Review of Literature
CHAPTER 2

REVIEW OF LITERATURE

“Human biology is actually far more complicated than we imagine.....Most biology will come from the complex interaction of all the proteins and cells working with environmental factors, not driven directly by the genetic code” — Craig Venter

2.1 CANCER- “AN ECONOMIC KILLER”

HUMAN system is at the highest level of organisation which is driven by cascade of complex regulatory processes. In achieving this level of organisation, nature has faced numerous challenges from the past millions and billions of years. Evolution of living cell from abiotic elements was the milestone behind the present living system. Moreover, environment has played a very crucial role in the journey of origin of life. Persistence of this beautiful creation for longer run in healthy conditions was in the hand of its descendents. It was clear that disturbance at any level of this evolutionary development will definitely create an imbalance rendering towards destruction. Unfortunately, man has disobeyed the rule made for governing this creation. Although, adapting modernization, westernization in living and forgetting his origin has given temporary relaxation and relief but has sown the seed for many more devastating problems for the present and the future. Excessive use of chemicals in agriculture, food industry, transports, infrastructure development, research and domestic fields is increasing day by day. In the current scenario, human beings are being exposed to several xenobiotics leading to life threatening diseases and disorders. Altered lifestyle and polluted environment accounts for 90-95% of most of the cancer (Figure 2.1) (Anand et al., 2008; Theodoratou et al., 2014). Involuntary addition of hazardous xenobiotics in the environment contributes to the rising trend in cancer incidence (Jain et al., 2013). Cancer continues to be the most prevalent killer worldwide. In the past few decades environment has undergone many critical changes which have drastically altered the living conditions of mankind in which accumulation of new carcinogenic factors is one of the reasons. Evidences show that the environment has changed over the same time scale as the recent rise in cancer incidence.
Cancer is a multifactorial, multifaceted, multidimensional and multimechanistic condition. This disease is outcomes of dysregulated multiple molecular and cellular events that transform a normal cell to malignant neoplastic cell (Pietras and Ostman, 2010). Cancers in past were rare and considered to be the diseases of Western countries, but over last few decades it has been frequently diagnosed in less developed or economically transitioning countries. According to the report by Livestrong and the American Cancer Society (2010), cancer has the most devastating and largest economic impact amongst any cause of death in the world (Figure 2.2). Cancer is pressing a socio-medical problem as its rates rise in several countries. Beside the major socio-medical problem, cancer is the world's top "economic killer". Quantification of the economic loss due to cancer or other chronic diseases on a global basis has received a major interest. Disability-adjusted life year (DALY) has been introduced to describe the overall burden of the diseases i.e. death and disability dimensions of illness.

Recently, the International Agency for Research on Cancer (IARC) has presented the GLOBOCAN 2012 project and contributed in World Cancer Report 2014 revealing the latest estimates related to cancer. GLOBOCAN 2012 has provided contemporary estimates of the latest incidence, mortality and prevalence of 28 major types of cancer from 184 countries of the world (Ferlay et al., 2013; Siegel et al., 2013).
Figure 2.2: Economic loss from the Top 15 global causes of death. Data obtained from the WHO Global Burden of Disease estimate for 2008 (Reported by Livestrong and American Cancer Society, 2010)

Global cancer burden risen to 14.1 million new cases and 8.2 million cancer related deaths worldwide in 2012, compared with 12.7 million cases and 7.6 million cancer-related death in 2008 (Bray et al., 2013; Siegel et al., 2013). Striking rising patterns of cancer incidence worldwide in women is another new finding of GLOBOCAN 2012. 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). There also reported inequality in cancer control and care globally. The 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.
2.2 CHEMICAL CARCINOGENESIS

2.2.1 Historical Perspective and Carcinogens

According to global epidemiologic studies, exposures to chemicals (environmental and occupational) have been identified as the biggest contributors to the burden of human cancer (Loeb and Harris, 2008). Skin absorption, ingestion and inhalation are the basic routes for the greatest surface exposure to the environmental agents acting as carcinogens. Chemical carcinogenesis is a multistage process imitating several steps and stages undergone by a normal cell to evolve progressively to a neoplastic state. The field of chemical carcinogenesis involves the exposure of chemicals to normal cell in which they acquire distinct and complementary capabilities that enable tumor growth and metastasis. Improved understanding regarding cancer cause, development, treatment and prevention is possible by increasing the descriptive knowledge of the multiple steps of chemical carcinogenesis in various experimental models. Therefore it seems reasonable to explore the importance of chemical carcinogenesis and its prevention. Advancement in the areas of molecular biology, eukaryotic cell biochemistry, cellular immunology, virology, cytogenetics, and cell culture have provided significant new insights into the mechanisms of carcinogenesis. However, still there is a gap in understanding the complete cascade of events leading to cancer and mechanisms critically targeted by anticancer agents. Thus, to fill the gap, many novel ideas and concepts are needed to be introduced into the field and the results of several provocative experiments are yet to be disseminated and shared. Multidisciplinary research in the field of chemical carcinogenesis allows predictions of possible outcomes and might open new doors to the development of alternatives or strategies with reduced risks.

The concept of chemically induced carcinogenesis was introduced in 16th century somewhere in Austria. In 1567, Theophrasti Paracelsi proposed “wasting disease of miners” might be attributed to the exposure to natural ores such as realgar (arsenic sulphide) and unraveled occupational carcinogen. Later a report published a systematic account on the work-related ‘peculiar diseases’ in several fields (Ramazzini, 1700). The English physician John Hill in mid 18th century demonstrated that snuff users developed nasal cancer more frequently than the general population (Sreedharan et al., 2007). Since then several studies have reported association between cancer and occupational exposures at the end of the 19th century. In the early 20th century, physicians and pathologists started inducing cancer in laboratory animals using chemical carcinogens. For the first time, cancer was experimentally produced by the
application of coal tar to the ear of rabbits (Yamagiwa and Ichikawa, 1951). Marked transition into the modern era of experimental cancer research began after this pioneer experiment. Sir Ernest Kennaway and his co-workers at the Royal Cancer Hospital in London, opened the door of chemical synthesis of carcinogens such as polycyclic aromatic hydrocarbons (PAH), aromatic amines etc. (Kennaway and Hieger, 1930; Waller, 1994). Parallel studies with aminoazo dyes in rats provided evidences for the experimental hepatocarcinogenicity (Kinosita, 1936). However, among various cancer models, development of multi-stage chemical carcinogenesis in mouse skin has been established and reported (Abel et al., 2009).

Simultaneously, Boveri’s theory of somatic mutation in cancer cells in 1914, laid down the genetic basis of tumor development. Later in 1980s and 1990s, the findings via molecular biology revealing proto-oncogenes and tumor suppressor genes supported above theory (Cohen, 1998). Normal cell experience many thousands of DNA damaging events per day. As expected, $10^{-5}$ and $10^{-6}$ spontaneous genetic error occur during cell division. Reactive species generated in normal cell resulted in the replacement of numerous nucleotides per cell per day. However, normal cell is equipped and evolved with an armamentarium of efficient DNA repair system counteracting the extensiveness of DNA damage by excising altered residues from DNA and maintaining the genome. Moreover, during such conditions cell also activates checkpoint signaling pathways resulting in cell cycle arrest or undergo apoptosis by recruiting immunologic and inflammatory defense system. In chemical carcinogenesis, a compound is termed as carcinogenic when its administration to experimental animals induces a statistically significant increase in the incidence of one or more histological types of malignancies or tumors when compared to the control group (Oliveira et al., 2007). Initially cancers produced from carcinogens were thought to be the result of the interactions with proteins in cells (Miller and Miller, 1952). However, at the end of 1960s the correlation between carcinogen-DNA interactions and biological potency clarified the concept of onset of carcinogenesis via genotoxic and non-genotoxic mechanism (Figure 2.3) (Luch, 2005). For initiating carcinogenesis chemical has to contribute a relative amount of DNA damage. So, that minute fraction of DNA damage escapes DNA repair system at time of replication and directs the incorporation of non-complementary nucleotides. Escaped DNA lesions initiates mutagenesis by obstructing DNA replication fork or are carried over by error-prone DNA polymerases (McCulloch et al., 2008). Such incorporations can result in causing mutation in genome stimulating carcinogenesis.
Various research groups have classified factors responsible for carcinogenesis as exogenous and endogenous (Gutierrez and Salsamendi, 2001). Exogenous factors include nutritional habits, socio-economic status, lifestyle and physical, chemical and biological agents. Excess alcohol consumption, exposure to excess of drugs, inhalation of tobacco products, ingestion of contaminated food are included in unhealthy lifestyle habits. Endogenous factors are immune system disorder, inflammation, uncertain aetiology, genetic makeup, age, endocrine balance, and physiological conditions (Oliveira et al., 2007). Great diversities of compounds (as pure chemicals or present in mixtures) have been well demonstrated as carcinogenic in humans till date. Carcinogenic compounds can be direct-acting carcinogens (do not require metabolic activation or chemical modification to induce cancer) or indirect-acting carcinogens also known as pro-carcinogens (require metabolic activation) (Weston and Harris, 2000). Alkylation agents including several anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others) and acylating agents (1-Acetyl-imidazole, Dimethylcarbamyl chloride, etc) are few examples of direct-acting carcinogen. Few examples of procarcinogens that require metabolic activation are listed as follow.
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- Polycyclic and heterocyclic aromatic hydrocarbons
  - Benz[a]anthracene
  - Benzo[a]pyrene
  - Dibenzo[a,h]anthracene
  - 3-Methylcholanthrene
  - 7,12-Dimethylbenz[a]anthracene
- Aromatic amines, amides, azo dyes
  - 2-Naphthylamine (β-naphthylamine)
  - 2-Acetylaminofluorene
  - Dimethylaminoazobenzene (butter yellow)
- Natural plant and microbial products
  - Aflatoxin B1
  - Griseofulvin
  - Betel nuts
- Others
  - Nitrosamine and amides
  - Vinyl chloride, nickel, chromium
  - Insecticides, fungicides
  - Polychlorinated biphenyls (PCBs)
  - Arsenic
  - Asbestos

2.2.2 Stages of Carcinogenesis

After conducting several in-vivo, in-vitro studies and epidemiologic assays, investigators concluded that neoplastic pathogenesis or carcinogenesis is a multistage complex process and can be divided into three distinct stages (Trosko, 2001; Oliveira et al., 2007). In last 50 years, the sequence of events has been systematically studied and this contribution has foretold some of the key concepts on the mechanisms of chemical carcinogenesis. An overriding concept has emerged that carcinogenesis requires the malignant conversion of hyperplastic cells from a benign to malignant state (Cairns, 1975). Malignant conversion includes three different and distinguished stages defined experimentally as initiation, promotion and progression, as demonstrated in figure 2.4. Alterations in the genome architecture and changes in gene expression along with several hallmarks occur across the three stages of
neoplastic development (Oliveira et al., 2007). Although human lives under very different conditions as from laboratory animals, the process of carcinogenesis is similar. However, humans are exposed to these chemicals throughout their lives thus altering the speed of the process (carcinogenesis) and frequency of mutation. Rate of phenotypical expression of the altered or mutated genes may also vary. Moreover, individual’s susceptibility and defense system may also influence the interaction and hence may modify different neoplastic stages.

**INITIATION**

The concept of multiple steps specifically first step involved in carcinogenesis was demonstrated after the seminal publication of Berenblum and Shubik (1947). The observations from several experiments enabled to conclude that initiation of carcinogenesis is due to the irreversible alterations in the genetic material. These permanent genetic alterations enforce the susceptible normal cells to malign evolution and aid in attaining immortality (Shacter and Weitzman, 2002; Trosko, 2003; Oliveira et al., 2007; Loeb and Harris, 2008). The initiated cell has taken first step towards neoplastic transformation after facing successive genotypical and phenotypical alterations (Trosko, 2003). The interactions of chemical carcinogen with DNA serve as kick-starts event for chemical carcinogenesis (Santella et al., 2005). Cell has well equipped DNA repair system; however proliferating cells have less time to repair the damaged DNA. Such mistakes lead to the establishment of covalent bond between chemical and DNA forming DNA-adducts (Poirier, 2012). The latency period of such initiated cells varies from weeks to many years. During successive cell division such initiated cells form clonal cells with mutated genetic material. As cell division is essential for sustaining the DNA damage before repair systems, therefore stem cells are more prone to carcinogenesis (Trosko, 2001). Moreover, this is an additive stage as neoplastic development depends on the dose of carcinogens. Increasing carcinogen exposure increases the incidence and the multiplicity of carcinogenesis and simultaneously reduces the latency of its manifestation. Another essential factor determining the transformation of normal cell into neoplastic cell is the position where mutation occurred in the DNA. Mutations in the genes regulating the terminal differentiation and apoptosis are essential for the neoplastic transformation (Trosko, 2001; Oliveira et al., 2007; Poirier, 2012). Besides exogenous exposure (genotoxic mechanisms) sometimes normal cells get spontaneously initiated via endogenous agents or spontaneous mutations. Spontaneously initiated cells are present in all living organisms however such initiation is less common (Gomes-Carneiro et al., 1997).
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PROMOTION

The seminal publication of Berenblum and Shubik (1947) has also introduced the concept of promotion when chemical having low carcinogenic activity were found to develop cancer in experimental animals. This stage is accompanied by increased cell proliferation so as to contribute in fixing mutations and enhance the alterations in genetic expression. In case of chemical with low carcinogenic activity, several compounds are used as promoter along with carcinogens (Williams, 2001). Promoters do not interact directly with DNA and without being metabolically activated increases cell proliferation. Indirectly promoters may damage DNA by oxidation. Earlier promoter activity was associated with the epigenetic mechanism but nowadays investigators have reported that promotion also involves genetic alterations (Hanahan and Weinberg, 2000). Up-regulation of multiple intracellular signaling pathways, including cascades involved in survival, proliferation, and cell cycle progression has resulted in uncontrolled tumor cell proliferation (Logue and Morrison, 2012). Unlike initiation stage, promotion stage is a reversible stage. Disappearance of promoter may causes regression in cell proliferation probably by apoptosis. This stage can be regulated by physiological factors and hence the extent of chemical carcinogenesis can be modulated. Large number of promoters have been investigated and some are found to be tissue specific (Gutierrez and Salsamendi, 2001). There are studies where prolonged exposure and high dose of promoter agents have shown development of neoplasia without initiation. Cells which are stimulated or dividing or have survived apoptosis take part in promotion stage when promoters are exposed to tissue (Gutierrez and Salsamendi, 2001; Trosko, 2001). Histopathology of tissue lesions during initiation and promotion are demonstrated as pre-neoplastic lesions and /or benign (Gutierrez and Salsamendi, 2001).

PROGRESSION

This stage is the most extended one and is the last stage of carcinogenesis where transformation of pre-neoplastic to neoplastic lesions occurs (Klaunig et al., 2000). The transformation into neoplastic (or malignant) lesions is accompanied by genetic and epigenetic mechanisms (Shacter and Weitzman, 2002). Irreversibility, genomic instability, invasion, metastasis, and faster growth are some of the characteristic features of progression stage. Cell proliferation during this stage is independent of any stimulus (Gutierrez and Salsamendi, 2001; Oliveira et al., 2007). During progression stage, transformed cell undergoes several biochemical, metabolical and morphological changes leading to distinct genotypical and phenotypical characteristic. Angiogenesis is one of the important
This epigenetic phenotype contributes to malignancy and any inhibition in angiogenesis may delay the neoplastic development (Hawighorst et al., 2001).

**Figure 2.4: Stages of Chemical Carcinogenesis** (Adapted from Oliveira et al., 2007)

**HALLMARK OF THREE STAGES OF CARCINOGENESIS**

- Exposure to endogenous and exogenous carcinogens
- Carcinogen dose dependent
- Carcinogen-DNA adduct formation
- Oxidative DNA adduct formation
- Irreversible genetic alterations
- Inefficient DNA repair
- Different cell population
- Selective clonal expansion
- Reversible in absence of promoting agent
- Cell proliferation (hyperproliferation)
- Inhibition of Apoptosis
- Tissue remodeling
- Differentiation
- Inflammation
- Genetic heterogeneity and instability
- Irreversible and progressive alterations
- Neoplastic (malignant) phenotype
- Cell proliferation
- Independent of stimulus
- Invasion and Metastasis
- Biochemical, metabolic and morphological alterations
- Long latency period
2.3 N-NITROSAMINES

About 100 years ago, N-Nitrosamines were introduced in the chemical literature. However, they became the subject of intensive research after the report of Magee and Barnes demonstrating the carcinogenicity of these compounds (Anselme, 1979; Magee and Barnes, 1956). Since then, investigation into the chemistry and biological effects of these compounds has accelerated. After that approximately 300 of these compounds were tested and 90% of nitrosamines were found to be genotoxic chemical carcinogens. N-Nitrosamines have nitroso group attached directly to the amine nitrogen (Figure 2.5) and exist in the following configurational forms (Figure 2.6). N-Nitrosamines are derivatives of dialkyl, alkaryl, diaryl, or cyclic secondary amines. Few examples of nitrosamines compounds have been shown in figure 2.7 such as N-Nitrosodimethylamine (NDMA), N-Nitrosomethyl ethylamine (NMEA), N-Nitrosopyrrolidine (NPYR), N-Nitrosodiethylamine (NDEA), N-Nitrosopiperidine (NPIP), N-Nitrosomorpholine (NMOR), N-Nitrosodi-n-Propylamine (NDPA), N-Nitrosodi-n-Butylamine (NDBA).

Nitrosamines are polar stable compounds which slowly decompose in light or aqueous acid solutions. The physical properties of nitrosamines depend upon the substituent groups in their molecular structures (Brown, 1999). The degree of carcinogenicity among nitrosamines varies significantly depending upon the group attached. Moreover, most of the nitrosamines are mutagens, transplacental carcinogens and organ specific. Nitrosamines have been linked to carcinomas of lung, liver, kidney, mammary gland, stomach, pancreas, bladder, or oesophagus (Tyagi et al., 2014). European Commission (2007) reported N-Nitrosodimethylamine (NDMA) and N-Nitrosodiethylamine (NDEA) to be the most potent carcinogen among the Nitrosamines, and Nitrosodiphenylamine (NDPA) to be least potent. Nitrosodimethylamine (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).

![Figure 2.5: Structure of Nitrosamine where R1 and R2 are alkyl or aryl groups](image)

Figure 2.5: Structure of Nitrosamine where R1 and R2 are alkyl or aryl groups
N-Nitrosamines are ubiquitously present in the environment and are emerging pollutants (Tricker and Preussmann, 1991; Mhlongo et al., 2009). Ender and co-workers (1964) for the first time reported confirmation of the environmental occurrence of NDMA in nitrite-preserved herring used in sheep fodder. Unequivocal proof of endogenous formation of nitrosamines reported by Sander (1967) reinforced the concern over the possible health risk posed by nitrosamines. Various nitrosamine detectors have reported the occurrence of N-Nitroso compounds in air at a wide range of concentration. The atmosphere around industrial area is highly contaminated with nitrosamines enriched omissions or due to their formation from secondary amines and nitrogenous oxides. Relatively high concentration of volatile nitrosamines have been observed in ambient air and in waste fluid of industries like leather tanning, metal working, and rubber and tyre manufacture (Scanlan, 1983). During food processing, preservation and preparation, oxides of nitrogen are formed which further react with other nucleophiles to produce different nitroso compounds. Critical analysis shows that foodstuffs most commonly contaminated with N-Nitroso compound are cured meat products, bacon, cheese, smoked fish and meat products, beer and whisky, low-fat dried milk products,
spices and pickles. Moreover, nitrosamines in rubber pacifiers and/or baby feeding rubber nipples are posing a major health concern for infants (Tricker et al., 1991). Endogenous formation of nitrosamines (carcinogenic molecule) takes place in the gastrointestinal tract (Crew, 2010). Precursor amines either from diet or endogenous protein react with nitrite to form corresponding N-Nitrosoamines under acidic condition in the mammalian stomach (Vermeer and van Maanen, 2001).

NDEA has been classified as a Group B2 carcinogen i.e. probable human carcinogen. As limited human data are available, related to the carcinogenic effects of NDEA. Tobacco, cosmetics, pharmaceutical products, agricultural chemicals, colour fixatives and flavouring preservatives are the major sources of NDEA exposure (Sadik et al., 2008; Wang et al., 2011). Human carcinogenicity data includes the cases of liver cancer in individuals being exposed in occupational settings, ingestion (cutting oils, tobacco products etc). However, there exists large database on the carcinogenicity of NDEA in various animal models such as rats, mice, hamsters, guinea pigs, rabbits, fish, dogs and monkeys. NDEA model of hepatic tumor is mainly used in basic research and demonstrates cancer development both in rats and mice (Brown-Peterson et al., 1999; Awuah et al., 2012; Bharati et al., 2012; Sharma and Janmeda, 2014). NDEA has been reported to be administered at different ages, however the outcome found to be varied in terms of efficiency and efficacy (De Minicis et al., 2013).

**Physical properties of NDEA (CAS Database Reference: 55-18-5)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (g)</td>
<td>102.14</td>
</tr>
<tr>
<td>Boiling point (°C/mm Hg)</td>
<td>176.9/760</td>
</tr>
<tr>
<td>Density (g/mL)</td>
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</tr>
<tr>
<td>Refractive index</td>
<td>1.4386</td>
</tr>
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<td>Storage temperature</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Physical appearance</td>
<td>yellow liquid</td>
</tr>
<tr>
<td>Solubility</td>
<td>Water, alcohol and ether</td>
</tr>
</tbody>
</table>
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Metabolism of NDEA

NDEA like any other N-Nitrosodialkylamines is inactive and stable under physiological conditions. The basic routes of potential exposure in human population are via ingestion, inhalation and dermal contact. Polluted air, diet and smoking contribute to human exposure of 1mg NDEA/ day (Glory and Thiruvengadam, 2012). The carcinogenic potential of NDEA is attributed to its ability of alkylating DNA structure. NDEA requires metabolic activation for presenting mutagenic and carcinogenic properties. The first step in the metabolic degradation or metabolic activation of NDEA by enzymes of mixed function cytochrome P450 (CYP)-dependent microsomal mono-oxidase system is α-hydroxylation of the alpha-carbon atom to the N-nitroso group (Zimmerman, 1993; Inami et al., 2009). The enzymes involved in the introduction of a hydroxyl group are human esophagus and liver CYP2A3 and CYP2E1 (Aiub et al., 2011a and b). The highest activities of these enzymes lie in the centrilobular hepatocytes. α-hydroxy nitrosamine thus formed undergoes spontaneous elimination of an aldehyde group resulting in the formation of ethyldiazohydroxide (Figure 2.8).

Other N-Nitrosoamide and their related compounds are chemically reactive under physiological pH forming alkyl diazohydroxide species. This intermediate compound produces electrophilic alkyl diazonium ion which is the ultimate carcinogen reacting with nucleophilic sites in DNA forming DNA adducts (Inami et al., 2009). DNA alkylation by NDEA results in various modified bases and these mutated bases serve as the critical cellular target for carcinogens. Mutated bases and adduct formation play a crucial role in stimulating the initiation of hepatocarcinogenesis. NDEA degradation or metabolism results in the formation of ethylene free radical as carbonium ion which is highly reactive species. Ethylation of N and O atoms of most bases from DNA is the triggering stimuli for tumor initiation. The transformed bases such as O°-ethylguanine and O°-ethylguanamine, if not repaired leads to tumor formation in animals (Aiub et al., 2011a). NDEA induces tumor mainly in the liver, however the exact reasons is under investigation but it has been hypothesized that presence of certain enzyme in liver is responsible for the metabolic activation of NDEA (Aiub et al., 2011b). Moreover, NDEA works in dose-dependent manner as single low initiation dose generally does not result in the formation of tumor (Teoh et al., 2008). High dose or multiple low doses induces HCC after a period of latency (Williams et al., 2000). Cancer research using mouse models has gained insights for better
understanding of the mechanism underlying the pathogenesis of HCC. Beside its DNA alkylating mechanism, oxidative stress caused by NDEA also plays an important role in the development of HCC (Qi et al., 2008; Heindryckx et al., 2009). Free radical generation and reactive oxygen species (ROS) produced during bio-activation of NDEA using CYP metabolism might be responsible in inducing oxidative stress. Hydrogen peroxide and superoxide anion are produced during CYP dependent enzymatic system and are responsible in contributing oxidative stress. 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, peroxisomes and inflammatory action (Klaunig and Kamendulis, 2004; Pizzimenti et al., 2010). ROS induced lesions in DNA include base modifications, strand breaks and abasic sites (Bont and van Larebeke, 2004). Oxidative damage to DNA, protein and lipid during NDEA metabolism is a crucial step in hepatic cancer development (Valko et al., 2006). NDEA model in experimental animals proves to be a good representation of hepatocarcinogenesis associated with poor prognosis (Lee et al., 2004).

![Metabolic activation of N-Nitrosodialkylamine](adapted from www.osha.gov)

**Figure 2.8:** Metabolic activation of N-Nitrosodialkylamine (Adapted from www.osha.gov)
2.4 LIVER - THE TARGET ORGAN

Liver is the second largest organ, accounting for approximately 2% to 3% of average body weight. Generally with age, the weight of liver relative to body weight decreases. It is also the largest gland located in the right upper quadrant of the abdominal cavity beneath the right hemi-diaphragm. Liver possesses smooth and shiny surface, reddish brown in colour, highly vascular, fragile with a soft consistency.

Gross Anatomy

Liver possesses three surfaces, viz., facies superior, facies inferior and facies posterior. The anterior and posterior view of liver shows four lobes i.e. right lobe, left lobe, caudate lobe (the more superior-under facies posterior) and the quadrate lobe (the more inferior-under facies inferior) (Figure 2.9). The superior surface is convex and is attached to the diaphragm and anterior abdominal wall by a triangular or falciform fold of peritoneum. Diaphragm separates liver from the lower parts of lungs and pleurae, heart and pericardium and the right costal arches. Traditional gross anatomy of liver reveals two lobes from the superior surface i.e. left and right lobes. Facies inferior or visceral surface is uneven, concave, directed downward, backward and to the left. It faces the stomach and duodenum, the right colic flexure, the right kidney and suprarenal gland. The inferior surface is completely invested by the peritoneum except the part where gall bladder is attached to liver and at the porta hepatis. The facies posterior lies behind the right lobe and its large part is not covered by the peritoneum and is in direct contact with the diaphragm.

Figure 2.9: Diagram representing the gross anatomy of liver. Adapted from Encyclopedia Britannica, Inc (2010)
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Rib cage protects and maintains the position of liver through peritoneal reflections, referred to as ligamentous attachment. Ligamentous attachments although, are not true ligaments as these are avascular in nature. Falciform ligament (round ligament) arising at or near the umbilicus courses cranially along the anterior surface of liver, dissect into the hepatic peritoneal covering coursing posterosuperiorly into anterior left and right coronary ligaments. This ligament is surgically important as the hepatic vein (inferior vena cava, IVC) lies below this ligament (Jamieson, 2006). Morphologically it seems that the falciform ligament divides liver into left and right lobes, however it’s not true. The IVC through IVC ligaments maintain the intimate relationship to the caudate lobe and right hepatic lobe (Kogure et al., 2007). The gastrohepatic ligament is an attachment of connective tissue between the lesser curvature of the stomach and the left hepatic lobe at the ligamentum venosum. The hepatoduodenal ligament and porta hepatis formed direct association between duodenum and liver.

Microscopic Anatomy

The structural organization reveals that the liver is a complex 3-D structure that comprises epithelial (parenchymal) and mesenchymal elements arranged in repetitive microscopic units. The structural organization and its related functional diversity are still under debate. The quantitative distribution of the number of different types of cells in liver consists of hepatocytes (78%), endothelial sinusoidal cells (2.8%), Kupffer cells (2.1%), Stellate cells (1.4%) and rest is extracellular space i.e. 16%. The microscopic anatomy of liver tissue is conceptualized in two most common models as hepatic acinus and hepatic lobule (Krishna, 2013).

Figure 2.10: Diagrammatic representation of hepatic acinus with different zones (1, 2 and 3). Where, portal vein is represented by ‘v’, hepatic artery as ‘a’, bile duct as ‘d’ and central vein as ‘t’. Adapted from Suriawinata et al., (2007)
The liver acinus consists of a small portal tract at the center and central vein at the periphery (Figure 2.10). It is considered to be the smallest functional unit and is divided in three zones i.e zone 1 (periportal); zone 2 (midportal) and zone 3 (pericentral) (Krishna, 2013). Oxygenated and nutrient rich blood from the portal tract flows through these zones and descends in the central vein with decreasing oxygen and nutrient gradient. Alternatively, the hepatic lobule model is the most accepted one in which the central vein is the central structure and the periphery is delineated by portal tracts. Liver stromal components include Glisson’s capsule (thin layer of moderately dense connective tissue) lined by the visceral peritoneum (a simple squamous epithelium). Moreover, connective tissue septa subdivide liver parenchyma into lobules and ensheathes most of the blood vessels, lymphatics, bile ducts, and nerves of liver parenchyma from Glisson’s capsule.

Figure 2.11: Diagrammatic representation of hepatic lobules and other components within hepatic lobule. Adapted from Anatomy & Physiology, Connexions (2013)

Classic liver lobule is considered as the smallest morphological unit of liver parenchyma. The approximate diameter of the liver lobule measures 0.7-2 mm. Millions of hepatic lobules like small polyhedral prism fit into each other (Figure 2.11). Transverse section of the lobules reveals regular penta- or hexagonal structure with a central vein in the middle and portal tracts at the corners. Portal tracts are channels which originate at the hilum and course
through the organ in a branching pattern. Portal tract structure includes bile ducts and ductules, hepatic artery, hepatic portal vein, lymphatic vessels, nerve fibers and inflammatory cells.

Liver receives up to 25% of total cardiac output, more than any other organ. It has a unique blood supply from two different systems i.e., it receives 25% to 30% blood supply through hepatic artery and remaining 70% to 75% from portal vein. Before draining into the systemic circulation, arterial and portal blood mixes within the hepatic sinusoids (Figure 2.12) (Blumgart and Belghiti, 2007). Arterial vasculature of liver is highly variable and complex receiving oxygenated blood through common hepatic artery. The bulk of the nutritive blood supply is provided by the portal vein to liver. The main portal vein receives blood from superior mesenteric vein, splenic vein, coronary vein, cystic vein and tributaries of the right gastric and pancreaticoduodenal veins.

The main portal vein enters the liver in form of right and left portal vein. The venous drainage is through the intrahepatic veins that drain into the IVC superiorly. Intrahepatic biliary system is comprised of multiple ducts forming biliary tree that are responsible for the formation and transport of bile from liver to the duodenum and typically follows the portal venous system. The bile is carried to the gall bladder by the cystic duct or poured directly into the duodenum where it aids in the digestion. Hepatic lymph is formed in the space of Disse and enters lymphatics via hepatic lymph nodes. The lymphatic runs in close association with hepatic veins in portal tracts. Lymphatics follow the course of the hepatic veins and convey lymph to lymph nodes around the inferior vena cava.

![Diagram showing flow of arterial and portal blood](image)

**Figure 2.12:** Diagram shows flow of arterial and portal blood into a sinusoid and then into a central hepatic vein branch. Flow of bile is in backward direction to the portal tract

Adapted from Tissupath (2014)
Hepatocytes

Dutrochet (1824) discovered the hepatocytes as “cellules vesicularies agglomerées” in liver tissue. In subsequent years F. Kieman (1833), J. Henle (1836) and J. E. Purkinje (1837) gave the description of hepatocytes (Kuntz and Kuntz, 2008).

Hepatocytes are epithelial cells overlapping each other to form a 3-D plate-like structure. Hepatocytes are radially arranged around central vein in cords of one or two cells thick separated by small capillary vessels called sinusoids. However, hepatocytes form a sheath-like layer bordering portal tracts. Structurally hepatocytes are large polygonal cells having abundant eosinophilic cytoplasm and a central round nucleus with prominent nucleoli. The general properties of hepatocytes are as follows:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of liver cells</td>
<td>300 billion</td>
</tr>
<tr>
<td>Number of hepatocytes per g of liver</td>
<td>171 million</td>
</tr>
<tr>
<td>Diameter of hepatocytes</td>
<td>20-40 μm</td>
</tr>
<tr>
<td>Proportion of hyaloplasm in cell volume</td>
<td>54.9%</td>
</tr>
<tr>
<td>Lifespan of hepatocytes</td>
<td>150 (-200) days</td>
</tr>
<tr>
<td>Mitosis rate per 10,000-20,000 liver cells</td>
<td>1</td>
</tr>
<tr>
<td>Membrane surface of hepatocytes and organelles</td>
<td>33,000 m²</td>
</tr>
</tbody>
</table>

Hepatocytes are highly polarized and their membrane has differential morphological and functional cellular polarization (Wang and Boyer, 2004). Their plasma membrane is divided by tight junctions into sinusoidal-basolateral and canicular-apical domains. About 37% is sinusoidal surface (basolateral) with absorptive and secretory function. Presence of microvilli of sinusoidal membrane in Disse’s space increases the absorption and even protrudes through the fenestrae into sinusoids. Canicular surface (apical) of hepatocytes membrane comprises about 15% and is considered as the secretory pole of the cell. The smooth intercellular fissure which is connected with Disse’s space comprises remaining 50% of the external hepatocytes membrane. Tight junctions seal this fissure from the canaliculi and allow the exchange of only water and cations. Adhering junctions including intermediate junctions and desmosomes form adhesion areas between neighbouring hepatocytes facilitating the intercellular exchange.
Sinusoidal cells

Sinusoidal cells constitute 30-40% of the total liver cell number making only a relatively small proportion of the liver volume (6.3%) (Dancygier, 2010). Endothelial cells, Kupffer cells, Ito cells, and PIT cells are different mesenchymal cell types comprising the sinusoidal cells. 70% of the sinusoidal cells are flat endothelial cells forming a continuous lining of the sinusoids. However, endothelial cells have loose connections with both the neighbouring endothelial cells and the microvilli of the hepatocytes forming numerous intercellular spaces and/or pores. Such intercellular spaces, pores and fenestrae are essential for filtering the components of the blood; function as scavenger and exchanging fluid and material between the blood in the sinusoids and the hepatocytes. Moreover, endothelial cells form and secrete cytokines, matrix components, growth factors and vasoactive substances. Toxic exposure, alcohol consumption, hypoxia, viruses or increased pressure in the sinusoids cause damage to endothelial cells thus, exposing hepatocytes to all attacks. Kupffer cells belong to the mononuclear phagocytosis system (MPS) randomly distributed in the sinus-endothelium and constitute about 25% of the sinusoidal cells. Mostly, kupffer cells are star-shaped and possess villiform surface (fuzzy coat) therefore, also termed as “stellate cells”. Various functions possessed by kupffer cells includes: phagocytosis; pinocytosis; discharge of signal substances or proteins; clearance of toxins, antigens, etc. Ito cells (fat storing hepatic stellate cells or lipocytes) are long-lived cells with long thin strands, cytoplasm with abundant fat droplets and vitamin A and situated in Disse’s space. PIT cells are lymphocytes with large granules and rod-cored vesicles found particularly in the sinusoids and in the Disse’s space. Functionally, they are natural killer cells and help in evading tumour cells or foreign cells or necrosed cells.

Myriads of functions performed by liver have attracted the interest of physiologists, dieticians, pharmacists, and researchers from time immemorial (Hall and Guyton, 2010). Important functions performed by liver for the survival of human being include:

- **Regulation, Synthesis, Secretion and Storage**

Glucose, minerals and vitamins (including fat-soluble vitamins, folate, vitamin B 12, copper, iron etc) are taken up by liver from portal and systemic blood for storage. Most blood proteins (except for antibodies) including albumin, blood clotting factors, transporter proteins etc are synthesized and secreted by the liver. Bile (known detergent in digestion and absorption of dietary fats), cholesterol, lipoproteins and many phospholipids are synthesized
in liver and then secreted out for distribution to different parts of the body or excretion for removal from the body.

- **Purification, Transformation and Clearance**

Liver removes harmful substances (ammonia, toxins, and various drugs) from the blood and transforms them into less harmful compounds. Ammonia is converted to urea and is excreted out into the urine by the kidneys. The liver takes up bilirubin (the breakdown product of red blood cell haemoglobin) from the blood and conjugates it with glucuronic acid to form water soluble bilirubin that can be excreted into bile. Liver also plays important roles in hormonal modification and inactivation. Masculine hormone testosterone and the feminising hormone estrogen are metabolized and inactivated by the liver. Liver plays a very crucial role in activating, modifying or inactivating most of the drugs before they enter the systemic circulation. Moreover, liver is responsible for detoxifying many chemical agents and poisons including alcohol.

- **Regeneration**

Liver has a remarkable capacity to regenerate after injury and to adjust its size to match its host. The appearance of circulating mitogenic factors such as hepatocyte growth factors, TNF-alpha, Interlukin-6, epidermal growth factor, norepinephrine and insulin triggers the hepatic regeneration.

- **Immunity**

Liver also functions as an organ of the immune system through the function of kupffer cells. Kupffer cells are fixed macrophages and play an important role in cleaning the blood by capturing and digesting bacteria, fungi, parasites, worn-out blood cells, and cellular debris.

### 2.5.1 LIVER CANCER

Liver being an incredible complex organ continuously filters blood and knocks 90 percent of the poisonous and unwanted chemicals out from the body. Continuous exposure to toxins and carcinogens render liver the most susceptible organ for cancer. Based on the estimates reported by IARC (2012), incidence of liver cancer ranked seventh among the major types of the cancer worldwide (Siegel et al., 2013). Liver cancer is still a major problem of the less developed regions of the world where 83% of the estimated 782,000 new liver cancer cases occurred in 2012. Detailed survey revealed that it is the fifth most common cancer in men estimating 554,000 new cases and the ninth in women (228,000 cases). The more interesting
and surprising fact related to liver cancer is its mortality rate. Liver cancer is the second most common cause of cancer related death estimating 746,000 deaths worldwide in 2012. Moreover, the prognosis of liver cancer is very poor as is evident from the overall ratio of mortality to incidence i.e. 0.95. The estimated incidence and mortality rate of liver cancer in 2012 among all cancer is demonstrated in the figure 2.13. World cancer atlas also revealed similar geographic patterns of liver cancer incidence and mortality throughout different regions of the world.

Incidence of liver cancer has shown a tremendous increase in past decades. However, the exact cause of liver cancer is unknown. Elevated prevalence of chronic hepatitis B virus (HBV) infection in parts of Asia and sub-Sahara Africa accounts for high liver cancer rates in these regions (London and McGlynn, 2006). 60% of total liver cancer cases in developing countries and 23% in the developed countries are influenced by the HBV infection. Among the developing countries, China has a major share of deaths linked to the hepatic cancer i.e. nearly 50%. Interaction of aflatoxin B1 (AFB), hepatitis C virus (HCV), alcohol related cirrhosis and other chronic diseases have been noted to increase the liver cancer incidence (Siegel et al., 2013; El-Serag, 2007). Obesity epidemic and the rise in HCV infection through continued transmission during drug abuse are the leading factors behind the increasing incidence rates of liver cancer in many parts of the world including the Unites States and Central Europe (Bosetti et al., 2008).

![Graph: Estimated cancer incidence and mortality worldwide in 2012.](GLOBOCAN 2012 (IARC) Section of Cancer Surveillance (4/8/2014))

**Figure 2.13**: Estimated cancer incidence and mortality worldwide in 2012. Adapted from GLOBOCAN 2012 (IARC) Section of Cancer Surveillance (4/8/2014)
Because of different types of cells in liver, there occurs a chance of forming several types of tumors. Tumors of liver can be classified as benign (noncancerous) or cancerous. Benign growths are not generally considered as cancer and are successfully treated with or without surgery. Haemanigoma, hepatic adenomas, and focal nodular hyperplasia are the most common types of benign growths of the liver. Basis on the origin, tumors can be described as primary liver cancer (originated within the liver i.e. cancer of hepatocytes or intrahepatic bile ducts cells) and secondary tumors or liver metastases (liver tumor developing from cancer cells of different origin mainly colorectal, gastrointestinal and melanoma cancers).

Generally, primary liver cancer is of epithelial cell origin. Histologically primary epithelial liver cancer is classified in 6 types based on the origin and histological features (Hamilton and Aaltonen, 2000). Hepatocellular carcinomas (HCC) and intrahepatic cholangiocarcinoma are the two main subtype of epithelial liver cancer. HCC is the most common and sometimes also known as hepatoma or cancer of hepatocytes. Cholangiocarcinoma is the type of cancer that starts in the cells that line the bile duct and is more common in women than in men. Moreover, there are two rare types of primary liver cancer that includes Angiosarcomas (or Haemangiosarcomas develops in the hepatic blood vessels and occur in people above 70yrs) and Hepatoblastomas (an uncommon malignant liver neoplasm occurring and affecting young children). Few symptoms and signs of primary liver cancer includes hard lump on the right side just below the rib cage, discomfort in the upper abdomen, swollen abdomen, jaundice, east bruising or bleeding, unusual tiredness, loss of appetite, nausea, vomiting, weight loss and weakness.

2.5.2 HEPATOCELLULAR CARCINOMA

Among the various common malignancies in the world, HCC accounts for 85-90% of all primary liver cancers and is a major health problem worldwide (Gomez and Lobo, 2011; Siegel et al., 2013). The incidence of HCC has doubled in past 20 years i.e. from 2.6 to 5.2 per 100,000 populations (Altekruse et al., 2014). Moreover, the incidence of HCC is twice in developing countries than that in developed countries (Altekruse et al., 2014). High incidence and extremely poor prognosis are the two particular germane of concern in HCC. HCC affected patients have the shortest survival times and accounts for the high mortality rates in both males and females. Hepatitis viral infection, toxic industrial and occupational chemicals, food additives, alcohol, fungal toxins, air and water pollutants, are the major risk factors of
HCC (Herbst and Reddy, 2012; Hamed and Ali, 2013). Cirrhosis has been recognized as the strongest factor behind the significantly higher incidence of HCC (Malek et al., 2014).

HCC begins in the few transformed hepatocytes inside the liver where it is seldom detected and moreover, it is tolerated by the liver for a certain period of time. It is believed that hepatic stem cells are the originator of HCC in most of the cases (Alison, 2005). HCC progresses with local expansion, intrahepatic spread, and distant metastases. HCC is an aggressive tumor that is frequently associated with chronic disorders and is diagnosed at a symptomatic stage when tumor has grown and advanced and treatment becomes unfeasible (Bruix 2011; Clark, 2005). Usually HCC patients have no symptoms other than those related to their chronic liver disease. Ascites, encephalopathy, jaundice, or visceral bleeding are few symptoms heightened in HCC patients. Some patients may also suffer from mild to moderate upper abdominal pain, weight loss, early satiety, or a palpable mass in the upper abdomen, indicating advanced lesion.

**Histopathology of HCC**

With the advancement of diagnostic modalities, most valuable pathomorphologic information has been suggested in recent years. HCC is a multi-step process and in most of cases it arises as a well differentiated form which is followed by stepwise process of dedifferentiation (Brunt, 2012). Premalignant lesions or equivocal nodular lesions have been classified as ‘dysplastic nodules’ (DNs) and subclassified into ‘low-grade DNs’ and ‘high grade DNs’ (Roskams and Kojiro, 2010; Park 2011). Low-grade DNs corresponding to adenomatous hyperplasia are characterized by increase in cell density and marked iron deposits. Moreover, it is highly difficult to differentiate low-grade DNs with large regenerative nodule with no premalignant potential. Low-grade DNs are devoid of sinusoidal capillarization and are characterized by altered number of portal tracts. High-grade DNs are considered as borderline HCC and correspond to ‘atypical adenomatous hyperplasia.’ Architectural atypia with small cell change, increased cell density and nuclear crowding are typical features of high-grade DNs. Sometimes varying degree of diffuse fatty changes is observed in the nodules along with sinusoidal capillarization. High-grade DNs are hardly distinguishable with early well-differentiated stage except the absence of stromal invasion (Kojiro, 2005). Development of well-differentiated HCC may take place within the DNs and may emerge directly, but there
seems to have considerable confusion in the fact. After considering numerous cases, concept of ‘nodule-in-nodule’ appearance has been appreciated.

Well-differentiated HCC are divided into two types: a distinctly nodular type (tumor nodule surrounded with fibrous capsule and/or fibrous septa) and an indistinctly nodular type (vaguely nodular appearance with indistinct margins). Characteristic features of well-differentiated early stage HCC includes increased cell density with enhanced nuclear/cytoplasmic ratio; trabecular pattern; increased eosinophilic or basophilic staining intensity; frequent fatty changes. Temporal hypoxic condition persists with insufficient unpaired arteries leading to fatty changes. Early well-differentiated HCCs receive increase blood supply because of the presence of more numbers of portal tracts with-in tumors. Early-stage HCC advanced to well-differentiated HCC with gradual dedifferentiation. Less differentiated cells are always located inside the high differentiated tissue thus giving nodule-in-nodule appearance. Tumor thrombus in the portal veins, tumor size, intrahepatic metastasis, liver cirrhosis, necrosis and hemorrhage are various factors involving in transforming early-stage HCC to advanced HCCs. Moderately differentiated HCC have abundant eosinophilic staining and possess a thicker trabecular pattern with several layers than that of well-differentiated HCCs. Further dedifferentiated tumor cells are characterized with scanty cytoplasm and higher nuclear/cytoplasm ratio.

Inspite of progress in identifying risk factors, understanding disease etiology and developing anti-viral strategies, incidence of HCC is still in rising trend. Limited therapeutic options and poor survival after diagnosis urge in developing better preventive, diagnostic and therapeutic tools urgently. Establishing animal models for HCC is essential for basic and translational studies. Over the years several rodent models have been used to study HCC pathogenesis. Cancer research using mouse models has gained insights for better understanding of the mechanism underlying the pathogenesis of HCC. Small size, high breeding capacity, short lifespan, and physiologic, molecular and genetic similarities to humans, entirely sequenced genome, and high degree of genetic and biological similarity between the processes of neoplastic development with humans have made laboratory mouse the best model to study HCC in vivo. The multistage nature of HCC has been demonstrated in both rodents and human cancer (Yang et al., 2012; Bakiri and Wagner, 2013).
2.6 MANIFESTATION OF HEPATOCELLULAR CARCINOMA (Sequential cellular 
and molecular changes)

2.6.1.1 ALTERED CELLULAR REDOX STATUS

Cytochrome oxidases (cytochrome P450 family i.e. CYPs) form a superfamily of 
haemothiolate proteins throughout the eukaryotes. They act as mono-oxygenases, 
synthesizing and degrading endogenous steroid hormones, vitamins and fatty acids 
(“endobiotics”) and moreover metabolizing foreign compounds such as drugs, pollutants and 
carcinogens (“xenobiotics”) (Nelson et al., 1996). CYPs are included in phase I xenobiotics 
metabolizing defense system. The maximum content and expression of CYPs isoforms are 
found in the liver, however other extra-hepatic sites include nervous system, gastrointestinal 
tract, kidney, lungs and adrenal glands (Anzenbacher and Anzenbacherova, 2001). 
Cytochrome P450 isozymes (CYP2A3 and CYP2E1) transformed procarcinogen NDEA into 
carcinogenic molecules generating highly reactive species (ethyl free radicals) (Rose and 
Hodgson, 2004). These enzymes function as monooxygenases and catalyze the oxidation of 
lipophilic substrates such as chemical carcinogens to more polar, hydrophilic, water-soluble 
metabolites in an attempt to facilitate their excretion from the body. At the same time, 
induction of phase I enzymes is considered to be a potential risk factor because many of the 
phase I reactions such as hydroxylation, epoxidation etc. leads to the activation of pro-
carcinogens to their ultimate carcinogenic form which is rendered suitable for interaction 
with nucleophilic sites in DNA (Lampe et al., 2000). Cytochrome b5, is another heme-protein 
found in the endoplasmic reticulum of eukaryotic cells and has been known to augment some 
CYP monooxygenase reactions (Porter, 2002). NADPH is used to reduce the oxidized CYP 
so that there is a continuous source of reduced CYP to metabolize the xenobiotic. 
Cytochrome b5 can also act as the electron donor to replenish the reduced CYP store. Liver 
being the primary organ for CYP2A3 and CYP2E1 expression is the reason for the NDEA to 
be a specific hepatocarcinogen. Ethyl free radical thus formed result in ethylation of N and O 
atoms of most bases from DNA such as N7 and O6 of guanine and sometimes O4 position 
also. O\(^\text{6}\)-ethyguanine and O\(^\text{4}\)-ethyguanine, if not repaired may serve as an initiator of 
mutation and tumor formation (Aiub et al., 2003; Ferguson and Denny, 2007).

Exposure to oxidants (ROS) alters the delicate balance between the cellular oxidants and 
antioxidants leading to oxidative stress (Figure 2.14). Alterations in redox status and 
depletion of antioxidants stimulate carcinogenesis (Franco et al., 2009). NDEA mediated 
HCC is also attributed to oxidative stress (Qi et al., 2008). Certain essential biochemical 
reactions are associated with the production of free radicals (atoms or group of atoms with
one or more unpaired electrons) and ROS during NDEA metabolism (Valko, 2005). ROS has the dual character as it is important for many cellular processes and also possess deleterious effects. In all aerobic cells, ROS are the by-products of metabolic reactions. ROS play vital roles in regulation of signal transduction, gene expression, activation of several transcription factors, Ca^{2+} accumulation, apoptosis, microbial and cytotoxic action of immune system cells, macrophages and neutrophils, as well as in aging and age-related degenerative diseases (Cao et al., 2009). Free radicals are very reactive and unstable, making them highly efficient in capturing electron from other biomolecules and hence have potential to damage cells and tissues (Takahashi et al., 2006; Fruehauf and Meyskens, 2007). Consequences of high exposure of free radicals produced during NDEA metabolism include oxidative damage to cellular macromolecules during hepatocarcinogenesis (Sung et al., 2014).

Targeting macromolecules of the cell such as proteins, lipids and nucleic acids, free radical initiates chain reactions generating variety of reactive species. Oxidative stress condition has a major contribution in the initiation and development of cancer by altering cellular processes. Unsaturated bonds in cellular and organellar membrane lipids are the primary targets for ROS and free radicals. Phospholipids, glycolipids and cholesterol are attacked by ROS resulting in structural and functional changes. Membrane physiological characteristics are altered during oxidative lipid damage in membranes. Peroxidation of polyunsaturated fatty acids results in the formation of certain reactive lipid-derived radicals such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) (Tuma, 2002). The enhanced production of nitrosamine free radicals targets in same fashion as other ROS. NDEA exposure increases the lipid peroxidation in liver tissue and has shown correlation with hepatocarcinogenicity (Sa’ńchez-Pé’rez, 2005). MDA and other final products of peroxidation are highly reactive and are responsible for further cell membrane damage. Differential MDA concentrations found in many diseases has been considered as a biomarker for evaluating oxidative stress and disease development (Sung et al., 2014). These alterations may be correlated with the structural changes of the hepatocytes such as cell rounding, organelle atypia and distorted cellular membranes. Oxidative DNA damage can lead to the formation of DNA adducts and chromosomal aberrations resulting in mutagenesis, strand breakage, genomic instability, deoxyribose oxidation, removal of nucleotides, aneuploidy, modification of bases, DNA-protein cross links, and amplification of DNA. Several types of DNA adducts formed during oxidative stress have been identified such as 8-OHdG. ROS induces oxidative lipid damage products may react with DNA to form more deleterious products. Oxidative stress mediated DNA damage (mutations), genomic instability and cell proliferation are linked with the cancer initiation and progression (Hussain et al., 2003).
Cellular proteins are also targeted by ROS during oxidative stress conditions. The conformational changes of redox-related proteins (such as kinases, transcription factors and phosphatase) modify several important enzymatic activities or protein-protein interactions such as proteolysis, alteration in electric charge, aggregation of cross-linked products and peptide fragmentation.

Figure 2.14: Oxidative Stress mediated alterations at various cellular levels attributing to the initiation of carcinogenesis

2.6.1.2 ANTIOXIDANT DEFENSE SYSTEM
Reducing environment inside the cells helps in preventing oxidative damage. Basically, cellular antioxidants defense system combat oxidative damage via three different mechanisms (Landriscina et al., 2009). Firstly, antioxidant stabilizes the free radicals by donating protons, thus affecting the cell’s redox status directly. Antioxidants delays or prevents the oxidation of substrates thus constituting body’s primary defense against oxidative damage. Secondly, activation of detoxifying and antioxidant enzymes (phase II enzymes, glutathione peroxidase, superoxide dismutase and catalase) play an important role in strengthening antioxidant defense system. Thirdly, several antioxidants have specific effects by targeting transcriptome. Decreased antioxidant level and increased exposure of ROS has been suggested to play an important role in cancer initiation (Franco et al., 2009).
The thiol redox status in the cell governs the maintenance of the reducing environment as it helps in preventing protein misfolding or aggregation. Reduced glutathione (L-γ-glutamyl-L-cysteinyl-glycine; GSH) is a ubiquitous tripeptide playing principle role in balancing the intracellular redox status by not only acting as an antioxidant, but also participating in many metabolic processes. GSH is used in the detoxification of xenobiotics mainly electrophiles forming GSH-conjugates, either spontaneously or enzymatically (Traverso et al., 2013). Glutathione conjugates are subjected to further metabolism before excretion. The glutamyl and glycinyl groups of GSH are removed by specific enzymes, and an acetyl group (donated by acetyl CoA) is added to the amino group of the remaining cysteinyl moiety (Hayes et al., 2005). Besides maintaining reducing environment, GSH also participates in the protein and nucleic acid synthesis (Dickinson and Forman, 2002). High intracellular concentration of GSH in liver cells is involved in various cellular functions regulating carcinogenic mechanisms (Estrela et al., 2006). Deregulation of GSH biochemistry has been observed in many diseases including cancers (Ortega et al., 2008; Koul and Arora, 2010; Koul et al., 2012). The glutathione effectiveness depends mainly on (i) biosynthesis and concentration of GSH in the tissue; (ii) uptake of GSH by tissue; (iii) export of oxidized glutathione (GSSG).

Glutathione-S-transferases (GST) super family of multiple isoenzymes with functional polymorphic variations are phase II detoxification enzymes (McIlwain et al., 2006). GSTs play a major role in cytosolic biotransformation processes by catalyzing the conjugation of intercellular GSH to a wide variety of endogenous and exogenous electrophilic centers including ROS, toxins, carcinogens and drugs (Hayes et al., 2005). GSH conjugation to xenobiotics increases the hydrophilicity which facilitates the metabolism of xenobiotics transforming into eliminating form (Estrela et al., 2006). GST thus play an important role in cellular antioxidant defense system against oxidative stress. Other antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) contribute in maintaining the levels of free radicals molecules (Valkó et al., 2007). SOD catalyses the dismutation of superoxide radical into hydrogen peroxide and molecular oxygen. Cells in aerobic environment are prone to superoxide radicals hence SOD acts as an important antioxidant defense. Dysregulation in SOD activity has been associated with several types of cancer such as HCC (Elchuri et al., 2005; Ganger et al., 2010). CAT is another important enzyme in cell catalyzing the decomposition of hydrogen peroxide to water and oxygen. Alterations in the activity of CAT may lead to increase in hydrogen peroxide concentration in cell which further stimulates cancer promotion (Ganger et al., 2010). GR and
GPx are primarily involved in maintaining the intercellular GSH level. In addition to CAT, GPx also chemically detoxify hydrogen peroxide using GSH as a substrate and transforming GSH into oxidized glutathione (GSSG). GSH is regenerated from GSSG through reduction mediated by GR (Dickinson and Forman, 2002).

2.6.2 ALTERED METABOLIC PATHWAYS
Cancer is also known as a metabolic disease involving several tempospatial alterations in cell physiology. Oncogenic mutations occur in presence of metabolic oxygen radicals and moreover deactivation of tumor suppressor gene further alter the metabolism and induces aerobic glycolysis (Warburg effect) (Dang, 2012). Neoplasia (abnormal cell growth) the characteristic feature of carcinogenesis has been associated with the impaired cellular energy metabolism. The Warburg effect represents robust metabolic hallmark for all cancers regardless of the tissue or cell origin (Diaz-Ruiz et al., 2011).

In normal differentiated cell, much more energy is obtained from respiration, rather than from fermentation. In the biological system, energy is stored in the form of ATP and is obtained upon hydrolysis of the terminal phosphate bond. Respiration involves aerobic glycolysis converting glucose into pyruvate followed by oxidative phosphorylation and generating high amount of ATP (Figure 2.15). On the other hand fermentation is considered as anaerobic glycolysis which takes place in normal cell during hypoxia i.e. at low oxygen concentration. Fermentation is the energy deficient process as too little ATP is formed.

Over a century, Otto Warburg (1956a) has presented the underlying phenomenon of cancer cell metabolism where he introduced the concept of “Warburg effect”. According to the work published by Warburg, cancer cell faces excessive demand of energy required by vital processes such as biosynthesis, replication, ion gradients generation, structural maintenance and proliferation. Cancer cells display enhanced lactate production (i.e. glucose fermentation) for ATP generation in place of mitochondrial oxidative metabolism (i.e. decreased respiration). Moreover, cancer cell undergoes abnormal “Pasteur Effect” as in cancer cell lactic acid production continued even in the presence of oxygen (Diaz-Ruiz et al., 2011).

In cancer cells, latency period exists in which fermentation is increased after irreversible respiration damage. Enhanced ROS in initial phase of cancer cell may be involved in injuring the mitochondrial metabolic enzymes of Kreb’s cycle. Intrinsic oxidative stress leads to
suppression of oxidative phosphorylation thus, forcing the cancer cells to increase aerobic glucose fermentation to compensate the ATP requirement (Figure 2.16). Emerging evidences have displayed certain interesting advantages in enhanced glycolytic activity with impaired oxidative phosphorylation in cancer cells. Inspite of energy deficient pathway, glucose fermentation metabolism is favored by cancer cells for compensating energy loss because of irreversible respiration damage. Fermentation energy also aids in transforming differentiated body cells into undifferentiated cells having abnormal cell division potential and decreased organelle (Gatenby and Gillies, 2004; Diaz-Ruiz et al., 2011). This metabolic switch also plays a key role in enhancing cell proliferation in hypoxic environments. Suppressed oxidative metabolism has been hypothesized for assisting cancer cells in escaping from apoptosis (Gogvadze et al., 2010).

Figure 2.15: Glucose metabolism in normal differentiated cell. Blood supplies glucose and oxygen to tissue where through diffusion process cell receives required nutrient. Inside the cell, aerobic glycolysis takes place where hexokinase converts glucose into glucose-6-phosphate which is further converted to pyruvate generating 2 ATP per glucose. In the presence of oxygen, pyruvate undergoes oxidative phosphorylation into HCO₃ and producing 36 additional ATP per glucose (Gatenby and Gillies, 2004)
High glycolytic rates indicate the increased level of glucose in cancer cell and are associated with the over-expression of glucose transporters (Gogvadze et al., 2010). Moreover, increased glycogen metabolism is correlated with the enhanced glucose utilization (Favaro et al., 2012). Upregulation of glycogen phosphorylation (PYGL) and downregulation of glycogen synthase (GYS1) in cancer cell facilitate increased glucose utilization. Moreover, increased activity of glycolytic enzymes may contribute in the high glycolytic fluxes (Pelicano et al., 2006). Conversion of nonionic glucose to an anion glucose-6-phosphate via ATP-dependent phosphorylation is primary and the rate-limiting reaction in glycolysis. This rate limiting step is catalyzed by hexokinase and the product glucose-6-phosphate (G6P) serves as a substrate for the glycolytic pathway and pentose phosphate pathway. Increased hexokinase activity in cancer cell may contribute to elevated glycolytic flux (Pelicano et al., 2006). Phosphoglucoisomerase (PGI) is a multifunctional ubiquitous cytosolic protein playing a key role in glycolysis. In the extracellular milieu it acts as a potent cytokine/mitogen and is also known as autocrine motility factor. It has been playing an important role in glycolytic and gluconeogenesis pathways, cell motility, proliferation, metastasis and angiogenesis. Emerging evidences have correlated PGI activity with cancer
progression in addition to its contribution in enhanced glycolytic pathway (Funasaka et al., 2005; Tsutsumi et al., 2009).

![Diagram of metabolic pathways]

**Figure 2.17:** Schematic representation of pentose phosphate pathway i.e. a metabolic biosynthetic pathway (Adapted from [www.t3portal.org](http://www.t3portal.org))

Cancer cell also requires metabolic sources for DNA duplication and cell growth during abnormal cell proliferation in addition to energy pool. Normal nucleotide reservoirs do not serve the demand of abnormal cancer cell replication. Upregulation of biosynthetic pathways is an urge for the continuous cell cycle in cancer cells. **Glucose-6-phosphate dehydrogenase (G6PD)** is a rate limiting enzyme catalyzing the metabolic biosynthetic pathway i.e pentose phosphate pathway (PPP) and has been associated with multiple tumors (Wang et al., 2012; Stanton, 2012; Rodriguez-Rdorriguez et al., 2013). G6PD is the major source for nicotinamide adenine dinucleotide phosphate (NADPH) which is required in many cellular system including antioxidant pathways, cytochrome P450 system and others (Figure 2.17). The major product of oxidative stage of PPP catalyzed by G6PD generates ribose-5-phosphate, basic substrate required for nucleotides and nucleic acid biosynthesis and NADPH. G6PD activity has been linked in various pathophysiological disorders such as diabetes, cancer, malaria, and other diseases (Stanton, 2012).
Chapter 2  
Review of Literature

2.6.3 CELL PROLIFERATION, HYPOXIA AND CHROMOSOMAL INSTABILITY

Cancer including HCC is a phenotypic outcome of abnormal cell proliferation. Abnormal hepatocytes proliferation plays an important role in all the stages of carcinogenesis i.e. initiation, promotion and progression (Farber, 1991). Chemical exposure stimulates regenerative hepatocytes to proliferate initiating carcinogenesis and further clonal proliferation activates nodular formation. Cell proliferation aids in the interaction of carcinogen with DNA or protein causing mutation in the nucleotide sequence. Moreover, cell proliferation is also essential in fixing the mutated nucleotide sequence in new replicated cell leading to abnormal cell population. Tumor transformation process thus includes cell proliferation as an important feature. Assessment of altered proliferative activity of liver during transformation from normal to HCC may contribute in controlling the increasing incidence. Cell cycle progression is regulated by the family of protein kinases known as cyclin-dependent kinases (CDKs) (Figure 2.18) and the protein product of the tumor suppressor Retinoblastoma susceptibility gene (pRb). The dysregulation is a common phenomenon in the tumor development. The characteristic features of different four phases of cell cycle are:

1) S phase (synthesis phase) – DNA replication occurs to divide the cell in two daughter cells.
2) M phase (mitotic phase) – Segregation of fully replicated chromosome in two daughter nuclei.
3) G\textsubscript{1} phase (gap 1 or preparatory phase) – The length of gap 1 phase is highly variable and the cell is in highly metabolic state. Cells prepare to duplicate their DNA and enter the cycle.
4) G\textsubscript{2} phase (gap 2 or preparatory phase) – Cell growth continues and protein required for replication and DNA repair are synthesized.

Phosphorylated pRb plays a central role in the transition of G\textsubscript{1}/S phase and activates CDKs. During G\textsubscript{1} phase CDK4, 6/cyclin D and CDK2/cyclin E complexes are required for the progression into S phase. Further activated CDK2/cyclin A and CDK1/cyclin B complexes enforces the checkpoints and aids in S/G\textsubscript{2} and G\textsubscript{2}/M transitions. Studies related to cyclin complexes revealed that CDK2 deficient tumor cells proliferate normally (Gladden and Diehl, 2003; Hinds, 2003). Cyclin D1 has been considered as proto-oncogene and has been associated with the development and progression of several cancers (Hughes, 2006; Yamamoto et al., 2006). Overexpression of cyclin D1 is a common event in the tumor...
development and thus, agents targeting cyclin D1 may be useful in developing anti-cancer drug.

**Figure 2.18:** Four phases of cell cycle include synthesis (S phase) and mitosis (M phase) and two preparatory or gap phases (G\textsubscript{1} and G\textsubscript{2}). Figure also represents key drivers of the cell cycle and checkpoints at which CDK inhibitors are active by Hughes, 2006)

Proliferating cell nuclear antigen (PCNA) is a nuclear protein synthesized during G1/S phase and serves as a molecular marker for determining the proliferative activity (Gramantieri et al., 2003; Mun et al., 2006). PCNA, a 36-kilodalton protein, is a central protein associated with DNA synthesis and repair. PCNA is a ring shaped homotrimeric DNA polymerase processivity factor acting as a “sliding clamp” aiding in localizing proteins to DNA (Essers et al., 2005). PCNA expression has been linked with different liver diseases and has provided different angles in understanding hepatocytes proliferation during non-neoplastic to neoplastic transformation (Lake-Bakaar et al., 2002).

Hypoxia (i.e. areas with low pO\textsubscript{2} \textless 2.5mmHg) acts as a pivotal factor of tumor biology since it can promote tumor progression and resistance to therapy. Tumor progression and development is based on the clonal selection of cancer cells having potential of surviving in adverse microenvironment. Abnormal reduction in oxygen supply in locally advanced solid tumors is a major obstacle in cancer cell survival (Masson and Ratcliffe, 2014). Imbalance between the supply and consumption of oxygen leads to the hypoxic area (Figure 2.19).
Hypoxia has been contributing majorly in chemoresistance, tumor metastasis, altered metabolism, vasculogenesis, and other essential processes (Funasaki et al., 2005; Chaturvedi et al., 2014). Moreover, hypoxia is associated with advanced stages of malignancy and hypoxic tumor is enriched with poorly to undifferentiated cancer and stromal cells (Kim et al., 2009). Hypoxia in solid tumors are developed through major pathogenic mechanism such as severe structural and functional abnormalities of microvessels, deterioration of diffusion geometry, tumor associated anemia leading to reduced oxygen transport capacity of the blood. Persistent hypoxia suppresses the proliferation of cells and arrest the cycle in G1/S phase. In contrast, hypoxia also stimulates the growth of tumor via inducing expression of hexokinase and insulin-like growth factor-2. This represents that hypoxia participates like “Janus face” character (Vaupel and Mayer, 2007). Hypoxia induces autophagy through a variety of different mechanisms. Moreover, the hypoxic regions are associated with altered cellular metabolism and poor prognosis (Xu et al., 2013).

Hypoxia-inducible factor-1 (HIF-1) is a ubiquitous protein mediating hypoxia-induced signaling. HIF-1 consists of two factors, HIF-1α (O2-regulated) and HIF-1β (O2-insensitive) maintaining O2 homeostasis (Harris, 2002). HIF-1β is also termed as aryl hydrocarbon receptor nuclear translocator (ARNT). ARNT is a constitutively expressed component of HIF-1 whereas HIF-1α is degraded by ubiquitination during normoxia. In hypoxic conditions,
HIF-la is translocated to nucleus where it binds with ARNT. Active expression of HIF-la induces expression of many genes involved in angiogenesis, glucose metabolism, survival and tumor progression. Adaptive responses occur in cancer cell due to hypoxia involves the production of vascular endothelial growth factor required for vascularization or blood vessel growth and other angiogenic cytokines, thereby enhancing tissue oxygenation and glycolytic enzymes (Semenza, 2007). Enhanced HIF-1 activity is indeed correlated with various tumor developments such as renal cell carcinoma, pancreatic cancers, breast cancer etc (Couvelard et al., 2005).

HCC is an outcome of multistep progression accumulating several inherited and somatic mutations in gatekeepers (oncogenes and tumor suppressor genes) and caretakers (genomic integrity) genes. Chromosomal instability (CIN), a main type genetic instability, is a characteristic manifestation associated with abnormal proliferation in HCC (Michor, 2005). Rapid hepatocytes proliferation in the abnormal environment may lead to DNA damage causing tumorigenesis. Abnormalities in number of chromosomes including gain or loss in the genes have been associated with cancer formation and progression. In other words, CINs are the silent features in the development of HCC (Feitelson et al., 2002). Defects in mismatch repair mechanism during constitutive proliferation may be responsible in inducing chromosomal aberrations. Moreover, mounting evidences suggest that CINs emerge during early stages of HCC formation (Aly et al., 2010). Occurrence of allelic imbalance on specific chromosome containing tumor suppressor gene and/or oncogenes has been linked with HCC development (Nishimura et al., 2002). HCC progression through common cascades of molecular events irrespective of its wide geographical variations in the incidence remains controversial.

Mitotic checkpoint defects, cohesion defects and merotelic attachment attributed to abnormal cell proliferation results in abnormal chromosome breakage from centromeric region generating daughter cells with different sets of chromosomes (Figure 2.20). Missegmentation of chromosome thus produces aneuploidy daughter cells (Holland and Cleveland, 2009). Moreover, rapid and abnormal cell proliferation results in random breaks in chromosomes. Cells with reactive chromosome arms with and without centromere contribute in early stages of carcinogenesis. Small arm without centromere is easily removed during cell divisions or is translocated to other chromosomes. Arms with centromere persist during subsequent cell divisions and are involved in forming reactive species (Martinez and van Wely, 2010). Recently several chromosomal aberrations have been analyzed and are suggested to be
associated with tumor progression, metastasis, vascular invasion and poorly differentiated phenotype. Transformation of initiated tumor cells into a more aggressive form may be related to chromosomal aberrations, however the correlation is still obscure. Moreover, understanding the extent of aberrant chromosomal regions during HCC can serve as an efficient prognostic marker predicting the stage of HCC and selecting surgical therapy for HCC management.

Figure 2.20: CIN showing arm of chromosome with and without centromere; dicentric chromosome; and pseudocentric chromosome (Martinez and van Wely, 2010)

2.6.4 APOPTOSIS AND CANCER
Liver homeostasis in normal physiological conditions is maintained by highly coordinated and tightly controlled events including proliferation, growth and programmed cell death (Pellettieri and Alvarado, 2007; Walsh, 2014). Apoptosis or programmed cell death is not an accidental event in nature but it is an orchestra of cellular process that occurs under physiological and pathological conditions to remove damaged hepatocytes. Accidental stimulation of the apoptotic machinery may result in enhanced and decreased cell death
leading to various liver disorders. Hence understanding the underlying mechanism of apoptosis may play a pivotal role in managing liver diseases. HCC is one of the scenarios where too little apoptosis is responsible for abnormal cell growth leading to malignant transformation of the affected cells (Weber et al., 2010). Under physiological conditions lack of growth factors and changes in hormonal environment act as physiological stimuli for apoptosis induction.

Apoptosis is characterized by several morphological, biochemical features and possess certain physiological significance in liver homeostasis (Wong, 2011). Cell shrinkage with membrane blebbing, but with no loss of integrity; Nuclear fragmentation and chromatin condensation; Shrinkage of cytoplasm (pyknosis); Cell fragmentation into smaller apoptotic bodies; formation of membrane bound vesicles (apoptotic bodies); and retraction of pseudopoles are the major morphological features associated with apoptosis. Biochemical features of apoptotic cells include nuclear scaffold and cytoskeleton breakdown; vital cellular protein cleavage; activation of apoptosis related enzymes (caspases); energy (ATP)-dependent process; nuclear DNA degradation; release of various factors into cytoplasm; and alterations in membrane asymmetry and recognition by phagocytic cells. Apoptosis has a physiological significance in various involution processes such as embryonic development, regression of the lactating breast, replacement proliferation in gut epithelium, thymus development in early age and removal of unwanted or aged cells. Apoptosis have been linked with various pathological conditions including AIDS associated cell death and depletion of CD4+ cells, occurrence of Hepatitis B or C, immune rejection process (cytotoxic T cell stimulated cell death), oxidative stress induced cell death, initiator of degenerative diseases (Alzheimer’s and Parkinson’s disease) and heart diseases such as myocardial infarction associated cell death.

Mechanism of Apoptosis
Cascades of caspases (cysteine aspartyl proteases) aid in implementing cell death by degrading a variety of cellular substrates (Li and Yuan, 2008). Caspases are the prime initiator and executioner in the mechanism of apoptosis. Caspases can follow three initiation pathways of apoptosis i.e. intrinsic (mitochondrial); extrinsic (death receptors) and intrinsic endoplasmic reticulum pathway (O’Brien and Kirby, 2008). Intrinsic and extrinsic are
commonly described initiation pathways of apoptosis eventually leading to a common executive pathway.

Extrinsic pathway begins with the binding of death ligands such as tumor necrosis factor (TNF-α) and FasL to death receptors (TNFR1 and CD95 respectively) (Elmore, 2007). The binding results in the formation of death-inducing signaling complex (DISC) which further stimulates the assembly and activation of pro-caspase 8. Active caspase then initiates apoptosis via cleaving executioner downstream caspases (O’Brien and Kirby, 2008).

Intrinsic pathway begins in the presence of various internal stimuli such as hypoxia, genetic stress, severe oxidative stress and cytosolic Ca^{2+} ions (Karp, 2008). Internal stimuli result in increased mitochondrial permeability and release of pro-apoptotic molecules in cytoplasm. This mitochondrial pathway is a principle integrating sensor for multiple death insults resulting in the release of cytochrome c and apoptosis inducing factor (AIF) which further orchestrates the assembly of an apoptosome complex.

Intrinsic pathway is regulated by members of Bcl-2 family consisting of both pro-apoptotic proteins (Bax, Bad, Bid, Bim etc.) and anti-apoptotic proteins (Bcl-2, Bcl-XL, Bcl-W etc.). Apoptotic factors released from mitochondrial may activate caspase 3 via apoptosome
formation or binding to inhibitors of apoptosis proteins (Kroemer, 2007). Caspase 3 then
cleaves the inhibitor and triggers nuclear apoptosis. Protein kinases, DNA repair protein,
inhibitory subunits and cytoskeleton are attacked by downstream caspases contributing in
morphological alterations (Li and Yuan, 2008). Intrinsic endoplasmic reticulum (ER)
pathway is not well understood and is hypothesized that the cellular stresses like free radicals,
oxen starvation or glucose depletion causes injury to ER leading to cell death.

**Figure 2.22**: Diagrammatic representation of mechanism of apoptosis (Wong, 2011)

Cancer is an outcome of a succession of genetic changes resulting in the transformation of
normal cell into a malignant one. Several factors contribute in the mechanism of
carcinogenesis, among these factors evasion of apoptosis play a major role in the cancer
development (Ghobrial et al., 2005). The mechanisms contributing in evasion of apoptosis and development of carcinogenesis basically include over-expression of anti-apoptotic proteins and underexpression of pro-apoptotic proteins; impaired caspases functions (reduced expression or activity); disrupted receptor signaling pathway (reduced expression of death receptor or death signals); defects or mutations in tumor suppressor genes (such as p53) and increased expression of inhibitors of apoptosis (IAPs) (Wong, 2011). Ratio of anti-apoptotic and pro-apoptotic members of Bcl-2 family play a major role in regulating apoptosis sensitivity rather than the expression level of one particular protein.

Recently, Bcl-2 has also been associated with the regulation of the intracellular redox status (Fulda, 2010). The exact mechanism behind the protective effect of Bcl-2 is still obscure; however following are some possible anti-apoptotic approach: reduction in the generation of ROS; preventing lipid peroxidation; inhibiting the release of cytochrome c from mitochondria; altering cellular glutathione pool (Lee et al., 2001). Study revealed that Bcl-2 is a known mitochondrial membrane protein and it prevents the leakage of hydroperoxides and cytochrome c from mitochondria instead of preventing hydroxide formation (Lee et al., 2001).

2.7 HEPATOCELLULAR CARCINOMA (LIVER CANCER): TREATMENT AND THERAPY

Nowadays various treatments are available with the advancement in surgical techniques and instrumentation including curative resection, ablation, liver transplantation, trans-arterial chemoembolization, radiofrequency ablation, systemic targeted agent (Raza and Sood, 2014). However, attainment of optimal liver cancer management is difficult because of high mortality in few months. Cancer management depends on the consideration of several factors such as tumor morphology, tumor vasculature, tumor metastasis, aggressiveness of the tumor, functional status of the patients and available treatment facilities. According to the Cancer Research UK, HCC undergoes four stages (www.cancerresearchuk.org). Stage 1, reveal single tumor without metastasis. Stage 2, HCC includes multiple tumors less than 5cm but with no metastasis. Stage 3, HCC multiple tumors with at least one larger than 5cm or grown into the blood vessels or has spread to organ closer to liver but not has spread to any other part of the body or lymph nodes. Stage 4, the most aggressive stage where tumors have grown into blood vessels or have spread to other part of the body. At present, sorafenib is the
only approved therapy for patients with advanced HCC, but research is continued discovering novel systemic molecular targeted agents. Stage of HCC, availability of treatment resources, severity of liver disease and clinical facilities with expertise are primary factors determining the success of the therapy (Bruix, 2011; El-Serag, 2011). Liver resection or lobectomy, transplantation and local ablation treatments are included in potentially curative approach, but are associated with 75% of 5-year survival rate (El-Serag et al., 2008). Potentially curative approach serves as a primary approach for patients with early stage HCC. Surgery for primary liver cancer is possible when cancer hasn’t spread. Liver resection including removal of the liver area containing tumor nodules may cure the HCC (Ryder, 2003; Taura et al., 2007). Up to 80% of the liver can be removed as liver has a high regenerating capability. Local ablation treatment (destroying the cancer cells) can be classified as chemical ablation and thermal ablation. Patients with small HCC are recommended for ablation therapy. Ethanol and acetic acid are frequently used chemicals for local ablation treatment. Thermal ablation uses radiofrequency, microwaves, cryoablation, lasers, and ultrasound. Transarterial therapy is based on cutting off the tumor main blood supply by providing obstruction with or without regional chemotherapy (Tsochatzis et al., 2010). Potentially curative treatments are applicable to less than 20% HCC patient because of a shortage of liver donors, late tumor stage, or liver dysfunction (Davila et al., 2012).

Chemotherapy is recommended at advanced stage of cancer where it is primarily aimed to shrink the tumor or to stop the growth of cancer. Chemotherapy usually includes chemicals with alkylating agent, anti-metabolite and anti-tumor antibiotics (Figure 2.23). Unfortunately, these chemicals aren’t biased and may attack healthy cells. However, in case of HCC, chemotherapy has limited benefits and may cause severe side effects (Lin et al., 2012). Palliative chemotherapy is used to extend the life and alleviate symptoms. Although palliative and symptomatic treatments are available for majority of HCC patient, the duration of survival for patients is of 3 years. Unfortunately, despite many advances, the treatment of HCC is unsatisfactory. The maximum 5-year survival rate provided by these treatments is of 40%. Late diagnosis, poor prognosis and asymptomatic nature of HCC are the major reason behind this failure. Moreover, improvement in the HCC treatment is observed in resource-rich countries, hence the effort does not contribute in reducing the overall mortality rate of the tumor. The answer to combat HCC may, therefore, lie with the prevention rather than cure.
2.8 AN OUNCE OF PREVENTION IS BETTER THAN A POUND OF CURE

World Cancer Report 2014 states that global cancer burden is growing at an alarming pace and thus, emphasizes the need for urgent implementation of efficient prevention strategies to curb this disease. Cancer development in humans can be divided in two phases i.e. pre-clinical phase and clinical phase. In pre-clinical phase human being is exposed to certain...
unknown insults resulting in the onset of disease without any sign or symptoms. However, clinical phase include the period after the human shows the onset of symptoms and signs. Thus, natural history of the disease ‘cancer’ indicates that cancer evolves over time and as time passes several pathological changes occurs and become irreversible. The ultimate aim of prevention is to retard or inhibit the progression. Being a multi-factorial disease, cancer management requires a multi-target approach for combating carcinogenesis. The success obtained in pre-clinical studies involving chemoprevention of cancer provides a strong mandate for adopting this approach for cancer prevention in humans. Cancer chemoprevention is a pharmacological intervention using synthetic or natural agents to prevent, block or reverse the process of carcinogenesis at its early stages (Sporn, 1976; Steward and Brown, 2013). This concept was first developed by Sporn in 1986 and was based on the two basic theories: Field Cancerisation and Multistep theory of carcinogenesis (Sporn and Suh, 2000). Chemoprevention is a prevention strategy that does not require prior knowledge of specific etiological factors (Surh, 2003). Cancer chemoprevention can prove to be beneficial in high-risk population including groups with high exposure of carcinogen or individuals who are genetically predisposed to cancer development or those with premalignant lesion (Wu et al., 2011).

Primary prevention targets early stages of pre-clinical phase i.e. preventing the exposure to risk factor or increasing individual’s resistance to them. In other words, primary prevention involves complete eradication of etiological agents initiating carcinogenesis which is possible only theoretically. However, primary chemoprevention can work by administrating certain agents to the general population or to cancer-prone population with particular risk factors (Steward and Brown, 2013). Secondary prevention is applied during the pre-clinical phase through detection or screening of cancer at an early stage so that, the treatment is more effective. Screening activities play an important component in secondary prevention. Secondary chemoprevention involves identification of individuals with early stage i.e. with pre-malignant lesions and administration of preventive agent to retard or inhibit the progression to invasive cancer. Tertiary prevention is appropriate in the clinical phase where use of treatment programmes improve the outcome of illness. Administration of preventive agents to inhibit recurrence of primary cancer in particular individual who have been successfully treated lies in tertiary chemoprevention (Steward and Brown, 2013).
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Chemopreventive agent must be safe, nontoxic, economical, highly available and effective. Absolute classification of chemopreventive agents is not possible because of unknown mechanisms possessed by these agents in demonstrating the effect. Attempts have been made to classify broadly chemopreventive agents according to the effects they have on different phases of carcinogenesis (Tamimi et al., 2002; De Flora and Ferguson, 2005).

During initiation of carcinogenesis, procarcinogen is metabolized to carcinogenic form reacting with DNA and causing irreversible damage to the genetic material. Inhibiting the formation of nitrosamines inhibits the initiation of carcinogenesis, thus can act as a chemopreventive action. Few examples of chemopreventive agents inhibiting nitrosamine formation are ascorbic acid, ferulic, gallic acid etc. Modulating the activity of cytochrome P450 enzyme has shown to exhibit chemopreventive activity. Disulfiram, the first inhibitor of cytochrome P450 has shown chemopreventive effect against dimethylhydrazine induced colon cancer (Fiala et al., 1977). Similarly, dietary phenethyl isothiocyanate, diallyl sulphide, ellagic acid etc inhibitors of phase I enzymes have shown chemopreventive effects (Wattenberg, 1987; Morse et al., 1993). In few cases, inducers of cytochrome P450 also act as blocking agents as phase I enzymes are also involved in metabolic activation of carcinogens in non-carcinogenic form or by enhancing oxidative detoxification. However, inducers of phase II enzymes are more potent chemopreventive agents than inhibitors of cytochrome P450 (Zhang et al., 1992; Zhang et al., 1994). Scavengers of electrophiles or free radicals directly have shown anti-carcinogenic effect in animal models, such as N-acetylcysteine (NAC), ellagic acid, β-carotene, phenolic antioxidants, and vitamin E (Gerhauser et al., 2003). Agents such as inducers of cell differentiation, modulators of signal transduction, inhibitors of oncogene activity and inducers of apoptosis may also be explored for producing more efficient chemopreventive agents (Tamimi et al., 2002).

Chemoprevention by natural or synthetic agents is an effective means of controlling cancer and is gaining importance as a preventive strategy (Surh, 2003). Discovering potential chemopreventive agent requires the approval of various stages or trials (Steward and Brown, 2013) (Figure 2.24). Systematic strategy based on epidemiological, laboratory, and clinical research has to be applied for exploring the compounds both naturally occurring and synthetic possessing chemopreventive potential. Various chemopreventive agents have been identified with diverse structural and functional chemical classes. Hypothesized
chemopreventive agents have to pass through preclinical and clinical testing. Preclinical testing includes a primary assessment of the chemopreventive efficacy using \textit{in vitro} and \textit{in vivo} animal models of carcinogenesis. The compounds with high efficacy and low toxicity in animal studies are approved for testing in humans.

Figure 2.24: Possible mechanism of chemoprevention. (Adapted from Tamimi et al., 2002)

Clinical chemopreventive trials include three phases for approving the chemopreventive agent. Because of the pharmacological safety of natural chemopreventive agents, they can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy induced toxicity. There is a dire need for discovery of new agents having an acceptable safety profile.
An ideal chemopreventive agent is one that is non-toxic throughout the period, effective at lower dose, orally taken, low cost and easily available. There are common three basic approaches that can be applied in producing chemopreventive agent. Pharmaceutical approach generates a chemically synthesised drug which mimics a natural substance that helps the body in restoring the normal functioning and preventing malfunctioning. These twisted forms of the natural chemicals may have similar effect but possess many undesirable effects (side effects). The nutraceutical approach also attempts to produce the required natural chemical in laboratory but through extraction or biosynthetic pathway (Saldanha and Tollefsbol, 2011). Nutraceuticals are thus more effective, less expensive and have fewer side effects. However, in nature a particular chemical is found in a package with the other chemicals producing synergistic effect. Taking high amounts of one nutraceutical that is found in the biological pathway and is protective may also cause tremendous problem in other way. Recently, for HCC chemoprevention many pharmaceutical and nutraceutical approaches have also been exploited (Shimizu et al., 2014). Although there are many reports in literature where pharmaceutical and nutraceutical have shown promising strategies for
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preventing carcinogenesis and improving prognosis of this deadly disease, yet the best way to health approach is by enriching the diet.

Among natural and synthetic agents, synthetic agents are more toxic and possess inherent resistance towards cancer therapeutic approach. Chemically synthesized and highly purified medicines exhibit pronounced effects because of their enhanced potency. However, when used for a longer duration, they may exert undesirable side effects that may sometimes be lethal (Hasler, 2003). Cancer therapy is most often accompanied by life threatening side effects rendering the treatment process highly disadvantageous to the patient. The current treatment options for cancer using cytotoxic drugs suffer from the disadvantage of being highly toxic to a wide spectrum of tissues such as heart, lungs, bone-marrow, kidney, brain etc (Sporn and Suh, 2000). Therefore, modifying diet with supplements or correcting a dietary deficiency may work as a ‘natural, intuitive or holistic’ approach. Dietary intake of fruits and vegetables hold great promise in preventing and suppressing cancer (Benetou et al., 2008). Natural products constitute a great chemical diversity offering several approaches for cancer chemoprevention due to their presence in the diet, wide availability and tolerability. Traditional medicines which are based largely on products derived from plants and animals are the primary source of health care in the rural areas of developing countries (Alves and Rosa, 2007). 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). Besides the ancient medical reports, presently all clinicians are advising natural products in cancer therapy as anticancer agents.

Researchers are now focusing on establishing a new technology on natural-product based drug discovery approach. Large number of evidences reveals the association of phytochemicals with reduced risk of developing chronic diseases, such as cancer (Mehta et al., 2010; Singh et al., 2014). Few examples of plant derived natural drugs in cancer research are vincristine, vinblastine, paclitaxel, irinotecan, and etoposide (Newmann and Cragg, 2007). Commonly known drugs from microbial sources are actinomycin D, doxorubicin, L-asparaginase, mitomycin C and bleomycin. Citarabine is the first drug originated from a marine source (Pramanik and Pandey, 2013). Apart from the aforementioned natural anticancer drugs, recently researchers have developed other promising natural agents for cancer prevention such as tea polyphenols, curcumin, capsaiacin, iso-thiocyanate, resveratrol, lycopene, luteolin, genistein etc. (Amin et al., 2009; Wang and Jiang, 2012). Moreover,
chemically synthesized pure component of natural origin may sometimes have distorted original physiological properties that are essential for their medicinal value. Extracts have the benefit of being less toxic and being more or equally powerful in combating the disease. The investigators reported that the presence of myriad number of compounds in the extract enhances the medicinal properties of active components through synergistic effect (Stahl and Sies, 2005; Chandra et al., 2012). Therapeutic activity of a medicinal plant does not lie exclusively in one single component or a few components. However, one substance is so dependent on the presence of another substance that the plant or part of the plant when used in its entirety often yields better results than any single component if used in isolation. There are many components that are considered as physiologically inert in the plant but are present in bulk are playing an indispensable factor as they contribute significantly in enhancing the medicinal value of some other component of the plant. 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. Tumor cells use multiple cell survival pathways to prevail and thus agents that can suppress multiple pathways have great potential for treatment of cancer (Pratheeshkumar et al., 2012).

Crude herbal preparations may not work like magic bullets but definitely exert beneficial effects with little or no side effects. They also tend to be more effective for long standing health problems that do not respond well to synthetic medicines. Therefore there is a need to reassess the therapeutic potential of herbal preparations supported by scientific methodology, which can lead to their rational use. The identification of such therapeutic targets involving the use of extracts would bolster the ongoing efforts directed in exploiting the beneficial effects of extracts in treatment of diseases without any fear of undesirable side effects.

2.9 LYCOPENE- THE POSTER CHILD OF TOMATO

Tomato is an incredible food with low calorie and high nutrition, and is considered as one of the world’s healthiest foods. It is the fruit of the plant Lycopersicon esculentum (also, known as Solanum lycopersicon). The tomato is a versatile food having thousands of different varieties varying in shape, size, and colour (Figure 2.26). Being the most popular fruit worldwide it is referred as “love apple” in French, “pomodoro” or “golden apple” in Itlay and so (Gentilcore, 2010). The tomatoes are originally cultivated in Mexico, most likely in Aztec civilizations. The word “tomato” is originated from the Aztecan word “tomatl” which means
“the swelling fruit”. At present tomatoes are enjoyed worldwide with the consumption of about 130 million tons per year. China is the largest tomato producing country followed by the United States, Turkey, India and Italy. Tomatoes offer several health benefits and are a treasure of riches because of their antioxidant benefits.

Figure 2.26: Pictorial representation of tomato (Adapted from www.fruitsinfo.com)

According to USDA National Nutrient database the raw nutrition value per 100g of tomatoes include:

<table>
<thead>
<tr>
<th>Nutritional Value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy</strong></td>
<td>18 Kcal</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td>3.9g</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>0.9g</td>
</tr>
<tr>
<td><strong>Total Fat</strong></td>
<td>0.2g</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>0mg</td>
</tr>
<tr>
<td><strong>Dietary Fibre</strong></td>
<td>1.2g</td>
</tr>
</tbody>
</table>

- **Phytonutrients**
  - **Lycopene** 2.5mg
  - **Carotene-β** 0.45mg
  - **Carotene-α** 0.10mg
  - **Lutein-zeaxanthin** 0.12mg

- **Vitamins**
  - **Folates** 0.02mg
  - **Niacin** 0.60mg
  - **Pyridoxine** 0.08mg
  - **Thiamine** 0.04mg
  - **Vitamin A** 833IU
  - **Vitamin C** 13mg
  - **Vitamin E** 0.54mg
  - **Vitamin K** 0.008mg

- **Electrolytes**
  - **Sodium** 5mg
  - **Potassium** 237mg

- **Minerals**
  - **Calcium** 10mg
  - **Iron** 0.3mg
  - **Magnesium** 11mg
  - **Manganese** 0.15mg
  - **Phosphorous** 24mg
  - **Zinc** 0.17mg
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Lycopene is the red colored pigment and a unique phytochemical present in the tomato in high amount. Lycopene is considered as the signature carotenoid of the tomato (Canene-Adams et al., 2005). Millardet in 1876 discovered this red colored pigment in the tomato and later it was named lycopene by Schunck (Vogele, 1937). Moreover, lycopene is a nutritionally important carotenoid present in selected red-colored fruits and vegetables other than tomatoes such as watermelon, guava, papaya, apricots, and pink grapefruits and also found in bacteria, fungi and algae (Straub, 1987; Palozza et al., 2011). Lycopene being a natural colored pigment is drawing great attention worldwide in place of synthetic compounds in food, cosmetics and pharmaceuticals. Food industry has accepted lycopene as a food additive for its health benefits and due to its preventive effects against numerous diseases (Giovannucci, 1999; Bhuvaneswari and Nagini, 2005; Seren et al., 2008).

2.9.1 BIOLOGICAL PROPERTIES AND BIOSYNTHESIS OF LYCOPENE

Lycopene is a polyunsaturated acyclic hydrocarbon with a chemical name-(all-E)-2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30 dotriacontatridecaene (Figure 2.27) and common names include \( \psi, \psi \)-carotene, all-trans-lycopene and (all-E)-lycopene (Shi and Le Maguer, 2000).

![Structure of lycopene](supplementscience.org)

Figure 2.27: Structure of lycopene (Adapted from supplementscience.org)

Naturally, it occurs as all-trans form with 11 linear conjugated and 2 non-conjugated double bonds. Its structure lacks the terminal \( \beta \)-ionic ring found in the basic structure of vitamin A hence lycopene is not the precursor for vitamin A. Lycopene possess hydrophobic characteristics due to its acyclic structure and is a lipophilic compound. Lycopene is tightly bound to vegetable fiber and thus thermal processing aids in enhancing the bio-accessible lycopene content from tomato (Colle et al., 2010; Jockaert et al., 2012).
**Physical Properties of Lycopene**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>$\text{C}<em>{40}\text{H}</em>{56}$</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>536.85 Da</td>
</tr>
<tr>
<td>Melting point</td>
<td>172-175°C</td>
</tr>
<tr>
<td>Appearance</td>
<td>Dark reddish brown</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil</td>
</tr>
<tr>
<td>Stability</td>
<td>Sensitive to light, high temperature, acids, catalyst, oxygen and metal ions</td>
</tr>
</tbody>
</table>

**Table 1: Basic properties of lycopene (Shi et al., 2002)**

Lycopene possesses highest free-radical scavenging property due to the presence of extensive conjugated double bonds in the structure (Palozza et al., 2012). In plant, lycopene plays a very important role in different metabolic pathways besides being an attractant of the fruit.

- Aids in the antioxidant protection of photosystem during photosynthesis from damage caused by excessive light (Demmig-Adams and Adams, 2002).
- Provides a substrate for the synthesis of phytohormones (Auldridge et al., 2006).
- Serves as a precursor for the synthesis of a number of biologically important compounds (Bouvier et al., 2003).
- Speculated to draw attraction, consumption and seed dissemination by pollinators and herbivores.
- Acts as a scavenger for singlet oxygen and peroxy radicals and deactivates DNA chain-breaking agents (Palozza et al., 2012).

Lycopene like any other carotenoid is synthesised by nuclear-encoded enzymes in the plastids via 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway that starts with the reaction between pyruvate and glyceraldehyde-3-phosphate (Botella-Pavia et al., 2004). Biosynthesis of lycopene thus, depends on the poly-cis pathway converting Geranyl-geranyl diphosphate (GGPP) to phytoene and phytoene to lycopene. The poly-cis pathway is catalyzed by phytoene synthase (PSY), phytoene desaturase (PDS), $\zeta$-carotene isomerase (ZISO), $\zeta$-carotene desaturase (ZDS) and prolycopene isomerase (CrtISO). Further, lycopene cyclization is catalysed by lycopene cyclase resulting in the formation of other phytomolecules including carotene and lutein. Thus, downregulation of lycopene cyclase gene is responsible for the accumulation of lycopene and hence, providing red colour to
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tomatoes. During biosynthesis, lycopene is formed in all-trans isomeric form. Light, thermal energy and chemical reactions can induce cis-isomerisation as several cis-isomers of lycopene have been identified in several processed tomato products and biological fluids and tissues (Shi et al., 2004). Moreover, cis-isomers are more stable and possess higher antioxidant potential compared to the other form (Chasse et al., 2001).

![Schematic representation of a compressed version of carotenoid biosynthesis](Cunningham and Gantt, 1998)

Figure 2.28: Schematic representation of a compressed version of carotenoid biosynthesis

(Cunningham and Gantt, 1998)
2.9.2 BIOAVAILABILITY AND METABOLISM OF LYCOPENE IN HUMANS

The main dietary source of lycopene is predominated by all-trans isomer of lycopene. However blood, plasma and tissues contain higher concentration of cis-isomer of lycopene (Campbell et al., 2007). Thermal and food processing of tomato induces the release of lycopene from food matrix and isomerisation of trans isomer to cis-lycopene. Food processing affects majorly the bioavailability of lycopene and increase the absorption. Tomato sauce, cooked tomatoes in olive oil and processed tomato products greatly increase the lycopene levels in human plasma due to higher absorption (Karakaya and Yilmaz, 2007). Cis-lycopene is more bioavailable because they have higher solubility and better absorption in intestinal lumen than trans-lycopene (Boileau et al., 2002). In-vivo study revealed that lycopene isomerisation may take places along the digestive tract in the acidic condition in gastric milieu (Fraser, 2001). Lycopene is a fat soluble compound and thus has a similar absorption in human digestive system as dietary fat. Lycopene is separated from the food matrix in the stomach and duodenum and subsequently mixed in the lipid phase. Bile salts and pancreatic lipases aid in the formation of droplets from the lycopene lipid phase and further forms multi-lamellar lipid vesicles in duodenum (Clinton, 1998; Krinsky and Johnson, 2005). Absorption of lycopene multi-lamellar lipid vesicles in small intestine is mediated via passive or diffusion process. Recently specific epithelial transporters such as scavenger receptor class B type I protein (SR-B1) transporter were discovered playing role in the absorption of lycopene (Krinsky and Johnson, 2005; Lobo et al., 2010). Small intestinal membrane possesses the major amount of SR-B1 than liver, adrenals, ovaries, placenta, kidneys, prostate and brain. Lycopene is parceled from intestinal mucosa into triacylglycerol-rich chylomicrons and flushed into lymph transport system (Roldán-Gutiérrez and Dolores Luque de Castro, 2007). Lipoprotein lipase degrades chylomicrons in the bloodstream forming chylomicrons remnants. Lycopene is a lipophilic molecule and is prone to accumulate in the plasma hydrophobic compartment of membrane or lipoprotein. After the uptake in the plasma, lycopene lipoprotein is distributed depending on its chemical structures and polarity of metabolites. The half-life of plasma lycopene ranges from 12-33 days (Rock et al., 1992). Again cis-lycopene has higher ability to be incorporated in lipoprotein due to its shorter chain length (Boileau et al., 2002). Lycopene distribution in organs and tissues is partly governed by the non-covalent interactions of lycopene with specialized protein (Liu et al., 2006). Figure 2.29 demonstrates the proposed metabolism of lycopene in human body as described by Wang (2012). Lycopene concentration varies in different organs with higher amount.
present in liver, adrenal and reproductive tissues i.e. ten times higher than other tissues (Table (Erdman, 2005). Few metabolites of lycopene and other carotenoids have also been identified in human serum and milk.

Figure 2.29: Simplified schematic representation of lycopene metabolism in human body.
(Adapted from Wang 2012)

In liver, chylomicron remnants are diffused from the blood in the hepatocytes by chylomicron receptor. Thus, liver serve as the primary organ for the accumulation of lycopene metabolites where it is incorporated into very low-density lipoproteins (VLDL) and cleared back into the blood. Further uptake of lycopene into other organs depends on the activity of LDL receptors present in the tissues. Liver, adrenal glands and testes possess higher activity of LDL receptors. Several investigations have revealed various lycopene metabolite products during different oxidizing system in vitro i.e. cleavage and oxidative products similar to those identified in serum and tissue (Lindshield et al., 2007; Ferreira et al., 2003). Central cleavage at the 15, 15' double bond by carotene 15, 15'-monoxygenase (CMO1) has been illustrated in vitro for provitamin A carotenoids leading to vitamin A formation (Figure 2.30) (Memitz and Wang, 2007). However, another asymmetric cleavage pathway by carotene-9',10'-monoxygenase (CMO2) demonstrates enzymatic cleavage of both β-carotene and lycopene. These investigations also reveal that iron is an essential cofactor for the enzymatic cleavage activity of carotenoids (Hu et al., 2006).
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All-Trans Lycopene

5-Cis Lycopene

13-Cis Lycopene

Figure 2.30: Schematic illustration of proposed lycopene metabolism and its oxidative Metabolites. Adapted from (Memitz and Wang, 2007)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Lycopene (nmol/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>0.2-1.3</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.9-21.6</td>
</tr>
<tr>
<td>Brainstem</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Breast</td>
<td>0.8</td>
</tr>
<tr>
<td>Colon</td>
<td>0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>1.3-5.7</td>
</tr>
<tr>
<td>Lung</td>
<td>0.2-0.6</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.3</td>
</tr>
<tr>
<td>Prostate</td>
<td>0.8</td>
</tr>
<tr>
<td>Skin</td>
<td>0.4</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.2</td>
</tr>
<tr>
<td>Testis</td>
<td>4.3-21.4</td>
</tr>
</tbody>
</table>

Table 2: Lycopene levels in human tissues. Data adapted from Clinton et al. (1996)
2.9.3 BIOLOGICAL FUNCTIONS AND MECHANISM OF ACTION

Several recent human epidemiologic, cell culture and animal model studies illustrate the possible pathway of lycopene metabolism and biological activities of lycopene metabolites (von Lintig, 2010; Kelkel et al., 2011). However, more research is needed for the detailed characterization of the biological activities of lycopene to provide invaluable insights into the underlying mechanism of lycopene effects in human. The in vitro study using HL-60 human promyelocytic leukaemia cells showed the growth inhibitory effect of the mixture of lycopene oxidative products, suggesting that lycopene metabolites may be acting against different stages of carcinogenesis (Ben-Dor et al., 2005). In biological systems, the reactivity of lycopene depends on a number of factors such as molecular and physical structure of metabolites, influence of other antioxidants, site of action within tissue or cells, and concentration and the partial pressure of oxygen (Britton, 1995; Young and Lowe, 2001). The mechanism behind the chemopreventive potentials of lycopene may involve alterations in pathways leading to cell growth or cell death.

Antioxidant capabilities possessed by lycopene has been ascribed for its primary biological activity. Lycopene is known to have the strongest quenching and scavenging ability towards free radicals among naturally occurring carotenoids (Young and Lowe, 2001; Palozza et al., 2012). Polyene structure with electron-rich system has made lycopene as an eligible target for electrophilic reagents. Length of the conjugated double bonds in the lycopene is related to its energy transfer reactions and hence, lycopene displays strong antioxidant capacity. Lycopene has the most efficient singlet oxygen quenching ability. Lycopene during singlet oxygen quenching reaches the triplet state from where it releases thermal energy while dispensing vibrational and rotator interactions with the solvents (Krinsky, 1998). The quenching ability of lycopene is twice as possessed by β-carotene and 10 times higher than α-tocopherol (Cantrell et al., 2003; Palozza et al., 2012).

Beside its singlet oxygen quenching ability, lycopene also accounts for another mechanism for the antioxidant activity i.e. by reacting with free radicals. Three possible mechanisms behind the free radical scavenging ability of lycopene are:

1. Adduct Formation \( (\text{R}^* + \text{Lycopene}) \rightarrow \text{R-Lycopene}^* \)
2. Electron transfer to the radical \( (\text{R}^* + \text{Lycopene}) \rightarrow \text{Lycopene}^{**} + \text{R}^* \)
3. Allylic hydrogen abstraction \( (\text{R}^* + \text{Lycopene}) \rightarrow \text{Lycopene}^{**} + \text{RH} \)

Source: Krinsky and Johanson, (2005) where \( \text{R}^* \) is any free radical.

Several reports demonstrated the protective effect of lycopene against lipid peroxidation and oxidative damage in mammalian cell. Intake of lycopene rich diet resulted in enhanced serum
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lycopene level and lowered amount of lipid peroxides acting as an in vivo antioxidant (Rao and Aggarwal, 1998; Matos et al., 2000). 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).

In past few years, greater knowledge regarding the metabolism of lycopene has attributed in understanding the action of lycopene metabolites in modulating antioxidant/detoxifying enzymes. Lycopene cleavage products have shown significant effects in the induction of detoxifying phase II enzymes via nuclear factor E2-related factor 2 (Nrf2) signaling (Ben-Dor et al., 2005). Induction of phase II enzymes by lycopene is mediated through the activation of cis regulatory DNA sequences known as antioxidant response elements (AREs) by Nrf2 transcription factor (Wang et al., 2010). Lycopene cleavage products such as apo-10'-lycopenal, apo-10'-lycopenoic acid and apo-10'-lycopenol were found to be effective in activating the Nrf2-mediated induction of hemeoxygenase-1 (HO-1) (Lian and Wang, 2008).

Thus, dietary lycopene plays an important cellular defense in response to oxidative insults through induction of detoxifying phase II enzymes. Upregulation of ARE stimulates the production of antioxidant enzymes such as superoxide dismutase, catalase, glutathione-S-transferase and quinine reductase (Ben-Dor et al., 2005).

Lycopene metabolite have been associated with chromatin condensation, DNA fragmentation, decreased Bcl-2 expression in leukaemia cells suggesting apoptosis induction. Lycopene has shown potential anticancer properties in multiple cancer cell lines (Palozza et al., 2011). 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).

In human Hep3B cells lycopene induces cell cycle arrest Park et al., (2005). Decreased cyclin D and c-myc expression may be associated with the cell-cycle arrest induced by lycopene in addition to G0/G1 and S phase arrest. Studies have reported that lycopene mediates apoptosis via activating death receptors (such as Bax and Fas ligand) in human colon cancer cells (Velmurugan et al., 2005). Variety of cellular processes is the outcome of cell signaling cascades which in turn are activated by the binding of growth factors to their cognate receptors present on the cell surface. Steroid hormones (such as androgens and estrogen) and growth factors (such as insulin-like growth factor 1-IGF-1, vascular endothelial growth factor-VEGF, epidermal growth factor-EGF, and platelet-derived growth factor-PDGF) are hypothesized to play a role in the biological action of lycopene (Herzog et al., 2005). Several studies have suggested that IGF signaling pathway plays a critical role in regulating lycopene
mediated cell-cycle progression, survival and transformation. Functional studies have suggested that PDGF is inhibited by lycopene and hence may contribute in inhibiting proliferation and migration (Lo et al., 2007; Chen et al., 2010).

Figure 2.31: Pictorial illustration of plasma membrane with gap-junction (Pearson Prentice Hall, Inc. 2005)

The gap junctional channels are specialised membrane structures mediating cell-to-cell communication and allowing the transfer of molecules less than 1000Da (such as ions, aminoacids, nucleotides, metabolites and second messengers). It consists of hemichannels called as connexons constituting of 6 subunits of connexins (Cx) (Machado-Santelli and Ionta, 2012). A wealth of knowledge is available regarding gap junction intercellular communications (GJIC) and connexin expression status in different conditions. Liver has offered a suitable system to study GJIC and modulation during cell proliferation, differentiation, cell death, and hepatocarcinogenesis.

The assumption, that GJIC plays an important role in the development of carcinogenesis and in growth control is an old concept (Loewenstein and Kanno, 1966). Malignant transformation is characterized by an inhibition of GJIC and its improvement may prevent the malignant process. Chemoprevention by carotenoids is found to be related with the improvement of GJIC which is independent of the antioxidant properties of the carotenoids (Liu et al., 2009). Among various carotenoids, lycopene was found to be much more potent in inhibiting cell proliferation and stimulating cell-to-cell communication (Livny et al., 2002). However, the mode of mechanism by which lycopene enhances GJIC in cancer cells is unclear but may be correlated with the increased expression of protein connexins.
2.10 LYCOPENE AND CANCER CHEMOPREVENTION

Several epidemiological studies have reported the inverse relation between the intake of tomatoes and cancer incidence (Heber, 2000; Mariani et al., 2014). As early as in 1950s, for the first time in vivo effects of lycopene demonstrating increased survival and lower incidence in radiation-induced peritoneal tumors was reported (Forssberg et al., 1959; Lingen et al., 1959). Then a report published in 1999 by Giovannucci and his co-workers stated that tomato based food might be playing protecting role in reducing cancer. After that lycopene and tomato based products have attracted the attention of various research groups focussing on cancer preventive as a potential cancer fighting foods. As a result, large number of observations from various studies has influenced the scenario about lycopene and cancer prevention particularly for prostate cancer. There are reviews and research studies reporting positive, neutral (inconclusive) and negative correlation of lycopene intake and cancer. Some observational studies have reported a protective effect with the use of tomato products (Jain et al., 1999; Tzonou et al., 1999). However, few observations failed to report the protective effect of lycopene in prostate cancer (Key et al., 1997; Cohen et al., 2000). Moreover, there were also few controversial facts regarding the intake of tomatoes that is whether the results would be consistent with all tomato products and preparations. A review by Miller et al., (2002), summarizes the accumulated research correlating lycopene, tomato products and prostate cancer risk, by reporting that consuming tomato products as a part of a health dietary pattern might be responsible for decreasing the risk of prostate cancer or other chronic diseases. Also a meta-analysis of observational studies exploring the association of lycopene and tomato products in the prevention of prostate cancer conducted by Etminan et al., (2004) reported an inverse association between lycopene consumption and the risk of prostate cancer. These studies also confirmed the strong preventive effect of cooked tomato products than for high intakes of raw tomatoes. A more recent epidemiological study confirmed the findings that lycopene is associated with 30-40% lowered risk of developing prostate cancer (Giovannucci, 2002; Kirsh et al., 2006; Ilic et al., 2011). In prostate cancer, lycopene has delayed the development of high-grade prostate intraepithelial neoplasia into tumor (Mohanty et al., 2005). In vitro studies have also reported that lycopene in 20-60μM concentration had inhibited the proliferation of prostate cancer cells (Gunasekera et al., 2007; Kanagaraj et al., 2007).

In addition to prostate cancer, increasing evidences suggest that lycopene is associated with decrease risk of lung, breast, colon, leukemic and digestive tract cancers (Heber, 2002; Salman et al., 2007; Kirsh et al., 2006). In lung cancer chemoprevention, originally β-
carotene was the most extensively studied agent. Auto-catalytic pro-oxidant activity observed in the lungs of smokers has raised the negative issue against its use in heavy smokers and is no longer being used for lung cancer chemoprevention (Omenn, 1998; Cohen and Khuri, 2003). However, Stahl and Sies (1996) examined lycopene as a potent antioxidant and had found a positive correlation between lycopene consumption and lower lung cancer risk. Several studies have reported that lycopene molecule possesses preventive measure against the formation and the development of lung cancer (Palozza et al., 2011). Studies involving breast cancer in Chinese women have shown an inverse relation of lycopene consumption with risk of breast cancer (Huang et al., 2007). In cell culture analysis, lycopene has shown to inverse the proliferation of human colon carcinoma, erythroleukemia, Burkii lymphoma cell lines and chronic lymphocytic leukemia.

As mentioned above, various observational and clinical researches have suggested that dietary intake of lycopene is associated with reduced risk of several human cancers, but still this protective association remains to be explored with HCC. Till date very few epidemiological studies, clinical studies, in vivo studies and in vitro studies are available relating the chemopreventive effect of lycopene but still the results are ambiguous (Ip and Wang, 2014). Supportive evidence stemmed from epidemiological studies where, lycopene rich diet is associated with cancer mortality but is yet to be discovered with HCC (Giovannucci et al., 2002). Moreover, few studies have also reported the preventive effect of lycopene (1-10μM) in inhibiting the proliferation of human liver cancerous cells and preventing metastatic process (Hwang and Lee, 2006). Liver chemoprevention with lycopene has limited in vivo studies. Lycopene supplementation has shown inhibitory effect against liver-specific carcinogen NDEA induced HCC in animal experimental models (Wang et al., 2010). Moreover, depending on the HCC experimental models, dietary lycopene has shown variable effect in ameliorating liver cancer development (Watanabe et al., 2001). Besides positive outcomes i.e. reports illustrating the inverse association between consumption of tomato and/or lycopene and cancer risk, there are reports showing negative outcomes. Differences in the route of lycopene incorporation form of lycopene and vehicle solvent may have resulted in no preventive effect of lycopene in cancer. Moreover, not enough literature is available regarding the therapeutic application of lycopene. In contrast, there are studies revealing no beneficial effects of lycopene intake and correlation with the reduced risk of cancers. Despite promising reports, there is not enough data demonstrating the complete benefits of lycopene supplementation (Etminan et al., 2004; Seren et al., 2008). Therefore, these effects are modest. Despite the preventive benefits of lycopene found in these studies,
the existing evidences are not overwhelming enough to recommend the use of lycopene supplements in the cancer prevention. Moreover, lycopene research lacks clear clinical evidences demonstrating it as a suitable antitumorigenic drug (Rackley et al., 2006). In addition to considerable interest in lycopene as a therapeutic agent, qualitative studies are required for supporting and providing strength so that a promising preventing agent can be established.

Single antioxidants generally are not as effective as combination of phytochemicals. It is the synergistic interactions between phytochemicals which finally attributes for their health benefits as seen in case of pure lycopene and tomato lycopene extract (Sarkar et al., 2012). Although, among commonly occurred dietary carotenoids, lycopene has the most potent antioxidant potentials, but still combinations of other carotenoids with lycopene are even more effective. Synergism between different carotenoids enhances the protective effect of lycopene (Heber, 2002). Tomatoes are a rich source of lycopene along with vitamins C and E, β-carotene, and flavonol. And all these components exert antioxidant effects in different levels. Clinical trials using β-carotene, vitamin C and vitamin E supplements alone did not reduce cardiovascular disease risk. Moreover, pure lycopene has shown less chemopreventive effect as it is less efficiently absorbed than the lycopene-rich tomato carotenoid. In addition, the matrix in which lycopene is administered play a crucial role in lycopene uptake (Tanaka et al., 2012).

2.11 LYCOPENE AND HEALTH BENEFITS

Pathogenesis of various chronic diseases such as cancer, diabetes, cardiovascular disease and neurodegenerative disorders have been associated with the damage caused by free radicals or reactive oxygen species (ROS). In this context, cellular macromolecules (lipids, protein and nucleic acids) are the targets of free radicals/ROS. Excessive free radical generation or accumulation in the cell eventually leads to oxidative stress which acts as a triggering factor in the initiation of various disorders (Rao and Rao, 2007). In oxidative stress induced disorders, antioxidants provide an additional effective means to combat the deleterious effects of free radicals or ROS. Agents scavenging free radicals or enhancing cellular defense system have thus, gained a lot of importance in recent years as potential prophylactic and therapeutic agents in many diseases. Epidemiologic studies have reported reduced risk of chronic diseases associated with the intake of tomato or tomato products (Agarwal and Rao, 2000). Lycopene rich foods in diet have shown an inverse relation with oxidative DNA damage and its protective effects against DNA damage has been reported in the lymphocytes (Zhao et al.,
2006; Watters et al., 2007). Reduced lipid and protein oxidation was observed in human having lycopene in their diet (Rao et al., 2007).

Lycopene, a unique carotenoid has found capabilities in improving skin cellular functions by defending some of the basic factors of skin aging and damage caused by UV-light exposure (Rao and Rao, 2007). In the battle against skin aging lycopene confer protection by acting as antioxidant, free radical scavenger, reducing inflammation, blocking UV light, encouraging cell renewal and inhibiting DNA damage. Topical application of lycopene aids in defending the harmful effects of UVB radiation through suppression of an enzyme known as ornithine decarboxylase (ODC) (Fazekas et al., 2003). ODC is an initiator and rate limiting for polyamine biosynthesis and development of photo-carcinogenesis. Hydrophobic nature and small molecular size of lycopene give advantage in easily absorption in skin lipid-rich environment when applied topically (Lopes and Reed, 2010). Lycopene plays a vital role in influencing the thickness, strength and fluidity of skin cell membranes by preventing oxidative damage in lipid membrane (Shi et al., 2004). Moreover, lycopene also acts as a cell communication enhancer thus, improving skin texture and keeping skin younger. Lycopene has also been associated with the decreased activity of enzymes involved in collagen destruction and hence strengthening the skin integrity (Huang et al., 2007).

In recent past years, epidemiological and experimental studies have demonstrated the protective effect of lycopene in diabetes management (Suzuki et al., 2002; Ali and Agha, 2009; Kuhad et al., 2008). Diabetes is an outcome of chronic metabolic disorder eventually leading to disturbed carbohydrate, lipid and protein metabolism. Research shows an inverse association between serum lycopene and plasma glucose and fasting insulin concentration (Coyne et al., 2005). Administration of lycopene (90mg/kg body weight) in hyperglycaemic rats caused a significant decrease in glucose levels, decrease in oxidative stress, increased antioxidant status and an increase in insulin concentration (Ali and Agha, 2009). However, lycopene lack specific and detailed data regarding relationship between lycopene and other diabetic complications. Moreover, a report from Wang et al., (2006) showed low evidence for a protective association between plasma lycopene and the risk of diabetes in middle aged and older women.

Numerous epidemiological investigations have reported the protective role of lycopene in various cardiovascular diseases including coronary heart disease, cerebrovascular diseases, congestive heart failure, hypertension and congenital heart disease (Rao and Rao, 2007). Higher blood lycopene levels have been connected with lower risk of heart attack (Hak et al., 2004). Elevated low density lipoprotein cholesterol (LDL-C) levels, lifestyle and genetic
factor are key risk factors for cardiovascular diseases (Vasan et al., 2004). These risk factors eventually lead to the oxidative stress playing a role in the pathogenesis of these diseases. Lycopene being an oxygenated carotenoid has proven to be the great antioxidant in reducing cardiovascular risk. Lycopene slows the progression of atherosclerosis by inhibiting the tissue damage deriving to oxidative process (Riccioni et al., 2007a). LDL oxidation is a basic step in atherosclerotic plaque formation and several studies reported that intake of tomato supplement products retards the oxidation of LDL particles (Hadley et al., 2003; Burton-Freeman et al., 2012; Silaste et al., 2012). Study has shown decreased serum lipid peroxidation and LDL oxidation after having lycopene rich diet (Sesso et al., 2004). Serum lycopene have been associated with the decreased atherosclerotic plaques in the aorta compared to the control group (Hu et al., 2008). Lycopene has also been linked with decreased blood cholesterol levels which may be due to its inhibitory action on HMG-CoA reductase (Ried and Fakler, 2011). Moreover, several anti-inflammatory actions of lycopene may be responsible for its preventive effect against excessive proliferation of vascular smooth muscle cells (Palozza et al., 2010 a and b).

Lycopene has also exerted a protective effect against several toxicities such as testicular, spermatoxicity, cardiotoxicity, hepatotoxicity and nephrotoxicity (Yilmaz et al., 2006; Beştaş et al., 2008; Koul et al., 2010). Lycopene acts as a protector in DNA damage against $\gamma$-radiations (Srinivasan et al., 2007). Dietary supplementation or adequate intake of lycopene along with vitamin A rich foods may be beneficial in asthmatic subjects (Riccioni et al., 2007b). Lycopene has also shown the modulatory effect against 7,12-dimethylbenz (A) anthracene induced hepatic clastogenicity in murine model (Koul et al., 2010). Moreover, recently a study has also investigated the protective role of lycopene in periodontal disease also. Bacterial colonization in periodontal tissues induces ROS-mediated tissue damage including damage to gingival hyaluronic acid and proteoglycans and others. Lycopene has also found effective in lowering the effect of elevated ROS level in periodontal tissue as reviewed by Bhardwaj et al., (2013).

Male infertility is another disorder increasing nowadays, and that is associated with the high rate of oxidative damage as sperms are highly vulnerable to lipid peroxidation (Said et al., 2004; Durairajanayagam et al., 2014). Elevated ROS levels are a major factor behind idiopathic male factor infertility. Few studies have reported the antioxidant therapy using lycopene in managing the male factor infertility (Mohanty, 2001; Durairajanayagam et al., 2014). Moreover, there are studies where lycopene has prevented the elevation of Sertoli cellular apoptosis and hence aided in treating infertility (Krishnamoorthy et al., 2013).
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Lycopene helps in boosting sperm concentration, sperm motility and functional characteristics. Neurodegenerative is also an oxidative stress induced disorder as neurons are vulnerable to free radical damage. Again lycopene in few studies has been used as protective agent however no conclusive outcome is reported.

2.12 Lacunae
HCC is a major public health problem in many parts of the world and is also considered as a multifactorial disease. Persistent oxidative stress due to various xenobiotics is an important contributor in HCC as it causes alteration in many cellular processes such as proliferation, signaling, cell death, inflammation and others. The remedy to this problem lies in the chemoprevention where an agent with multi-faceted biological actions has strong capability against cancer. Lycopene has recently become the poster child of bioactive substances found in food that demonstrate health benefits. Epidemiological and in-vitro evidences suggest that lycopene possess multifaceted biological actions and hence it has a potential to fight against multifactorial disease i.e. HCC. Till now the strongest clinical evidences for the benefits of lycopene in cancer have been reported for prostate cancer and lung cancer on the basis of clinical and animal studies. Moreover, there is enough experimental evidence to support that consumption of tomato extract rather than pure lycopene has anticancer potential. The identification of therapeutic targets involving the use of extracts would bolster the ongoing efforts directed in exploiting the beneficial effects of extracts in treatment of diseases without fear of undesirable side effects. Considering the above mentioned facts, it seems worthwhile to explore the involvement of lycopene extracted from tomatoes in hepatic cancer prevention.