Chapter-2

Review of Literature

2.1 Cancer

Cancer refers to the uncontrolled proliferation of abnormal cells in the body. It develops when the body's standard control mechanism stops functioning. Old cells fail to die and grow beyond the control, producing abnormal cells. These cells can form a mass of tissue known as a tumor.

2.1.1 Causes

Cancer is caused in different body parts by varying factors. Nevertheless, it is treated as one of the leading causes of death (Karim-Kos *et al*., 2008).

- 5-10% of cancer is due to genetic defects inherited from the patient's parents, while 90-95% of cancer is due to environmental and lifestyle factors (Anand *et al*., 2008).
- Infection by certain viruses like hepatitis B, hepatitis C, *Helicobacter pylori*, human papillomavirus (HPV), and human immunodeficiency virus (HIV) is responsible for 15% of cancer (Plummer *et al*., 2016).
- There are also specific physical, chemical and biological agents in interaction with genetic factors resulting in cancer (WHO, 1996).

2.1.2 Physical agents

UV-rays, X-rays and γ-rays; they can induce mutation and lead to genomic instability. For example, in 1986 Popescu and his colleagues conducted an experiment where they treated human foreskin fibroblasts invitro irradiated with UV rays and observed structural chromosomal abnormality as well as numerical chromosomal alterations. (Popescu *et al*., 1986).

The mineral fibres such as asbestos are primarily found in foodstuffs like meat, vegetables, and oils and are found in the cement used for construction. These asbestos fibres can stimulate genetic mutations, chromosomal abnormalities, neoplastic transformation, exchange of sister chromatids and chromosome deletions

(Ault *et al*.,1995). They can induce various types of cancer (Hansen and Mossman, 1997). Also, in factories, the workers inhale materials of silica fibres having carcinogenic properties leading to lung cancer (Hoet *et al*., 2004).

2.1.3 Chemical agents

Chemical agents may be of natural origin or manufactured synthetic ones. They differ in structure and fall into various chemical classes such as organic, inorganic, plastic, fibres, hormones etc.. They originate from natural as well as industrial processes. For example, Yamagawa and Ichikawa in 1915, produced tumor in rabbits by frequent application of coal tars on their skin. It turns out to be a pioneer in experimental cancer research (Vaessen *et al*., 1988).

Chemical carcinogens are again classified based upon their biological activities. They are genotoxic carcinogens which are DNA reactive and non-genotoxic carcinogens that are epigenic or non-DNA reactive. Genotoxic carcinogens are further classified into direct carcinogens that are active without any metabolic activation and indirect or procarcinogens that are active only after metabolic activation (Choudhuri *et al*., 2012).

Table: 1. List of some chemical carcinogens and the organs affected by them

Chemicals/ suspected carcinogens	**Organs affected**
Aflatoxin	Liver
4-Aminobiphenyl	Bladder
Arsenicals	Lung, skin
Diesel exhaust	Lung
Benzene	Leukemia
Cigarette smoking	Lung
Pipe smoking	Lip
Soot	Scrotum
Dyes (aromatic amines)	Urinary bladder
Nickel compounds	Lung
Vinyl chloride	Liver
Radium (radioactive watch colors)	Bone
Formaldehyde	Nose
Snuff	Nose
Diethylstilbestrol	Genital tract

Marquardt (1999)

2.1.4 Biological agents

Certain steroid hormones, bacteria, and viruses can increase the risk of cancers. Certain steroid hormones that activate cell growth and reproduction in somatic and sexual organs are observed to cause breast, prostate and endometrial cancer (Soto and Sonnenschein, 1985). Certain bacteria like *Heliobacter pylori* that cause peptic ulcers can increase gastric cancer risk (Versalovic, 2003). Then, the fungi *Aspergillus flavus* living on stored foods produce a fungal toxin named Aflatoxin B1 (AFB) which can cause infection and act as a risk factor for hepatocellular carcinoma and liver carcinoma (Abarca *et al*., 2000; Madden *et al*., 2002) where it degenerates the DNA double helix leading to mutations or deletions of nitrogenic bases during DNA synthesis. Rous Sarcoma is the first virus discovered to cause cancer in animals in 1911. Presently, numerous viruses can induce cancer and known as tumor viruses (Russel, 1998). These viruses can activate the primary tumor genes abnormally owing to cause cancer (Steven and Lowe, 2000).

2.1.5 Characteristics of cancer

Unlike normal cells, cancer cells undergo uncontrolled proliferation leading to tumor formation. Normal cells communicate with each other and respond to various signals, while cancer cells fail to interact with other cells and do not respond to signals. Normal cells can undergo either repair mechanisms or apoptosis (programmed cell death), but cancer cells cannot undergo repair mechanisms and apoptosis. Normal cells remain stuck together by the substances secreted by them, but cancer cells lack these substances. They can travel to nearby locations or distant tissues in the body via the lymph channels or bloodstream. Normal cells have the sticking property to stay together at one location. Cancer cells can migrate to neighbouring tissues and other regions, as they lack the adhesion molecules and form

a tumor in the new location (Klerkx *et al*., 2013; Aoyagi *et al*., 2013; Fukui *et al*., 2013). Normal cells can reproduce and stop when there are required cells, but cancer cells can reproduce rapidly at a high rate even before the existing cells mature. Normal cells can attain maturity whereas, cancer cells remain immature. In normal cells, the immune system can detect the damaged cells and remove them. However, cancer cells have the ability to trick the immune system thus can form a tumor. Normal cells have specific functions to perform and can act according to it; at the same time, cancer cells are non-functional. Normal cells show a similarity in size, whereas cancer cells vary in size and have an abnormal shape. Then the nucleus of cancer cells is even darker and more prominent with excess DNA. The chromosomes of cancer cells are abnormal in number and are packed in a disorganized manner. Normal cells undergo angiogenesis (forming new blood vessels for the supply of oxygen and other nutrients) only when necessary, but cancer cells undergo excessive angiogenesis even when growth is unnecessary (Gopinadh, 2015).

2.2 Carcinogenesis

Carcinogenesis is the result of insult to cells caused by anyone or combined action of physical, chemical, biological or genetic agents. It involves the changes that occurred to the genome of the neoplastic or transformed cell. Such genetic changes can result from mutations such as insertions, transitions, transversions, deletions, chromosomal rearrangements, or gene amplifications (Harris, 1991; Schwab and Amler, 1990; Bell *et al*., 1991). The process of carcinogenesis is divided into 3 stages: Initiation, Promotion and Progression (Pitot, 1993).

2.2.1 Initiation

The first stage of carcinogenesis. It is the irreversible changes or genetic alterations that happen to the target somatic cells. It can be simple mutations,

transitions, transversions or deletions in the DNA segment, creating the ability for neoplastic development (Cox, 1994), which transforms the damaged cell and its progenies to succeeding neoplastic transformation. Oncogenes are the human DNA sequences accountable for transformation. Numerous oncogenes have been isolated through molecular cloning, e.g. Burkitt's lymphoma, human bladder carcinoma, lung carcinoma, breast carcinoma, and many others. Even though two or more oncogenes appear to be essential for transformation, one study reveals that one-hit kinetics may activate initiation (Bishop, 1982). For example, in the case of human bladder carcinoma, Ha-ras protooncogene is converted to a potent oncogene by a single point mutation. It was the first recognized human oncogene mutation (Tabin *et al*., 1982). Such oncogene mutations can disrupt normal cellular behavior and response and damage the genes involved in controlling the proliferation of the cell. Even though a single mutation can initiate neoplastic cellular development, it requires subsequent gene mutation or varying cellular environments to express neoplastic transformations fully (UNSCEAR, 2000).

2.2.2 Promotion

Till further stimulation that disturbs the cellular balance, the transformed cell remains harmless. The other changes of the transformed cell due to neoplastic transformation involve two or more steps and require prolonged and continuous exposure to promoting stimuli (Upton *et al*., 1986). The intracellular and extracellular environments have an impact on neoplastic development. In promotion, however, the molecular structure of DNA remains unchanged. Only the expression of the genome is altered. Tumor promotion involves the "selective clonal expansion of initiated cells." Because the rate of cell division is directly proportional to the mutations accumulated, thus creating a larger quantity of cells at risk of undergoing further

changes in the genome and malignant conversion (Cairns, 1975; Verma and Boutwell, 1980).

2.2.3 Progression

Foulds in 1954 originally designated progression as the whole process of carcinogenesis following the initiation stage. Nevertheless, as the stage promotion is reversible, the actual concept of Foulds should be modified. However, when carcinogenic agents are present in sufficiently high doses, the idea is acceptable in certain conditions. More precisely, cells in the promotion stage develop to the progression stage. The main features of this stage are that malignant neoplasms started to appear with crucial genetic alterations and involve structural modifications confining the karyotype of cells. Such changes include the viral genome and fragment incorporation into the host cell genome due to infection.

The infected host cells with oncogenic viruses in them can bypass the promotion stage as a whole or in part. The cells can evolve with specific features like an invasion, anaplasia, metastatic growth, and a higher degree of malignancy during progression (Pitot, 1989; Pitot, 1993).

2.3 Cancer Genes

For neoplasia development, there are three classes of genes that are prone to be the molecular targets. They are protooncogenes (Temin, 1974), oncogenes (Garrett, 1986) and tumor suppressor genes (Marshall, 1991).

2.3.1 Protooncogenes

They are the class of normal cellular genes that have many different functions in the cells. They are confined in separate compartments of the cell and are expressed in the cell cycle at different stages. They have an essential role in the regulation of cellular growth like proliferation, differentiation and apoptosis. Protooncogenes are

transformed into oncogenes by different genetic mutations like insertions, substitutions and deletions (Bishop, 1991), gene amplification and chromosomal rearrangements (Bishop, 1989). In the cellular genome, many protooncogenes were identified through viral transformation, such as c-erbB, c-myc, c-mos, c-myb, C-H-ras (reviewed by Bishop, 1987).

2.3.2 Oncogenes

In normal cells, they are inactive as protooncogenes. However, in the process of carcinogenesis, they are the positive regulators. Protooncogenes can be activated by gene mutations and resulting in a gain of function. Moreover, thus converting them into oncogenes thus contributes to cancer development. The oncogene mutations mostly seen in human tumors are commonly detected in the ras family (Rodenhuis, 1992), such as K-ras (50% colon carcinomas, 90% pancreatic carcinomas), N-ras and H-ras (in hematologic malignancies). Chromosomal rearrangements are often noticed in some solid cancers and hematologic malignancies (Solomon *et al*., 1991). Furthermore, it is mainly due to chromosomal translocations, and chromosomal inversions.

2.3.3 Anti-oncogenes or tumor suppressor genes

They belong to the negative growth regulators. In normal cells, during cell cycle progression, they control cell proliferation. Mutation in the tumor suppressor genes results in loss of gene function and aids carcinogenesis (Perkins and Stern, 1997). Many tumours in humans, e.g., colon carcinoma, retinoblastoma, and Wilm's tumor resulting from recessive mutation, cause cancer in both homologs (Knudson, 1993). The commonly studied anti-oncogenes are the p53 and Rb genes. The proteins encoded by them inhibit the cell cycle progression. A mutation to the Rb gene leads to the failure to inhibit cell cycle progression, leading to enhanced cell proliferation.

Genetic modifications that cause loss of apoptotic signals would intensify the malignant transformation (Symonds *et al.*, 1994). p53 has a vital role in maintaining cellular equilibrium and genomic stability. p53 promotes apoptosis and controls the cell cycle via G1-S checkpoint control and initiates cell differentiation. After DNA damage, this gene, through a signal transduction pathway in the cell cycle, causes apoptotic cell death or a G1 arrest (Kastan and Skapek, 1997). So p53 mutations that are loss of function mutations will cause apoptosis suppression and encourage cell division by removing the G1-S block and stopping cell differentiation, owing to neoplasm development (Curtis, 1993). P53 gene mutations are considered the most common genetic alterations observed in numerous human malignancies; p53 abnormality is found in about 50% of all cancers occurring in humans (Hollstein *et al*., 1991). Many cancers like skin, lung, breast, colon, ovary, urinary bladder and lymphoid organs cancer have revealed mutations of this gene. For example, p53 mutations of more than 500 numbers have been recorded in breast cancer (Hartmann *et al*., 1997).

2.4 Oxidative stress

The term oxidative stress is defined as the condition in which the balance between free radical generation and antioxidant defences are altered and becomes unfavourable (Rock *et al*., 1996). This can cause damage to molecular species like proteins, lipids and nucleic acids (McCord, 2000). In tissues, short-term oxidative stress can be caused by infection, trauma, toxins, excessive exercise etc. These altered tissues can increase the generation of radical producing enzymes like cyclooxygenase, lipoxygenase etc., phagocyte activation, the liberation of iron, copper ions or causing damage to oxidative phosphorylation and eventually lead to the production of excess ROS. The different steps involved in cancer and the treatment of cancer have been

connected to the disparity between Reactive oxygen species and the antioxidant defence system (Rao *et al*., 2006). Oxidative stress stands as a significant causal factor for all sorts of inflammatory diseases, ischemic diseases, emphysema, hemochromatosis, acquired immunodeficiency syndrome, gastric ulcers, neurological disorders, hypertension, organ transplantation, smoking-related diseases, alcoholism etc. (Stefanis *et al*., 1997).

2.4.1 Free radicals

A free radical is a molecular species with an unpaired electron in the atomic orbital that can exist independently. Many free radicals are highly reactive and unstable. They can behave as oxidants or reductants by accepting an electron or donating an electron to other molecules (Cheeseman and Slater, 1993). Free radicals can attack specific vital macromolecules owing to cell damage and disruption of homeostasis. Their main targets in the human body include proteins, nucleic acid and lipids (Lobo *et al*., 2010). Hydroxyl radical, Hydrogen peroxide, Superoxide anion radical, Nitric oxide radical, Oxygen singlet, Hypochlorite, and Peroxynitrite radical etc., are some free radicals containing oxygen seen in many diseases' states. These radicals are highly reactive and can harm biological molecules such as proteins, DNA, lipids, and carbohydrates. (Young and Woodside, 2001).

Free radicals and Reactive Oxygen Species (ROS) are formed in the human body from normal critical internal metabolic processes or external sources like air pollutants, cigarette smoking, ozone, X-rays etc. (Bagchi and Puri, 1998). Free radical production constantly occurs in the cells due to enzymatic and non-enzymatic reactions (Liu *et al*., 1999). Some internally produced free-radical sources include mitochondria, peroxisomes, xanthine oxidase, inflammation, arachidonate pathways, phagocytosis, cigarette smoking, exercise, pollutants, certain drugs, ischemia etc.

(Ebadi, 2001). Free radical reactions are common and considered normal and it increases with aging. However, apart from these reactions, genetic and environmental differences can modulate the normal free radical reaction and can lead to free radical damage. These can be manifested as diseases caused by environmental and genetic factors at particular ages. Two important diseases resulting from free radicals that can lead to death are atherosclerosis and cancer. Cancer is associated with oncogene activation and chromosomal defects, which can be induced by endogenously produced free radical reactions, like those imposed by ionizing radiations that can lead to tumorigenesis (Lobo, 2010).

2.4.2 Oxygen radical

The biologically significant free radicals obtained from oxygen are hydroxyl radical ($^{\cdot}OH$), superoxide anion ($^{\cdot}O_2^-$), perhyroxyl radical ($HO_2^{\cdot}$) and singlet oxygen. Certain ROS having carcinogenic potential and have been linked with tumor formation are O_2^-, H_2O_2 and $^{\cdot}OH$ (Irani *et al*., 1997). Different ROS differ in their reactivity and oxidation potential and it may be represented as O_2- < H_2O_2 < $^{\cdot}OH$ (Fridovich, 1978). $^{\cdot}OH$ is the most reactive one and it is produced by the Fenton and Haber-Weiss reaction from H_2O_2 and O_2^- respectively (Knight, 1999).

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH \longrightarrow$ Fenton reaction

${}^{\bullet}O_2^- + H_2O_2 \longrightarrow {}^{\bullet}OH + OH^- + O_2 \longrightarrow$ Haber-Weiss reaction

Some sources of ROS in the cells are:

- Electron leakage from microsomal cytochrome P-450, mitochondrial electron transport chain, and enzymatic systems donate electrons to oxygen (Fridovich,

1989; Beal, 1997). Nearly 1 to 4 % of the oxygen taken by the human body is transformed into free radicals.

- The normal xenobiotics metabolism generates ROS as by-products in the living cells (Parihar *et al*., 1997).
- Radiation exposure or high-temperature exposure can produce ROS (Sen, 1995; Parihar and Dubey, 1996).
- During inflammation for certain beneficial causes ROS are produced from macrophages and activate phagocytes like eosinophils, monocytes and neutrophils (Babior and Woodman, 1990).
- During biochemical reactions, oxidation and reduction, enzymatic as well as non-enzymatic reactions O_2- is produced by the univalent reduction of the O_2. Numerous oxidase enzymes like tyrosine hydroxylase, monoamine oxidase (MAO), L- amino acid oxidase etc. in-vivo generates H_2O_2 additionally (Coyle and Puttfarcken, 1993).
- Various free radicals in higher quantity are present in the cigarette smoke (Halliwell and Gutteridge, 1989).

2.4.3 Nitrogen species

In 1992, nitric oxide having many molecular targets is known as the unorthodox messenger molecule (O'Dell *et al*., 1991). It is synthesized from nitric oxide synthases (NOS) family of enzymes utilizing L-arginine and molecular oxygen as the substrates. The three isoforms of $NO^{\cdot}$ synthases are Type 1 NOS (neuronal NOS), Type 2 NOS (Inducible NOS and Type 3 NOS (endothelial NOS). It has got a variety of functions such as, control neurotransmission (Schuman and Madison, 1991), manages mRNA translation and gene transcription (Khan *et al*., 1996) and can make post-translational modifications of proteins (Brune *et al*., 1994). In the cells Cu-

Fe proteins are the molecular targets of nitric oxide, which releases free Cu_2^+ and Fe_2^+ and generates O_2^- and $OH^\cdot$ which is highly reactive and can cause oxidative injury. The lipophilic nitric oxide free radical gas can quickly diffuse to the underlying smooth muscles through the plasma membrane and lead to the enhanced calcium extrusion by activating cytosol soluble cyclic GMP. And this reduced calcium concentration is the cause for NO intervened non-vascular and vascular relaxation and the signal transduction in PNS (peripheral nervous system) (Moncada *et al*., 1991). The inducible NOS can produce a comparatively large concentration of $NO^\cdot$ and it is responsible for many inflammatory conditions. The reaction between $NO^\cdot$ and superoxide anion can produce peroxynitrite ($ONOO^-$) and initiate Reactive Nitrogen Species (RNS) generation (Thuy *et al*., 2017). It is highly reactive and deleterious molecule with powerful nitrating and oxidizing properties (Radi *et al*., 1991) which can give rise to many other RNS like nitrogen dioxide and dinitrogen trioxide (Squadrito and Pryor, 1998). Peroxynitrite targets a wide range of substrates like lipids, aminoacid, thiols and DNA and can result in mitochondrial damage, disturbances in apoptosis, cell signalling, lipid peroxidation of membranes, post-translational modifications of proteins etc. (Pacher *et al*., 2007; Czaja, 2008).

2.4.4 ROS in Cancer

One of the primary reasons for cancer is considered to be the damage caused to DNA by ROS (Ames, 1983). It can lead to mutations. A significant fraction of mutations initiated by ROS can cause modifications to guanine, owing to G-T transversions (Higinbotham *et al*., 1992). If it happens to critical genes like oncogenes or tumor suppressor genes, tumor initiation or progression can occur (Ames *et al.*, 1993). In most human tumours, G-T transversion mutation in the p53 tumor suppressor gene is the most common one leading to cancer (Hollstein *et al*., 1991).

Indeed ROS has a more significant role in several steps of cancer development, especially in the initiation and progression of cancer (Moller and Wallin, 1998).

ROS are also the mediators in activating certain carcinogens via peroxyl radical-mediated hydroperoxide-dependent oxidation (Trush and Kensler, 1991). Oxidative DNA adduct can be formed by the direct reaction ROS or lipid peroxidation by-product MDA with DNA (Chaudhary *et al*., 1994). Base pair and frame-shift mutations are included in the mutations induced by MDA (Moller and Wallin, 1998).

Cigarette smoke is rich with carcinogens like polycyclic aromatic hydrocarbons and nitrosamines (Rodgman *et al*., 2000; Shields, 2000; Das, 2003), which can result in the deposition of 8-hydroxydeoxyguanosine (8-OHdG), and this could result in mutations even induced by free radicals of oxygen leading to fibrosis, inflammatory responses and tumor development (Zienolddiny *et al*., 2000). Urine collected from smokers shows highly elevated altered nucleotides that are said to be generated by ROS (Fraga *et al*., 1996). Urinary 8-OHdG is used as a biomarker to detect atherosclerosis, diabetes, cancer and oxidative stress (Wu *et al*., 2004).

Since ROS promotes mutagenesis, tumor promotion and progression, they are considered potential carcinogens. Even some types of cancer cells can generate high amounts of ROS. ROS production is initiated by several genes like H-Ras or mox1 having transformed phenotype after their expression. High levels of ROS and low levels of antioxidants or ROS scavengers can enhance the chances of developing cancer. ROS interrupts normal central cellular processes like apoptosis, senescence, proliferation etc. which are involved in cancer development (Waris and Ahsan, 2006).

2.5 Antioxidants

Antioxidants are known as "any substance that when present at low concentrations with those of an oxidizable substrate considerably delays or prevents oxidation of that substrate" (Halliwell and Gutteridge, 1995). As the above definition implies, the physiological function of antioxidants is to inhibit cellular damage due to chemical reactions involving free radicals (Young and Woodside, 2001). To elaborate, the biological activities of antioxidants include suppression of prostaglandin synthesis, activation of drug-metabolizing enzymes, suppression of carcinogen activated mutagenesis and free radical scavenging (Hirose *et al*., 1994). For instance, N- acetylcysteine, an antioxidant, has chemo preventive and antimutagenic activities in various organs like skin, lung, colon and liver (De Flora *et al*., 1995; Izzotti *et al*., 1994).

The antioxidant action mechanisms include,

- Oxygen removal
- Scavenging reactive nitrogen and oxygen species
- Suppressing ROS or RNS formation
- Enhancement of endogenous antioxidative defences (Halliwell, 1996)

Antioxidants have substantial effects on the capacity of many transcription factors to bind to DNA, including AP-1, NF- KB, Spl, and elk-l (Winyard and Blake, 1996). They can inhibit various stages of tumorigenesis such as tumor initiation, promotion, progression and transformation (Steele *et al*., 1990). In the smooth muscle cells, they can accelerate apoptosis independent of the oxidative reactions (Liu *et al*., 1998; Tsai *et al*., 1996). Modifications in antioxidant defence enzymes like catalase, superoxide dismutase (SOD), glutathione S-transferase (GST) and glutathione peroxidase (GPx) in cancer cells has been widely described (Cerutti *et al*., 1994).

2.5.1 Antioxidant enzymes

The enzyme system of antioxidants consists of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) etc. These enzymes work to protect the body from harmful oxygen radicals generated during metabolic processes and after oxidative stress (Sun, 1990).

Catalase (CAT)

Loew named one of the oldest known enzyme catalase in 1901 (Percy, 1984). It catalases the reaction:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

This enzyme is present in almost all aerobic cells. CAT is found in all main organs in animals, with a high concentration in the liver and erythrocytes. At subcellular level, CAT is mainly present in peroxisomes (80 percent) and cytosol (20 percent). It is made up of four protein subunits, each of which has a heme [Fe(lll)-protoporphyrin] group attached to its active site. The activity of the molecule is lost when it is dissociated into its subunits (Halliwell and Gutteridge, 2015). The human CAT gene is located on chromosome 11p13 (McAlpine *et al*., 1988).

Superoxide dismutase (SOD)

It was first discovered in 1969 by McCord and Fridovich (McCord and Fridovich, 1969). SOD can dismutate two molecules of $O2^-$ resulting in H_2O_2 and O_2. The reaction is given below:

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

There are four metalloforms for SOD family, two of which contain copper and zinc, one manganese, and one iron. Cu,ZnSOD is present in the cytosol of majority of eucaryotic cells (Fridovich, 1975); however, a distinct type of Cu,ZnSOD is present in extracellular fluids and is referred to as ECSOD (extracellular SOD) (Marklund,

1984; Marklund *et al*., 1982). MnSOD can be found in both the mitochondrial matrix and bacteria, while FeSOD is found in numerous aerobic bacteria (Fridovich, 1974; Sun, 1990). CuZnSODs can be inhibited by cyanide, which is a very strong inhibitor. The human genes for Cu, ZnSOD, and MnSOD are located on chromosomes 21q22.1 and 6q21 respectively (McAlpine *et al.*, 1988).

Glutathione peroxidase (GPx)

GPx was first discovered in 1957 by G C Mills. The enzyme GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. The reaction is shown below:

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

The enzyme is made up of four protein subunits, each of which has one selenium atom in its active site (Ding *et al*., 1998). It has a high level of activity in the liver, a moderate level of activity in the heart, lungs, and brain, and a low level of activity in the muscle. It is distributed in cytosol and mitochondrial matrix (Mills, 1960). Se-dependent GPx is encoded by a gene on chromosome 3p13-q12 (McAlpine *et al.*, 1988).

Glutathione reductase (GR)

Hopkins and Elliott discovered GR in the livers of various animals in 1931 (Hopkins and Elliott, 1931), and Mann isolated it from ox, sheep, and rabbit liver in 1932 (Mann, 1932). It catalyses the reaction,

$$GSSG + NADPH + H^+ \quad 2GSH + NADP^+$$

Has got 2 subunits, each of which contains the flavin FAD at its active site. As in GPx, it is also distributed in cytosol and mitochondrial matrix. 1,3-bis-2-chloroethyl-l-nitrosourea is an inhibitor of glutathione reductase (Ray and Prescott, 1975). The gene encoding GR is located on human chromosome 8p21.1 (McAlpine *et al.*, 1988).

2.5.2 Antioxidant vitamins

Vitamin C

Vitamin C (L- ascorbic acid) is a water-soluble micronutrient necessary for many biological activities. It is an important antioxidant that protects against many infections and iron absorption. It is one of the potent reducing agents and free radical scavengers in biological systems. It acts as a scavenger of the oxidizing free radicals and dangerous oxygen-derived species like hydrogen peroxide, hydroxyl radical and singlet oxygen (Tolbert *et al*., 1975; Packer, 1997; Hacisevki, 2009). It plays a role in the first place of antioxidant defence, preventing oxidative damage to lipid membranes and proteins. Vitamin C can neutralize free radicals and inhibit free radical damage both within and outside the cells as a water-soluble molecule. Also, it acts as an excellent source of electrons for free radicals looking out for an electron to achieve stability. Vitamin C can make free radicals stable and reduce their reactivity by donating electrons (Rouhier *et al*., 2008; Bindhumol *et al*., 2003). They can also act against lipid peroxidation as a scavenger of ROS and reduce one electron of lipid hydroperoxyl radicals through the vitamin E redox cycle (Sato *et al*., 1990; Halliwell and Gutteridge, 2015; Pehlivan, 2017).

Vitamin E

It is a fat-soluble vitamin seen in plasma which includes 4 tocopherols and 4 tocotrienols of which alpha-tocopherol is the most abundant one in nature, having the highest biological activity (Herrera and Barbas, 2001). Vitamin E acts as an antioxidant in cell membranes, preventing the proliferation of free radical reactions, though it has also been shown to have pro-oxidant activity. Vitamin E is a chain-breaking antioxidant that can specifically scavenge ROS and is the most abundant lipid-soluble antioxidant found in all cellular membranes, protecting lipid

peroxidation, membrane polyunsaturated fatty acids (PUFA), and low-density lipoprotein (LDL) (McCay, 1985). Vitamin E interacts directly with various oxy radicals such as the peroxy radical $ROO^{\cdot}$, $CC13^{\cdot}$, $^{\cdot}OH$, $O2^{\cdot}$, and singlet oxygen (Fukuzawa and Gebicki, 1983). From the chromane ring of vitamin E, it donates hydrogen to free radicals. By interacting with reductants that serve as hydrogen donors, the tocoperoxyl radical can be reduced to tocopherol.

$$ROO^{\cdot} + \text{Vit E-OH} \longrightarrow ROOH + \text{Vit E-O}^{\cdot}$$

$$\text{Vit E-O}^{\cdot} + AH^{\cdot} \longrightarrow \text{Vit E-OH} + A^{\cdot}$$

Thus, vitamin E can neutralize ROS and decrease oxidative DNA damage and mutations due to its antioxidant properties (Frei, 1994).

Apart from Vitamin C and E, Vitamin A (retinol), a fat-soluble vitamin and its active metabolite, retinoic acid (RA), are well-known for maintaining healthy cells and tissues. RA can regulate cell growth by slowing the cell cycle and inducing immature and transformed cells to differentiate into a more mature phenotype. (Ross, 2010). Retinoids- the vitamin A derivatives are potent regulators of cell proliferation, embryogenesis, epithelial cell differentiation and carcinogenesis (Easwaran *et al*., 1999).

2.5.3 Other non-enzymatic antioxidants

Metal-binding proteins (MBPs)

MBPs are the first endogenous antioxidants described. They include intra and extracellular proteins, like albumin, myoglobin, ferritin, ceruloplasmin, metallothioneins, lactoferrin and transferrin. The key contributors to plasma antioxidant potential are MBPs. Their ability to bind metal ions is one of their antioxidant properties (Schwab *et al*., 2014). These free-redox-active transition metal

ions (Cu 2+ and Fe 2+) have a high pro-oxidant potential, which means they are able to react with hydrogen peroxide and can catalyze the production of reactive species (ROS) in the Fenton reaction (Taverna *et al*., 2013). Some of these proteins can also serve as true reactive species scavengers, such as the free sulfhydryl groups of cysteine in albumin and metallothioneins, which can scavenge hydroxyl radicals (Mirończuk-Chodakowska *et al.*, 2018).

Glutathione

Glutathione (GSH) is a compound with low molecular weight. It is made up of 3 amino acids: glycine, cysteine and glutamic acid. GSH is found in all plant and animal cells. Reduced glutathione (GSH) and oxidized glutathione (OGS) are the two most common redox forms of glutathione found throughout the human body (GSSG) (Forman *et al*., 2009). GSH acts as an antioxidant by reducing reactive oxygen species (ROS) in enzymatic and non-enzymatic reactions. Other oxidized small molecule antioxidants, such as vitamin C and vitamin E, are regenerated by GSH (Rahman, 2007). It is involved not only in the scavenging of free radicals but also in repairing damaged cells. It also acts to recover protein molecules, nucleic acids and lipids damaged in peroxidation processes and maintain sulphydryl groups of protein in the reduced state (Alli *et al*., 2014; Chatterjee, 2013). Also, it has the ability to shield other thiol groups in proteins from oxidative damage due to the involvement of the thiol group in the molecule (Samuelsson, 2011). In cells, thiol groups (-SH) are among the most reactive chemical compounds (Mirończuk-Chodakowska *et al.*, 2018).

Flavonoids

Flavonoids are phenolic compounds isolated from various vascular plants, and there are over 8000 different known compounds. They have antioxidant,

antimicrobial, visual attractors, photoreceptors, light screening and feeding repellant properties in plants (Pietta, 2000). Flavonoids have been reported to act as antioxidants by scavenging reactive oxygen (Rice-Evans *et al*., 1996) and nitrogen species (Kerry and Rice-Evans, 1999; Pannala *et al*., 1998) and in some cases by chelating transition metal ions (Brown *et al*., 1998). The amount of phenolic hydroxyl groups and their positions bound to ring structures provide antioxidant property to flavonoids. They act as antioxidants by donating an electron to an oxidant depends on the radicals' reduction potentials and the radical's accessibility (Jovanovic *et al.*, 1998). Flavonoids, for example, are excellent scavengers of peroxyl radicals because of their lower reduction potentials than alkyl peroxyl radicals, and thus, in theory, potent lipid peroxidation inhibitors (Rice-Evans, 2001).

Uric acid

Uric acid (UA) is a low molecular weight organic compound produced during purine metabolism. UA is a water-soluble antioxidant that accounts for about two-thirds of the blood serum's overall oxygen scavenging operation (Lippi *et al*., 2008; Mirończuk-Chodakowska *et al.*, 2018). However, a high concentration of uric acid can result in gout.

Bilirubin

The mononuclear phagocyte system produces bilirubin (BIL) as a degradation product of haemoglobin and other heme proteins (Mirończuk-Chodakowska *et al.,* 2018). In biological investigations, bilirubin has been proven to have substantial antioxidant effects. (*Zelenka et al*., 2016; Ziberna *et al*., 2016). Bilirubin has been found to have strong antioxidant activity against peroxyl radicals in polar media such as aqueous lipid bilayers. (Hatfield and Barclay, 2004).

Ceruloplasmin

Ceruloplasmin (CP) is a 1000-amino-acid glycoprotein with a high molecular weight. CP is produced in a variety of organs and tissues, including the brain and liver, and it has a variety of functions (Mirończuk-Chodakowska *et al.*, 2018). It is known for its role as a chain-breaking antioxidant. It can protect myocardial tissue from oxygen-free radicals (Verma *et al*., 2005). It also protects polyunsaturated fatty acids in erythrocyte cell membranes from reactive oxygen species (ROS). It may also serve as an oxygen radical scavenger by securing cardiovascular tissues from ischemia-reperfusion injury (Paradis *et al*., 2010).

Some other non-enzymatic antioxidants include melatonin, coenzyme Q10 and some polyamines (Mirończuk-Chodakowska *et al.*, 2018).

2.5.4 Lipid peroxidation

Lipid peroxidation is called the process in which lipids having carbon-carbon double bonds, mainly polyunsaturated fatty acids (PUFAs), are attacked by the oxidants like free radicals (Ayala *et al*., 2014). It can seriously affect the cell membrane and cause reduced fluidity and breakdown of several secretary functions of the membrane. The autooxidation process can be induced by the peroxyl radical, hydroxyl radical and singlet oxygen and cannot be induced by the less reactive radicals like superoxide anions and hydrogen peroxide.

The overall lipid peroxidation process takes place in 3 steps: initiation, propagation and termination. In the first step, the pro-oxidants such as hydroxyl radical removes the allylic hydrogen from the carbon chain's methylene carbon-producing carbon-centered lipid radical (L·) In the next phase, this lipid radical soon generates a lipid peroxy radicals by reacting with oxygen (LOO·), and this radical produces another new lipid radical (L·) (in which chain reaction proceeds) and lipid

hydrogen peroxide (LOOH·) by abstracting hydrogen from a second lipid molecule. In the final termination step, to the peroxy radicals, a hydrogen atom is donated by the antioxidants such as vitamin E. It becomes a corresponding radical of vitamin E that can produce non-radical products by reacting with another LOO·. Once the lipid peroxidation starts, a chain reaction propagation occurs until the formation of termination products occurs (Yin *et al*., 2011; Girotti, 1998; Kanner *et al*., 1987). The end products are primarily hydrocarbons, aldehydes and different chemical residues like malondialdehyde (MDA) and 4- *hydroxynonenal* (O'Brien, 1969). These degradation products can disperse away from the chain reaction site, lead to cell edema, inflammation, and interrupt vascular permeability and chemotaxis.

2.6 Inflammation and cancer

Inflammation and cancer have long had a functional relationship. Back in 1863, Virchow proposed that cancer begins at the chronic inflammation sites, based in part on the concept that certain types of irritants, combined with the tissue damage and inflammation, induce and promote cell proliferation (Balkwill and Mantovani, 2001). While it is now clear that cell proliferation does not cause cancer in and of itself, persistent cell proliferation in an inflammatory cell-rich environment, activated stroma, growth factors and promoting agents of DNA damage amplifies and promotes neoplastic risk. Cell proliferation increases during tissue damage associated with wounding as the tissue regenerates, but inflammation and proliferation get reduced when the assaulting agent is expelled, or the repair is accomplished. On the contrary, proliferating cells that have been exposed to DNA damage and mutagenic attack (such as initiated cells) carry on with proliferation in microenvironments with abundant inflammatory cells and growth or survival factors that promote their growth.

In a way, tumors pretend to unhealed wounds or wounds that failed to heal (Flier et al., 1986; Coussens and Werb, 2002).

So, the development of cancer can be accelerated with inflammation. Upregulation of inflammatory mediators like cyclooxygenase (COX-2) leads to the formation of inflammatory cytokines and prostaglandins (PG), which reduce cell-mediated immune responses and elevate angiogenesis, creating the ideal environment for cancer development (O'Byrne and Dalgleish, 2001). When inflammatory cells like monocytes and macrophages are stimulated by cytokines, mitogens, serum, or endotoxins, COX-2 is produced. Inflammation enhances PG synthesis, at least in part because COX-2 is upregulated rather than COX-1. Inflammatory cells can also accelerate DNA damage by accelerating procarcinogens, leading to mutations like estrogen, aflatoxins, phenols by ROS- dependent mechanisms and aromatic amines activated by neutrophils (Rosin *et al*., 1994). Increased free radical activity is linked to the stimulation of neutrophil NADPH oxidase and the uncoupling of several redox systems, including endothelial cell xanthine dehydrogenase, at the site of inflammation (Winrow *et al.,* 1993).

Ulcerative colitis (UC) is known as a chronic inflammatory disease that affects the large intestine mucosa and has an uncertain cause. Colorectal cancer risk is ten times higher in people with UC than in the general population (Itzkowitz and Yio, 2004). Current evidence shows that inflammation signaling pathways appear to be constitutively involved in Inflammatory Breast Cancer (IBC) and breast cancer. The NF-B, COX-2, and JAK/STAT signaling systems, in particular, appear to play a crucial role in the tumor formation of IBC. In IBC preclinical studies, inflammatory molecules like gamma interferon, tumor necrosis factor-alpha (TNF-α) and interleukin-6 seems to be the factors that can lead to malignant transformation, at the

same time, the TNF-α, interleukins 8 and 1β as well as transforming growth factor-β seems to have a significant role in the survival, proliferation, epithelial-mesenchymal transition, metastasis and invasion (Hartman *et al.*, 2011; Fouad *et al*., 2014).

2.7 Cancer treatment

Throughout history, cancer care has had its ups and downs, not only due to treatment ineffectiveness and side effects but also due to faith and complete recovery and cure in many situations. The most popular forms of cancer therapies available are chemotherapy, surgery, and radiotherapy. Angiogenesis inhibitor therapy, biological therapies (including interferons, interleukins, monoclonal antibodies, colony-stimulating factors, gene therapy, and nonspecific immunomodulating agents), bone marrow and peripheral blood stem cell transplantation, laser therapy, hyperthermia, photodynamic therapy, and targeted cancer therapy are some of the recent treatment methods (Arruebo *et al*., 2011). Antitumor medications and radiation, which have been the treatment of choice in some cases, are part of the clinical strategy, alongside surgery in the case of solid tumors.

2.7.1 Cancer surgery

With Miles' first abdominoperineal resection in 1908, cancer surgery techniques began at the turn of the twentieth century (Miles, 1908). Non-invasive techniques such as laparoscopic colectomy (for the extraction of colon cancer), video thoracoscopy, radiofrequency ablation, and radiosurgery techniques such as cyberknife® have largely replaced Halstedian techniques in modern surgery. To enhance aesthetic outcomes and prevent lymphedema, breast-conserving surgery with sentinel-node removal has been used (Singletary, 2001). The use of laryngoscopic laser surgery in early laryngeal cancer is another example of conservative surgery

(Genden *et al*., 2007). The Da Vinci®, a robotic device for prostate and kidney cancer removal, is considered the most recent advancement in 2011. (Arruebo *et al*., 2011).

2.7.2 Radiation treatment

Becquerel and Rontgen's discovery of X-rays and radiation in the late 1800s was the first move to radiation therapy. Marie Curie's contributions to the advancement of radiotherapy were significant. In 1898, the first cancer case was cured entirely by radiation. Moreover, various new technologies have been discovered over time. Ginzton and Kaplan used "Clinac 6" rotational linac radiotherapy to concentrate x-rays much intensely and reduce its side effects on the skin. Intensity-modulated radiotherapy (IMRT) was started to use after modern computers, enabling 3-D X-ray therapy from computed tonography (CT) scans using mapping information. In 2003, a new type of IMRT called the Tomotherapy system was developed. This treatment employs CT-guided IMRT, which guides the radiation source by spinning it around the patient, making it trouble-free to map the morphological limits of a tumor with the beam (Hall, 2006). Then later developed the 4-D conformal radiotherapy that can record the video sequence of tumor movement. Image-guided radiation therapy (IGRT) and image-guided adaptive radiation therapy (IGART) are two types of this treatment (IGART) (Murphy and Li, 2010). In summary, fractionated dosage distribution, technical advancements in X-ray processing and delivery, and improvements in computer-based treatment planning have all been areas of advancement.

2.7.3 Nano based technologies

The most recent treatment strategies include the use of nanoparticles for the targeted drug delivery and in the detection of tumors. Magnetic nanomaterials constitute a class of highly functionalizable platforms for cancer therapy. Because of

their intrinsic magnetic properties and multifunctional nature, they provide a multimodal theragnostic tool for cancer diagnosis, tracking, and therapy (Mukherjee *et al*., 2020). Furthermore, the truly intelligent multifunctional nanocrystal systems may combine diagnostic capabilities, antitumor therapy, and successful live monitoring of cancerous cells into a single multifunctional delivery system (Joseph and Singhvi, 2019).

2.7.4 Chemotherapy

Paul Ehrlich coined the term chemotherapy to describe the treatment of metastasis (Masood et al., 2016). Skipper and his colleagues developed the guiding principles of modern chemotherapy in the early 1960s, using the rodent leukemia L1210 as a guide (Chabner and Roberts, 2005). Cancer chemotherapy aims to destroy cancer cells while causing minimal damage to healthy cells. Since several cells divide in cancerous tumors, most drugs are designed to prevent cell growth and division by blocking DNA, RNA, or protein synthesis. Chemo drugs are classified based on their mechanism of action, chemical composition, and interactions with other medications. Some medications have multiple effects and can be classified into numerous classes. They are,

Alkylating agents

By damaging the cell's DNA, alkylating agents prevent it from proliferation. These medications target all stages of the cell cycle. They are used to treat various cancers, like lung, breast and ovarian cancers, as well as lymphoma, sarcoma, leukemia, Hodgkin disease, and multiple myeloma. As these drugs can damage the DNA, they can harm the bone marrow cells making new blood cells, resulting in leukemia in rare cases (ACS 2019) (Chu and Rubin, 2018).

Examples of alkylating agents include:

Cisplatin, Bendamustine, Altretamine, Cyclophosphamide, Carboplatin, Carmustine, Busulfan, Chlorambucil, Dacarbazine, Lomustine, Temozolomide, Mechlorethamine, Melphalan, Fosfamide, Trabectedin, Oxaliplatin, Thiotepa.

Nitrosoureas

Nitrosoureas are a class of alkylating agents with a unique mechanism of action. The other alkylating agents are unable to enter the brain, but nitrosoureas do. They can reach the brain because they can cross the blood-brain barrier, a unique barrier that prevents other drugs out of the brain (ACS 2019; Francisco *et al*., 2008).

Examples of nitrosoureas include:

Carmustine, Lomustine, Streptozocin.

Antimetabolites

Antimetabolites disrupt DNA and RNA by replacing the RNA and DNA's usual building blocks. When this occurs, the DNA cannot replicate itself, and the cell is unable to reproduce. They are widely used to treat leukemia, breast, ovarian, intestinal cancers and other forms of cancer (Scaife and Kerr, 2018; ACS 2019)

Some of these include: 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), Azacitidine, Floxuridine, Gemcitabine (Gemzar), Capecitabine (Xeloda), Clofarabine, Cytarabine (Ara-C), Cladribine, Thioguanine, Fludarabine, Hydroxyurea, Methotrexate, Nelarabine, Decitabine.

Antitumor antibiotics

These drugs are not the same as antibiotics, which are used to prevent infection. Instead, they function by altering the DNA of cancer cells to prevent them from multiplying and developing.

Anthracyclines: Antitumor antibiotics known as anthracyclines work by interfering with enzymes involved in DNA replication during the cell cycle. They

bind to DNA, preventing it from replicating and preventing a cell from reproducing. They are used to treat various types of cancers. Examples include Doxorubicin, Epirubicin, Idarubicin, Valrubicin etc..

The fact that these medications can permanently harm the heart if administered in large doses is a significant concern when administering them. As a result, lifetime dosage limits (also known as cumulative dose) are often imposed on these medications. Bleomycin, Dactinomycin, Mitomycin-C, and Mitoxantrone are examples of non-anthracycline antitumor antibiotics (Szucs and Jones, 2016; ACS 2019).

Topoisomerase inhibitors

These medications are also known as plant alkaloids. They obstruct topoisomerase enzymes, which help separate DNA strands so that they can be copied. Certain leukemias and gastrointestinal, ovarian, lung, colorectal, and pancreatic cancers are treated with topoisomerase inhibitors. Topoisomerase inhibitors are classified by the type of enzyme they target:

Inhibitors of topoisomerase I (Camptothecins)

Includes: Irinotecan, Irinotecan liposomal, topotecan etc.

Inhibitors of topoisomerase II (Epipodophyllotoxins)

Includes: Etoposide (VP-16), Teniposide, Mitoxantrone (also an anti-tumor antibiotic)

Inhibitors of topoisomerase II will raise the risk of second cancer (Szucs and Jones, 2016; ACS, 2019).

Mitotic inhibitors

Mitotic inhibitors are also known as plant alkaloids. This is because they are the compounds extracted from natural products, like plants. They function by preventing cells from dividing to form new cells, but they can damage cells at any

stage because enzymes are prevented from producing proteins required for cell reproduction.

Taxanes and vinca alkaloids are examples of mitotic inhibitors.

They treat various cancers, including breast cancer, lung cancer, myelomas, lymphomas, and leukemias. However, since these medications can cause nerve damage, the amount that can be given is restricted (Szucs and Jones, 2016; ACS, 2019).

Corticosteroids

Corticosteroids, also known as steroids, are naturally occurring hormones and hormone-like medications used to treat various cancers and other illnesses. When these medications are used in the cancer treatment, it is considered as chemotherapy drugs as well as effective in minimizing cancer-related pain. Prednisone, Methylprednisolone, and Dexamethasone are examples of corticosteroids. Steroids are also widely used to assist with chemo-induced nausea and vomiting. In addition, they are often used before some forms of chemotherapy to help avoid severe allergic reactions (Haywood *et al*., 2015).

Other chemotherapy drugs

Some chemotherapy medications have a unique action that does not fit neatly into any other groups such as, All-trans-retinoic acid, Asparaginase, Eribulin, Ixabepilone, Mitotane, Omacetaxine, Procarbazine etc. (ACS 2019).

2.7.5 Anticancer drugs of natural origin

There are many anticancer drugs of natural origin. Catharanthus alkaloids of plant origin from *Catharanthus roseus* like vincristine, vinblastine etc. are used in chemotherapy to treat various cancer of breast, ovary, lung and soft tissue. They are also used for treating various types of lymphomas, sarcomas and leukemia (Moudi *et*

al., 2013; Almagro *et al*., 2015). They have cytotoxic properties due to their affinity to bind tubulin. The vinca alkaloids hinder the assembly of microtubules and cause the mitotic spindle dissolution due to their high affinity binding to tubulin (Owellen *et al*., 1976). Viscotoxins (VT) and lectins derived from the mistletoe plant (*Viscum album*) are another class of plant medications with cytotoxic properties. *Viscus album* extract and its compounds like viscotoxin can stimulate the immune system, can interact with tumoural angiogenesis, they also exhibit growth-inhibiting and tumor reducing properties (Kienle *et al.*, 2009). Paclitaxel is a type of medicine derived from the *Taxus baccata* and *Taxus brevifolia* plants. It is a mitotic inhibitor that belongs to a class of compounds known as taxanes. Taxanes have anticancer properties that are comparable to vinca alkaloids and are linked to their effect on microtubules, which are made up of heterodimers of α-tubulin and β-tubulin. On the other hand, taxanes belong to the second category of microtubule-interacting agents, which are microtubule-stabilizing agents that promote microtubule polymerization (Mukhtar *et al*., 2014).

An anticancer compound Psammaplin A (PsA) is derived from *Poecillastra* and *Jaspis* species. It was first discovered in sea sponges called *Psammaplin aplysilla*. PsA and biprasin are found in marine cyanobacteria, cyanobacteria, and heterotrophic bacteria that live in close proximity to invertebrates (e.g., sponges, soft corals and tunicates) (Darkin-Rattray *et al*., 1996). This medication shows antitumor properties and blocks aminopeptidase N, a crucial factor in tumor cell invasion and angiogenesis (Shim *et al*., 2004). Psammaplin A has been shown to reduce leukemia cell proliferation by inducing apoptosis, and also cell growth in Bap1-null cells, while exhibiting low harm in SKN human neuroblastoma cells (García *et al*., 2011).

Likewise, didemnin, dolastatine and Ecteinascidin are all marine natural products obtained from marine species and they exhibit anticancer properties.

Many macro fungi have also been used as antitumor agents. Different bioactive compounds have been isolated from various fungi. These mainly include polysaccharides, glycopeptide/protein complexes, proteoglycans, proteins, triterpenoids etc.. The active compounds like polysaccharides and triterpenoids from *Ganoderma lucidum* exhibit anticancer, antioxidant, anti-inflammatory, antimicrobial properties etc. (Ahmad, 2018). *Antrodia cinnamomea* have compounds like polysaccharides, triterpenoids, ubiquinone derivatives, succinic and maleic derivatives, showing antitumor, anti-inflammatory, antioxidant properties, etc. (Zhang *et al*., 2019). *Pleurotus* sp contain polysaccharides, peptides, amino acids, phenolics, terpenes, sterols and fatty acid esters which exhibit antioxidant, anti-inflammatory, antidiabetic, anti-aging properties and so on (Correa *et al*., 2016). *Polyporus confluens, Agaricus bisporus, Flammulina velitipes, Auricularia auricular* are some other macro fungi with excellent medicinal activities. (Kim *et al*., 2003; Moradali *et al*., 2007).

2.7.6 Mycosynthesis of nanoparticles

Nanostructured particles with sizes ranging from 1 to 1000 nm have made headlines in the field of nanobiotechnology in recent years. As the physical and chemical methods for the synthesis of nanoparticles are hazardous and expensive biosynthesis of nanoparticles are considered the best option. Along with the green synthesis of nanoparticle using plants, mycosynthesis of nanoparticles using fungi are also considered as the most preferred route because it is undoubtedly environmentally benign, cost-effective, manipulable, and compatible with any type of biomedical research (Banerjee and Ravishankar, 2017). The metal nanoparticles like silver and

gold have shown tremendous biological applications. They are used as antimicrobials, catalysts, antioxidants, anticancer agents and so on (Siddiqi & Husen, 2016).

2.8 *Sclerotium stipitatum*

Berkeley, in 1862 identified a rare endemic fungus from South India and named it *Sclerotium stipitatum* Berk. *et*. Curr. (Berkeley, 1862). Using molecular phylogenetic techniques, the nilamanga specimen was suspected as *Xylaria acuminatilongissima*, a termite-associated species initially described from Taiwan (Latha *et al*., 2015). They are seen in various shapes and sizes, including oval, oblong, pyriform, irregularly spherical, and so on. The rind is black and somewhat wrinkled on the outside, but the interior is white and pithy (Shortt, 1867). This species is found solely in underground termite nests with holes. In Ayurveda and 'Parabharyavydyam,' it has outstanding medicinal properties. Ethnobotanical investigations conducted by Balakrishnan and Anilkumar (2001) prove that 'Nilamanga' is beneficial in treating various illnesses, including dehydration, stomach discomfort, earache, stomach cancer, and jaundice. 'Nilamanga' has an excellent potential for producing new commercially viable medicines. Due to the unavailability and lack of resources for this species, only a few particular studies have been undertaken (Anto *et al*., 2015).