2

REVIEW OF LITERATURE

Medicinal plants are natural resources, used in traditional treatment of human diseases, which constitutes one of the potential sources of new products and bioactive compounds for drug development (Ruparao*,* 2017). According to the World Health Organisation (WHO) some nations still rely of plant-based treatment as their main source of medicine and developing nations are utilizing the benefits of naturally sourced compounds for therapeutic purposes. They have been used for thousands of years in folk medicines in Asian and African populations and many plants are consumed for their health benefits in developed nations (Greenwell and Rahman, 2015).

The review of literature pertaining to the study entitled "Phytochemical Characterization, Antiproliferative and Apoptotic Efficacy of Green Synthesized Silver Nanoparticles Using *Tabebuia roseo-alba* (Ridl) sand against Lung Cancer" is presented under the following headings.

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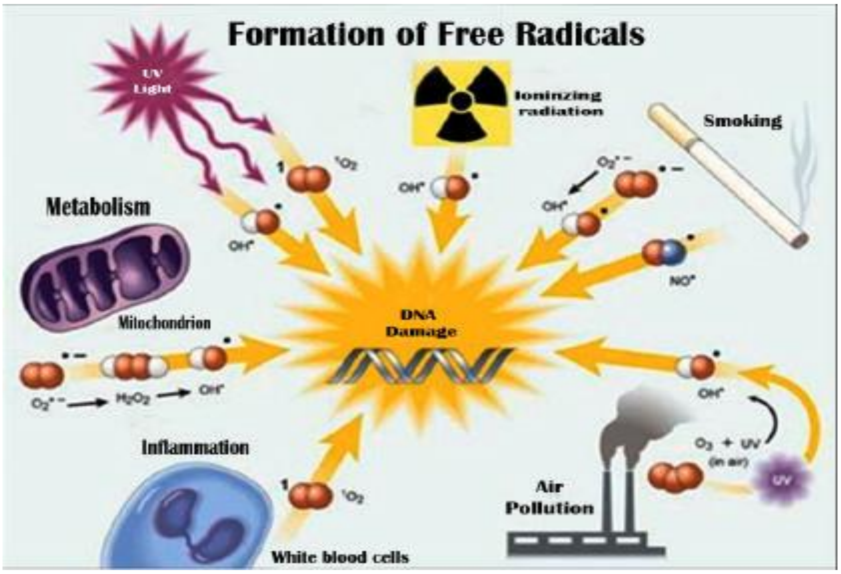
2.1 Generation and Sources of free radicals

Free radicals can be formed from both endogenous and exogenous substances. They are continuously forming in cell and environment (Kehrer and Klotz, 2015). Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress, and aging are all responsible for endogenous free radical production. Exogenous free radical production can occur as a result from exposure to environmental pollutants, heavy metals (Cd, Hg, Pb, Fe, and As), certain drugs (cyclosporine, tacrolimus, gentamycin, and bleomycin), chemical solvents, cooking (smoked meat, used oil, and fat), cigarette smoke, alcohol, and radiations. When these exogenous compounds penetrate the body, they are degraded or metabolized, and free radicals are generated as by-products (Pizzino *et al*., 2017).

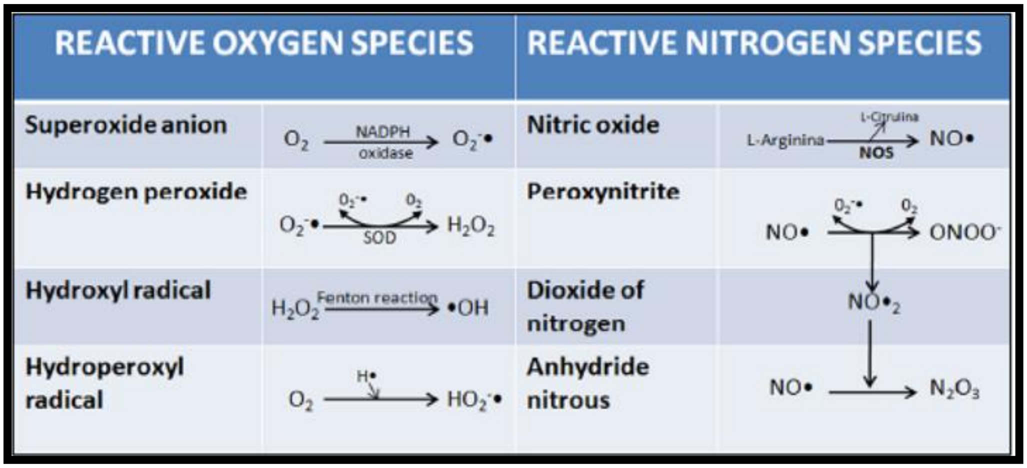
Figure 1. Formation of Free Radicals (Adly, 2010)



2.2 Types of free radicals

Free radicals are classified as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). ROS are oxygen centered free radicals which include radicals such as superoxide anion, hydroxyl radical, alkoxyl and peroxyl, non-radicals namely hydrogen peroxide and singlet oxygen. RNS are nitrogen centered radicals including nitric oxide, nitric dioxide and peroxynirite (El-Bahr, 2013).

Figure 2. Reactive oxygen and nitrogen reactive species (Ríos-Arrabal *et al.,* 2013)



REACTIVE OXYGEN SPECIES		REACTIVE NITROGEN SPECIES	
Superoxide anion	$O_2 \xrightarrow[\text{oxidase}]{\text{NADPH}} O_2^{-}\bullet$	**Nitric oxide**	$\text{L-Arginina} \xrightarrow[\text{NOS}]{\text{L-Citrulina}} NO\bullet$
Hydrogen peroxide	$O_2^{-}\bullet \xrightarrow[\text{SOD}]{O_2^{-}\bullet \quad O_2} H_2O_2$	**Peroxynitrite**	$NO\bullet \xrightarrow{O_2^{-}\bullet \quad O_2} ONOO^{-}$
Hydroxyl radical	$H_2O_2 \xrightarrow{\text{Fenton reaction}} \bullet OH$	**Dioxide of nitrogen**	$NO\bullet_2$
Hydroperoxyl radical	$O_2 \xrightarrow{H\bullet} HO_2^{-}\bullet$	**Anhydride nitrous**	$NO\bullet \longrightarrow N_2O_3$

2.2.1 Reactive Oxygen species

2.2.1.1. Superoxide anion

Formation of the $O_2^{\bullet-}$ can be considered to be the initial step for the subsequent formation of other ROS. It is generated by the single electron reduction of molecular oxygen (O2). Superoxide, compared with other free radicals, is a poorly reactive species and can exist in solution for a considerable time (and thus diffuse) before reacting with other free radicals or with specific clusters of iron–sulfur in target proteins. Being a charged species, superoxide cannot freely cross biological membranes but may do so via anion channels (Newsholme *et al.,* 2012)**.** Superoxide anion $O_2^{\bullet-}$ is constantly generated through endogenous processes and exogenous sources can cause the formation of several other reactive species through different pathways or chain reactions. It can directly produce hydroxy radical or indirectly from hydrogen peroxide which results from its dismutation by SOD through Fenton reaction which occurs in the presence of Fe^{2+}. Both $O_2^{\bullet-}$ and OH• can individually act on lipid membranes to promote the formation of lipid radical which in the presence of oxygen is capable of generating lipid peroxy radical. These radicals attack lipid membranes to initiate the most devastating effect of oxidative stress which is membrane lipid peroxidation (Ighodaro and Akinloye, 2018)

2.2.1.2. Hydrogen Peroxide

This is a product of dismutation reaction of superoxide radicals. Under physiological conditions, peroxisomes are the major producers and consumers of H_2O_2. They are not really free radicals but contain free electrons. They are therefore considered reactive oxygen species because of ability to react with biomolecules and be harmful to cells. Furthermore their high solubility in aqueous solution makes them easily penetrate biological membranes conferring on them highly deleterious properties. The enzyme catalase is also found in peroxisome and helps to decompose H_2O_2 hence maintaining a homeostatic balance. A disruption of this balance can lead to direct degradation of heme proteins by H_2O_2, release of iron, inactivation of enzymes and oxidation of DNA, lipids, -SH groups of proteins and keto acids. H_2O_2 also serve as a source for more toxic species such as OH and HCIO (Ifeanyi, 2018).

2.2.1.3. Hydroxyl radical

Hydroxyl radical (•OH) is formed not only by the interaction between hydrogen peroxide and the reduced forms of metal ions, *i.e.*, Cu^{2+} and Fe^{2+}, but also by reduction of hydrogen peroxide as well as the interaction of superoxide with hydrogen peroxide. The hydroxyl radical is particularly unstable and is the most reactive of the free radical molecules. In addition, it is capable of reacting rapidly and non-specifically with most biological molecules. Despite having very short half-lives of nanoseconds, they can cause severe damage to cell and other intracellular structures because they can cause covalent crosslinking of a variety of biological molecules. They cause cell damage by initiating chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins. Damage to DNA can cause mutations and possibly lead to cancer, if not reversed by DNA repair mechanisms. Also, damage to proteins causes enzyme inhibition, denaturation and protein degradation (Aprioku, 2013).

2.2.1.4. Peroxy radical

$RO^\bullet$ and $ROO^\bullet$ can be produced by decomposition of alkyl peroxides (ROOH). UV rays or transition metal ions can produce the same action on ROOH. $ROO^\bullet$ and $RO^\bullet$ are good oxidizing agents and can abstract hydrogen from other molecules with lower standard reduction potential. This reaction is frequently observed in the propagation stage of lipid peroxidation. $RO^\bullet$ formed from this reaction can react with oxygen to form another peroxyl radical, resulting in chain reaction. Some peroxyl radicals break down to liberate superoxide anion or can react with each other to generate singlet oxygen (El-Bahr, 2013).

2.2.1.5. Singlet oxygen

It is an electronically high excited, meta-stable state of molecular oxygen and is a highly reactive toxic reactive oxygen species. It is produced *in vivo* by the activation of neutrophils and eosinophils. It is also formed by some of the enzymatic reactions catalyzed by enzymes such as lipoxygenases, dioxygenases, and lactoperoxidase. It is a highly potent oxidizing agent that can cause DNA damage and tissue damage (Phaniendra *et al.,* 2015).

2.2.2 Reactive nitrogen species

Reactive nitrogen species are various nitric oxide-derived compounds, including nitroxyl anion, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols, and dinitrosyl iron complexes. RNS have been recognized as playing a crucial role in the physiologic regulation of many, if not all, living cells, such as smooth muscle cells, cardiomyocytes, platelets, and nervous and juxtaglomerular cells. They possess pleiotropic properties on cellular targets after both posttranslational modifications and interactions with reactive oxygen species. Elevated levels of RNS have been implicated in cell injury and death by inducing nitrosative stress (Martínez and Andriantsitohain, 2009).

2.2.2.1. Nitric oxide (NO•)

NO• is synthesized through the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS). The NO radical (NO•) is produced in higher organisms by the oxidation of one of the terminal guanido-nitrogen atoms of L-arginine. Depending on the microenvironment, NO can be converted to various other reactive nitrogen species such as nitrosonium cation (NO^+), nitroxyl anion (NO^-) or peroxynitrite ($ONOO^-$). At physiological concentrations, NO radical controls mitochondrial respiration, causing reversible inhibition of respiration by altering cytochrome C oxidase (Complex IV) activity, which is the terminal enzyme of the mitochondrial respiratory chain. NO radical also binds to soluble guanylate cyclase in the vascular endothelium to control vascular tone (Aprioku, 2013). •NO and its metabolites stimulate and inhibit lipid peroxidation reactions, modulate enzymatically catalyzed lipid oxidation, complex with lipid-reactive metals, and alter proinflammatory gene expression (Bloodsworth *et al.,* 2000).

2.2.2.2. Peroxynitrite

Superoxide anion and nitric oxide react and produce peroxynitrite anion ($ONOO^-$) which causes DNA fragmentation and lipid oxidation (Shastri *et al.,* 2016). It is highly toxic and can directly react with CO_2 to form other highly reactive nitroso peroxo carboxylate ($ONOOCO_2^-$) or peroxynitrous acid (ONOOH). The ONOOH further undergo homolysis to form both OH• and NO_2 or rearrange to form NO_3. $OONO^-$ can oxidize lipids, oxidize methionine and tyrosine residues in proteins and oxidizes DNA to form nitroguanine. The nitrotyrosine residues are considered as marker of peroxynitrite induced cellular damage (Phaniendra *et al.,* 2015). Peroxynitrite reacts with the aromatic amino acid residues present in the active site of the enzyme, result in the nitration of the aromatic amino acids which leads to the enzyme inactivation. Peroxynitrite is an important cytotoxic effector molecule in the defense against tumor cells, various protozoa, fungi, helminthes, and mycobacteria. The biological chemistry of peroxynitrite is modulated by endogenous antioxidant mechanisms and neutralized by synthetic compounds with peroxynitrite-scavenging capacity (Nimse and Pal, 2015).

2.3 Molecular targets of free radicals

When there is an imbalance between the free radical production (ROS/RNS) and antioxidant defenses, the former will be produced in higher concentrations leading to oxidative stress and nitrosative stress. Since these free radicals are highly reactive, they can damage all the three important classes of biological molecules including nucleic acids, proteins, and lipids (Phaniendra *et al.,* 2015).

2.3.1.Oxidative damage to DNA

ROS can lead to DNA modifications in several ways, which involves degradation of bases, single or double stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Most of these DNA modifications are highly relevant to carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases **(**Birben *et al.,* 2012). It has been reported that mitochondrial DNA are more susceptible to oxidative damage that have role in many diseases including cancer (Lobo *et al.,* 2010).

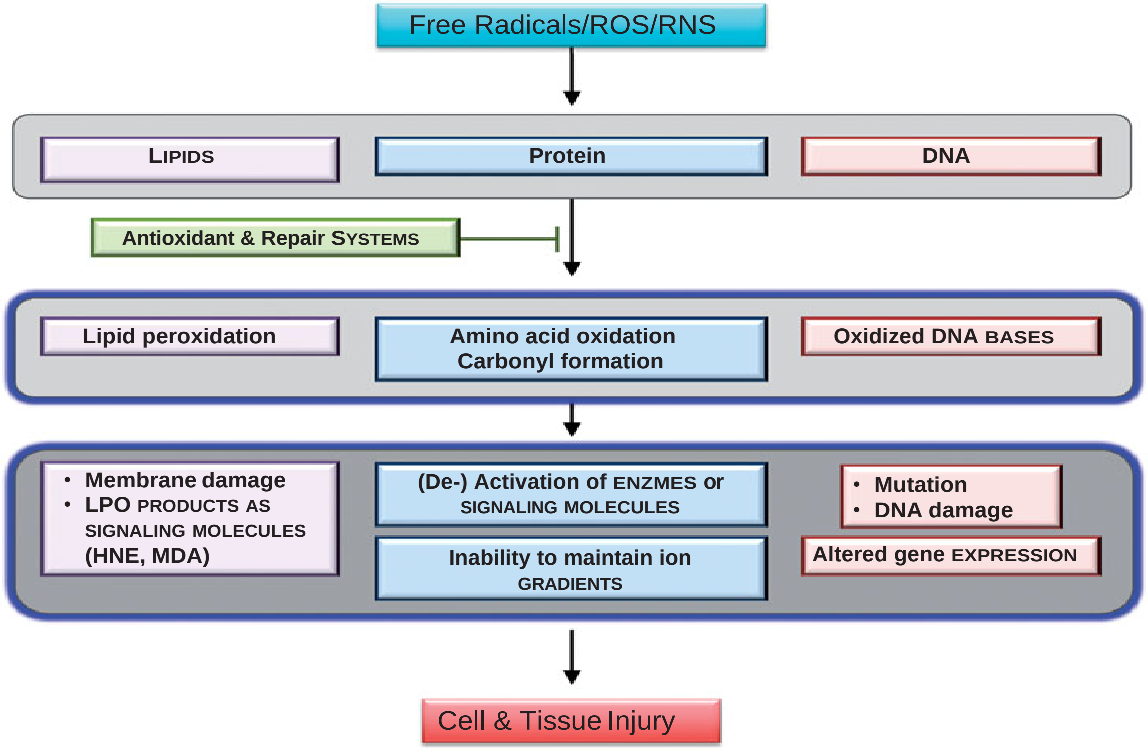
2.3.2. Oxidative damage to Lipids

Although Lipid peroxidation (LP) affects many cellular components, the primary action sites involve membrane associated poly unsaturated fatty acids (PUFA). Peroxidation of membrane associated fatty acids and cholesterol will alter cell membrane fluidity and permeability characteristics and may eventually induce widespread membrane damage. Lipid peroxides arising as a consequence of tissue damage can propagate further LP locally and at sites distant to areas of initial damage. The lipid peroxidation chain can lead to an elevated steady state concentration of lipid peroxides at the expense of oxygen and unsaturated lipids (Adly, 2010). Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages. Lipid peroxidation products, such as isoprostanes and thiobarbituric acid reactive substances, have been used as indirect biomarkers of oxidative stress, and increased levels were shown in the exhaled breath condensate or bronchoalveolar lavage fluid or lung of chronic obstructive pulmonary disease patients or smokers (Birben *et al.,* 2012).

2.3.3.Oxidative damage to proteins

Free radicals can cause direct damage to proteins which can directly interfere with enzyme activity and the function of structural proteins. Oxidation of proteins by ROS/RNS leads to production of stable as well as reactive products such as protein hydroperoxides that can further generate additional free radicals particularly upon interaction with transition metal ions. Mostly these oxidised forms of proteins are rapidly removed however their accumulation over a period of time can contribute to the damage associated with ageing as well as various diseases (Sharma, 2014)**.**

Figure 3. Mechanism of free radicals mediated damage (Kehrer and Klotz, 2015)

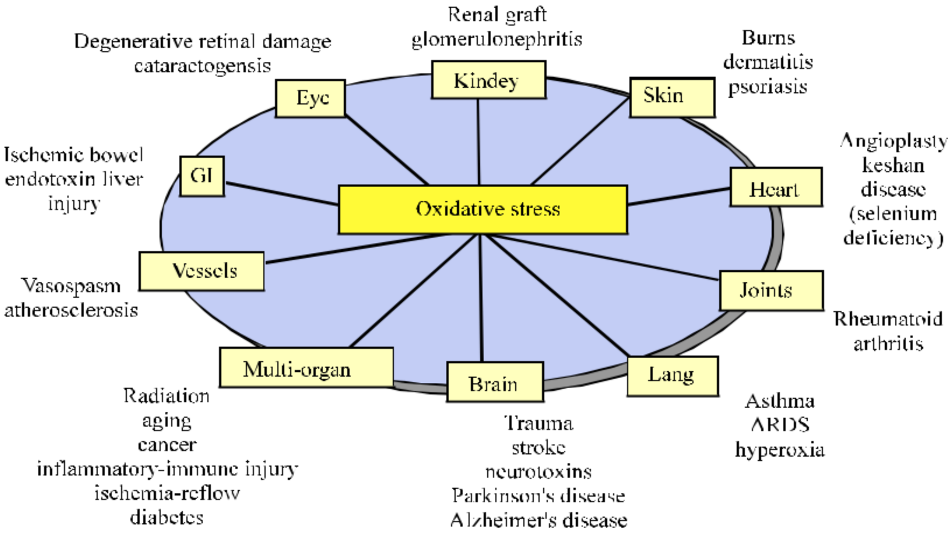


Further, oxidative attack on proteins can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, site-specific amino acid modification, aggregation of cross linked reaction products, and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases (Kumar, 2011).

2.4 Oxidative Stress and Diseases

Attack of free radicals on the critical cellular components such as lipids carbohydrates, proteins and DNA have been implicated in various pathological conditions such as cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, cataract, inflammatory bowel disease, rheumatoid arthritis, respiratory diseases, autoimmune diseases, liver diseases and ageing. These diseases can be categorized into two groups: (i) the first group involves diseases characterized by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance—the so called "mitochondrial oxidative stress" conditions (cancer and diabetes mellitus); (ii) the second group involves disease characterised by "inflammatory oxidative conditions" and enhanced activity of either NAD[P]H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of ROS (implicated in ischemia and reperfusion injury). The process of ageing is largely due to the damaging consequence of free radical action (lipid peroxidation, DNA damage, protein oxidation) (Sharma, 2014).

Figure 4. Oxidative stress and disease (Adly, 2010)

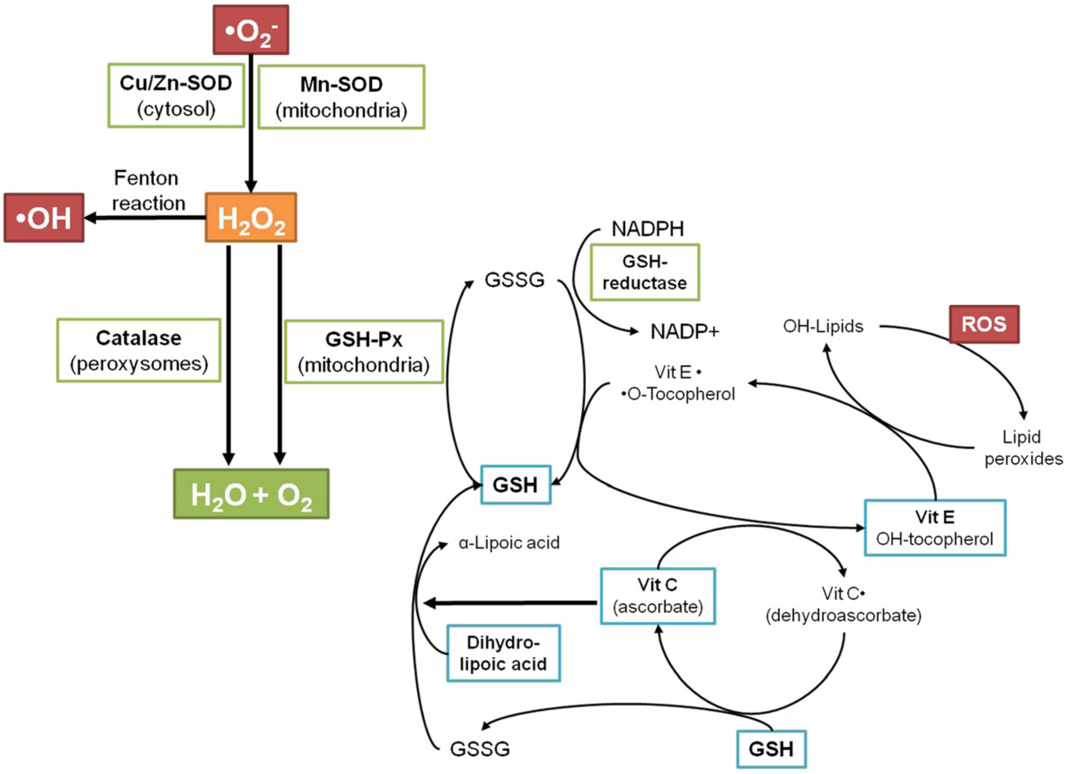


2.5 Cellular defense against oxidative stress

The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ* (endogenous) or externally supplied through foods and/ or supplements (exogenous). Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS; and therefore can enhance the immune defense and lower the risk of various diseases and cancer (Gupta *et al.,* 2014). Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents.

Two principle mechanisms of action have been proposed for antioxidants. The first is a chain- breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation.

Figure 5. Antioxidants defense against free radicals (Kurutas 2016)



An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to modulate the free radical reactions (Lobo *et al.,* 2010)**.** The most efficient enzymatic antioxidants contain glutathione peroxidase, catalase and superoxide dismutase, the first line of defense, act as the preventive antioxidants and suppress the formation of free radicals that leads to oxidative stress (Haida and Hakiman, 2019).

2.5.1 Antioxidant defense system

2.5.1.1 Enzymatic antioxidants

2.5.1.1. 1 Superoxide Dismutase

SOD, the first line of defense against free radicals, catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O2), consequently rendering the potentially harmful superoxide anion less hazardous. SOD is a metalloenzyme and hence, requires a metal cofactor for its activity. On the basis of the type of metal ion required as cofactor by SOD, various forms of the enzyme exist. The metal ions which are normally bound by SOD are iron (Fe), zinc (Zn) copper (Cu) and manganese (Mn). In this regard, SODs are classified into three forms and these include (i) Fe-SOD which is commonly found in prokaryotes and chloroplasts of some plants (ii) Mn-SOD which is present in prokaryotes and mitochondria of eukaryotes and (iii) Cu/Zn-SOD is predominant in eukaryotes and more distributed, localized basically in cytosol but also found in chloroplasts and peroxisomes **(**Ighodaro and Akinloye, 2018).

2.5.1.1.2. Catalase

Catalase, the heme-containing enzyme found in aerobic eukaryotes is important in the removal of H_2O_2 generated from peroxium by oxidizing the fatty acids, light respiratory, and purine demolition (AL-Aloosy *et al.,* 2019). Catalase exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H_2O_2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by H_2O_2 as it is reduced to water. (Birben *et al.,* 2012)**.** Reduced CAT activity leads to ROS generation and oxidative stress resulting in DNA damage and genome instability promoting cancer development (Snezhkina *et al.,* 2019).

2.5.1.1.3. Glutathione

Glutathione system includes glutathione S-transferases, glutathione peroxidases, and glutathione reductase. Glutathione peroxidase and glutathione reductase enzymes are found in a cell that metabolizes hydrogen peroxide to water while glutathione *S*-transferases are another class of glutathione-dependent antioxidant enzymes that show high activity with lipid peroxides (Adwas *et al.,* 2019).

Glutathione Peroxidase (GPx) is an important selenium-containing intracellular enzyme that breakdown hydrogen peroxides (H_2O_2) to water; and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol. The enzyme plays a more crucial role of inhibiting lipid peroxidation process, thus protecting cells from oxidative stress (Ighodaro and Akinloye, 2018) and has been found to be more effective than catalase at removing intracellular peroxides under many physiological conditions (Lubos *et al.,* 2011)**.**

Glutathione reductase is a flavoprotein that contains two FAD molecules as a prosthetic group, which is reducible by NADPH. GR is one of the thermostable enzymes. GR belongs to the defense system protecting the organism against chemical and oxidative stress. Deficiency of GR is characterized by hemolysis due to increased sensitivity of erythrocyte membranes to H_2O_2 and contributes to oxidative stress which plays a key role in the pathogenesis of many diseases (Adwas *et al.,* 2019). GR is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays key roles in the cellular control of reactive oxygen species (Couto *et al.,* 2016)**.**

Glutathione-S-Transferases (GSTs) (EC 2.5.1.18), another antioxidant enzyme family, inactivate metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. (Ladner *et al.,* 2004). GSTs are typically small proteins (200–250 amino acids) that are activated in response to oxidative damage and/or exposure to a large variety of toxins. GST plays an important role in the detoxification of ROS and limiting oxidative damage in tissues by catalyzing conjugation of GSH with various electrophiles, metabolites, and xenobiotics. The coupling of the xenobiotic to GSH increases the solubility of the toxin, thus facilitating its excretion. (Roncalli *et al.,* 2015, Robaczewska *et al.,* 2016).

2.5.1.2 Non Enzymic Antioxidants

2.5.1.2.1. Vitamin E (α -Tocopherol)

Vitamin E (α-tocopherol), is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). It functions to intercept lipid peroxyl radicals (LOO•) and to terminate the lipid peroxidation chain reactions (Nimse and Pal, 2015)**.** Lipid soluble Vitamin E prevents lipid peroxidation chain reactions in cellular membranes by interfering with the propagation of lipid radicals. Vitamin E and its derivatives inhibit growth and induce apoptotic cell death of cancer cells (Ryan *et al.,* 2010)**.**

2.5.1.2.2. Vitamin C

Another important antioxidant vitamin is vitamin C (ascorbic acid), a potent and effective hydrophilic antioxidant. Vitamin C is an electron donor (*i.e.*, reducing agent), and by donating its electrons, it prevents other compounds from being oxidized, while it is oxidized in the process. Thus, one of the mechanisms of antioxidant action of vitamin C is through the scavenging or quenching of free radicals. Vitamin C is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. In addition, the antioxidative effects of vitamins C and E have been shown to be complimentary or synergistic (Aprioku, 2013)**.**

2.5.1.2.3. Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. Due to its high concentration and central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Lobo *et al.,* 2010).

2.6 Cancer

Cancer is one of the major health issues among the population all over the world, resulting in millions of diagnosis every year and increasing deaths resulting from this dreadful disease (Sharmila and Padma, 2013). Carcinogenesis is a complex and multistep process that involves the accumulation of successive transformational events driven by genetic mutations and epigenetic alterations that affect major cellular processes and pathways such as proliferation, differentiation, invasion and survival. Massive deregulation of all components of the epigenetic machinery is a hallmark of cancer. These alterations affect normal gene regulation and impede normal cellular processes including cell cycle, DNA repair, cell growth, differentiation and apoptosis (Schnekenburger *et al.,* 2014)**.** Genetic changes can occur at many levels, with gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. There are two broad categories of genes which are affected by these changes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promotes the malignant phenotype of cancer cells. Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell (Knudson, 2001**).**

Lung cancer is currently the malignant tumor with the highest mortality rate worldwide, often because it is not detected until there has been substantial progression of the illness, which leads to a significant reduction in quality of life of the patient. The most important risk factor is smoking and the incidence rates of lung cancer are generally higher among men than women. Lung cancer is classified into nonsmall cell lung carcinoma (NSCLC), including squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, which represents 80% of all lung cancer cases, and the remaining cases are small cell lung carcinoma (SCLC) (Monteiro *et al.,* 2014).

Lung cancers develop through a multistep process involving development of multiple genetic and epigenetic alterations, particularly activation of growth promoting pathways and inhibition of tumour suppressor pathways. Lung cancer mutations have been identified in v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), BRAF and the parallel phosphatidylinositol 3-kinase (PI3K) pathway oncogenes and more recently in MEK and HER2 while structural rearrangements in ALK , ROS1 and possibly rearranged during transfection (RET) provide new therapeutic targets. Amplification is another mechanism of activation of oncogenes such as MET in adenocarcinoma, fibroblastgrowth factor receptor 1 (FGFR1) and discoidin domain receptor 2 (DDR2) in SCC. Intriguingly, many of these genetic alternations are associated with smoking status and with particular racial and gender differences, which may provide insight into the mechanisms of carcinogenesis and role of host factors in lung cancer development and progression (Cooper *et al.,* 2013).

2.7 Apoptosis

Apoptosis is a highly conserved, precisely regulated cascade of biochemical and molecular events for eliminating aged and sub-lethally damaged cells, and can be induced by various chemical and mechanical damages. Morphological changes in the apoptotic cells include membrane blebbing, loss of membrane asymmetry and attachment, cell shrinkage, chromatin condensation, and chromosomal DNA fragmentation (Vardhini *et al.,* 2013) and can be induced through extrinsic and/or intrinsic pathways. The extrinsic pathway is initiated by the interaction of death receptors and specific signalling molecules, while the intrinsic pathway is primarily stimulated by cellular sensing of extracellular and/or intracellular stresses, both of which require appropriate stimuli to trigger (Pumiputavon *et al.,* 2017).

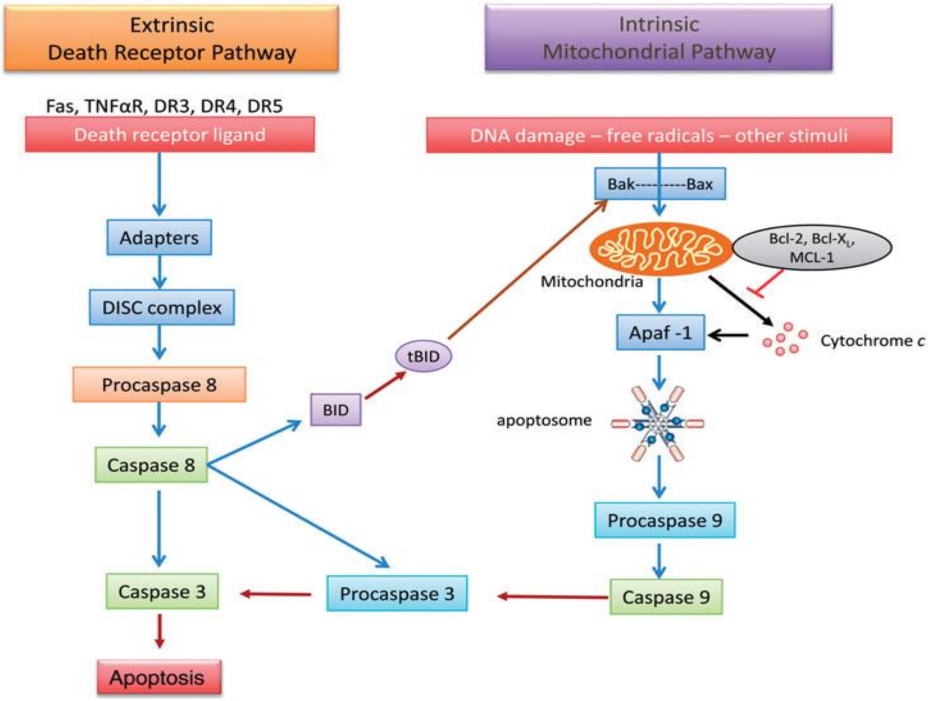
2.7.1 The extrinsic death receptor pathway

The extrinsic death receptor pathway, as its name implies, begins when death ligands bind to a death receptor. Although several death receptors have been described, the best known death receptors is the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95) and their ligands are called TNF and Fas ligand (Fas L) respectively. These death receptors have an intracellular death domain that recruits adapter proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), as well as cysteine proteases like caspase 8. Binding of the death ligand to the death receptor results in the formation of a binding site for an adaptor protein and the whole ligand-receptor-adaptor protein complex is known as the death-inducing signalling complex (DISC). DISC then initiates the assembly and activation of pro-caspase 8. The activated form of the enzyme, caspase 8 is an initiator caspase, which initiates apoptosis by cleaving other downstream or executioner caspases (Wong, 2011).

2.7.2 The intrinsic mitochondrial pathway

Internal stimuli such as irreparable genetic damage, hypoxia, extremely high concentrations of cytosolic Ca^{2+} and severe oxidative stress are some triggers of the initiation of the intrinsic mitochondrial pathway. Regardless of the stimuli, this pathway is the result of increased mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm. This pathway is closely regulated by a group of proteins belonging to the Bcl-2 family, there are two main groups of the Bcl-2 proteins, namely the pro-apoptotic proteins (e.g. Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim and Hrk) and the anti-apoptotic proteins (e.g. Bcl-2, Bcl-XL, Bcl-W, Bfl-1 and Mcl-1). While the anti-apoptotic proteins regulate apoptosis by blocking the mitochondrial release of cytochrome-c, the pro-apoptotic proteins act by promoting such release. The balance between the pro- and anti-apoptotic proteins that determines whether apoptosis would be initiated. Other apoptotic factors that are released from the mitochondrial intermembrane space into the cytoplasm include apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac), direct IAP Binding protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2). Cytoplasmic release of cytochrome c activates caspase 3 via the formation of a complex known as apoptosome which is made up of cytochrome c, Apaf-1 and caspase 9.

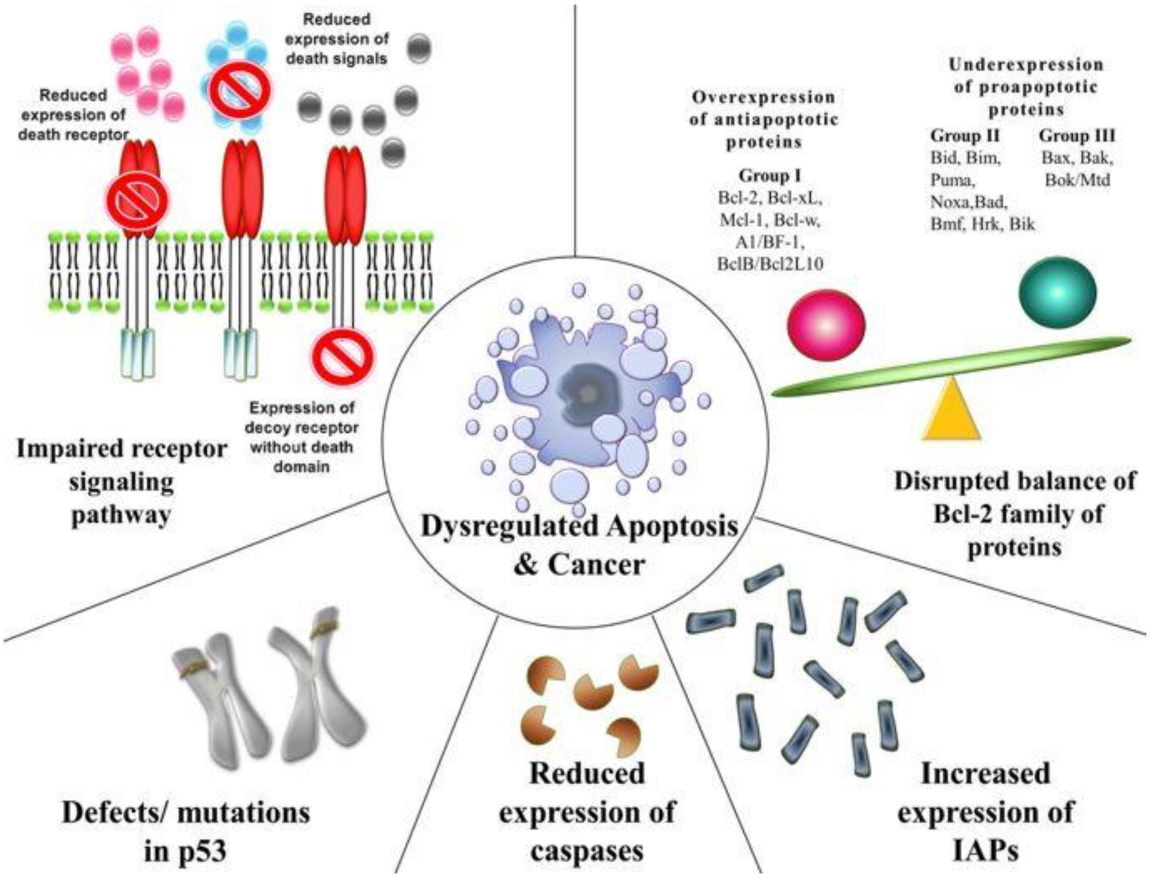
Figure 6. Intrinsic and Extrinsic Apoptotic pathway (Kehrer and Klotz, 2015)



2.8 Apopotosis and carcinogenesis

The hallmarks of cancer are present in all cancer cells regardless of the cause or type; these include uncontrolled growth, angiogenesis and apoptosis evasion. The loss of apoptotic control allows cancer cells to survive longer and gives more time for the accumulation of mutations which can increase invasiveness during tumor progression, stimulate angiogenesis, deregulate cell proliferation and interfere with differentiation. There are many ways in which cancer cells which evade apoptosis: caspase function can be inhibited or the trigger for apoptosis can be disabled. Disrupted balance of pro-aopptotic and anti-apoptotic proteins, impaired death receptor signaling (Wong, 2011, Pfeffer *et al.,* 2018).

Figure 7. Mechanisms pertaining to evasion of apoptosis and carcinogenesis (Wong, 2011)



One way of treating cancer is to gain control or possibly terminate the uncontrolled growth of cancer cells. Additionally, targeting apoptosis is the most successful non-surgical treatment. Targeting apoptosis is also effective for all types of cancer, as apoptosis evasion is a hallmark of cancer and is nonspecific to the cause or type of the cancer. There are many anticancer drugs that target various stages in both the intrinsic and extrinsic pathways. Two common strategies for therapeutic targeting are stimulation of proapoptotic molecules and inhibition of antiapoptotic molecules. The upregulation of antiapoptotic BCL-2 proteins and loss of BAX and/or BAK are the predominant methods of evasion (Pfeffer *et al.,* 2018).

2.9 Concept of Nanotechnology

Nanotechnology is a multidisciplinary field that combines science and technology with aims to develop new and improved materials with significant functions, physical and chemical properties (Jasmani *et al.,* 2020). Advances in Nanoscience are promptly enabling the evolution of nanoparticles with distinct properties which address the limitations of traditional disease diagnostic and therapeutic agents (Rajendran *et al.,* 2015). Nanomaterials have been reported to be the ‘‘material of the 21st century” because of their unique designs and property combinations compared with conventional materials. There is a wide range of applications of NPs such as in human health appliances, industrial fields, medical applications, biomedical fields, energy science, drug and gene delivery, mechanics, space industries, engineering, electronics, and environmental studies. Recently, enormous attention has been focused on the use of nanoparticles such as nanotubes, nanowires, fullerene derivatives, and quantum dots to create new types of analytical tools in the fields of life science and biotechnology (Akter *et al.,* 2018).

Nanoparticles (NPs) are usually between 10 and 100 nm in diameter and, when compared with the parent material they often exhibit new properties at nanoscale, compared to their respective particles at higher scales, like increased bioavailability due to improved solubility, longer biological half-life, increased reactivity or stability in a chemical process, enhanced mechanical strength and increased catalytic activities as a result of the higher surface to volume ratio. Thus, the main benefit is that a reduced concentration is needed to produce a desired response with less overall toxicity to normal cells (Alqahtani *et al.,* 2016). Nanomaterials are “deliberately engineered” to guide the improvement of special properties at the nanoscale level. They may have superior bioavailability than larger units, ensuring in greater utilization of individual cells, tissues, and organs. Nanomaterials that gain admission to our bodies just penetrate biological membranes and access cells, tissues, and organs (Beyenea *et al.,* 2017).

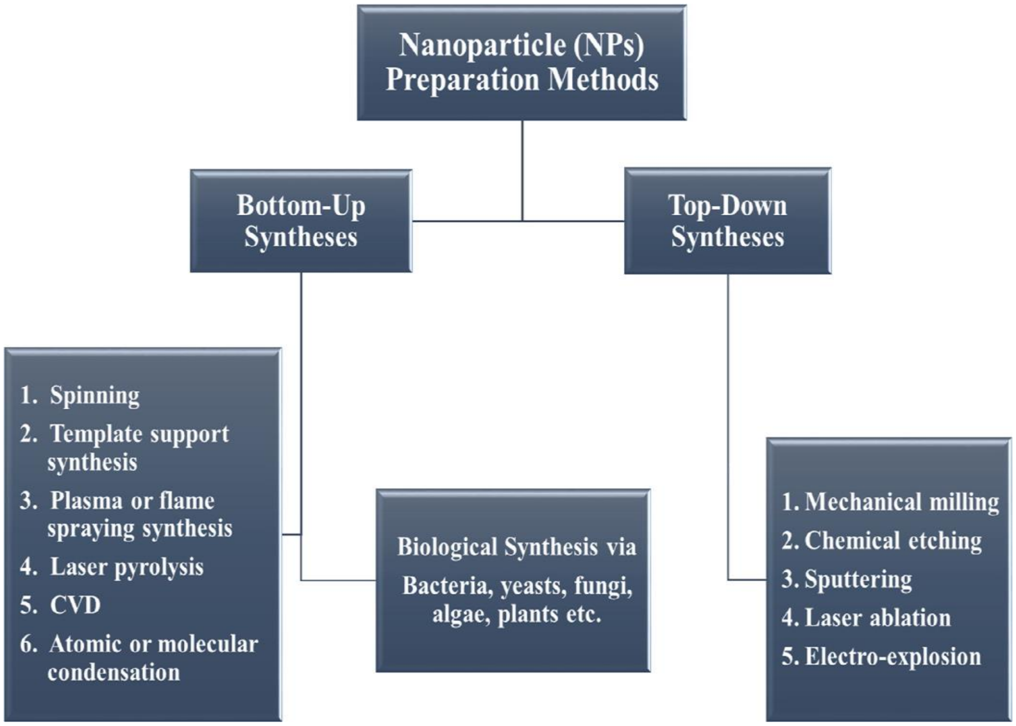
Nanoparticles have many functional platforms that can be utilized for imaging and therapeutic functions. These platforms can be prepared from various inorganic and organic materials, but the inorganic platforms are very important for simultaneous therapy and diagnosis because of their easy modification, high drug loading capacity and stability. Nanoparticles can be used in drug delivery and in the determination of drugs in pharmaceuticals (Alaqad and Saleh, 2016).

Metal nanoparticles have received great attention from researchers across the world because of a plethora of applications in agriculture and the biomedical field as antioxidants and antimicrobial compounds (Gahlawat and Choudhury, 2019). Metallic nanoparticles are emerging as new carriers which provide way to site-specific targeting and drug delivery by these nanoparticles. The exclusive physical–chemical properties of metal NPs give them high multifunctionality. Noble metallic nanoparticles, such as AuNPs, AgNPs, PtNPs, offer high stability, easy chemical synthesis and tuneable surface functionalization (Vinci and Rapa, 2019). Among the various inorganic metal NPs, Silver Nanoparticles (AgNPs) have received substantial attention for various reasons. AgNPs are one of the promising products in the nanotechnology industry (Ankanna *et al.,* 2010). Among the noble metals (Ag, Pt, Au and Pd), Silver (Ag) is the metal of choice for potential applications in medicine due to its unique properties such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity, increased oral bio-availabilty (Lee and Jun, 2019). Ag is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications; it has even been shown to prevent HIV binding to host cells (Badawy, 2014). Silver nanoparticles have become the focus of intensive research owing to their wide range of applications in various other areas such as catalysis, optics, antimicrobials, and biomaterial production. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution (Awwad *et al.,* 2013). Besides, silver nanoparticles could induce apoptosis-dependent programmed cell death in the absence of the tumor suppressor p53 while conventional cancer therapy often fails to cause cell death in p53-deficient cancer cells. Moreover, nanoparticles of size 5-35nm primarily induced cell death through the mitochondrial structure and function targeting (Abdel-Fattah and Ali, 2018).

2.10 Methods of synthesis of nanoparticles

Generally nanoparticles are produced and stabilized either by a “top down” or “bottom up” strategy. In the “bottom up” strategy, nanoparticles are synthesized via self-assembly of atoms into nuclei which further develop into nanoscale particles. This approach includes chemical and biological methods of production whereas in the “top down” strategy, bulk material is broken down into small particles by size reduction using various physical and chemical techniques. The physical methods include grinding, milling and thermal ablation. On the other hand, the chemical approach of nanoparticles synthesis includes electrochemistry, chemical reduction, and photochemical reduction techniques (Khan *et al.,* 2017).

Figure 8. Methods of nanoparticle synthesis (Khan *et al.,* 2017)



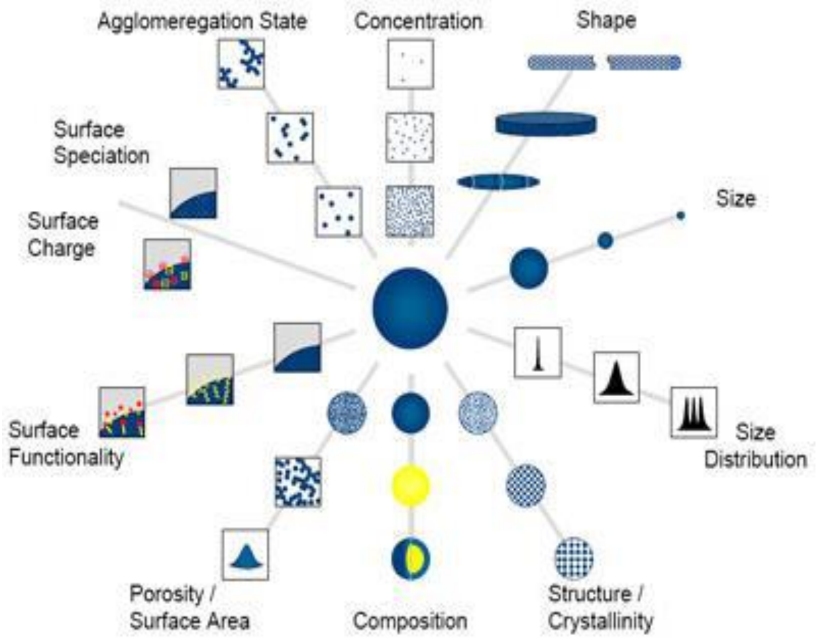
The physical approaches require high amount of energy which makes these types of processes more capital intensive. Another drawback of physical methods is the lower production yield of nanoscale materials. Over the past years, the chemical processes have been most preferable approach for nanoparticles synthesis because of requirement of less energy during reduction step and formation of homogenous particles with high preciseness in size and shape. However, chemical methods are environmentally hazardous because of the use of various perilous chemicals (hydrazine or potassium bitartrate) which are responsible for carcinogenicity, genotoxicity, and cytotoxicity (Gahlawat and Choudhury, 2019). Apart from these disadvantages, the manufactured particles are not of expected purity, as their surfaces were found to be sedimented with chemicals. It is also very difficult to prepare AgNPs with a well-defined size, requiring a further step for the prevention of particle aggregation. In addition, during the synthesis process, too many toxic and hazardous byproducts are excised out (Zhang *et al.,* 2016). Thus the use of chemical methods for synthesis of nanoparticles for biomedical application have been restricted because of toxicity, instability and less biocompatibility. Therefore developing an environment friendly approach that effectively modulates the size, morphology, stability and charecteristics is presently the main focus of the research on nanoparticle synthesis (Gahlawat and Choudhury, 2019).

Biological synthesis of AgNPs from herbal extract has appeared as a promising route for synthesis of biocompatible and stable nanoparticles as these methods have several advantages over the chemical and physical methods of synthesis. Indeed these routes are simple, cost effective, eco-friendly and easily scaled up for high yields and or production. Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and several types of secondary metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-product (Siddiqi *et al.,* 2018) Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability. Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research (Zhang *et al.,* 2016).

2.11 Charecterization of nanoparticles

The physicochemical properties of nanoparticles are important for their behavior, bio-distribution, safety, and efficacy. Therefore, characterization of AgNPs is important in order to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including UV-vis spectroscopy, X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) (Zhang *et al.,* 2016).

Figure 9. Characterization of nanoparticles (Ealias and Saravanakumar, 2017)



2.11.1. UV-Visible Spectroscopy

UV-VIS spectroscopy is an exceptionally valuable and essential for characterization of nanoparticles. UV-VIS spectroscopy is quick, simple, basic, and specific for various sorts of NPs, needs just a brief period of time for estimation. In AgNPs, the conduction band and valence band lie near each other in which electrons move openly. These free electrons offer ascent to a surface plasmon reverberation (SPR) assimilation band, this is occurring because of the aggregate swaying of electrons of silver nanoparticles in reverberation with the light wave. The assimilation of AgNPs relies upon the molecule estimate, dielectric medium, and synthetic surroundings (Roy, 2017).

2.11.2. Dynamic Light Scattering (DLS)

DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. It is possible to extract the size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function (Pal *et al.,* 2011). The size obtained from DLS is usually larger than TEM, which may be due to the influence of Brownian motion. DLS is a nondestructive method used to obtain the average diameter of nanoparticles dispersed in liquids. It has the special advantage of probing a large quantity of particles simultaneously; however, it has a number of sample-specific limitations (Zhang *et al.,* 2016).

2.11.3. Zeta potential

The nature and intensity of the surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The colloidal stability is analyzed through zeta potential of nanoparticles. This potential is an indirect measure of the surface charge. It corresponds to potential difference between the outer Helmholtz plane and the surface of shear. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion. High zeta potential values, either positive or negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The extent of surface hydrophobicity can then be predicted from the values of zeta potential. The zeta potential can also provide information regarding the nature of material encapsulated within the nanocapsules or coated onto the surface (Pal *et al.,* 2011). Nanoparticles with a zeta potential above (±) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles (Nikam *et al.,* 2014).

2.11.4. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) reveals vibrational stretch frequency of metal-oxygen bonds. To use the Fourier Transform Infrared Spectroscopy, a continuum source of light is used to produce light over broad range of infrared wavelengths (Srivastava, 2012). In FTIR spectroscopy, the light is directed on to the sample of interest, and the intensity is measured using an infrared detector. FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academic and industrial research. Furthermore, FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process. Therefore, FTIR is a suitable, valuable, non-invasive, cost effective, and simple technique to identify the role of biological molecules in the reduction of silver nitrate to silver (Zhang *et al.,* 2016).

2.11.5. X-Ray diffraction (XRD)

X-ray diffraction (XRD) is one of the most extensively used techniques for the characterization of NPs. Typically, XRD provides information regarding the crystalline structure, nature of the phase, lattice parameters and crystalline grain size. The latter parameter is estimated by using the Scherrer equation using the broadening of the most intense peak of an XRD measurement for a specific sample. An advantage of the XRD techniques, commonly performed in samples of powder form, usually after drying their corresponding colloidal solutions, is that it results in statistically representative, volume-averaged values. The composition of the particles can be determined by comparing the position and intensity of the peaks with the reference patterns available from the International Centre for Diffraction Data (ICDD, previously known as Joint Committee on Powder Diffraction Standards, JCPDS) database (Mourdikoudis *et al.,* 2018). XRD is based on the principle of constructive interference of X-rays and the sample concerned which should be crystalline. The X-rays which are generated by a CRT are filtered, collimated and then directed towards the sample. The interaction that follows produces constructive interference based on Bragg's law which relates wavelength of the incident radiations to the diffraction angle and lattice spacing (Boddolla and Thodeti, 2018)

2.11.6. Scanning electron microscope (SEM)

Scanning electron microscopy was extremely useful for the determination of topology and observations of surfaces as they offer better resolution and depth of field than optical microscope and it provides all available information about the NPs at nanoscale level. SEM is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. The types of signals produced by a SEM include secondary electrons (SE), back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons (Raliya and Tarafdar, 2014).

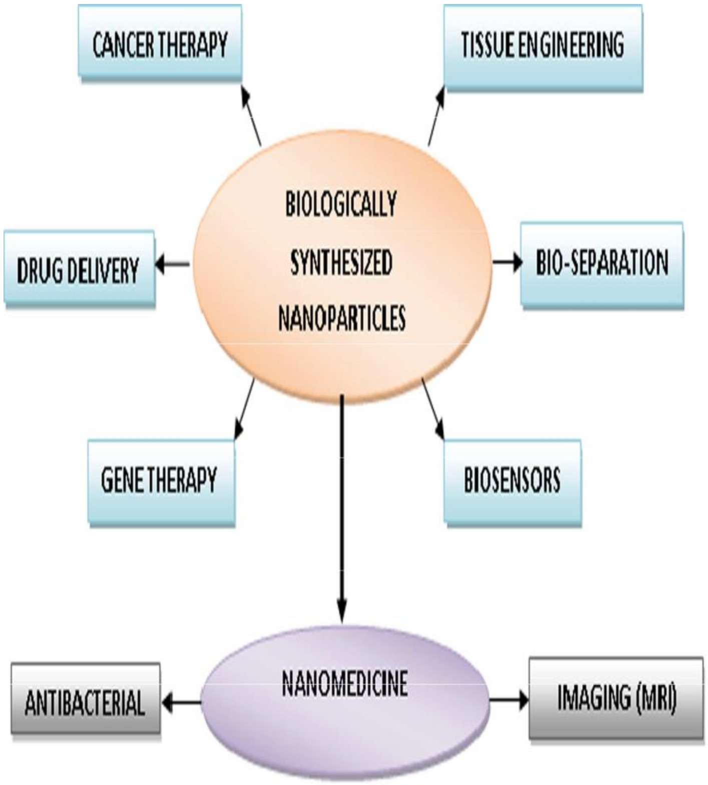
2.11.7. Transmission electron microscope (TEM)

TEM is based on electron transmittance principle, so it can provide information of the bulk material from very low to higher magnification (Khan *et al.,* 2017). In TEM analysis, an incident beam of electrons are generated by the process known as thermionic discharge or by field emission; they are then accelerated by an electric field and focused by electrical and magnetic fields onto the sample and then projected on a screen to generate a electron diffraction, amplitude-contrast image, a phase-contrast image, or a shadow image of varying darkness according to the density of unscattered electron (Srivastava, 2012). Transmission electron microscopy techniques can provide direct imaging, diffraction and spectroscopic information, chemical composition, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution.

2.12 Applications of Nanoparticles

The biologically synthesized nanoparticles are multi-functional with diverse biomedical applications. They are widely used in therapeutics, drug delivery systems, tissue regeneration, separation techniques and sensors. They are potentially used in nano-medicine (Nirmala *et al.,* 2013)**.**

Figure 10. Applications of biologically synthesized nanoparticles ((Nirmala *et al.,* 2013)



2.12.1 Cosmetics and Sunscreens

The conventional ultraviolet (UV) protection sunscreen lacks long-term stability during usage. The sunscreen including nanoparticles such as titanium dioxide provides numerous advantages. The UV protection property of titanium oxide and zinc oxide nanoparticles as they are transparent to visible light as well as absorb and reflect UV rays found their way to be used in some sunscreens. Some lipsticks use iron oxide nanoparticles as a pigment (Ealias and Saravanakumar, 2017).

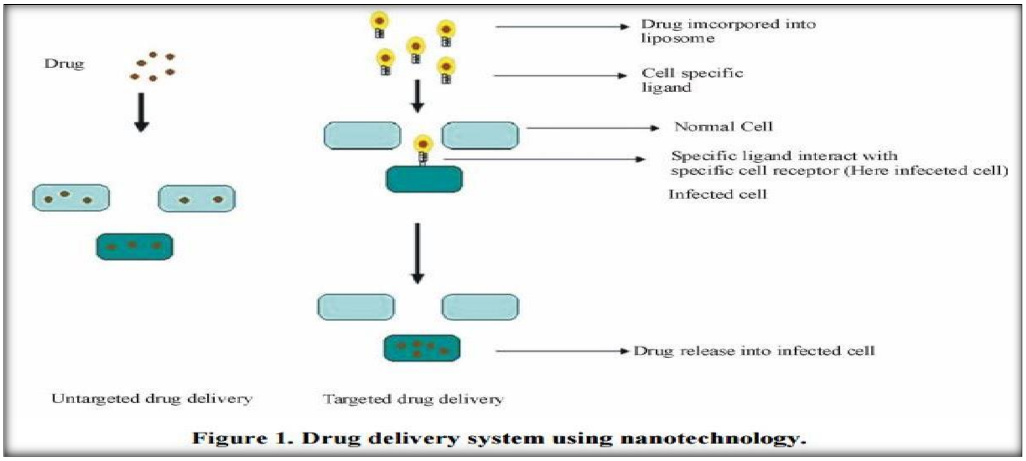
2.12.2 Nanoparticles for Gene delivery

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cellmediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system (Nikam *et al.,* 2014).

2.12.3 Drug Delivery system using AgNPs

The drug is incorporated into the liposome binds with their specific cell containing the targeted receptor. After that the drug is released inside the whole body using those infected cell. The nano sized carriers like micro nano suspension, liposome, dendrimer, ocular inserts, hydrogels are useful in oculardrug delivery which reduce toxicity. This method of approach will also increase the efficiency of drug delivery than conventional delivery system **(**Gupta, 2017).

Figure 11. Nanotechnology based Drug delivery system (Gupta *et al.,* 2017)



2.12.4 Nanotechnology in the treatment of neurodegenerative disorders

One of the most important applications of nanotechnology is in the treatment of neuro degenerative disorders. For the delivery of CNS therapeutics, various nano carriers such as, dendrimers, nano gels, nano emulsions, liposomes, polymeric nano particles, solid lipid nano particles, and nano suspensions have been studied. Transportation of these nano medicines has been effected across various *in vitro* and *in vivo* BBB models by endocytosis and/or transcytosis, and early preclinical success for the management of CNS conditions such as, Alzheimer's disease, brain tumors, HIV encephalopathy and acute ischemic stroke has become possible (Nikalje, 2015).

2.12.5 Nanotechnology in stem cell biology and medicine

Nanotechnology has made excellent contribution in the field of stem cell research. For example, Magnetic Nanoparticles (MNPs) have been successfully used to isolate and group stem cells. Quantum dots have been used for molecular imaging and tracing of stem cells, for delivery of gene or drugs into stem cells, nano materials such as carbon nano tubes, fluorescent CNTs and fluorescent MNPs have been used. Unique nanostructures were designed for controllable regulation of proliferation and differentiation of stem cells is done by designed unique nano structures. All these advances speed up the development of stem cells toward the application in regenerative medicine. The recent applications of nanotechnology in stem cell research promises to open new avenues in regenerative medicine. Nanotechnology can be a valuable tool to track and image stem cells, to drive their differentiation into specific cell lineage and ultimately to understand their biology. This will hopefully lead to stem cell-based therapeutics for the prevention, diagnosis and treatment of human diseases.

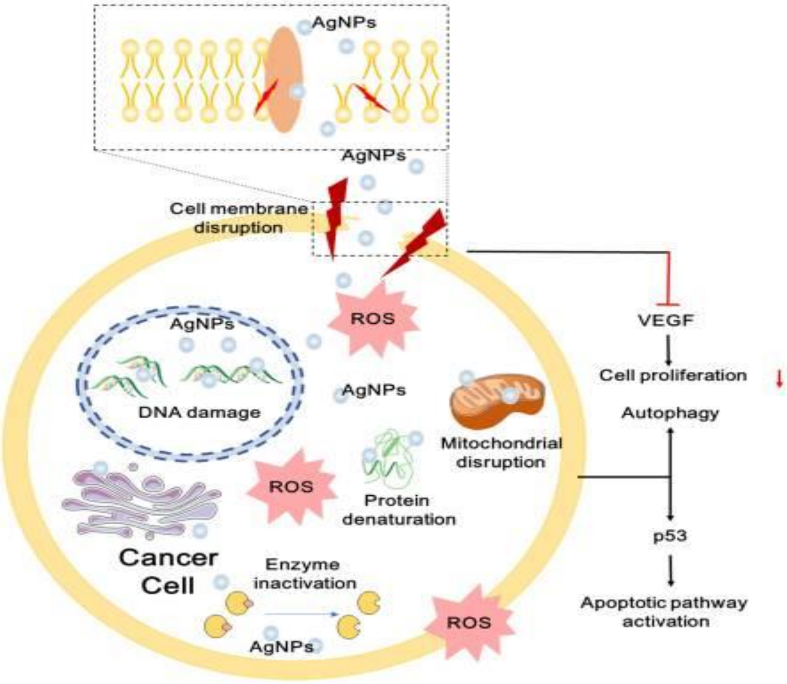
Nano devices can be used in stem cell research in tracking and imaging them. It has its applications for basic science as well as translational medicine. Stem cells can be modulated by mixing of nano carriers with biological molecules Nano devices can be used for intracellular access and also for intelligent delivery and sensing of biomolecules. These technologies have a great impact in stem cell microenvironment and tissue engineering studies and have a great potential for biomedical applications (Nikalje, 2015).

2.12.6 Silver nanoparticles in cancer therapeutics

Nanoparticles synthesized from green method are generally more toxic than those obtained from the non-green method. Some pathogens are more prone to nanomaterials, especially AgNPs than others due to the presence of both the Ag ions released and AgNPs. The cytotoxicity by Ag NPs is believed to be produced through reactive oxygen species as a consequence of which a reduction in glutathione level and an increase in ROS level occur (Siddiqi *et al.,* 2018)

Silver nanoparticles can enter cells by endocytosis and their localization inside the cell can be determined as the perinuclear space of cytoplasm and endo-lysosomal compartment. Besides, silver nanoparticles can enter the mitochondria and produce reactive oxygen species by affecting the respiration of cells. In summary, the mechanisms of AgNPs as toxic can lead to DNA damage, oxidative stress, induction of apoptosis, and mitochondrial damage to cancer cells. Furthermore, there are studies that AgNPs affect the function of the Vascular Endothelial Growth Factor (VEGF). It is also known as vascular permeability factor and plays a major role in the angiogenesis within tumors. These results support AgNPs have anti-cancer properties that can be used as an alternative for cancer therapy and angiogenesis inhibitor therapy (Yesilot and Aydin, 2019).

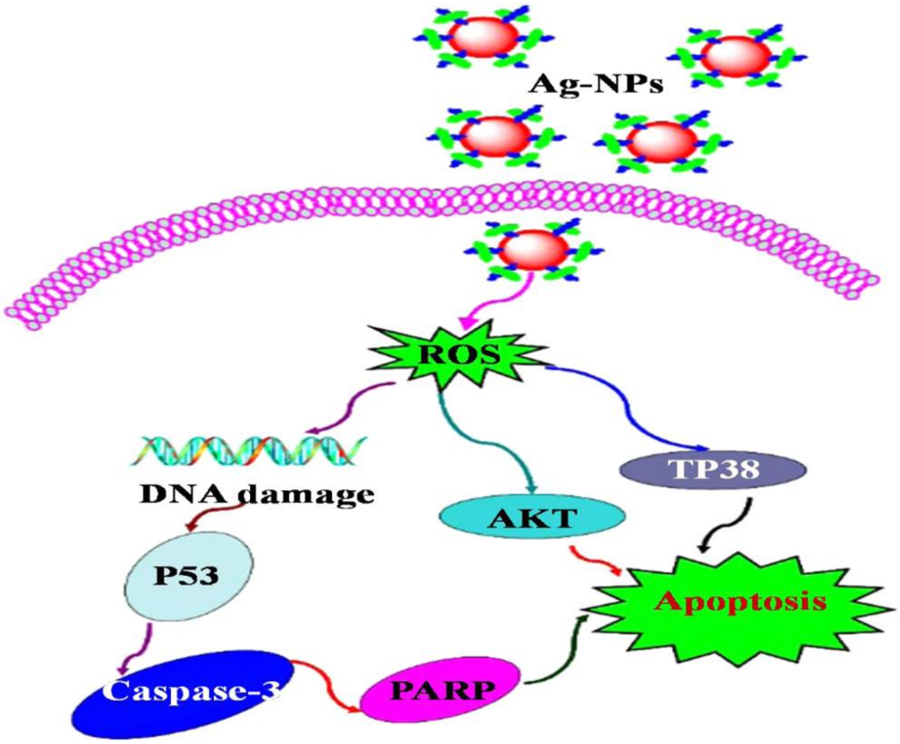
Figure 12. Mechanism of anticancer effect of silver nanoparticles (Xu *et al.,* 2020)



2.13 Silver nanoparticles in Apoptosis

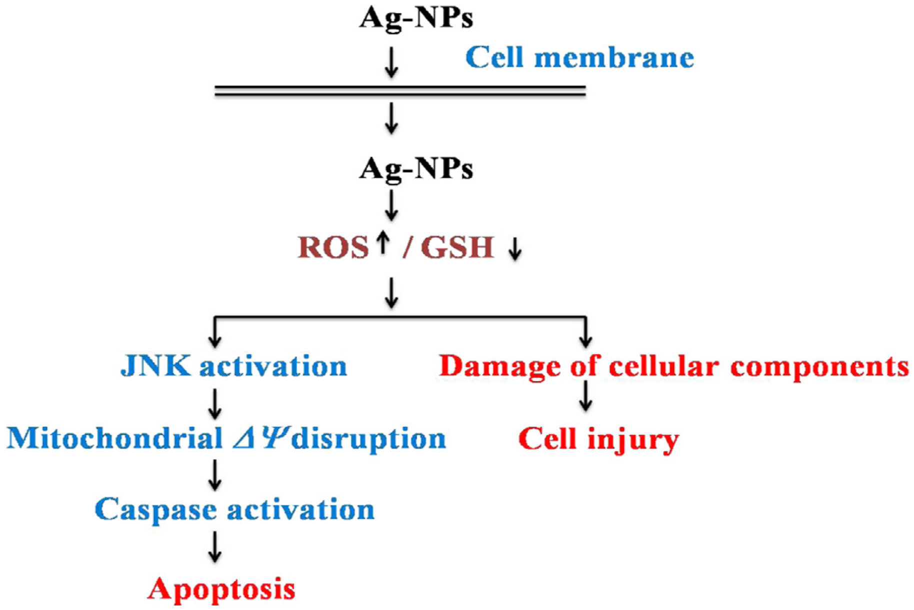
Overproduction of intracellular ROS, which activates cell death-regulating pathways such as p53, AKT, and MAPK signaling apoptotic pathways. Over production of ROS causes the down regulation of total AKT, which increases the expression of proapoptotic kinase p38. Meanwhile, decrease in PARP (Poly ADP Ribose Polymerase) expression resulting significant increase of caspase-3, H2X, p-p53, and total p53 expressions. Thus nanosilver can induce apoptosis by following p53 signaling pathway.

Figure 13. Apoptosis inducing pathway by p53, AKT, MAPK activation to suppress ROS generated by Ag-NPs (Li *et al.,* 2016)



Effect of Ag-NPs on mitochondrial membrane permeability could cause loss of mitochondrial integrity, which may regulate JNK mediated caspase dependent apoptosis. Loss of mitochondrial membrane potential regulate down-regulation of Bcl-2, upregulation of BAX and release of cytochrome c into the cytosol. Down-regulation of Bcl-2 can be influenced by JNK (Jun amino – terminal kinases). JNK is a member of MAPK family, which participate in apoptosis via phosphorylation of Bcl-2, consequent inactivation of Bcl-2. Release of cytochrome c into the cytosol initiates a cascade that leads to the initiation of caspase 3 through apaf-1 and caspase 9. Thus AgNPs can induce apoptosis via mitochondria and caspase dependent pathway mediated by JNK. Epigenetic dysregulation can also be induced by AgNPs, which may have long term effects on gene expression reprogramming. AgNPs could have effect on the cell cycle and induction of DNA hypermethylation following the p53 or p21 pathway, which may have effect on epigenomic level (Akter *et al.,* 2018).

Figure 14. A proposed pathway for Ag-NPs induced ROS generation and apoptosis (Piao *et al.,* 2011)

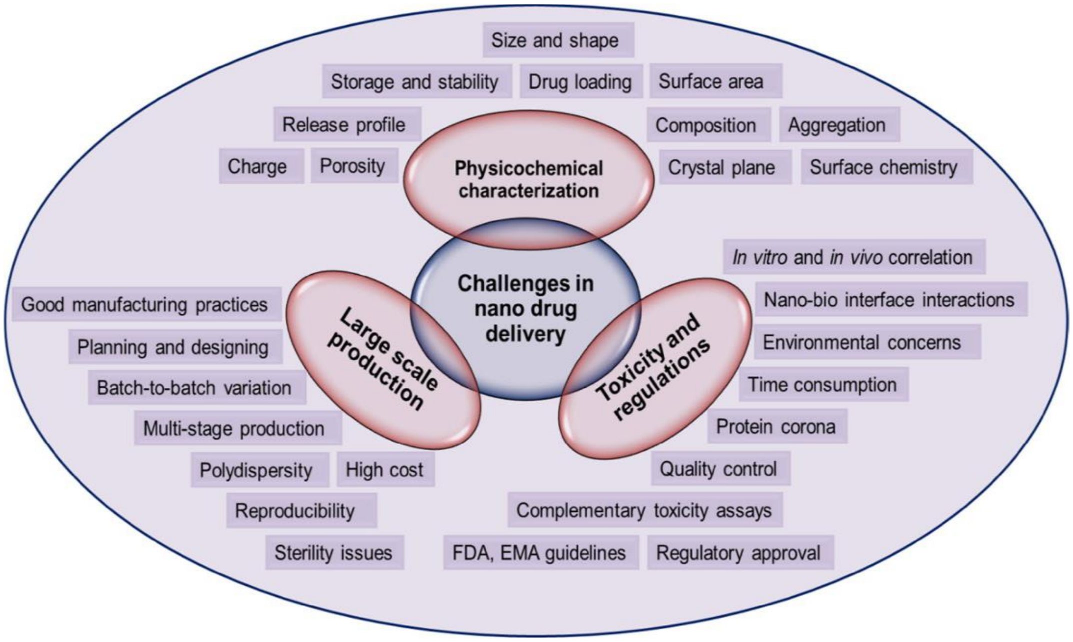


2.14 Challenges for Cancer Therapy Using AgNPs

Nanomedicine is as one of the fast developing and promising strategies to combat cancer using metallic nanoparticles. Nanoparticles have the ability to target through passive or active targeting of particular diseased cells or tumor tissues by the encapsulation of therapeutic agents with nanoparticles, and they have been used as drug delivery systems. Although many nanoparticle-mediated strategies have been developed, heterogeneity of the tumor and its stroma is a significant challenge for nanotechnologists and clinicians to come up with specific formulations to precisely target specific cancer cells. To achieve higher specificity, reduction in toxicity, biocompatibility, safety, better efficacy, and to overcome the limitations of conventional chemotherapy, nanoparticle-mediated therapy is the most suitable and alternative therapeutic strategy in cancer treatment.

However, there is a need to address the challenges and limitations of using nanoparticles for cancer therapy; these include physiological barriers, limited carrying capacity, enhanced permeability and retention effect (EPR), variability of nanoparticles, and regulatory and manufacturing issues (Zhang *et al.,* 2016). Since the physicochemical properties of nanomaterials play a significant role in the biocompatibility, and toxicity in the biological systems, synthesis and characterization of the nanomaterials for drug delivery need to be carefully performed to avoid the potential unwanted toxicity of nanocarriers to healthy cells. In conjunction to physicochemical properties, the nanomaterial storage and stability may also have an influence on their pharmacological performance. In addition, the use of nanocarriers in the treatment of cancer may result in unwanted toxicity through unfavourable interactions with biological entities. Furthermore, the manufacturing of nanomedicine products for commercialization is a key obstacle, as large scale-production is technically challenging. The involvement of complicated multi-stage processes of production of nanotherapeutics and the high cost of raw materials renders these nanotherapeutics an expensive option. Thus, to mitigate the problems associated with nanomaterial-based therapeutic agents for cancer treatment, design and development strategies need to be employed before they are used in medicine for better treatment and human life (Navya *et al.,* 2019).

Figure 15. Illustration of challenges in the delivery of cancer nanotherapeutics (Navya *et al.,* 2019)



2.15 Medicinal Plants / *T. roseo-alba*

Natural products especially medicinal plants have been used fot treatment of various diseases since the dawn of civilization. Herbal medicines are considered as a vast source of several impressive modern drugs. The medicinal plants play a vital role in the therapeutic armory of mankind. About 80% of world's population in developing countries totally depends on medicinal plants for their primary health care. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Nearly 3, 00,000 medicinal plants have been identified in the world, of which only 15% of them have been explored completely. Therefore, such plants need to be investigated to understand their properties, safety and efficacy (Kalita *et al.,* 2015).

The *Tabebuia roseo-alba* (Ridl) Sand, known as White Trump pet tree, belongs to the family Bignoniaceae and is considered as an economically important species owing to its ornamental use and its ethnopharmacological components. Earlier studies with the leaves and stem bark of this plant showed antimicrobial activity against microorganisms associated with nosocomial infections and cytotoxic activity of this plant species has also been proved (Silva *et al.,* 2017).

Kingdom : Plantae
Sub kingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Asteridae
Order : Scrophulariales
Family : Bignoniaceae
Genus : Tabebuia
Species : *roseo-alba*

Plate 1. *Tabebuia roseo-alba*

Further several species of *Tabebuia* have been reported to have a range of biological activities and is accepted as a remedial alternative for treating different diseases. *Tabebuia rosea* is used as an antipyretic, anti-inflammatory, antibacterial, antifungal, anti-cancer, and anti-diabetic agent. Stem bark of *T. avellanedae* is used in the treatment of snake bites. Also, *T. heterophylla*, *T. aurea*, *T. argentea* and *T. caraiba* are used as anticancer, anti-inflammatory, and antimicrobial agents (Rahman *et al.,* 2015).

The present study was carried out in four different phases and the methodology adopted for each phase is explained precisely in the following chapter.