated or excreted out can be toxic for cellular macromolecules and sometime can stimulate carcinogenesis.

Normally, reactive oxygen species (ROS) such as superoxide (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$) are generated continuously under aerobic conditions during oxidative phosphorylation, P450 metabolism, respiratory pathway in peroxisomes and inflammatory action (Klaunig and Kamendulis, 2004; Pizzimenti et al., 2010). ROS are appropriate example for “Two-Faced” molecules i.e. contributing both beneficial and harmful character. The detrimental/beneficial effects of ROS depend upon the state/type of tissue and the concentration of the ROS generated. ROS may interact with multiple cellular targets including membranes, proteins and nucleic acids with potentially deleterious effects (Jomova and Valko, 2011). There are studies that have established that DNA damage by ROS is responsible for mutagenesis, oncogenesis and aging (Fruehauf and Meyskens, 2007). ROS induced lesions in DNA include base modifications, strand breaks and abasic sites (Bont and van Larebeke, 2004). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are enzymes of antioxidant defense system maintaining the concentration of ROS inside the cell (Klaunig and Kamendulis, 2004). Direct scavenging or quenching by molecules such as vitamins, β-carotene, glutathione, and coenzyme Q present a comprising non-enzymatic components of antioxidant defense system. At high levels, ROS are responsible for causing damage to cellular macromolecules including DNA, triggering
senescence and causing permeabilization of the mitochondrial membrane (Fruehauf and Meyskens, 2007).

Empiric measures of observation and experience have resulted many developed tools and approaches in medical science for the treatment of cancer. But still man has to embark a new era in the battle against cancer. The approach to combat HCC may therefore lie in exploring some novel preventive agents rather than searching new curative technology. Primary prevention involving eradication of etiological agents sounds effective but remains the theoretical approach only. Being a multi-factorial disease, cancer management requires a multi-target approach for combating carcinogenesis. Cancer chemoprevention is an analogue of clinical prevention, reducing the cancer burden through chronic administrations of synthetic, natural or biological agents to reverse, suppress, or prevent the occurrence of malignancy (Steward and Brown, 2013). Wu et al., (2011) have reported 10 FDA approved agents for the chemoprevention of breast, prostate and colon cancer hence increased the interest in exploring new agents for other cancers. Dietary derived and multi-targeted agents have attracted major attention by cancer biologists as they have the capability of affecting throughout the carcinogenic process (Wu et al., 2011). Application of natural products or nutraceuticals in modern medicine has become an important aspect for the prevention or treatment of cancer. Phyto-medicine still offers primary health care for 75-80% of the world population, mainly in the developing countries due to their cultural acceptability, better efficacy, lesser side effects, and better compatibility with the human body (Goyal, 2012). Both crude extracts and isolated principles from these plants have shown their anticancer activity in various animal models (Balunasa and Kinghorn, 2005; Gupta et al., 2008). In certain cases isolated components have shown increased potency, but in most of the cases when used for a longer duration, showed severe side effects. However, crude extracts based on traditional medicines are found to be more compatible to human body with minimal side effects (Koul et al., 2005; Mehta et al., 2010). Such observations are specifically attributed to wide range of chemical compounds present in the crude extract and their synergistic effect.

The presence of myriad chemical contributes in the action of extracts to work in a miraculous way affecting numerous evolutionary pathways of tumour development at the same time.

Large number of evidence reveals the association of phytochemicals with reduced risk of developing chronic diseases, such as cancer (Mehta et al., 2010; Singh et al., 2014). Combinatorial efforts of phytochemicals and nutritional agents with advanced cancer therapies have shown a complementary and a safe approach (Singh et al., 2014).
Administration of dietary agents has an enhancing therapeutic efficacy as it aids in mitigating the adverse effect of conventional cancer treatments. Randomized epidemiological studies and various clinical trials have yielded mixed results regarding the association of dietary intake of antioxidants and cancer incidence (Bennett et al., 2012). Chemopreventive agents possessing antioxidant property can curtail the damaging effect of ROS (or free radicals) inducing carcinogenesis. Chemopreventive agents may act by preventing the interaction between carcinogens or free radicals and DNA, or by reducing the levels of damage and mutations contributing not only in initiation but also in cancer progression (Valko et al., 2007; Yu and Kong, 2007). Chemopreventive agents may also act as tumor-suppressing agents either by altering gene expression of tumor promoting proteins or by inhibiting cell proliferation or by inducing terminal differentiation or apoptosis (Steward and Brown, 2013).

Dietary supplementation with rich fruits and vegetables can be an effective chemopreventive pathway for cancer. Some examples of promising diet-derived chemopreventive agents are folate, curcumin, genistein and tea catechins (Amin et al., 2009; Wang and Jiang, 2012). The NCI has identified about 35 plant-based foods that possess cancer-preventive properties. These include garlic, soybeans, onion, turmeric, tomatoes, ginger and cruciferous vegetables (Surh, 2003). It is important to investigate the dietary constituents for its structural design, and to incorporate parallel preclinical studies of the food source. Moreover, investigation of the isolated agent in terms of efficacy, toxicity, biological mechanisms, and pharmacokinetics is must. Carotenoids, a ubiquitous group of isoprenoid pigments, are very efficient physical quencher and scavenger of singlet oxygen and other ROS (Edge and Truscott, 2010; Fiedor and Burda, 2014). The property of carotenoid can be exploited in maintaining uncontrolled generation of ROS level in the body. Carotenoids and some metabolites have shown a protective role in ROS-mediated diseases such as, cardiovascular diseases, cancer, neurological as well as photosensitive or eye disorders. Although humans and animals are incapable of synthesizing the carotenoids, they are found in the blood and tissue. Among 700 known carotenoids, only 50 are constituents of the human diet and further 20 are present in human blood and tissues (Britton et al., 2004; Khachik, 2006). β-carotene, α-carotene, γ-carotene, lycopene, lutein, zeaxanthin, phytofluene, phytoene, cryptoxanthin, and neurosporene are the most important carotenoids present in human plasma and tissue.

Lycopene, a nutritionally important carotenoid exhibits health beneficial effects by virtue of its antioxidant activity with minimal side effects (Seren et al., 2008). It imparts red color to tomatoes, guava, watermelon, rosehip and pink grapefruit (Vogele, 1937; Egydio et al.,
and has drawn much attention as a colouring and antioxidant agent in the food industry. The structural property of lycopene has attributed to its biological function. Structurally lycopene is a linear acyclic form of β-carotene and is a non-provitamin carotenoid as it lacks β-ionone ring structure at the end. It possesses highest free-radical scavenging property due to the presence of extensive conjugated double bonds in the structure (Palozza et al., 2012). Lycopene is present in all-trans geometric form in raw tomatoes which is thermodynamically more stable. Light, heat or chemical reaction however, introduces kinks along the conjugated double bonds and contribute in the transformation of all-trans isomer into various cis-isomers (Shi et al., 2004). Lycopene metabolism in human body produces several metabolites that circulate in serum and accumulate in tissues (Ford and Erdman, 2012).

Several reports demonstrated the protective effect of lycopene against lipid peroxidation and oxidative damage in mammalian cells (Matos et al., 2000; Rizwan et al., 2011). Intake of lycopene rich diet resulted in enhanced serum lycopene level and lowered amount of lipid peroxides acting as an in vivo antioxidant (Shi et al., 2004; Akdemir et al., 2012). Consumption of tomato sauces rich in lycopene has blocked the mitochondrial DNA damage caused by ROS generation through UV radiation (Rizwan et al., 2011). Lycopene being hydrophobic in nature lies predominantly parallel with the membrane surface consistent with its protective effect (Liu et al., 2006). Besides its antioxidant property, non-oxidative mechanisms have been reported for the action of lycopene such as regulation of gap junction communication, hormonal, immune systems and metabolic pathways of xenobiotics (Bhuvaneswari and Nagini, 2005; Krishnamoorthy et al., 2013).

In association with these properties, lycopene has been found to be protective against chronic diseases such as cancer, cardiovascular disorders and degenerative diseases. Several epidemiological studies have reported the inverse relation between the intakes of tomatoes and cancer incidence like the prostate gland, stomach and lung (Liu et al., 2006; Mariani et al., 2014). Lycopene has also exerted a protective effect against several toxicities such as testicular, spermotoxicity, cardiotoxicity, hepatotoxicity and nephrotoxicity (Yilmaz et al., 2006; Koul et al., 2010; Krishnamoorthy et al., 2013). Palozza et al., (2011) have reported the cancer preventive activities of lycopene and its positive relation in lung chemoprevention. Lycopene has shown potential anticancer properties in multiple cancer cell lines. Lycopene
treatment has found to promote cell cycle arrest as evident by decreased cell viability in the majority of cell lines especially prostate cell lines (Teodoro et al., 2012).

Lycopene derived from tomato has antioxidant nutrients and has the advantage of low toxicity, therapeutic potential and is protective when administered at pharmacological doses. FAO/WHO (2006) also reported the occurrence of mixture of carotenoids and non-carotenoids such as fatty acids, acylglycerols, phospholipids and waxes in tomato extract. However, as reported lycopene is the primary constituent present in the carotenoid fraction. Moreover, research in the chemopreventive exploration of lycopene demonstrated higher protective impact of lycopene in phytocomplex mixture in comparison to purified lycopene (Stacewicz-Sapuntzakis and Bowen, 2005). According to such reports the phytochemicals of tomato may work in synergism with lycopene and potentiate the protective effects and may help in maintaining the bodily homeostasis. The results of several epidemiological, in-vitro and in-vivo studies have revealed a role of tomato products and lycopene in cancer prevention and treatment.

Therefore, further studies are warranted to understand its mechanism of action so as to completely investigate and validate lycopene as an anti-cancer agent. Considering the dreadful impact of HCC and the need for discovering potent preventive agent, lycopene extracted from tomatoes can be exploited against HCC in animal model, so as to specify the beneficial properties of lycopene in HCC prevention and treatment. Therefore, exploring the chemoprotective effect of lycopene extracted from tomatoes in HCC development and the mechanism underlying its action may present a novel study in the field of HCC prevention. The present investigation was thus, designed to address the biochemical, histological, morphological and molecular changes that occur during the liver carcinogenesis and its intervention using lycopene extracted from tomatoes.

The study has been designed with following specific objectives:

- To extract lycopene from red tomatoes (LycT) and to quantify it using UV-visible spectroscopy.
- To characterize LycT using different spectrophotometric techniques such as UV-VIS, $^1$H NMR and FT-IR.
- To demonstrate the *in vitro* antioxidant and free radical scavenging property of LycT.
- To induce hepatic carcinogenesis in female Balb/C mice, using N-diethylnitrosamine (NDEA) and to classify it histologically using Hematoxylin and Eosin (H&E) staining.
To demonstrate statistically the chemopreventive effect of the lycopene, LycT against NDEA induced hepatocarcinogenesis.

To investigate the morphological and structural changes induced due to tumorigenesis in the liver using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) and their modulation by LycT.

To evaluate the modulation of carcinogen biotransformation enzymes (Phase I and Phase II) during the process of hepatic carcinogenesis and its intervention using LycT at different stages.

To determine the modulatory effect of LycT on non-enzymatic and enzymatic antioxidant defense system (reduced glutathione, glutathione redox ratio, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase) during the process of liver tumorigenesis at different stages.

To demonstrate the effect of LycT in modulating lipid peroxidation and reactive oxygen species level during NDEA induced hepatocarcinogenesis.

To determine the modulatory effect of LycT on non-enzymatic and enzymatic antioxidant defense system (reduced glutathione, glutathione redox ratio, glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase) during the process of liver tumorigenesis at different stages.

To demonstrate the effect of LycT in modulating lipid peroxidation and reactive oxygen species level during NDEA induced hepatocarcinogenesis.

To demonstrate the genotoxic behavior of NDEA and its amelioration by LycT in hepatocytes by following experiments
  - Assessing the formation of micronuclei in hepatocytes
  - Assessing chromosomal aberration in hepatocytes

To demonstrate apoptosis using DNA fragmentation, TUNEL assay and COMET assay, and to determine the modulatory effect of LycT on mRNA and protein expression of apoptosis associated genes like caspase 3, caspase 9, bcl-2 and bax.

To determine the modulatory effect of LycT on mRNA and protein expression of cell proliferation associated genes like PCNA, p53, p21, and cyclin D1.

To determine the effect of NDEA and/or LycT on membrane physiological parameters of hepatocytes.

To assess the protective effect of LycT on the liver damage by estimating liver tumor markers such as Aspartate aminotransferases (AST), Alanine aminotransferases (ALT), Alkaline Phosphatase (ALP) and Lactate Dehydrogenase (LDH) in serum and tissue.

To determine the protective effect of LycT on carbohydrate metabolizing enzymes by estimating activity of hexokinase, phosphoglucoisomerase, aldolase, and glucose -6-phosphatedehydrogenase and glycogen content in the liver.

To assess the effect of NDEA and/or LycT on hypoxia inducible-factor (HIF-1α) in HCC.