2

REVIEW OF LITERATURE

Biomedical nanotechnology is an evolving field having enormous potential to positively impact the health care system. Important biomedical applications of nanotechnology that may have potential clinical applications on targeted drug delivery, detection/diagnosis and imaging (Singh *et al*., 2011). Noble metalNPs such as gold, silver and platinum are particularly interesting due to their size and shape dependent unique optoelectronic properties. These noble metalNPs, have elicited a lot of interest for important biomedical applications because of their ease of synthesis, characterization and surface functionalization. Furthermore, recent investigations are demonstrating another promising application of these nanomaterials as self-therapeutics. To realize the potential promise of these unique inorganic nanomaterials for future clinical translation, it is of utmost importance to understand their interaction with the cells at the molecular level, biodistribution, pharmacokinetics influenced by their surface, routes of administration, mechanism of their detoxification, clearance and therapeutic efficacy in appropriate disease model (Arvizo *et al*., 2012).

Cancer remains one of the world's most devastating diseases and current cancer treatments include surgical intervention, radiation and chemotherapeutic drugs, which often also kill healthy cells and cause toxicity to the patient. Both the field of drug design and cancer imaging are going to gain from a development that is generally regarded as a fundamental breakthrough in medicine, namely the introduction of nanotechnology (Peer *et al*., 2007). Progression of tumors has been attributed to the cumulative accumulation of multiple alterations throughout the genome, which is manifested by genomic instability. Ubiquitously targeting cells within a tumor is not always feasible because some drugs cannot diffuse efficiently and the random nature of the approach makes it difficult to control the process. This lack of control may induce multiple-drug resistance (MDR), a situation where chemotherapy treatments fail in patients owing to resistance of cancer cells towards one or more drugs. The emerging trend of using NPs as drug carriers has exploited the potential of NPs to revolutionize cancer therapy (Viswanathan *et al*., 2003).

To the possible extent sincere efforts have been made to collect the relevant literature of the study. After thorough reviewing of all possible sources, it was observed that no studies on the antioxidative and antitumor role of methanolic extract of *Gloriosa superba* and its AgNPs have been conducted. So, the review of literature pertaining to the present research entitled "**Antioxidant and Antitumorigenic efficacy of methanolic extracts of *Gloriosa superba* and Silver Nanoparticles of methanolic extracts of *Gloriosa superba* to DLA tumor cells"** is appropriately presented under the following headings:

2.1 Concept of Nanotechnology

2.2 Synthesis of Silver NPs (AgNPs)

- 2.2.1 Organic layer protected AgNPs
- 2.2.2 Seconary metabolites protected AgNPs

2.3 Detection of AgNPs

- 2.3.1 UV-Visible absorbance Spectroscopy
- 2.3.2 Fourier transform infrared (FTIR) spectrum of AgNP
- 2.3.3 Scanning Electron Microscope (SEM)
- 2.3.4 Transmission Electron Microscope (TEM)

2.7 Medicinal plant selected for the study (*Gloriosa superba)*

2.1 Concept of Nanotechnology

The history of nanomaterials is quite long, nevertheless, major developments within nanoscience have taken place during the last two decades. In 1970 Norio Taniguchi first defined the term nanotechnology. "Nanotechnology is the processing of separation, deformation and consolidation of material by one atom or one molecule is significance in nano scale and properties of particles are far different than bulk scale properties (Buzea *et al*., 2007; Behera *et al*., 2011). Nanotechnology is briefly termed as "The ability to fabricate, characterize, and manipulate artificial structures, whose features are controlled at the nanometer level" (Parak *et al*, 2004). This may involve design, synthesis and preparation of NPs to create products with novel properties. In recent years, there has been much interest in the application of nanomaterials for biological purposes. These materials include NPs, nanotubes (Stefania *et al*., 2006) nanowires (Law *et al*., 2004), and quantum dots (Rhyner *et al*., 2006).

The NPs are being viewed as fundamental building blocks of nanotechnology. The most important and distinct property of NPs are that they exhibit larger surface area to volume ratio. The most effectively studied NPs today are those made from noble metals, in particular Ag, Pt, Au and Pd (Raveendran *et al*., 2006).

The NPs are being used in different fields including optical, catalytical electromagnetic, electrical, biological textile and chemistry in which shape and size of colloidal metal particles play crucial role in different applications including preparation of magnetic, electronic devices wound healing, antimicrobial, gene expression and in the preparation of bio composites (Mohanpuria *et al*., 2007; Song *et al*., 2008).

Nanotechnology is significant on account of its preeminence upon the comprehension, use, and control of matter at magnitudes of a minute scale, akin to approaching atomic levels, with which to manufacture new substances, instruments, and frameworks also known as ‘Molecular Manufacturing’. It is an emergent diversity of technologies in which medicine and engineering come together with physics and chemical science which are opening up many brand new possibilities especially within the medical arena in terms of implantable transmission methods, which are often favoured to the application of injectable medicines (Sulaiman *et al*., 2012).

Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications. The development of NPs for the delivery of therapeutic agents has introduced new opportunities for the improvement of medical treatment. NPs are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures (Thakkar *et al*, 2010).

Among the various inorganic metalNPs, AgNPs have received substantial attention for various reasons. Among the noble metals (Ag, Pt, Au and Pd), Ag is the metal of choice for potential applications in the field of biological systems, living organisms and medicine. AgNPs are one of the promising products in the nanotechnology industry (Ankanna *et al*., 2010). The absorption range of Surface Plasmon resonance (SPR) band for spherical AgNPs is 380-440 nm (Varshney *et al*., 2009).

2.2 Synthesis of Silver nanoparticles (AgNPs)

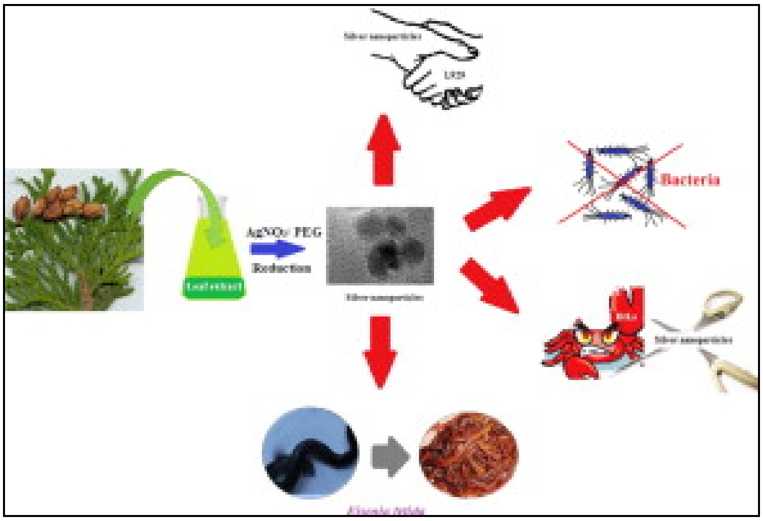
The AgNPs have emerged as an arch product from the field of nanotechnology. Over the last few years due to its good conductivity, chemical stability, catalytic and antibacterial activity silver has gained much of the interest. The diversity and importance of their applications has generated a great deal of interest in developing versatile methods to synthesize AgNPs with well-defined and controlled properties (Dorjnamjin *et al*., 2008). Colloidal silver solutions have an increased interest due to their antimicrobial properties, with large applications including pharmacology, human and veterinary medicine, food industry and water purification. For a long time silver has been known to have a disinfecting effect and has found applications in traditional medicines. Silver has known to be a metal that come into use even before Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8th century. Thus, NPs of silver have aptly been investigated for their antimicrobial properties.NPs of silver have thus been studied as a medium for antibiotic delivery (Sadowski *et al*., 2008).

To prolong the life span of metalNPs it is vital to select stabilizing agents and pathways that are environmentally friendly, non toxic and easy to implement. Plants are nature's "chemical factories". They are cost efficient and require little or no maintenance. A vast repertoire of secondary metabolites is found in all plants which possess redox capacity and can be exploited for biosynthesis of NPs (Ahmad and Ahmad 2011). One of such promising process is green synthesis. AgNPs can be synthesized by several physical, chemical and biological methods. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality (Wang *et al*., 2008). The "green" route for nanoparticle synthesis is of great interest due to eco-friendliness, economic prospects, feasibility and wide range of applications in nanomedicine, catalysis medicine, nano-optoelectronics (Salam *et al*., 2012).

The use of environmentally benign materials like plant leaf extract, for the synthesis of AgNPs offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications. The NPs synthesized using the plant system have applications in the field of medicines, cancer treatment, drug delivery, commercial appliances and sensors (Bashir *et al*., 2011).

Figure 1

Green route of nanoparticle synthesis



http://www.sciencedirect.com/science/journal/09277765

Many reports are available on the biogenesis of AgNPs using several plant extracts, particularly leaf extract of *Diopyros kaki* (Song and Kwon 2010), (*J. curcas* latex) Jatropha (Bar *et al*., 2009), Hibiscus, (Philip,2010) and *Achyranthus aspera* (Daniel *et al*., 2011c), neem leaf (Azadirachta indica), Pelargonium Graveolens, Geranium leaves, Medicagosativa (Alfalfa), Aloe vera and Emblica officinalis (Amla, Indian Gooseberry).

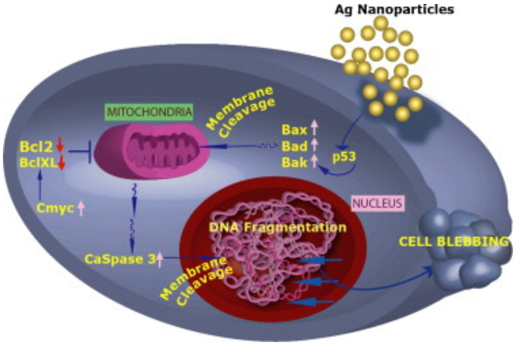
Ramteke *et al*., (2013) studied the rapid synthesis of AgNPs using aqueous leaves extract of Tulsi to have enhanced antimicrobial activity against well-known pathogenic strains, namely Staphylococcus aureus and E. coli. Philip (2009) studied mushroom mediated green chemistry approach towards the synthesis of Au, Ag and Au–Ag.

2.2.1 Organic layer protected AgNPs

Tiopronin AgNPs (Castillo *et al*., 2008), BSA capped Ag- Pt alloy NPs (Singh *et al*., 2009) and AgNPs protected with Na^+-poly γ -glutamic acid (Yu, 2007) are not cytotoxic (Table 1), while, by contrast bare AgNPs have been found to be rather toxic (Kim *et al*., 2009).

Figure 2

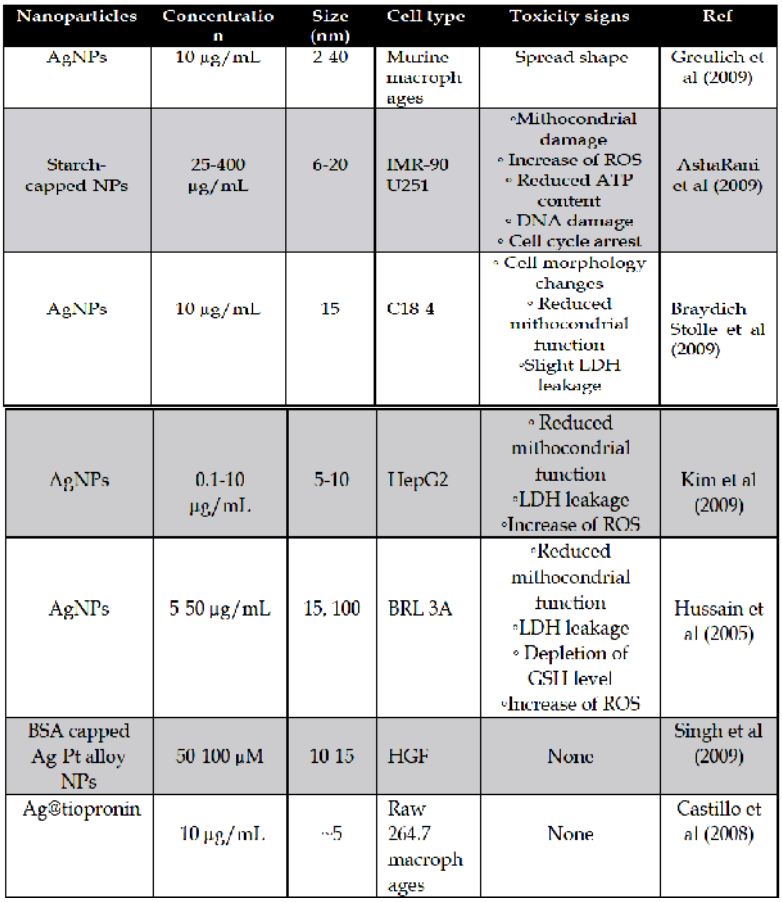
The AgNP induced apoptotic pathway



http://www.sciencedirect.com/science/article/pii/S0927776510000731

Table 1

In vitro cytotoxicity studies of AgNPs on different cell lines



Nanoparticles	Concentration	Size (nm)	Cell type	Toxicity signs	Ref
AgNPs	10 µg/mL	2 40	Murine macrophages	Spread shape	Greulich et al (2009)
Starch-capped NPs	25-400 µg/mL	6-20	IMR-90 U251	◦Mithocondrial damage ◦ Increase of ROS ◦ Reduced ATP content ◦ DNA damage ◦ Cell cycle arrest	AshaRani et al (2009)
AgNPs	10 µg/mL	15	C18 4	◦ Cell morphology changes ◦ Reduced mithocondrial function ◦Slight LDH leakage	Braydich Stolle et al (2009)
AgNPs	0.1-10 µg/mL	5-10	HepG2	◦ Reduced mithocondrial function ◦LDH leakage ◦Increase of ROS	Kim et al (2009)
AgNPs	5 50 µg/mL	15, 100	BRL 3A	◦Reduced mithocondrial function ◦LDH leakage ◦ Depletion of GSH level ◦Increase of ROS	Hussain et al (2005)
BSA capped Ag Pt alloy NPs	50 100 µM	10 15	HGF	None	Singh et al (2009)
Ag@tiopronin	10 µg/mL	~5	Raw 264.7 macrophages	None	Castillo et al (2008)

http://www.sciencedirect.com/science/article/pii/S0927776510000731

This supports the idea that the toxicity is associated to the presence of bare metallicNPs surfaces, while particles protected by an organic layer are much less toxic except starch capped NPs which present mithocondrial dysfunction, induction of reactive oxygen species (ROS), DNA damage and cell cycle arrest (AshaRani *et al*., 2009).

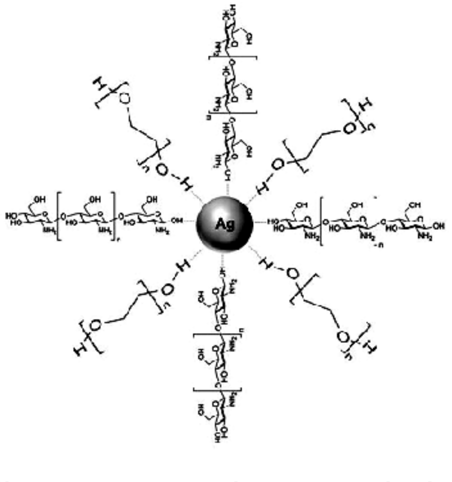
The AgNP induced p53-mediated apoptotic pathway through which most of the chemotherapeutic drugs trigger apoptosis (programmed cell death). Thus AgNPs could be attributed as therapeutic agent for biomedical and pharmaceutical applications (Gopinath *et al*., 2010).

2.2.2 Seconary metabolites protected AgNPs

Polyols extract of *Trianthema decandra* are mainly responsible for the reduction of Ag ions, whereby they themselves get oxidized to unsaturated carbonyl groups (Geethalakshmi and Sarada 2010). Carboxylate content in *Ocimum tenuiflorum* which may be responsible for the reduction of metal ion to metalNPs (Daniel *et al*., 2011b).

Figure 3

Capping and stabilization of AgNPs



http://www.mdpi.com/1422-0067/12/8/4872

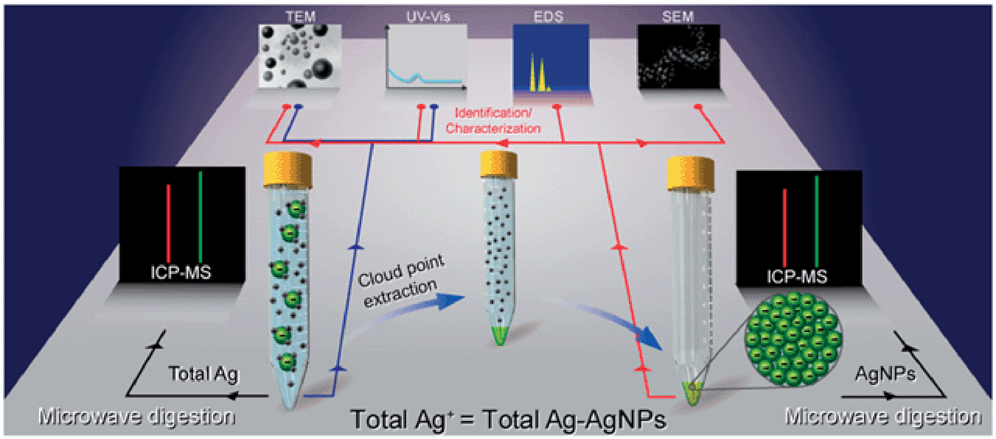
D-sorbitol capped AgNPs formed through electrostatic attraction or bind to the protein groups of Polyalthia longifolia leaf extract via hydrogen bond and increased the stability of the AgNPs. It indicated that the functional groups in biomolecules are mainly responsible for the reduction of silver ions (Kaviya *et al*., 2011). Reducing $AgNO_3$ in different stirring times of reaction at a moderate temperature using green agents, chitosan (Cts) and (PEG) polyethylene glycol (Ahmad *et al*., 2011).

2.3 Detection of AgNPs

A number of different measurement techniques can be used to detect and characterize the absorption maxima, size, shape, intensity and stability of AgNPs.

Figure 4

Characterization and detection of AgNPs



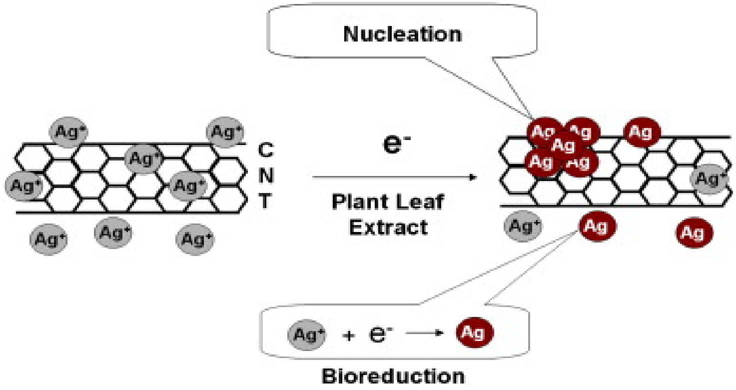
http://pubs.rsc.org/en/content/articlelanding/2013/em/c2em30595j/unauth

A number of approaches are available for the synthesis of AgNPs, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route.

The synthesis of AgNPs will be based on a wet chemical method. The starting point of the synthesis is the production of a silver nitrate ($AgNO_3$) solution. When silver nitrate is dissolved it splits into a positive silver ion (Ag^+) and a negative nitrate ion ($NO^-{}_3$). In order to turn the silver ions into solid silver, the ions have to be reduced by receiving an electron from a donator. Figure 26 illustrate the reduction of the silver ions by the addition of an electron in a solution of ethanol. After the silver germ has been formed it starts to grow and continue the growth until the equilibrium between the final NPs and the (Ag^+) of the solution is reached.

Figure 5

Biosynthesis of AgNPs by plant leaf extract



(Vivekanandhan *et al*., 2012).

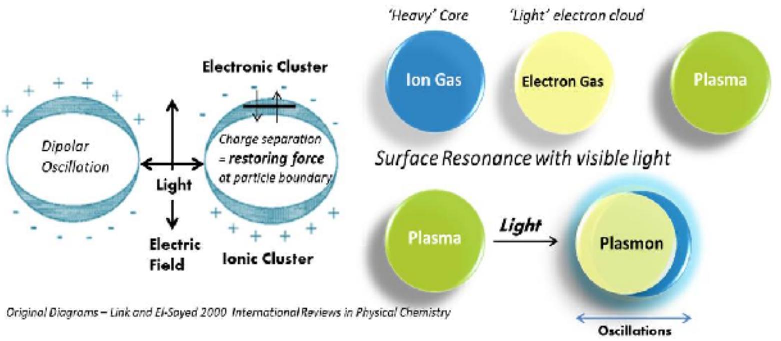
2.3.1 UV-Visible absorbance Spectroscopy

Optical properties of metallic NPs are dependent on their dimension. This section will describe the size dependence of the Localized Surface Plasmon Resonance (LSPR) for metallic NPs. LSPR is an in-phase oscillation of electrons lead by the coherent excitations of all free electrons in the conduction band. A LSPR is generated when the size of a metallic nanocrystal is smaller than the wavelength of incident radiation. A simple illustration of the surface plasmon oscillation is shown in Figure. The LSPR is a dipolar excitation between the negatively charged electrons and the positive charge lattice in the particle (Haes and Duyne, 2004).

The electric field of the incoming light wave induces a polarization of the free electrons relative to the cationic lattice. The net charge difference is only induced at the surface of the spherical metallic nanoparticle. This acts as an restoring force, causing a dipolar oscillation of the electrons with a period T. (Cao, 2004).

Figure 6

Localized Surface Plasmon Resonance



http://www.chemguide.co.uk/analysis/uvvisible/analysis.html

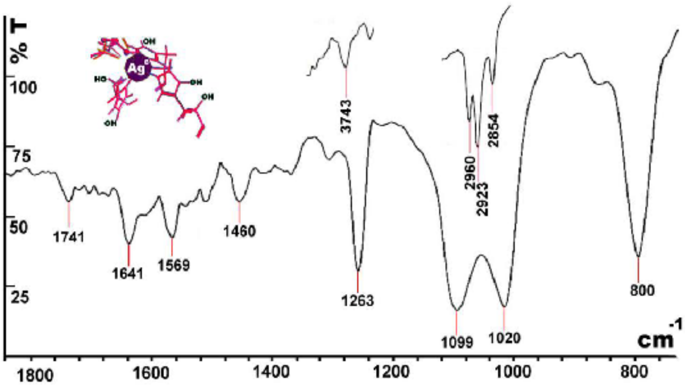
UV-Visible absorbance spectroscopy is used to determine the absorbance maxima to confirm the presence of metalNPs (Sergey and Bozhevolnyi 2005).

2.3.2 Fourier transform infrared (FTIR) spectrum of AgNPs

Secretion of some soluble organic components of carom seeds which could have contributed for the important role in the reduction and functionalization of the metalNPs. Consequently the organic moieties adsorbed on the NPs confer the stability. The polyphenols like terpenoids (thymol, which is a major constituent of the essential oils) of carom seed show characteristic absorption peaks and the same are responsible for bioreduction and capping process (Das *et al*., 2011; Singh *et al*., 2012).

Figure 7

FTIR absorption spectra of the biomoieties of aqueous ethanolic carom seed extract



2.3.3 Scanning Electron Microscope (SEM)

The microscope works by the same principle as an optical microscope, but instead of photons it measures the electrons scattered from the sample. When high-resolution images of the surface of a sample is desired, the SEM is a valuable instrument. Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than the one of photons. This makes the SEM capable of magnifying images up to 200.000 times. At the same time it is possible to achieve high resolution pictures of the surface, making the instrument very useful in determining the size distribution of NPs (Jiang *et al*., 2004; Christensen *et al*., 2011).

2.3.4 Transmission Electron Microscope (TEM)

The transmission electron microscope (TEM) forms an image by accelerating a beam of electrons that pass through the specimen. In TEM, electrons are accelerated to 100 KeV or higher (up to 1MeV), projected onto a thin specimen (less than 200 nm) by means of the condenser lens system, and penetrate the sample thickness either undeflected or deflected. The greatest advantages that TEM offers are the high magnification ranging from 50 to 10^6 and its ability to provide both image and diffraction information from a single sample (http://www.gitam.edu/eresource/nano/nanotechnology/tem.htm).

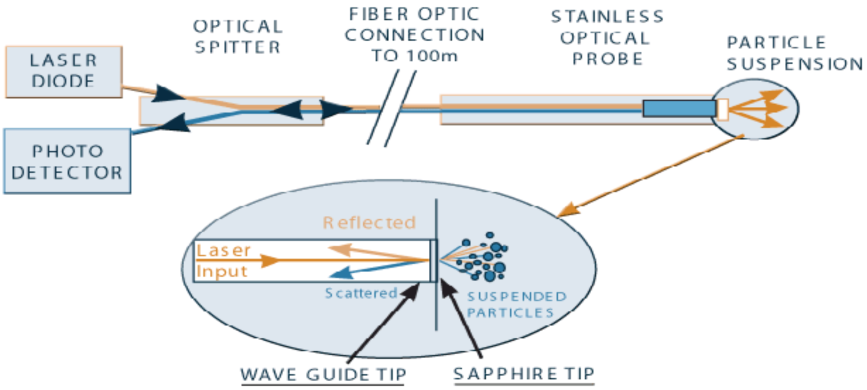
In addition to the capability of structural characterization and chemical analyses, TEM also has been explored for other applications in nanotechnology. Examples include the determination of melting points of nanocrystals, in which, an electron beam is used to heat up the nanocrystals and the melting points are determined by the disappearance of electron diffraction. Another example is the measurement of mechanical and electrical properties of individual nanowires and nanotubes. This technique allows a one-to-one correlation between the structure and properties of the nanowires (Yu and Yam 2005).

2.3.5 Dynamic Light Scattering (DLS)

The DLS technique uses light to determine the size of particles in a solution. Light at a given frequency is sent through the solution from a laser. When the light interacts with the moving particles in the solution and is scattered, the frequency of the light is also changed. This change of light frequency is directly related to the size of the particles in the solution and the smaller the particles, the greater the shift in the light frequency. This difference in the light shift is used to determine the size of the particles in the solution. DLS is capable of measuring particles in the size range from a few nanometers to a few micrometers. It is therefore applicable for determining the size of AgNPs (Proteinchemist.com 2005).

Figure 8

DLS apparatus



http://www.matter.org.uk/diffraction/x-ray/default.htm

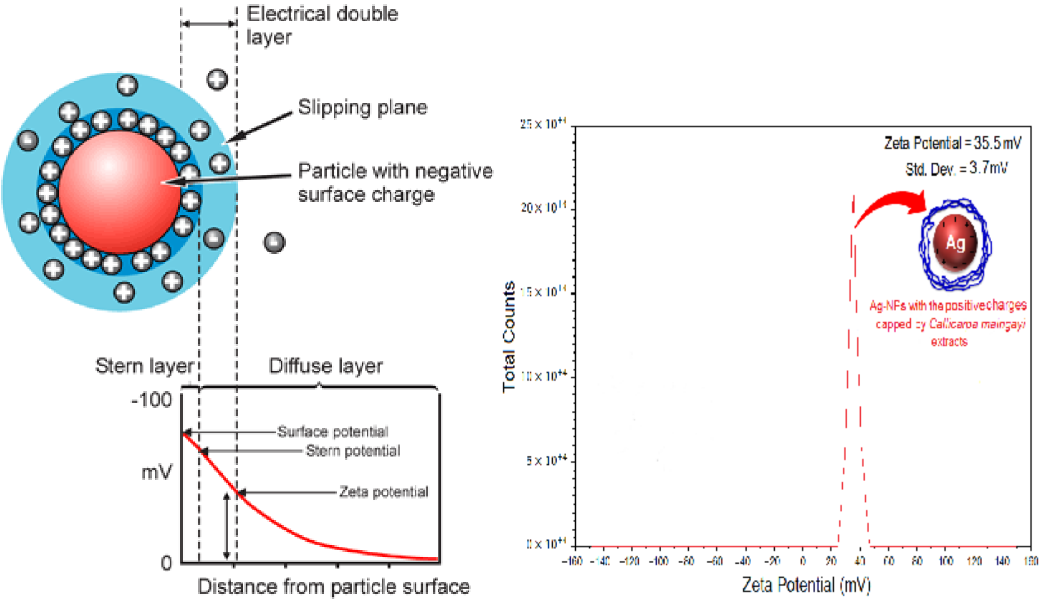
A laser diode emits light through the tube towards the solution. Part of the beam is reflected back towards the detector, while the other part is send into the solution where it is reflected and pointed back to the detector (Microtrac.com, 2005).

2.3.6 Zeta potential

Zeta potential (also known as the electrokinetic potential) is a measure of the “effective” electric charge on the NP surface, and quantifies the charge stability of colloidal NPs. When a NP has a net surface charge, the charge is “screened” by an increased concentration of ions of opposite charge near the NP surface. This layer of oppositely charged ions moves with the NP, and together the layer of surface charge and oppositely charged ions are referred to as the electrical double layer. The Zeta potential is a measure of the difference in potential between the bulk fluid in which a particle is dispersed and the layer of fluid containing the oppositely charged ions that is associated with the NP surface. Particles with a positive Zeta potential will bind to negatively charged surfaces, and vice versa. The magnitude of the Zeta potential provides information about particle stability, with higher magnitude potentials exhibiting increased electrostatic repulsion and therefore increased stability (Bonde 2011).

Figure 9

Zeta potential



(Sadowski *et al*., 2008)

2.3.7 X-RAY Diffraction (XRD) Analysis

X-ray diffraction analysis is the method by which multiple beams of x-ray create a threedimensional picture of the density of electrons of any crystalline structure. The purpose is to identify with a high degree of certainty—the composition of the molecules, on an atomic scale. X-ray powder diffraction is most widely used for the identification of unknown crystalline materials. Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology (Dutrow and Barb 1997).

2.4 Role of AgNPs in Oxidative Stress

In general, when using a targeting agent to deliver nanocarriers to cancer cells, it is imperative that the agent binds with high selectivity to molecules that are uniquely expressed on the cell surface. It is also possible to increase binding affinity and selectivity to cell surface targets by engineering proteins that detect a specific conformation of a target receptor (Ghosh *et al.*, 2008).

2.4.1 Oxidative stress and consequences

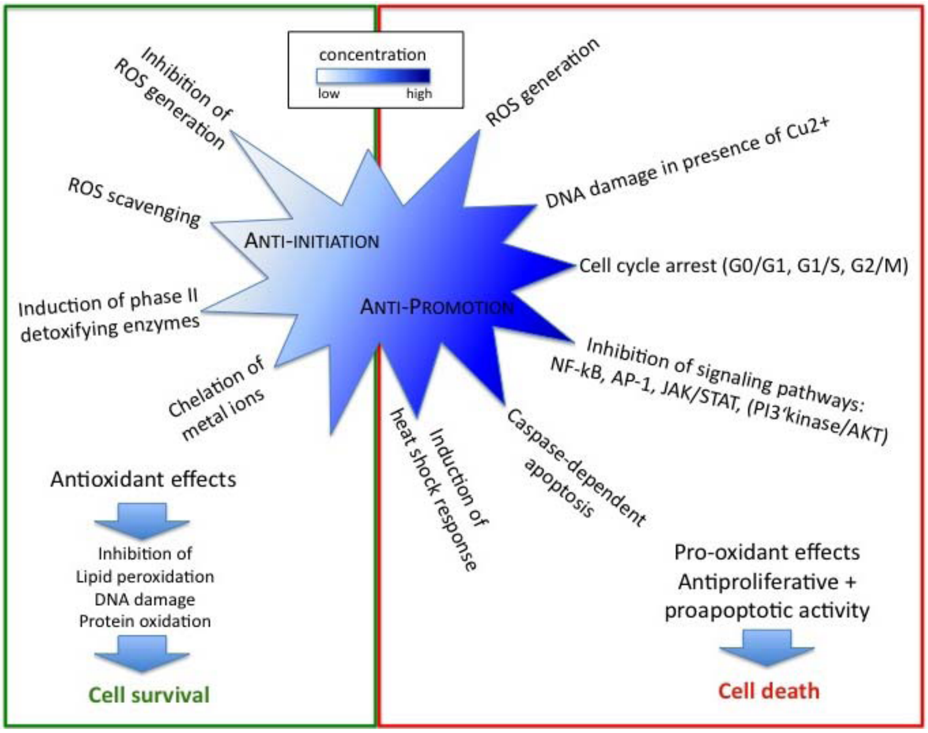
Body constantly reacts with oxygen as we breathe and the cells produce energy. As a consequence of this activity, highly reactive molecules are produced within our cells known as free radicals and oxidative stress occurs.

In a healthy situation, there must be a necessary balance between production of ROS/RNS/RSS and their clearance by antioxidants. Several situations could then contribute to oxidative stress, namely (i) diminished antioxidants, e.g., reduction in antioxidant defenses like antioxidant enzymes; (ii) over production of ROS/RNS/RSS e.g., elevation of O_2 or presence of radical-forming xenobiotics and toxins. ROS/RNS/RSS may have a triggering effect in cell damage. It has been suggested that tumors are in a “pro-oxidant” state generating more free radicals, which is usually not accompanied by up-regulation of DNA repair mechanisms causing mutations and modification of gene expression. According to current knowledge, ROS/RNS/RSS can participate at multiple levels in carcinogenesis, either by direct effects on DNA or by modulating signaling pathways leading to increase in cell proliferation. Nonetheless direct role of ROS/RNS/RSS in carcinogenesis is mostly related to initiation and promotion, whereas rest of tumor stages can be indirectly related to free radicals through the chronic inflammation-related deregulation of cytokines or other factors released by macrophages (Karin, 2006).

The effects of oxidative stress depend upon the size of changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death while more intense stresses may cause necrosis (Sumazian *et al*., 2010).

Figure 10

Oxidative stress in cellular signaling



http://www.sciencedirect.com/science/article/pii/S0006295206004199

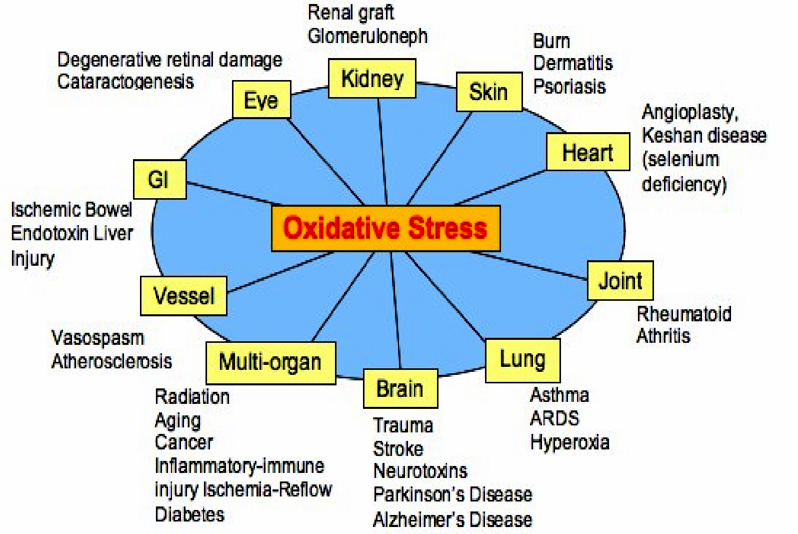
A particularly destructive aspect of oxidative stress is the production of free radicals. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage. The major portion of long term effects is inflicted by damage on DNA. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart (Huang *et al*., 2008).

When our protein-controlled antioxidant-response doesn't keep up oxidative stress causes oxidative damage that has been implicated in the cause of many diseases and also has an impact on the body's aging process (Pinnell 2003).

Oxidative stress is likely to be involved in age-related development of cancer. The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and it may also suppress apoptosis and promote proliferation, invasiveness and metastasis (http://en.wikipedia.org/wiki/Oxidative_stress - cite_note-cancer-3). Infection by *Helicobacter pylori* which increases the production of reactive oxygen and nitrogen species in human stomach is also thought to be important in the development of gastric cancer (Stanly *et al*., 2011).

Figure 11

Oxidative stress in health disorders



ARDS- Acute respiratory distress syndrome

http://www.oxidativestressresource.org/

A free radical is defined as a molecular species capable of independent existence and which contains one or more unpaired electrons. ROS are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and damage complex cellular molecules such as fats, proteins, or DNA. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress. which is apparent in pathology associated with aging and many age-related chronic diseases, including atherosclerosis, diabetes mellitus, rheumatoid arthritis, and neurodegenerative diseases (Sen *et al*, 2010).

Free radical species indirectly formed by the action of xenobiotics, pollutants or other carcinogens are indeed responsible for oxidative damage of macromolecules that finally ends up in nucleotide mutations in DNA. Moreover, during the past two decades extensive research has revealed the role of oxidative stress in causing chronic inflammation which is directly linked to chronic diseases like cancer (Reuter *et al*., 2010). In this way Halliwell has elegantly addressed how redox control in an increasing oxidative situation might induce one of the following outcomes when cells move progressively from a highly reduced to a highly oxidized intracellular environment, increased proliferation, adaptation by up-regulation of antioxidant defense, cell injury, senescence and cell death (Halliwell 2000).

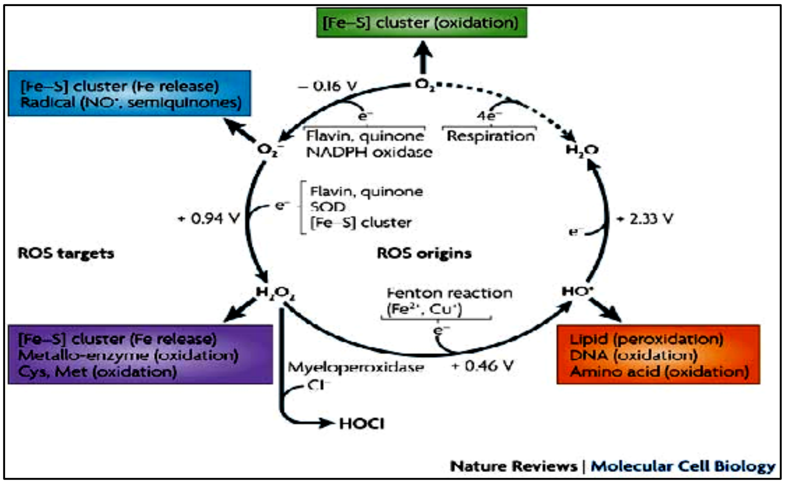
Most free radicals are coming from oxygen atoms and are called Reactive Oxygen Species (ROS). Sometimes reactive nitrogen atoms are involved and these free radicals grouped under Reactive Nitrogen Species (RNS). Nitric acid is the most important RNS. Some transitional metals, such as iron and copper, have many numbers of unpaired electrons and can also act as free radicals. These metals do not have that strong electron affinity but can easily accept and donate electrons (Li and Lin 2010). Sulphur-containing radicals are referred to as Reactive Sulphur Species (RSS). These result from the reaction of thiols with ROS. Both RNS and RSS result from reactions involving ROS (Chua *et al*., 2008).

2.4.1.1 Reactive Oxygen Species (ROS)

Reactive Oxygen Species is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen. The production of oxygen based radicals is the bane to all aerobic species. These molecules, produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation, have the potential to cause a number of deleterious events (Hancock *et al*., 2001).

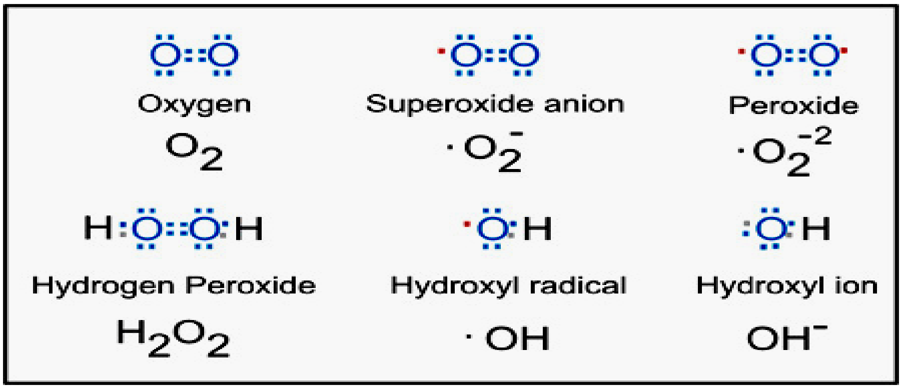
Figure 12

Formation of Reactive Oxygen Species



(D'Autreaux and Toledano 2007)

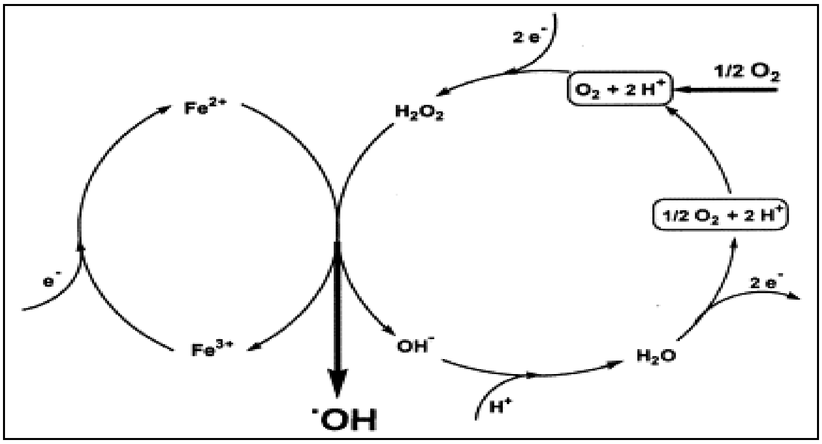
Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including superoxide, hydrogen peroxide, hydroxyl radical and hydroxyl ion.



http://www.biotek.com/resources/articles/reactive-oxygen-species.html

Hydroxyl radical ($OH^{\cdot}$)

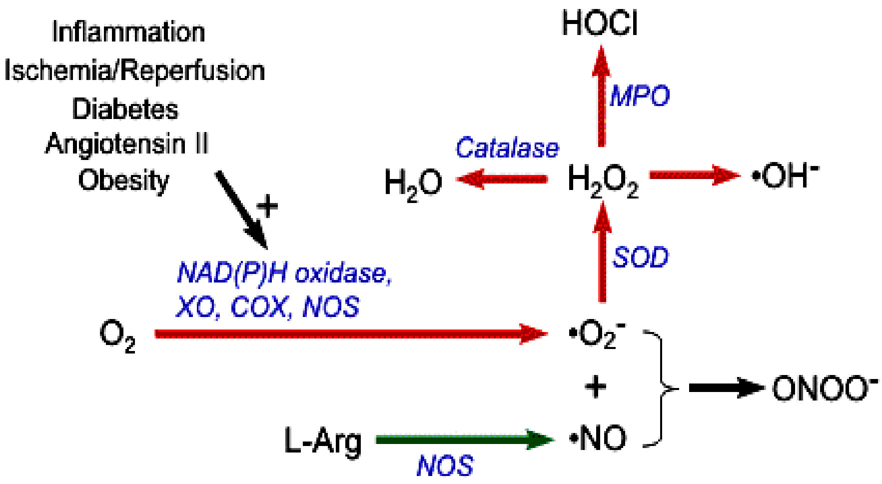
The primary free radical in most biological systems is $O_2^{\cdot}$ Although $^{\cdot}O_2$ - itself is quite unreactive compared to other radicals. The biological systems converted it into more reactive species, e.g., $OH^{\cdot}$ radicals. Free radical scavengers can scavenge the $OH^{\cdot}$ and protect the cell membrane from damage. $OH^{\cdot}$ can damage DNA, lipids and Proteins. The Fenton reaction generates $OH^{\cdot}$ radicals which degrade deoxyribose using Fe_2+ salts as an important catalytic component. Oxygen radical may attack DNA either at the sugar or the base, giving rise to a large number of products (Winterbourne and Kettle 2003).



http://www.sciencedirect.com/science/article/pii/S0022072801003692

Superoxide anion radical ($^{\cdot}O_2^{-}$)

The $^{\cdot}O_2^{-}$ radical, which is generated during the physiological metabolism related to the aging in human body, is known to toxic to cells and tissues, and thus stimulate tumors. It is also known to induce duodenal ulcer, diabetes, arthritis, Alzheimer disease and the aging of the skin. The compounds with superoxide dismutase (SOD) like activity and low molecular weight are belongs to phytochemicals. It is known that they protect cells from superoxide toxicity by inhibiting the $^{\cdot}O_2^{-}$ radical reaction (Kim and Park 2000).



MPO-myeloperoxidase

http://www.cvphysiology.com/Blood%20Flow/BF016.htm

Superoxide and its products can have vasoactive activities in addition to their tissue damaging effects. $^{\cdot}O_2^-$ has another property that makes it very important in cardiovascular pathology and pathophysiology (Shirwaikar *et al*., 2006). $^{\cdot}O_2^-$, with its unpaired electron, very rapidly binds to NO, which also has an unpaired electron. Because NO is a very important vasodilator substance, the reaction between superoxide and NO effectively scavenges NO, thereby reducing its bioavailability. This leads to vasoconstriction, increased platelet-endothelial cell adhesion, platelet aggregation and thrombus formation, increased leukocyte-endothelial cell adhesion, and morphologic changes in blood vessels, such as cell proliferation (Muller *et al*., 2007).

Hydrogen peroxide (H_2O_2)

The ability of H_2O_2 to initiate LPO is dependent on its ability to generate $OH^{\cdot}$ radical through the Fenton reaction. The Haber-Weiss reaction explains that both H_2O_2 and superoxide radical are required in the presence of metal catalyst for the formation of $OH^{\bullet}$ radical, the oxygen species largely responsible for the damage of macromolecules (Venkatesan and Rao, 2000).

The first step of the catalytic cycle involves reduction of ferric ion to ferrous:

$$Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2$$

The second step is the Fenton reaction:

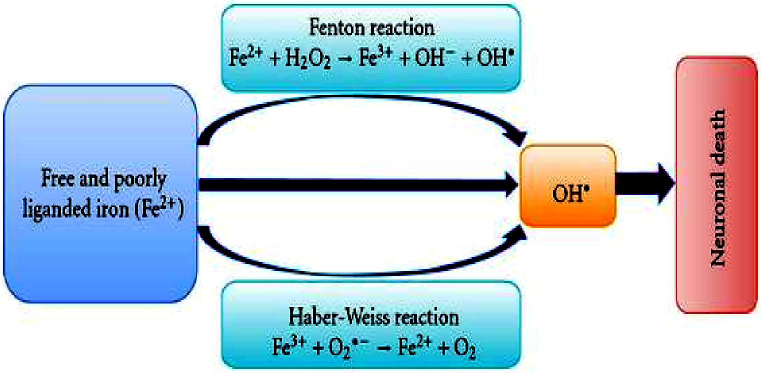
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$$

Net reaction:

$$\bullet O_2^- + H_2O_2 \rightarrow OH\bullet + OH^- + O_2$$

Figure 13

Generation of reactive and damaging hydroxyl radicals (OH•)



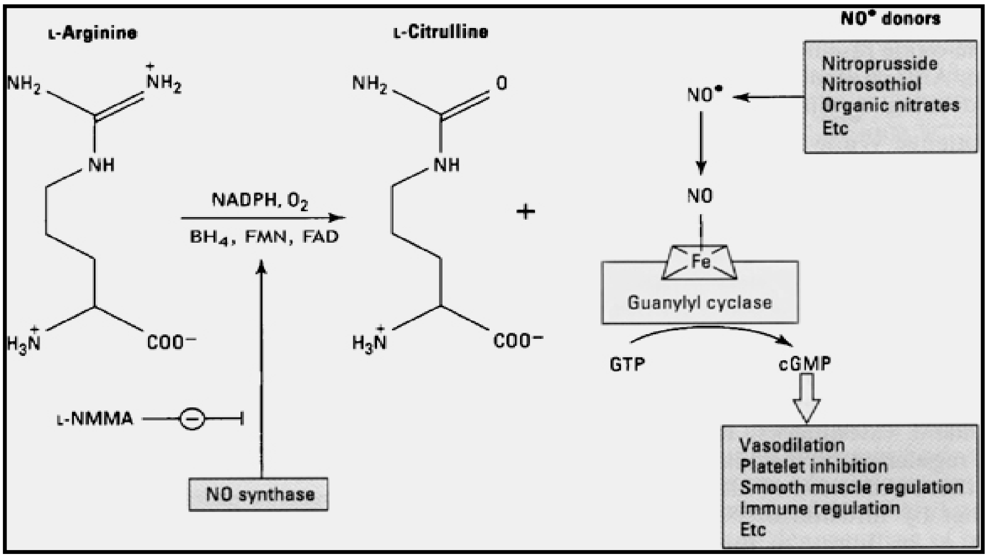
http://www.hindawi.com/journals/msi/2011/606807/fig2/

Free Iron (Fe^{2+}) reacts trough the Fenton reaction with H_2O_2, leading to the generation of very reactive and damaging $OH^\bullet$. Superoxide can also react with ferric iron in the Haber-Weiss reaction leading to the production of Fe^{2+}, which then again affects redox cycling. The highly reactive $OH^\bullet$ radicals lead to oxidative stress-induced LPO, mitochondrial dysfunction, and increase in intracellular free-calcium concentration, and finally causing neuronal death (http://www.hindawi.com/journals/msi/2011/606807/fig2/).

2.4.1.2 Reactive nitrogen species (RNS)

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Overproduction of NO can mediate toxic effects, *e.g.*

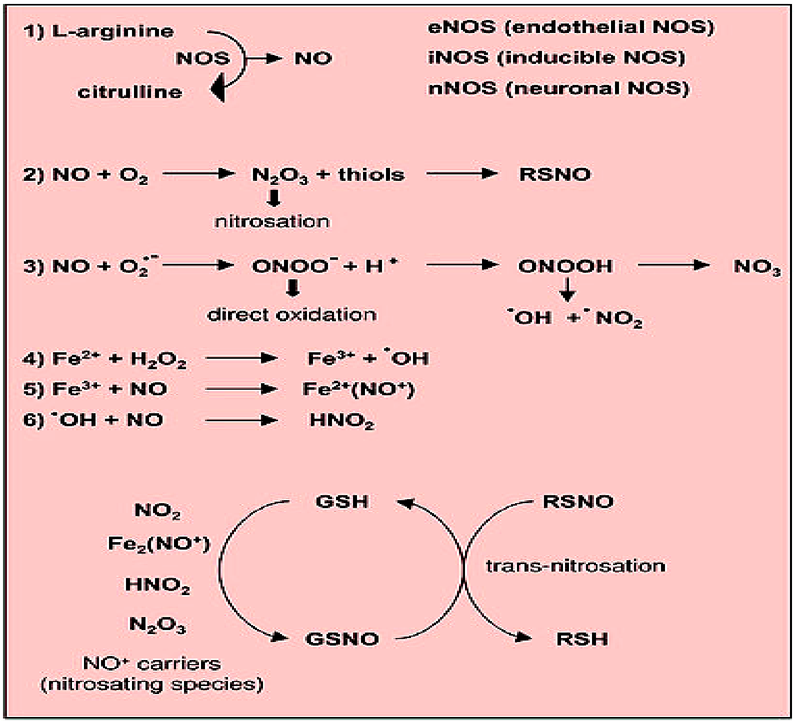
DNA fragmentation, cell damage and neuronal cell death. NO does not interact with the bioorganic macromolecules such as the DNA or proteins directly (Saha 2008).



http://www.nature.com/ijir/journal/v16/n6/fig_tab/3901256f1.html

During infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects like renal dysfunction and tumor growth. The peroxynitrite ($ONOO^-$) produced during the reaction of NO with $\cdot O^{2-}$ is probably responsible for genetic damage and LPO. Such permanent modifications of cellular macromolecules might ultimately result in carcinogenesis (Martindale and Holbrook 2002). It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities (Roberfroid and Calderon 2008). A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxide such as NO_2, N_2O_4, N_3O_4, NO_3- and NO_2- are very reactive. These compounds alter the structure and function of many cellular components (Valko *et al*., 2006).

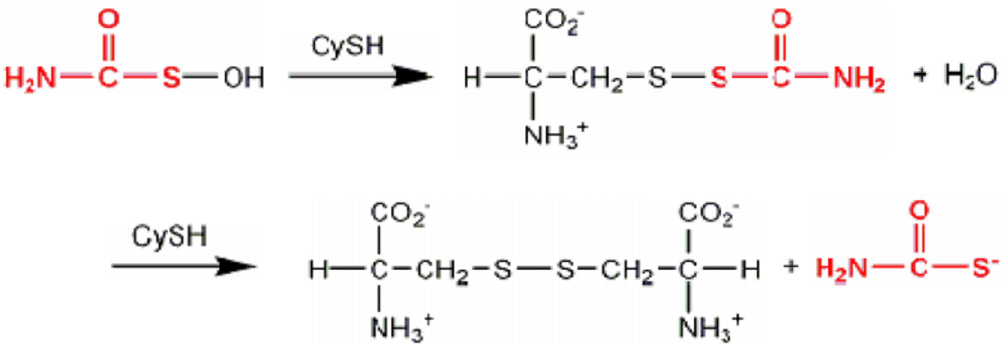
Besides ROS, RNS including nitric oxide (NO) play a role in oxidative damage of proteins via nitrosylation reaction. RNS are a family of antimicrobial molecules derived from nitric oxide (NO) and superoxide ($^{\cdot}O_2^{\cdot-}$) produced via the enzymatic activity of inducible NO synthase and NADPH oxidase respectively. NO synthase is expressed primarily in macrophages after induction by cytokines and microbial products, notably interferon-gamma and lipopolysaccharide (Ozcan 2003). RNS act together with ROS to damage cells, causing nitrosative stress. Therefore, these two species are often collectively referred to as ROS/RNS (Nicole *et al*., 2008).



http://en.wikipedia.org/wiki/File:Reactions_leading_to_generation_of_Nitric_Oxide_and_Reactive_Nitrogen_Species.jpg

2.4.1.3 Reactive sulfur species (RSS)

Sulfur is usually considered as part of cellular antioxidant systems. There is mounting evidence that reactive sulfur species (RSS) with stressor properties similar to the ones found in ROS are formed under conditions of oxidative stress. Thiols as well as disulfides are easily oxidised to sulfur species with sulfur in higher oxidation states. Such agents include thiyl radicals (RS., ArS.), disulfides, sulfenic acids and disulfide-S-oxides. They rapidly oxidise and subsequently inhibit thiol-proteins and enzymes and can be considered as a separate class of oxidative stressors providing new antioxidant drug targets (Giles and Jacob 2002).

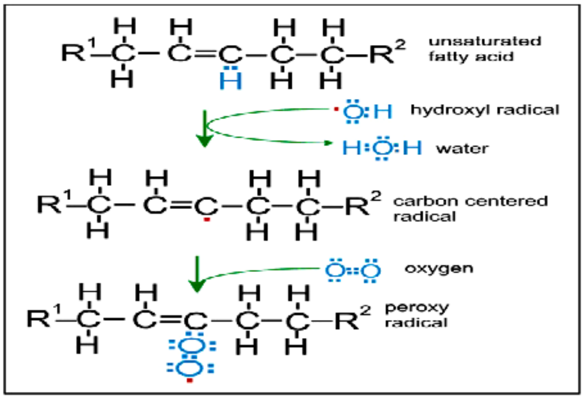


http://acswebcontent.acs.org/prfar/2008/AC4.HTM

2.4.1.4 Lipid peroxides (LO_2^o)

Malondialdehyde is a major end product of free radical reaction on membrane fatty acids. Although the cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products (Ravid and Korean 2003; Kato et al., 2007).

Lipid peroxidation



http://www.biotek.com/resources/articles/reactive-oxygen-pecies.html

Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. A dedicated balance between the availability of the substrate for lipid peroxidation (namely PUFA) in membranes and cellular antioxidant system, are essential to protect body against oxidative stress (Narayana, 2000). Anti - lipid peroxidation assay using goat liver homogenate and DPPH scavenging test has already established the anti - oxidant potency of the AgNPs (Konwarh et al., 2011).

2.5 Role of AgNPs as antioxidants in scavenging free radicals

Antioxidants are the substances which 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 cancer and degenerative diseases (Pham-Huy *et al*., 2008).

The AgNPs produced by polyethylene glycol and Aqueous extract of orange peel possess radical scavenging activity (Jokar *et al*., 2010). Synthesised AgNPs could play the role of a neoadjuvant antioxidant offering effective protection from free radicals in a wide range of conditions (Farooqui *et al*., 2010). Synthesized NPs using *Adhatoda vasica* showed high DPPH free radical scavenging activity and reducing power activity (Bandi and Vasundhara 2012). Cerium oxide NPs, Yttrium oxide NPs, Carbon NPs were found to possess antioxidant properties and have shown the ability of these NPs to offer protection against radiation damages (Daroczi *et al*., 2006). The reducing and scavenging ability of a compound depends on the presence of reductants which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Presence of reducers causes the conversion of the Fe_{3+}/ferricyanide complex used in this method to the ferrous form (Narendhirakannan and Limmy, 2010).

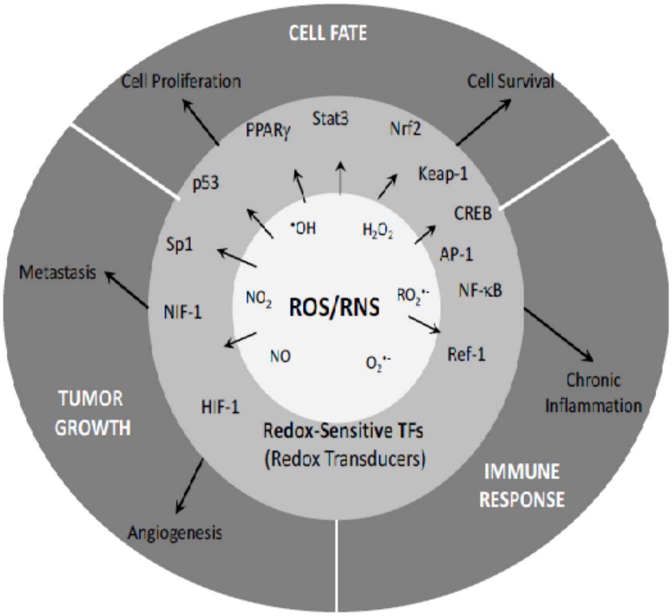
The biosynthesized *Syzygium cumini* seed extract AgNPs (ScSNPs) are found to have very potent reducing ability. The total antioxidant capacity of the

Sc and ScSNPs was based on the phosphomolebdnum method where the reduction of Mo(VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex.

Radiation is known to produce oxygen free radicals which are implicated in the process of DNA damage, cell killing, mutagenesis, and carcinogenesis; hence, it is reasonable to assume that agents capable of scavenging free radicals would play a significant role in modulating these processes. The radioprotection of normal cells by a number of synthetic and natural compounds are reported to be mediated through free radical scavenging activity (Devi 1998).

Figure 14

Redox status in Cancer



(Sainz *et al*., 2012).

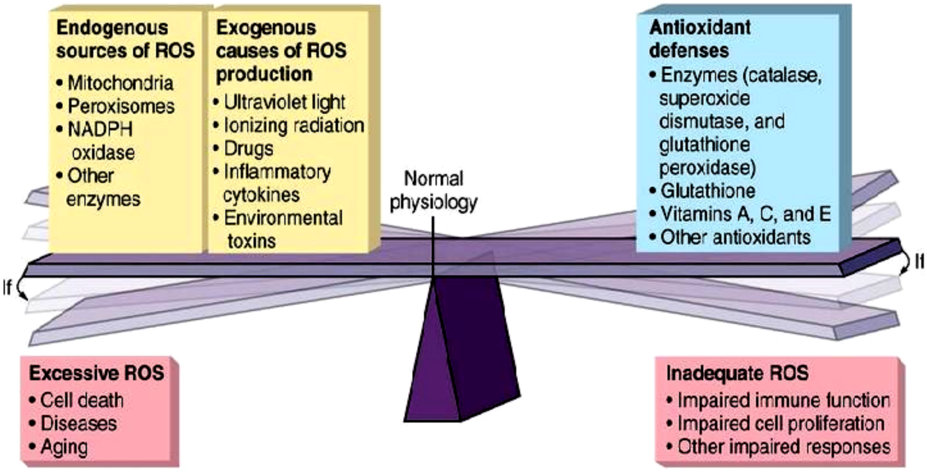
http://www.mdpi.com/2072-6694/4/2/442

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous and exogenous antioxidant enzymes. When the balance between ROS production and antioxidant defence is lost, ‘oxidative stress’ results, which through a series of events deregulates the cellular function leading to various pathological conditions (Bandyopadhyay *et al*., 1999). ROS/RNS/RSS control by antioxidants has been effeiciently proven to reduce tumor growth and progression (Atmani *et al*., 2009).

In order to prevent harmful accumulation of damaged DNA, lipids and proteins and subsequent initiation of carcinogenesis the cell possesses a complex and highly effective system of antioxidant defense that allows an immediate response to oxidative stress. Various enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) as well as non-enzymatic antioxidants Vitamin A and E and reduced glutathione (GSH) act together to render ROS/RNS/RSS and H_2O_2 harmless.

Figure 15

Antioxidants in ROS balance



http://humanphysiology2011.wikispaces.com/14.+Metabolism

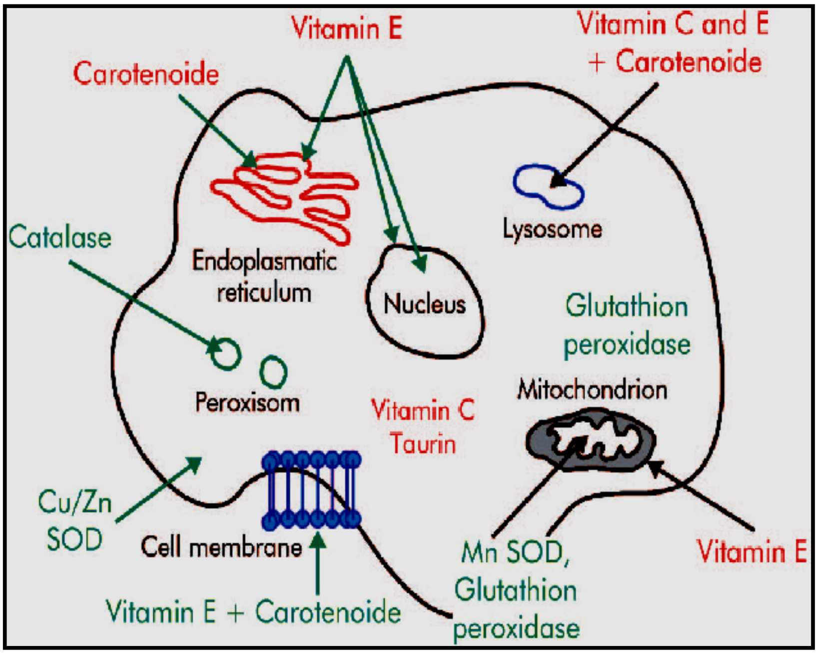
2.5.1 Endogenous antioxidants

The very most important way to fight the destructive power of free radicals is to produce antioxidants inside each cell of the body. That's why any true seeker of health will want to put a large focus on assisting his/her body to make more of the all-important endogenous antioxidants. (Endogenous means the body can make them in each cell of the body). Endogenous antioxidants are Catalase

(CAT), Superoxide dismutase (SOD), Glutathione *S*-transferase (GST), Glutathione peroxidise (GPx) and glutathione reductase (GR). Their role as protective enzymes are well known and have been extensively investigated both *in vivo* and *in vitro* model systems (Kelkel *et al*., 2010).

Figure 16

Defense against free radicals



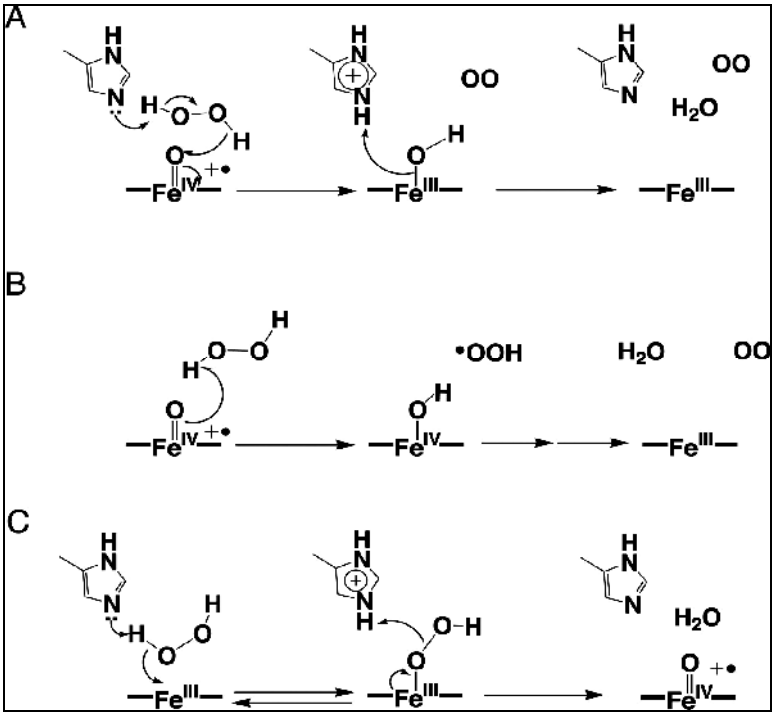
Sites of nutrients with antioxidative effect (red) and enzymes (green).
SOD = superoxide dismutase

www.royal-canin.de/uploads/pics/mono_3gif_01.gif

2.5.1.1 Catalase (CAT)

The CAT is a hemo protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to H_2O and O_2 and thus protecting the cell from oxidative damage by H_2O_2 and $OH^{.}$. The main function of catalase is to detoxify H_2O_2. Although catalase is significantly increased in rheumatoid arthritis its concentration is very low to expect considerable protection against H_2O_2 (Devi *et al*., 2007). Gupta *et al*., (2004) who showed significant increase in CAT activity of the methanol extract of *Caesalpinia bonducella* leaves in EAC bearing Swiss albino mice. Doxorubicin– poly (D,L-lactic-co-glycolic acid) conjugate was formulated into NPs had more activity to that of HepG2 cell line solid tumor in mice (Yoo *et al.*, 2000).

Mechanism of action of the catalase



A, ionic mechanism by utilizing a general acid-base catalyst.

B, radical mechanism.

C, role of a general acid-base catalyst on the formation of compound I.

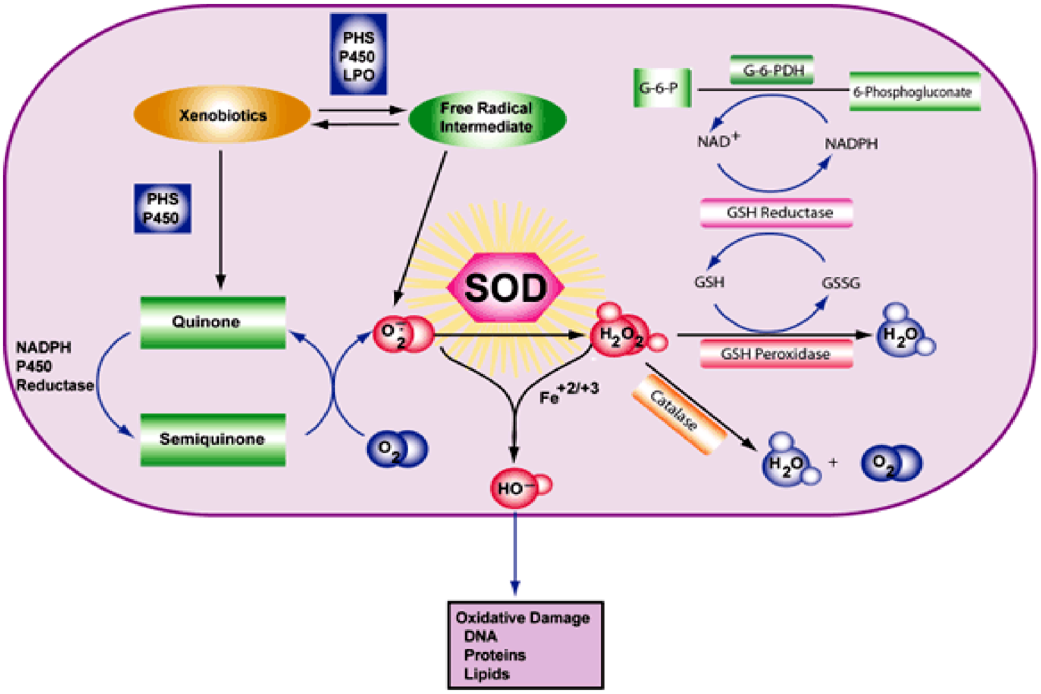
http://www.jbc.org/content/279/50/52376/F7.expansion

2.5.1.2 Superoxide dismutase (SOD)

Superoxide dismutase is an endogenously produced enzyme present both in prokaryotes and eukaryotes. SOD is a group of metallo enzymes with various prosthetic groups. SOD catalyses the dismutation of O_2- to dioxygen (O_2) and H_2O_2 Superoxide anion radical (O2 -•), a substance which has influence on aging, is generated during the metabolism process in the body, has a strong activity to react and destroy during the reduction of electrons, and thus induces the potent toxicity to cells and tissues. Due to such toxicity, various aging related diseases prevalent in adults, and arthritis are developed .It suppress the reaction of superoxide and protect the body from superoxide (Cadenas and Davies,2000). Polyphenols, which is a kind of phytochemical, were found to markedly induce apoptotic characteristics by reducing intracellular reactive oxygen species generation and by recovering the mitochondrial membrane potential (Park *et al*., 2007).

Figure 17

Mechanism of action of SOD

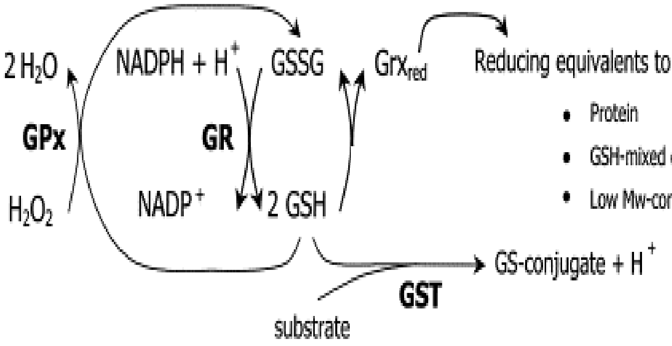


http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/cell-signaling-enzymes/superoxide-dismutase.html

2.5.1.3 Glutathione S-transferase (GST)

The GST composed of many cytosolic, mitochondrial, and microsomal proteins. GSTs are present in eukaryotes and in prokaryotes, where they catalyze a variety of reactions and accept endogenous and xenobiotic substrates. GSTs catalyse the conjugation of reduced glutathione — via a sulfhydryl group — to electrophilic centers on a wide variety of substrates. This activity detoxifies endogenous compounds such as peroxidised lipids, as well as breakdown of xenobiotics. GSTs may also bind toxins and function as transport proteins, which gave rise to the early term for GSTs of "ligandin" (Upadhya *et al*., 2004). Most mammalian isoenzymes have affinity for the substrate 1-chloro-2,4-dinitrobenzene (CDNB), and spectrophotometric assays utilising this substrate are commonly used to report GST activity. GSTs are considered, among several others, to contribute to the phase II biotransformation of xenobiotics (Allocati *et al*., 2009). Active oxygen species increase the level of glutathione transferase that metabolizes the toxic products of LPO. GST plays an essential role in the liver by eliminating toxic compounds by conjugating them with glutathione (Soyoz *et al*., 2004)

The major glutathione-associated antioxidant systems



http://www.sciencedirect.com/science/article/pii/S0891584901007249

2.5.1.4 Glutathione peroxidase (GPx)

The GPx1 is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. GPx4 has a high preference for lipid hydroperoxides; it is expressed in nearly every mammalian cell, though at much lower levels. GPx2 is an intestinal and extracellular enzyme, while GPx3 is extracellular, especially abundant in plasma. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans (Gupta *et al*., 2003).

The GPx is a selenium dependent enzyme has high potency in scavenging highly reactive free radicals. A specific isozyme, phospholipid hydroperoxide glutathione peroxidase, is the only major antioxidant enzyme that directly reduces phospholipid hydroperoxides within and lipoproteins and act together with alpha – tocopherol to inhibit lipid peroxidation involved and insufficient availability of GSH. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Ran *et al*., 2007).

$$2GSH + H_2O_2 \rightarrow GS\text{–}SG + 2H_2O$$

The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ is indicative of GPx activity, since GPx is the rate limiting factor of the coupled reactions (Bhabak and Mugesh 2010).

$$\text{R-OOH} + 2\ \text{GSH} \xrightarrow{\text{GPx}} \text{R-OH} + \text{GSSG} + H_2O$$

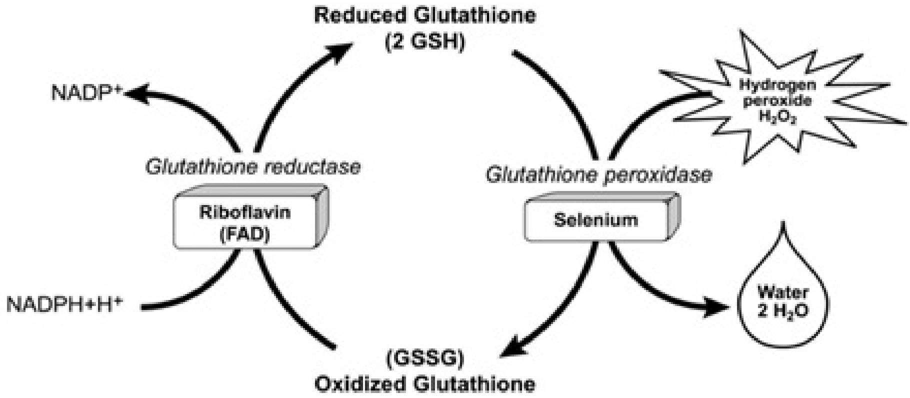
$$\text{GSSG} + \text{NADPH} + H^+ \xrightarrow{\text{GR}} 2\ \text{GSH} + \text{NADP}^+$$

2.5.1.5 Glutathione reductase (GR)

The GR, also known as an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. For every mole of oxidized glutathione (GSSG), one mole of NADPH is required to reduce GSSG to GSH. The enzyme forms a FAD-bound homodimer. The activity of glutathione reductase is used as indicator for oxidative stress. The activity can be monitored by the NADPH consumption, with absorbance at 340 nm, or the formed GSH can be visualized by Ellman's reagent. Alternatively the activity can be measured by the method of Michalak 2006 using redox-sensitive green fluorescent protein. Glutathione is a water soluble antioxidant it keeps cystein thiol group in the reduced state. If two thiol groups become oxidized, they can be reduced non- enzymatically by glutathione. GSSG is reduced by NADPH - dependent enzyme GR. A decrease in the activity of GR would further decrease the concentration of ascorbic acid. GRD is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of GSSG to reduced form (Takeoka and Dao 2003). Lima *et al*., (2005) who reported that drinking of Saliva *officinalis* tea improved liver antioxidants status by increasing the levels of glutathione reductase. Gupta *et al*., (2004) also reported that the administration of methanolic extract of *Bauhinia racemosa* elevated the levels of GSH in tumour bearing Swiss albino mice.

Figure 18

Glutathione Oxidation Reduction (Redox) Cycle



http://lpi.oregonstate.edu/infocenter/minerals/selenium/gsh.html

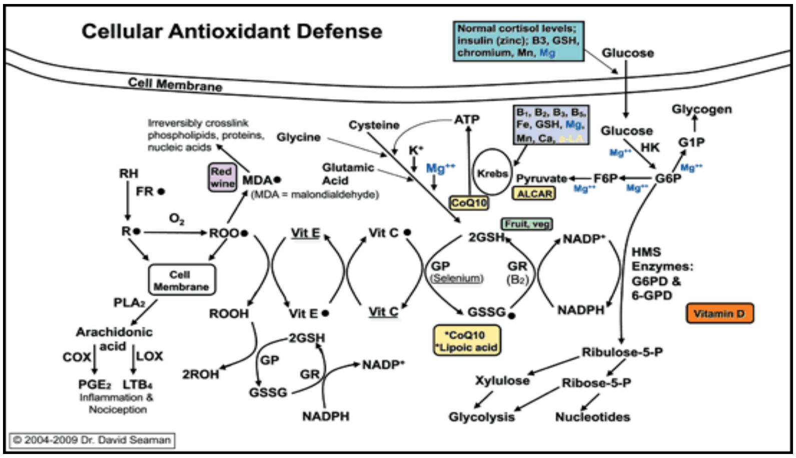
One molecule of hydrogen peroxide is reduced to two molecules of water, while two molecules of glutathione (GSH) are oxidized in a reaction catalyzed by the selenoenzyme, GPx. GSSG may be reduced by the flavin adenine dinucleotide (FAD)-dependent enzyme, GR.

2.5.2 Exogenous antioxidants

The antioxidant enzymes are complemented by small molecule antioxidants. The small molecule antioxidants are present extra and intracellularly and include fat soluble (Vitamins A and E), water (soluble Vitamin C) and GSH (Yen *et al*., 2008).

Figure 19

Non enzymatic antioxidants in the management of oxidative stress

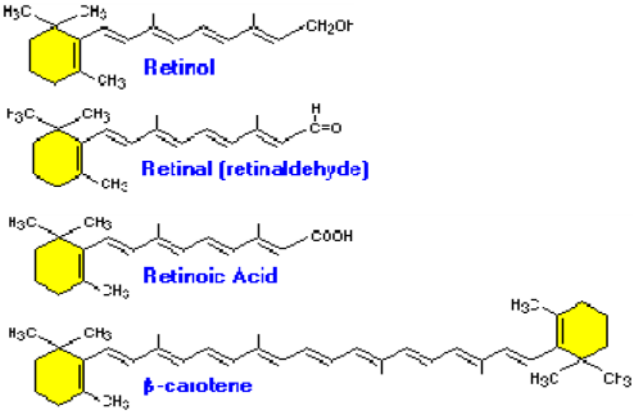


http://www.dynamicchiropractic.com/mpacms/dc/article.php?id=53885

2.5.2.1 Vitamin A

Retinol, the active form of Vitamin A, is rarely found in food. Instead, the body converts other substances found in the diet into Vitamin A. These substances are called precursors to Vitamin A. Another precursor to Vitamin A is found in the carotenoid beta-carotene. Vitamin A is essential to overall good health and individuals that do not get an adequate amount of Vitamin A in their diet have been shown to be more vulnerable to infection and infectious diseases, including AIDS, measles, bronchitis, yeast infections, and boils or abscesses (Kavimani and kumar 2000).

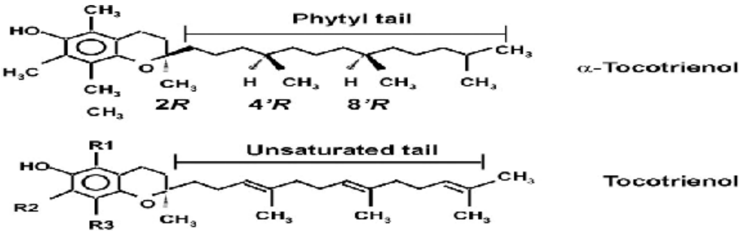
Structure Vitamin A (Retinol)



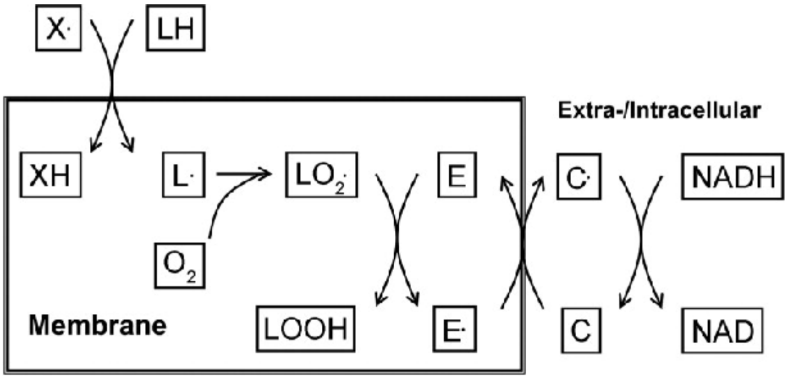
2.5.2.2 Vitamin E

Vitamin E, or alpha-tocopherol, is a leading antioxidant. It boosts the immune system's ability to fight off infectious diseases by increasing levels of interferon and interleukin, the biochemicals that are produced by the immune system. Vitamin E is being tested as a treatment for many chronic diseases for the elderly, including Alzheimer's, macular degeneration, osteoarthritis, and prostate enlargement (Mustacich *et al*., 2007). Vitamin C antioxidant that acts together with the membrane-localized Vitamin E in protecting membrane lipids from peroxidation as it regenerates oxidized Vitamin E (Knowles and Harris 2001).

Structure of the naturally occurring tocotrienols



Antioxidant function of Vitamin E



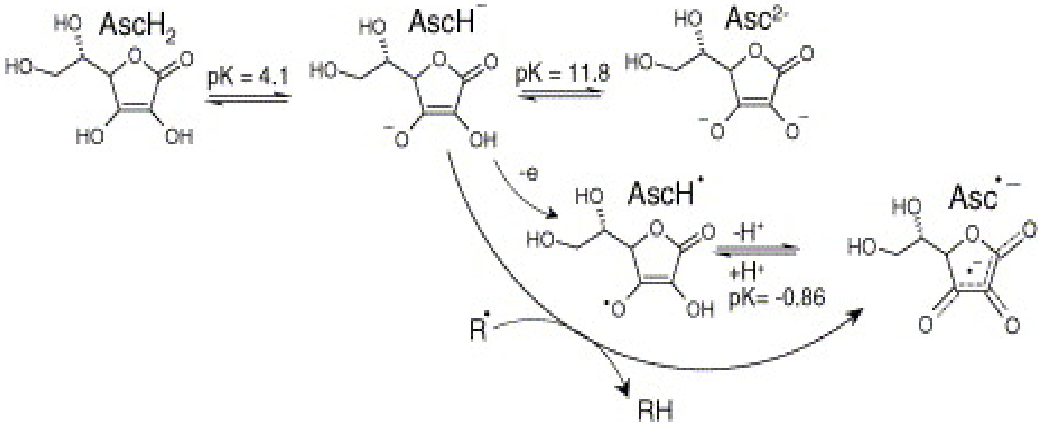
http://www.sciencedirect.com/science/article/pii/S0016508509014474

Located in the inner- and outer leaflet of the membrane, Vitamin E can stop the free radical–induced lipid peroxidation of membrane- forming unsaturated lipids. Either extra- or intracellular Vitamin C can reduce the Vitamin E–radical to form again Vitamin E. Finally, Vitamin C–radical is reduced via other water soluble redox systems (Biesalski, 2009).

2.5.2.3 Vitamin C

Vitamin C act as a direct scavenger of free radicals and reductant in enzymatic reactions. Vitamin E is the main lipid soluble antioxidant in membranes preventing lipid peroxidation. Vitamin C and E acts synergistically in scavenging a wide variety of ROS. Vitamin C can protect essential substances in the body such as proteins, lipids, carbohydrates and DNA and RNA from damage by free radicals (Padayatty *et al*., 2003 and Ball 2004). Vitamin C plays an important role in the human immune system by protecting various types of cells, such as phagocytes, lymphocytes and neutrophils, within the immune system (Bjelakovic *et al*., 2007). Vitamin C has multiplicity of antioxidant property and has been claimed to be most important antioxidant in human, because it disappears faster than other antioxidants when exposed to ROS. In arthritic condition, the level was found to be significantly decreased which may be due to decreased level of GSH and of enzyme system (NADH semidehydro ascorbate reductase) (Feng *et al*., 2001).

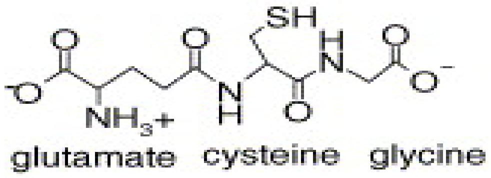
Various forms of ascorbic acid and its reaction with radicals (R.)



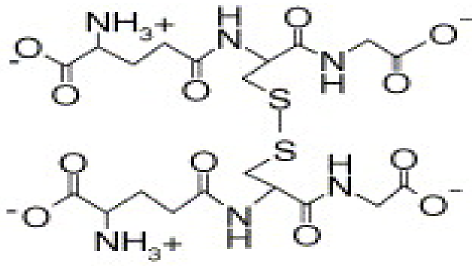
http://www.sciencedirect.com/science/article/pii/S0009279705004333

2.5.2.4 Reduced glutathione (GSH)

The GSH, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. GSH is a predominant low molecular weight thiol in the cytoplasm, which protects the tissue against *in vivo* toxicity of sulfhydryl – binding toxicants. The level of GSH appears to be reflux mechanism to protect against extracellular free radicals in chronic arthritis. Glutathione exist in the oxidized and reduced forms which are inter-convertable (Rajeshwar *et al*., 2005).



GSH



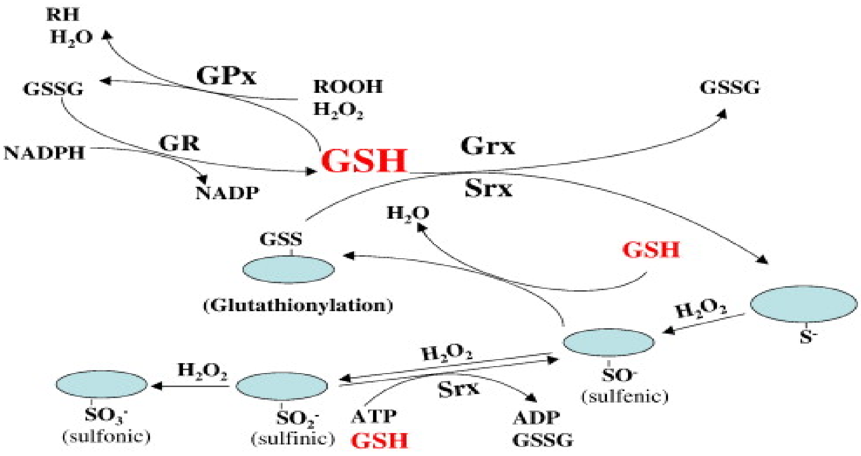
GSSG

http://www.sciencedirect.com/science/article/pii/S0009279705004333

The reduced GSH, in turn, keeps up the cellular level of the active form of Vitamin C. GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defence processes. Moreover, GSH serves as direct ROS scavenger and functions in the regeneration of oxidized forms of antioxidant Vitamins C and E. Due to the fact that GSH modulates activation and binding of transcription factors and as the cellular concentration of the reduced GSH is up to 100 fold higher than GSSG, minor increase in GSH oxidation can significantly affect the GSH:GSSG ratio and consequently influences signal transduction and cell cycle progression. The balance can be restored by NADPH-dependent glutathione reductase or the thioredoxin/glutaredoxin systems that catalyze the inverse reaction by reducing GSSG to GSH or by elimination of the oxidized GSSG from the cell (Benz and Yau 2008; Hussain *et al*., 2003). Reduced thiols have long been reported to be essential for recycling of antioxidants like Vitamin E and Vitamin C (Venukumar and Latha 2002).

Figure 20

Oxidative stress and glutathione



2.6 Role of AgNPs in medicine

In the past, silver was used for a variety of clinical conditions including epilepsy, venereal infections, acnes and leg ulcers. Silver foil was applied to surgical wounds for improved healing and reduced post-operative infections, while silver and lunar caustic (pencil containing silver nitrate mitigated with potassium nitrate) was used for wart removal and ulcer debridement (Klasen 2000). Although some centers still use these solutions, they have been shown to be very practical to use on small wounds or for short time periods due to instability. With nanotechnology, the availability of AgNPs has enabled the use of pure silver to achieve a rapid growth in medical practice. Since the size, shape and composition of AgNPs can have a significant effect on their efficacy, extensive research has gone into synthesizing and characterizing AgNPs (Shanmukh *et al*., 2008).

Studies on biomedical applications of NPs are growing with a rapid pace. Nevertheless, the biomedical applications of nanomaterials as cytotoxic or antimicrobial agents, biosensors and, drug carriers are growing with a rapid pace (Sondi and Sondi 2004).

In medicine, NPs may be the solution for multi-drug-resistance which is still a major drawback in chemotherapy of cancer. It has also been shown that the properties of noble metalNPs are strongly influenced by their shape and size. Surface modification allows for better and more accurate targeting of cancer cells compared to conventional methods and there is a great potential that NPs would meet the pressing need for better cancer therapy (Bharali *et al*., 2011).

Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery and gene delivery systems and artificial implants (Sachlos *et al*., 2006). The new age drugs are NPs of polymers, metals or ceramics, which can combat conditions like cancer (Farokhzad *et al*., 2006) and fight human pathogens like bacteria (Panacek *et al*., 2006).

The integration of nanotechnology with biotechnology and medicine means the ability to uncover the structure and function of biosystems, which intrinsically have an organizational level at the nanoscale (Kairemo *et al*., 2008). Nanotechnology may be translated into nanomedicine thereby referring to treatment and curing of diseases at a molecular scale. Indeed, the use of NPs (100 nm or smaller) for delivery and targeting of therapeutic and diagnostic agents is at the forefront of projects in cancer medicine. The targeting and accumulation of drugs to specific sites where the agent is released provides a means to reach high drug concentration at a designated area with far less systemic side effects (John *et al*., 2008).

2.6.1 Role of AgNPs in diagnosis and imaging

In terms of detecting cancer, Au–Ag nanorods were used in a recent study as a nano platform for multivalent binding by multiple aptamers, so as to increase both the signal and binding strengths of the aptamers in cancer cell recognition. The molecular assembly of aptamers on the nanorods was shown to lead to a 26-fold higher affinity than the original aptamer probes (Phanjom *et al*.,2012). Thus, these nanorod–aptamer conjugates are highly promising for use in specific cell targeting, as well as having the detection and targeting ability needed for cell studies, disease diagnosis, and therapy. It has some potential application like diagnostic biomedical optical imaging, biological implants (like heart valves) and medical application like contraceptive devices, surgical instruments and bone prostheses.

2.6.2 Role of AgNPs in wound dressings

Acticoat®, which is the first commercial wound dressing material made up of two layers of polyamide ester membranes covered with nanocrystalline silver ions, has been studied extensively (Fong and Wood 2006). Silver has long been recognized as having an inhibitory effect towards many bacterial strains and micro organisms commonly present in medical and industrial processes. The most widely used and known applications of silver and AgNPs are the topical ointments and creams containing silver to prevent infection of burns and open wounds (Murphy 2008).

2.6.3 Role of AgNPs in infections

Central venous catheters (CVC) are widely used in hospital practice, with around 5 million being inserted in the United States alone each year. However, the widespread use of CVCs is associated with potential infective complications, with the incidence of catheter-related bloodstream infection estimated at around 80000 cases annually. A new generation of silver-impregnated catheters based on the use of an inorganic silver powder, on which silver ions are bonded with an inert ceramic zeolite, has become available for clinical use. AgNPs polypropylene mesh has been studied recently. Similar to other studies using AgNPs, the results showed that AgNPs with polypropylene mesh had significant bactericidal efficacy against *Staphylococcus aureus*. Furthermore, it was shown that AgNPs could continue to diffuse off the mesh and had sustained activity (Cohen *et al*., 2007).

2.6.4 Role of AgNPs in orthopaedics

The AgNPs have been studied in artificial joints. For many years, ultra high molecular weight polyethylene has been the material of choice for fabrication of bearing inserts for joint replacement components (Alt *et al*., 2004).

2.6.5 Role of AgNPs in coating

Biologically synthesized AgNPs have many applications like coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials. Many major consumer goods manufacturers already are producing household items that utilize the antibacterial properties of AgNPs. These products include nanosilver lined refrigerators, air conditioners and washing machines (Mermel, 2000).

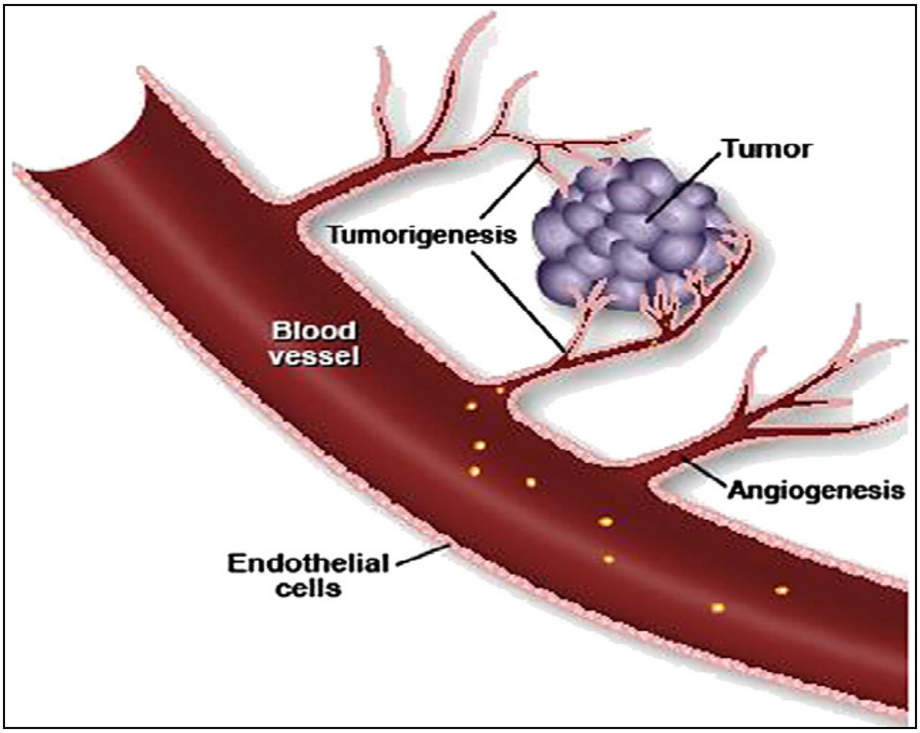
2.6.6 Role of AgNPs in cancer therapy

Carcinogenesis, which means the initiation or generation of cancer, is the process of dearrangement of the rate of cell division due to damage to DNA. Cancer is, ultimately, a disease of genes. In order for cells to start dividing uncontrollably, genes which regulate cell growth must be damaged. Proto-oncogenes are genes which promote cell growth and mitosis, a process of cell division, and tumor suppressor genes discourage cell growth, or temporarily halt cell division in order to carry out DNA repair. Typically, a series of several mutations to these genes are required before a normal cell transforms into a cancer cell. Cancer pathology is ultimately due to the accumulation of DNA mutations that negatively affect the expression of tumour suppressor proteins or positively affect the expression of proteins that drive the cell cycle (Nisa *et al*, 2011).

Solid tumors are angiogenic and require vascularization for growth. Angiogenesis, the formation of new blood vessels from existing ones, plays an important role in the growth and spread of cancer. New blood vessels “feed” the cancer cells with oxygen and nutrients, allowing these cells to grow, invade nearby tissue, spread to other parts of the body, and form solid tumors (Bhattacharya and Mukherjee, 2008).

Figure 21

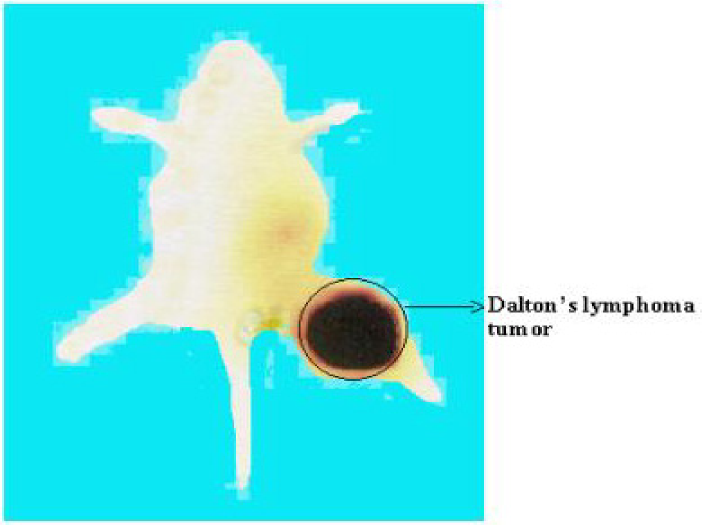
Normal angiogenesis and tumor angiogenesis



Four characteristics distinguished the cancer cell from normal cells: uncontrolled proliferation, dedifferentiation and loss of function, invasiveness and metastasis. Deoxyribonucleic acid mutation, which can be acquired or inherited, can turn a normal cell into a cancer cell but carcinogenesis is a complex multistage process involving two main genetic changes, the activation of proto oncogenes to oncogenes and the inactivation of tumor suppressor genes (Nipun *et al*, 2011). Angiogenesis is important in physiological process and plays a pivotal role in tumor growth, tissue invasion, and metastasis. Angiogenesis is a complex, multistep process involving interaction between tumor cells and endothelial cells, growth factors and extracellular matrix components (Van and Ming 2006).

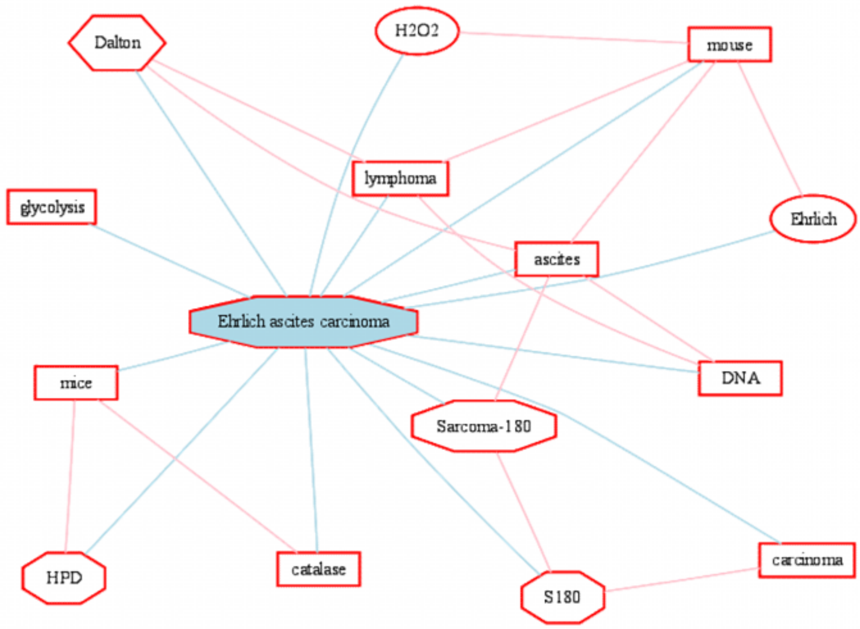
Figure 22

Gamma scintigraphic image of Etoposide loaded tripalmitin NPs after 48 h of intratumoral injection



(Reddy *et al.*, 2004)

Relational Network



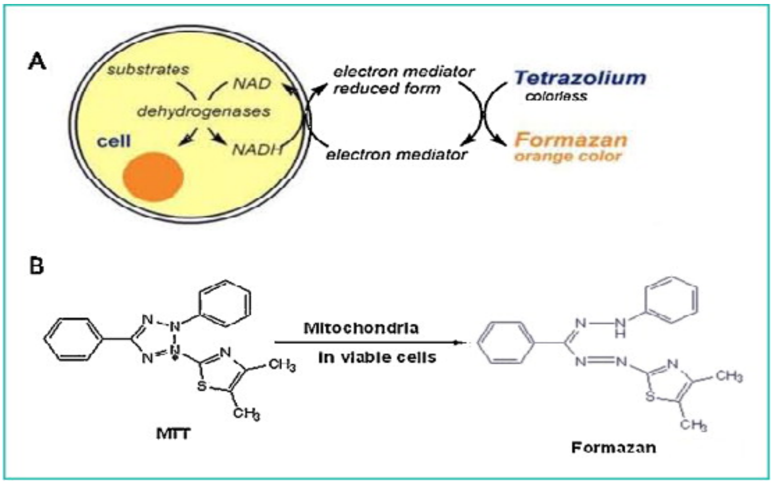
Copyright 2009, Research Foundation, Stony Brook

http://www.textmed.com/unknown/ehrlich-ascites-carcinoma.htm

Series of metallo (copper, cobalt, nickel and zinc) nanocomplexes engineered with a pre-prepared peptide [N,N′-(1,2-ethylene)-*bis*-hippuricamide] have been synthesized for damaging the DLA tumor model. Peptide bound zinc and cobalt nanocomplexes deliver excellent antitumor behavior against both the animal and human cancer cell lines (Sudharsan *et al*., 2013).

Biomedical applications of AgNPs need to be cytocompatible and have the capacity to restore natural morphology of the tissue in contact without triggering immunogenecity. Therefore, the evaluations of these effects are of high importance for future medical purposes. Prior to in vivo applications, in vitro methods are needed to evaluate cytocompatibility as a prerequisite. AgNPs studies work as basic tools for evaluating nanoparticle safety in order to foster the efficient movement of AgNPs products through preclinical and clinical development (Weyermann *et al*., 2005).

Figure 23
Principle of MTT assay



http://www.sciencedirect.com/science/article/pii/S0003450912001447

The MTT assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation and viability (Haldar *et al*., 2010). The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation (Afify *et al*., 2011). Cytotoxic effects of nanoparticle extracted from *Ficus Benjamina* have been studied on two cell lines, of HeLa and HEK 293 (Bhakat *et al*., 2012).

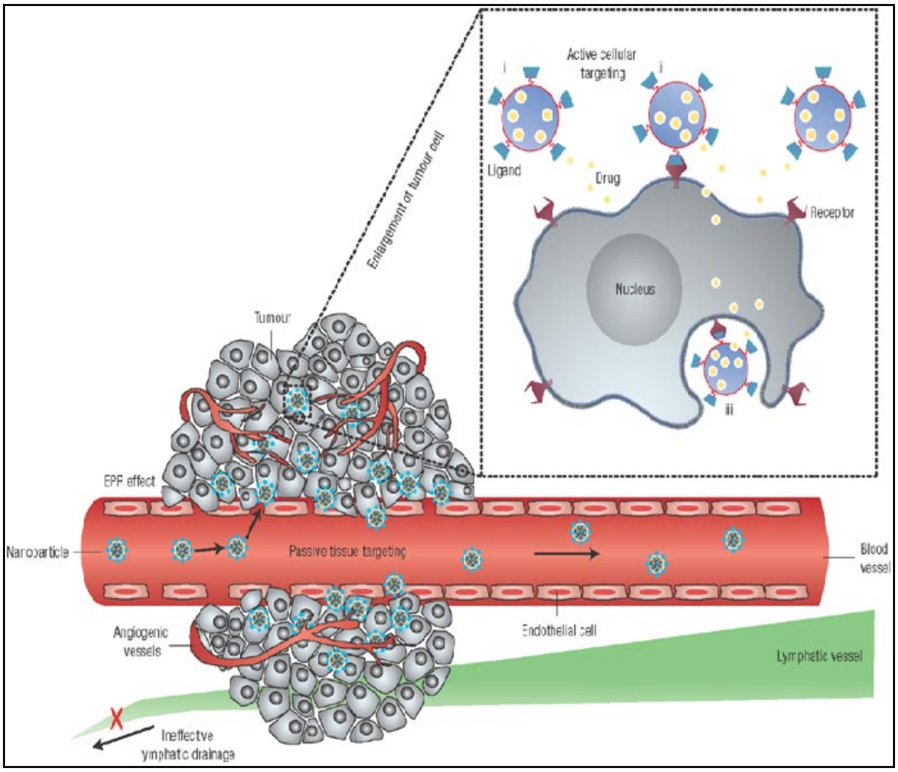
The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at 650nm by using a spectrophotometer. This reduction takes place only when mitochondrial reductase enzymes are active and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the antiproliferative effectiveness of the agent in causing death of cells can be calculated (Bhattacharya *et al*., 2011).

Plant based AgNPs possess considerable anticancer effect compared with commercial nanosilver. The use of AgNPs as drug delivery vehicles for anticancer therapeutics has great potential to revolutionise the future of cancer therapy. As tumour architecture causes AgNPs to preferentially accumulate at the tumour site, their use as drug delivery vectors results in the localisation of a greater amount of the drug load at the tumour site; thus improving cancer therapy and reducing the harmful nonspecific side effects of chemotherapeutics. In addition, formulation of these NPs with imaging contrast agents provides a very efficient system for cancer diagnostics. Given the exhaustive possibilities available to polymeric nanoparticle chemistry, research has quickly been directed at multi-functional NPs, combining tumour targeting, tumour therapy and tumour imaging in an all-in-one system, providing a useful multi-modal approach in the battle against cancer (Vlerken and Amiji, 2006).

Nanotechnology has become an enabling technology for personalized medicine in cancer detection, diagnosis, and therapy. The promises of nanotechnology in cancer research lie in the potential to overcome the drawbacks such as side effects and toxicity to healthy cells that come across in the current cancer treatment (surgery, radiation, and chemotherapy). Nanotechnology is significant on account of its preeminence upon the comprehensive use, and control of matter at magnitudes of a minute scale, akin to approaching atomic levels, with which to manufacture new substances, instruments, and frameworks also known as 'Molecular Manufacturing' (Cuenca *et al*., 2006).

Figure 24

Nanocarriers for cancer therapy



http://www.nature.com/nnano/journal/v2/n12/full/nnano.2007.387.html

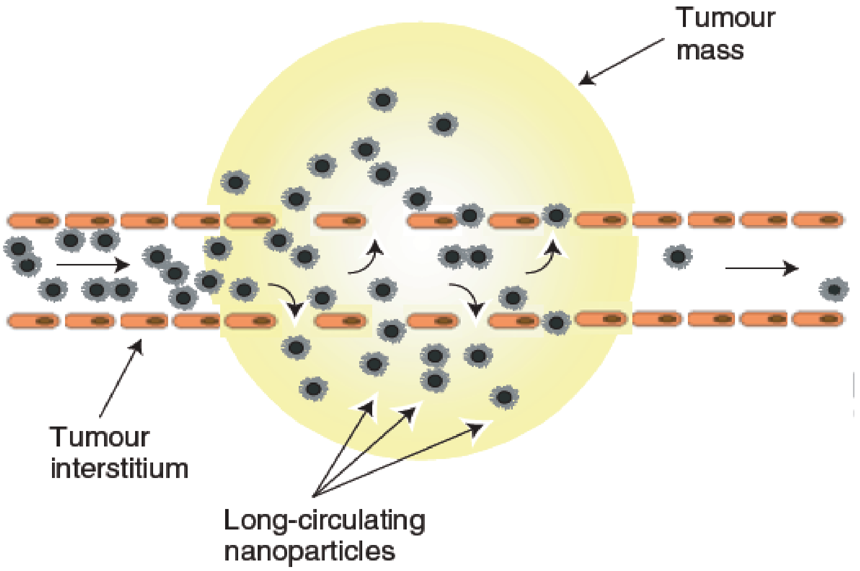
Rational design of NPs requires the knowledge of tumor-specific receptors that would allow endocytosis of NPs, tumor-specific biomarkers that facilitate identification of cancers, and tumor-specific homing proteins and enzymes that can permit selective uptake into cells or accumulation in tumor micro environments (Nie *et al*., 2007).

Several nanobiotechnologies mostly based on NPs, have been used to facilitate drug delivery in cancer. As tumor architecture causes NPs to preferentially accumulate at the tumor site, their use as drug delivery vectors results in the localization of a greater amount of the drug load at the tumor site; thus improving cancer therapy. It can target a tumor, carry imaging capability to document the presence of tumor, sense pathophysiological defects in tumor cells, deliver therapeutic genes or drugs based on tumor characteristics, respond to external triggers gene delivery offers the potentials to (a) replace missing or defective genes; (b) deliver genes that catalyze the destruction of cancer cells; (c) cause cancer cells to revert back to normal tissue (O'Connor *et al*., 2006).

2.6.6.1 As adjuvant for Cancer therapy

Figure 25

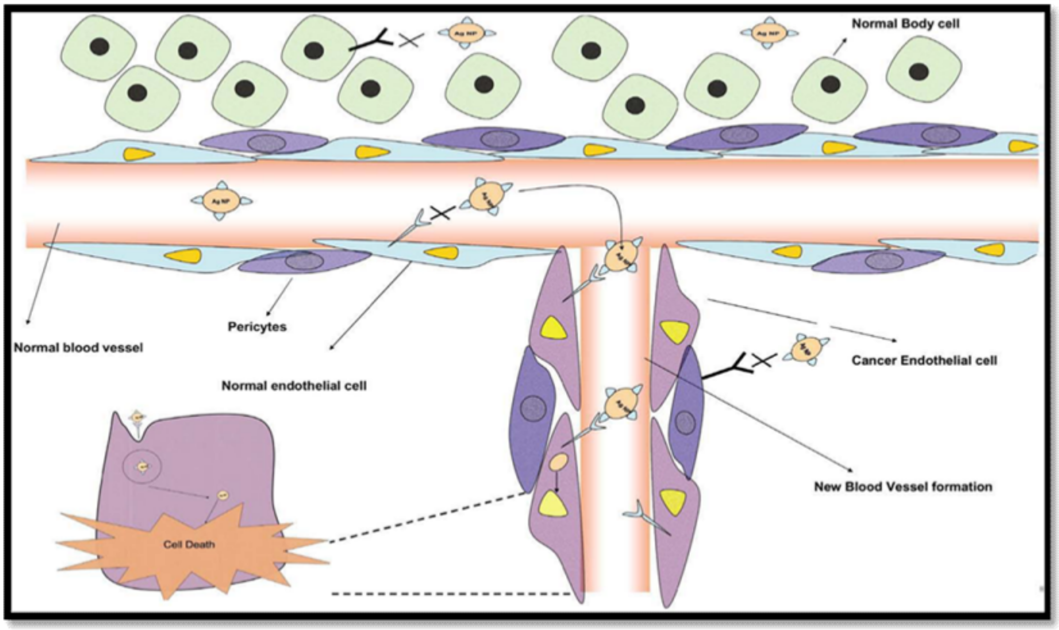
Nanoparticle localisation in solid tumours



(Loo *et al.*, 2005).

Figure 26

Targeted delivery of AgNPs in cancer therapy



http://www.sciencedirect.com/science/article/pii/S0168365910002270

Interaction of the functionalized AgNPs with target cells, leading to the cell membrane structure change and increase membrane permeability. With the specific properties and activities of the NPs, the AgNPs can be used as drug carriers, effectively facilitate anticancer drugs to enter into cancer cells and improve the drug concentration in the tumor cells. Thus, the function of AgNPs has the potential and important application in cancer clinical treatment.

The cytotoxic effect of AgNPs on cell viability has a major role in antitumor activity, thereby reducing disease progression. This is consistent with the effect of AgNPs on cell viability during vascular endothelial growth factor induced angiogenesis in retinal endothelial cells (Kalishwaralal *et al*., 2009) thereby inhibiting the development of angiogenic retinal disorders or complications. The cytotoxic effects of silver are the result of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA. The major criteria to be taken into consideration for any potential anticancer drug are its efficacy in prolongation of lifespan and decrease of tumor volume and viable tumor cell count (Arora *et al*., 2008).

The role of AgNP, in inhibiting DLA cell viability and proliferation will be similar to their potential to inhibit the permeability of endothelial cells by inactivating Src kinases which have been proven to have a role in retinal therapies (Furno *et al*., 2004). AgNPs serve as antitumor agents by decreasing progressive development of tumor cells. This may be due to their inhibitory activities in several signaling cascades responsible for the development and pathogenesis of the disease. Use of AgNPs should emerge as one of the novel approaches in cancer therapy (Vaidyanathan *et al*., 2009).

The antiproliferative effect of AgNPs and Ag^+ is evident from the reports of Okamoto *et al*., 2009; Ahamed *et al*., 2008; Rahman *et al*., 2009. Furthermore, the interaction of AgNPs and Ag+ with certain proteins has been the subject of extensive studies in recent years. Antiproliferative effect of silver in both ionic (Ag^+) and metallic (AgNPs) forms on parent and tamoxifen- resistant cells was investigated and reported both have effects towards T47D human breast cancer cells.

Liposomal NP therapeutics containing cytotoxic agents may provide the foundation for potentially more effective and less toxic anti-cancer treatment strategies due to their improved pharmacokinetics, reduced systemic toxicity, and increased intratumoral/intracellular delivery (Cho *et al*., 2008; Davis *et al*., 2008).

After internalization of AgNPs and Ag^+ into cells (which resembles the situation in parent cells), the cytotoxic effects of AgNPs is more than that of Ag^+, probably due to the fact that AgNPs may interfere with the proper functioning of cellular proteins and induce subsequent changes in cellular chemistry; as proposed previously by Rogers *et al*., 2008. This hypothesis is also in good agreement with experiments of Zolghadri and co-workers where they demonstrated that AgNPs provide a relatively high hydrophobicity inside bovine hemoglobin which causes a transition from alpha helixes to beta sheets and leads to partial unfolding and aggregation of the protein (Zolghadri *et al*., 2009). Other experiments suggest that AgNPs are likely to interact with thiolrich enzymes (Morones *et al*., 2005). Therefore, it sounds possible that once penetrated into cells, AgNPs may attack functional proteins of cells which results in partial unfolding and aggregation of proteins as it is the case in the bovine hemoglobin.

Interaction among Ag+ and nitrogen-containing-cytosine molecules which literally increases the stability of the parallel motif of the duplex DNA. One can not conceal the recent global attention towards discovering novel biomedical applications for metal-based NPs in both diagnosis and treatment of cancer (Lok *et al*., 2007 and Galanzha *et al*., 2009).

2.6.6.2 As specific ligand

Silver has been now recognized as a developing therapeutic molecule and will surely extend its use as a drug carrier. AgNPs can be used for both active and passive targeting of drugs. AgNPs have recently emerged as an attractive candidate for delivery of various payloads into their targets. The payloads could be small drug molecules or large biomolecules, like proteins, DNA or RNA. Efficient release of these therapeutic agents is a prerequisite for effective therapy. The release could be triggered by internal (e.g. glutathione (GSH), or pH or external (e.g. light stimuli) stimulus. This binding may be achieved by attaching targeting agents (ligands) to the surface of the nanocarrier by a variety of conjugation chemistries. Nanocarriers will recognize and bind to target cells through ligand–receptor interactions, and bound carriers are internalized before the drug is released inside the cell (Napier and DeSimone 2007; Williams *et al*., 2009).

Apoptosis is a tightly regulated and at the same time highly efficient cell death program which requires the interplay of a multitude of factors. Apoptotic pathways involve the activation of the downstream of mitochondrial proapoptotic events. The cancer cells can be targeted using AgNPs conjugated with a tissue specific ligand that can activate any of the Vascular Endothelial Growth Factor (VEGF) receptors and express its effect on any of the downstream molecules (Bhol and Schechter 2005). (−)-Epigallocatechin-3-gallate physically attached onto the surface of nanogold particles (EGCG–pNG) was confirmed to inhibitin MBT-2 murine bladder tumor cell growing by means of cell apoptosis (Hsieh *et al*., 2011).

The origin of nanoencapsulation based chemoprevention approaches could be traced the term nanochemoprevention for the first time. This group utilized the multi-functionality of biodegradable polylactic acid (PLA)–polyethylene glycol (PEG)NPs to incorporate the well recognized chemopreventive agent from green tea 'epigallocatechin gallate' (Bachrach and Wang 2002). The last decade has witnessed rapid advancement in the development of nanochemopreventive technology with the emergence of many nanoencapsulated formulations of different anticancer agents (Radwan *et al*., 2011).

Biodegradable chitosan NPs encapsulating quantum dots were prepared with suitable surface modification to immobilize both tumor targeting agent and chemokine on their surfaces. The interactions between immune cells and tumor cells were visualized using optical microscope. Use of Quantum dots in the treatment of cancer is a great advancement in this area. Quantum dots glow when exposed to UV light. When injected they seep into cancer tumour. The surgeon can see the glowing tumour (Lin *et al*., 2011).

2.6.6.3 As antiangiogenic agent

The AgNPs have been shown to have important antiangiogenic properties. So are attractive for study of their potential antitumor effects. Compounds possessing antiangiogenic properties are known for their potential ability to block the activity of abnormally expressed signaling proteins, such as Ras and Akt, cytokine-based therapies, DNA- or protein-based vaccines against specific tumor markers, and tyrosine kinase inhibitors which exhibit a consistent antitumor effect (Gurunathan *et al*., 2009).

One feature of DLA tumor growth related to the progression of angiogenic complications is enlargement of the cross-sectional area of peritoneal microvessels. The formation of enlarged microvessels early in the course of ascites tumor angiogenesis may relate to the fibrin stroma in which these vessels develop (Nagy *et al*., 1995). Tumor cells implanted into the peritoneal cavity secrete vascular permeability factor and thereby render the microvasculature supplying the peritoneal lining tissues hyperpermeable. With respect to vascular hyperpermeability, the ascites tumor model used resembles a solid tumor model, with angiogenesis and generation of a connective tissue stroma. AgNPs that have been proven to delay tumor progression in DLA cell lines and tumor models in vivo may have a potent antipermeability effect by inhibiting tubular formation in growth factor- and advanced glycation end product-induced vascular permeability and cytotoxic effects that inhibit existence of tumor cells, which may be due to their potent activation of the caspase enzyme (Chan *et al*., 2010).

The AgNPs established as an effective anti-angiogenic and anti-proliferative agent have been proposed as an economic alternative for treatment of various retinal angiogenic disorders. Their targeting potential to integrin αVβ3 receptors using arginine-glycine aspartate peptide and controlled release when engulfed within poly gamma glutamic acid affirms them as a trove (found treasure) for nanomedicine that tribute to therapeutic advances in retinal therapies (Kalishwaralal *et al*., 2010).

2.6.6.4 As cytotoxic agent via Cell cycle arrest and apoptotic activity

AgNPs have shown to be effective in triggering the activation of Caspase-3 molecule and thus resulting in the mediation and amplification of the death signal. The activation of the Caspase cascade could be through any of the three possible ways which include; granzyme B (granzyme 2, cytotoxic T-lymphocyte- associated serine esterase 1). mediated activation, death receptor mediated activation and the apoptosome (multimeric protein complex that mediates activation of an initiator caspase at the onset of apoptosis) mediated activation which includes the release of cytochrome c. The study on Caspase-3 alone gives a very transparent idea making the discussion very ambiguous and so examining the other caspases would be the next level of interest. There is yet another point of discussion which involves the evidence for the molecule leading to only programmed cell death and not necrosis, although both mechanisms result ultimately in cell death. The Caspase-3 activation makes it evident that it leads to cleavage of Caspase substrates, resulting in the fragmentation of the DNA. Thus, the triggering of the death inducing signal forms an important area of interest in the trafficking of the nano molecule.

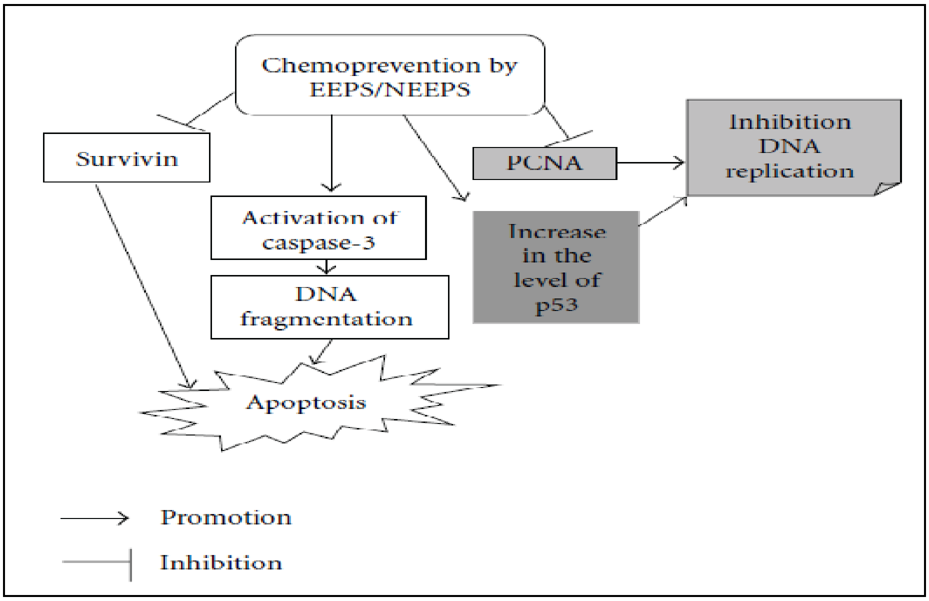
Caspase-3 has been identified as the main executioner of apoptotic response inside the cells. Cleavage of caspase-3 is a hallmark in the process of apoptosis. Activated caspase-3 induces the release of death substrate and lyses DNA in nucleus resulting in DNA fragmentation. Levels of caspase 3, a molecule which plays a key role in the apoptotic pathway of cells, were increased following the treatment with AgNPs. The cell lysates obtained from Hep2 cells treated with AgNPs activate caspase 3 suggested that AgNPs caused cell death through apoptosis, which was further supported by cellular DNA fragmentation. DNA ladders of the corresponding treated samples of silver nanopaticles synthesized from calli extract of Citrullus colocynthis confirmed apoptosis (Ji *et al*., 2011).

LY294002 prevented phosphorylation of protein kinase B (PKB/Akt) by inhibition of PI3K phosphorylation activity, thereby inducing G0/G1 cell cycle arrest and apoptosis in osteosarcoma. Apoptosis induction by LY294002 is accompanied by activation of caspase-9, caspase-3 and Poly (ADP-ribose) polymerase (PARP), which are involved in the mitochondrial apoptosis pathway. Therefore, PI3K inhibitors may represent a potential strategy for managing human osteosarcoma via affecting cancer stem-like cells (Gong *et al*., 2012).

Many anticancer compounds exert their growth inhibitory effect either by arresting the cell cycle at a particular checkpoint of cell cycle or by induction of apoptosis or a combined effect of both cyclic block and apoptosis.

Figure 27

EEPS/NEEPS-induced apoptosis of cancer cell (A549)

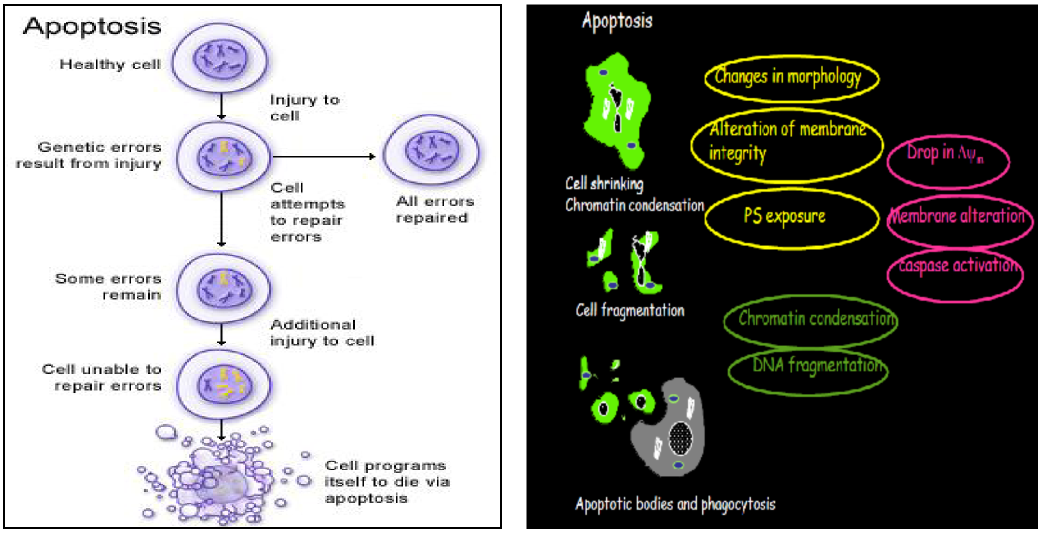


Apoptosis, a form of programmed cell death, is highly organized and evolutionary conserved process characterized by membrane blebbing, DNA fragmentation, loss of plasma membrane integrity, activation of proteases and endonucleases, enzymatic cleavage of DNA into oligonucleosomal fragments and segmentation of cells into membrane-bound apoptotic bodies. Apoptosis has been recognized to play a key role in the maintenance of tissue homeostasis by selective elimination of damaged or unwanted cells (Lin, 2001). Many anticancer agents exert growth suppressive effect on malignant cells by inducing apoptosis (Aravind *et al*., 2009).

Apoptosis of A549 cells by Ethanolic extract of *Polygala senega* (EEPS) and nanoencapsulated Ethanolic extract of *Polygala senega* (NEEPS) is associated with the down-regulation of survivin, PCNA mRNA expression. Caspase-3, the ultimate executioner that is essential for the nuclear changes, associated with apoptosis. EEPS and NEEPS-induced apoptosis of A549 was also related to the over expression of caspase-3 and p53. The molecular apoptotic pathway of NEEPS/EEPS-treated cells was found to be induced by activation of caspase-3 and p53 and suppression of survivin and Proliferating Cell Nuclear Antigen (PCNA) expression (Gong *et al*., 2004).

Figure 28

Stages of Apoptosis



(Lecoeur *et al*., 2001)

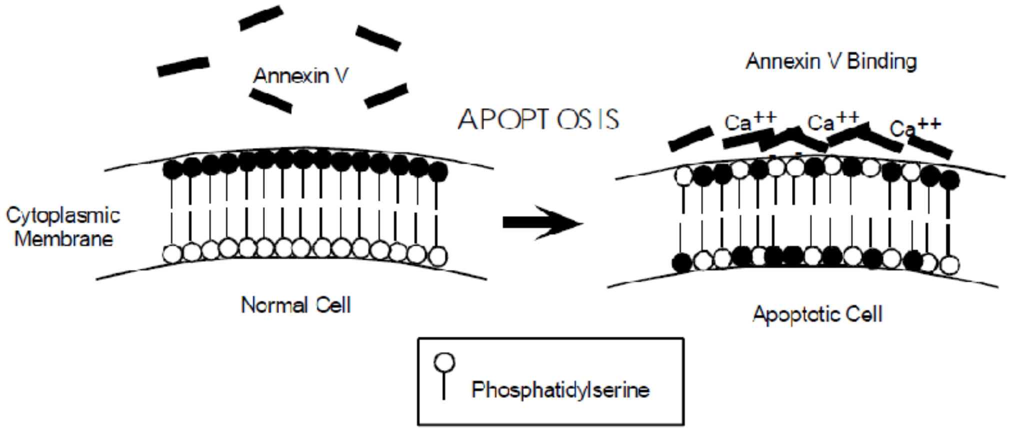
http://php.med.unsw.edu.au/cellbiology/index.php?title=Pre-Medicine_Program_-_Cell_Cycle

http://php.med.unsw.edu.au/cellbiology/index.php?title=Cell_Death_

Flow cytometric assay distinguish cells that are in the earlier stages of apoptosis (Annexin V positive, PI negative) from those that are in the later stages of apoptosis (Annexin V positive, PI positive) or already dead. Propidium iodide (PI) is excited at 488 nm and, with a relatively large stokes shift, emits at a maximum wavelength of 617 nm. Because of these spectral characteristics, PI can be used in combination with other fluorochromes excited at 488 nm such as fluorescein isothiocyanate (FITC) and phycoerythrin (PE). PI is a fluorescent vital dye that stains DNA. It does not cross the plasma membrane of cells that are viable or in the early stages of apoptosis because they maintain plasma membrane integrity. In contrast those cells in the late stages of apoptosis or already dead have lost plasma membrane integrity and are permeable to PI. PI is detected in the orange range of the spectrum using a 562-588 nm band pass filter (Dillon 2001; Poot *et al*., 2002).

Figure 29

Phospholipid flipping and binding of Annexin V during apoptosis

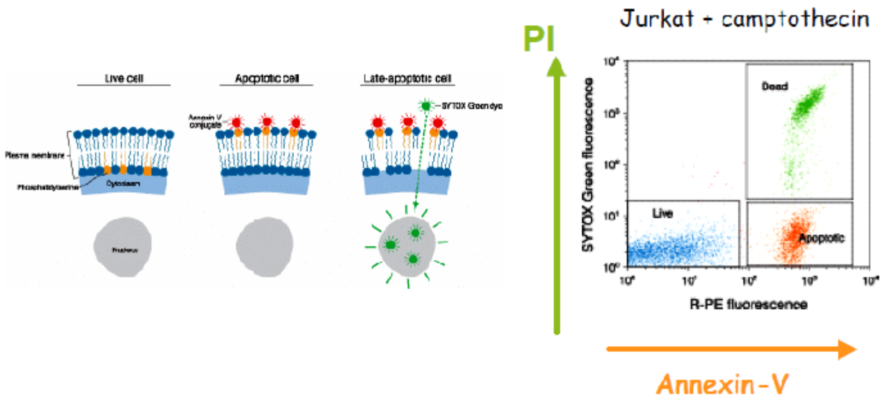


(Tait and Gibson 1992)

Annexin binding assay is a method permits the detection of the early phases of apoptosis before the loss of cell membrane integrity (Tao *et al*., 2004). The principle of Annexin V staining method used is the conjugation of Annexin V to phosphotidylserine of the apoptosis cells and in conjunction of dye Propodium Iodide which binds to cells at different stage and distinguishes apoptotic cells with necrotic cells (Craig 2007).

Figure 30

Detection of Apoptotic by Propidium iodide and Annexin V Staining



http://www.rndsystems.com/product_detail_objectname_annexin_v_kits.aspx

The prime feature of tumor development is the escape of cells from programmed cell death due to a metabolic abnormality or genetic mutation. Thus, another criterion for anticancer drug development is the ability of a compound to induce apoptosis in cancer cells (Mendoza *et al*., 2005).

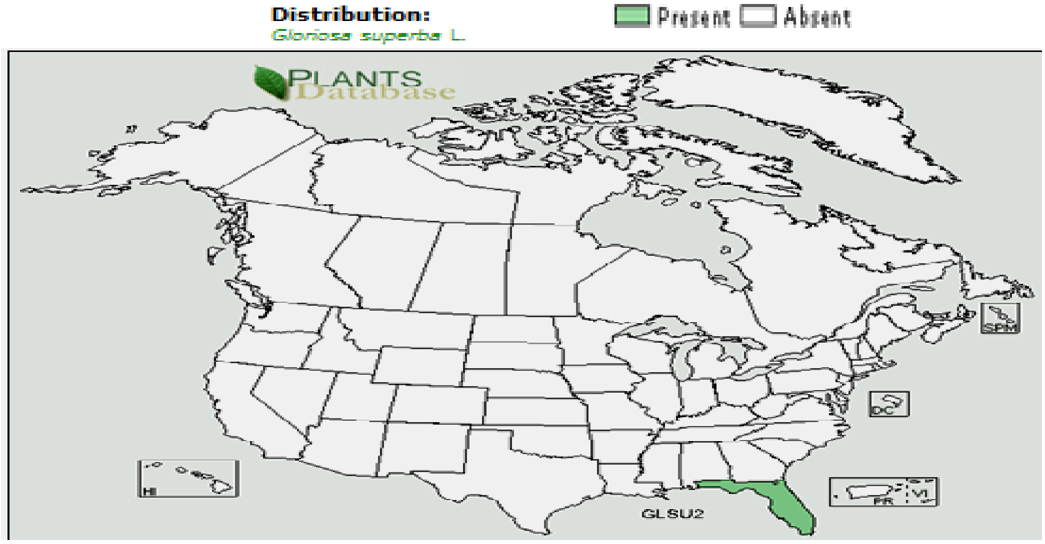
2.7 Medicinal plant selected for the study - *Gloriosa superba*

Gloriosa is a genus of ten species in the plant family Colchicaceae, and include the formerly recognised genus *Littonia*. They are native in tropical and Southern Africa to Asia, and naturalised in Australia and the Pacific as well as being widely cultivated.http://en.wikipedia.org/wiki/Gloriosa_(genus) - cite_note-Smith-1 The most common English names are flame lily, fire lily, gloriosa lily, glory lily, superb lily, climbing lily, and creeping lily. They are tender, tuberous rooted deciduous perennials, adapted to summer rainfall with a dormant dry season. All parts of the plant contain colchicine and related alkaloids and are therefore dangerously toxic if ingested, and contact with the stems and leaves can cause skin irritation. Various preparations of the plant are used in traditional medicines for a variety of complaints in both Africa and India (Pawar *et al*., 2010).

Gloriosa are perennial herbs that climb or scramble over other plants with the aid of tendrils at the ends of their leaves and can reach 3 meters in height. They have showy flowers, many with distinctive and pronouncedly reflexed petals, like a Turk's cap lily, ranging in colour from a greenish-yellow through yellow, orange, red and sometimes even a deep pinkish-red (Ade and Rai 2009). *Gloriosa superba* leaf extracts displayed anticoagulant properties by inhibiting thrombin-induced clotting (Kee *et al*., 2008). Glory lily have proved to have potent neutralizing effect of rattlesnake venom, when administered subcutaneously to mice (Ramar *et al*., 2008). The plant is known as 'Kalihari' in Hindi, 'Manthori khizangu' in Malayalam and 'Kazhappai kizhangu' in Tamil. Its stem is thin and grows at the rate of 20 feet per year. Leaves are ovate in shape about 6-8 inches long thread like at the apex that helps to climb on the trees. Gloriosine and colchicine are two commonly used phytochemicals for treatment of gout and rheumatism (Jomy *et al*., 2009; Hemaiswarya *et al*., 2009). Excellent antifungal sensitivity of glory lily has been reported against *Candida albicans* and *Candida glaberata* (Haroon *et al*., 2008) against adult cattle tick, malaria vector larvae, the various extract of glory lily showed inhibition of parasite in crude form (Zahir *et al*., 2009).

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Monocots
Order: Liliales
Family: Colchicaceae
Genus: ***Gloriosa*** L.

In Australia, "scattered naturalized populations exist in the understorey of coastal dry sclerophyll forest and sand dune vegetation throughout south-east Queensland and New South Wales". In India, *Gloriosa* is distributed in the Western Ghats but the density is rapidly decreasing due to excessive uprooting by the herbal medicine producers.



http://www.inchem.org/documents/pims/plant/pim245.htm