

Review of Literature

Medicinal plants are nature's priceless gift to human. Medicinal plants are now being widely used, either as a single drug or in combination for effective drug delivery system. Today's modern drugs have their origin in traditional medicinal plants. The development of modern medicine temporarily subdued the traditional herbal medicine. But now it has come back as blooming "herbal renaissance" across the world. 80% of the people in the developing countries rely on traditional medical practices (Bonifacio *et al.*, 2014). Medicinal plants are a rich source of bioactive constituents, which have antioxidative and antitumorigenic properties (Elansary *et al.*, 2018). Plant derived bioactive constituents are the resources for the green synthesis of nanoparticles which enhances the antioxidative and antitumorigenic potential (Mohanta *et al.*, 2017).

The term nanotechnology was first proposed by physicist Richard Feynman in 1959 in his talk entitled "There's Plenty of Room at the Bottom" presented at the American Physical Society meeting at the California Institute of Technology in 1959. The term nanotechnology was defined by Japanese scientist Dr. Nori Taniguchi in 1974 as "the processing of separation, consolidation, and deformation of materials by one atom or one molecule" (Guisbiers *et al.*, 2012; Mulvaney, 2015). Nanoscience, nanotechnology, nanomaterials, nanomedicine are some of the terms containing nano. The prefix "nano" came from the Latin word "nanus" literally means dwarf very small (Pal *et al.*, 2011). **Nanotechnology has rationalized the world of science and technology which deals with small things** that are less than 100nm in size (Theodore, 2014). Nanomaterials have significantly different properties than the same materials at larger scale. Nano sized particles have endless possibility due to their unique properties (<http://www.nnin.org>).

Among all the nanoparticles metallic nanoparticles have been used in drug delivery systems especially for the treatment of cancer and biosensors (Nikalje, 2015). In recent years, an exponential interest has developed for delivering novel drugs using

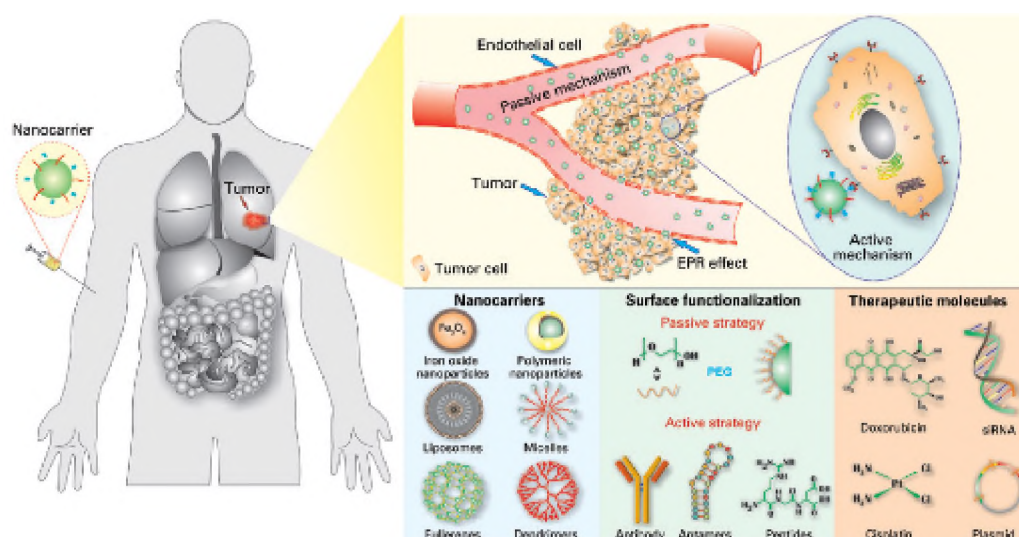
nanoparticles (Muller *et al.*, 2015). Nanoparticles can be used in different route of drug administration due to its high stability, high specificity, high drug carrying capacity, controlled release of drugs and the ability to deliver both hydrophilic and hydrophobic drug molecules in the diagnosis and treatment of diseases (Pal *et al.*, 2011). Bio-nano interactions showed the relations between nanoscale entities and biological systems such as peptides, proteins, lipids, DNA and cellular receptors (Lynch *et al.*, 2015).

In terms of global nanoscience India was ranked 4th position, possessing about 500 nanotechnology companies and 200 nano products commercially available in the market (www.researchandmarkets.com).

Cancer is the second leading cause of death next to cardiovascular disease. The current clinical treatments are unable to provide timely detection and curative therapy. To overcome these limitations nano oncology field plays a vital role. This field have designed new strategies to deliver chemotherapeutic drugs to the tumor site at higher concentrations with minimal damage to normal tissues (Shanmugasundaram *et al.*, 2017).

Figure 1

Nanocarriers for cancer treatment



Vieira and Gamarra (2016)

The review of literature pertaining to the present research entitled, “**Antioxidative and Antitumorigenic Potential of PEG Functionalized Silver Nanoparticles from Ethanolic Extract of *Volkameria inermis* Leaves to EAC Cells by *in vitro* and *in vivo* Studies**” is presented below.

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Green synthesis of nanoparticles using renewable natural sources like plant extract, microorganisms and biodegradable sources which acts as a reducing and capping agent have drawn tremendous attention towards nano field. For the rapid synthesis of nanoparticles plant sources are widely used as they fit for large scale biosynthesis. Various plant parts such as seed, leaf, stem, root and latex are commonly used for metallic nanoparticles synthesis (Kharissova *et al.*, 2013). Employing green technology for the synthesis of nanoparticles was found to be the best method, as it does not involve any harmful chemicals (Singh *et al.*, 2018; Gopinath *et al.*, 2013). Moreover, the synthesis of nanoparticle was influenced by the nature of the plant extract, pH, temperature, concentration of the metal salt and the contact time (Rossi *et al.*, 2014). It has been proven that, plant derived silver nanoparticles acted as a good antioxidant agent

as silver capped with the active functional groups present in the phytoconstituent of the plant extract (Hussen and Siddiqi, 2014).

2.2 METHODS FOR THE SYNTHESIS OF NANOPARTICLES

Basically there are two main approaches for nanoparticles synthesis i.e. the bottom up approach and the top down approach. Converting large material to the small material ie, the nanoscale size is recognised as top-down method. The top-down method includes physical, chemical and mechanical methods. The nanomaterial created from the atomic level is known as bottom-up method which comprises solid, liquid, gaseous and biological methods (Pacioni *et al.*, 2015).

2.2.1 Physical Methods

Synthesis of nanoparticles using physical methods includes radiolysis, microwave, ultrasonication, laser ablation and electrochemical methods (Sarsar *et al.*, 2014). Metal nanoparticles can be synthesized by evaporation–condensation mechanism, which was carried out using a tube furnace at atmospheric pressure. There are many draw backs associated with generating silver nanoparticles using a tube furnace. Tube furnace occupies a large space, and consumes large amount of energy by raising the environmental temperature around the source material. It is a time consuming process. A typical tube furnace needs power consumption of more than several kilowatts and a preheating time of several tens of minutes to attain a stable operating temperature (Abou El-Nour *et al.*, 2010; Makarov *et al.*, 2014).

2.2.2 Chemical Methods

In chemical methods, chemicals are used as the reducing agents. Chemically nanosilver can be synthesized by processes such as, reduction or oxidation of metal ions, inert gas condensation, or by the sol gel methods. Synthesis of nanoparticles by this method includes three main components: (i) metal precursors, (ii) reducing agents (iii) and stabilizing/capping agents (Thatai *et al.*, 2014; Thome *et al.*, 2015; Wei *et al.*, 2015). The size and the shape of synthesized nanoparticles are strongly dependent on these stages. Various chemicals methods namely chemical/electro chemical precipitation, brust-schiffirin method, two phase reduction method, citrate reduction, seeding growth method,

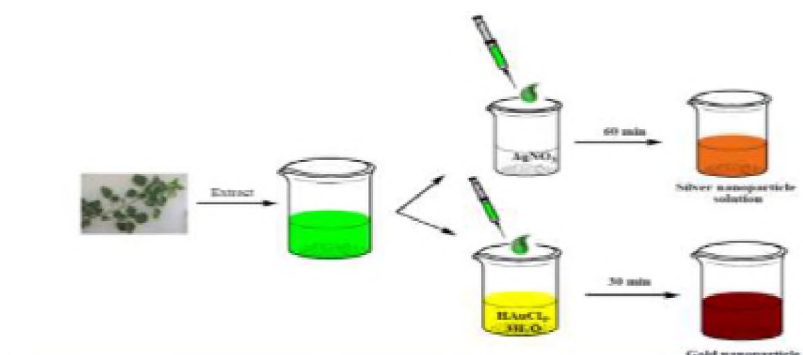
sol-gel process, aerosol pyrolysis, schimid method, thiodiglycol method and polyol process are used for the synthesis of nanoparticles (Singh *et al.*, 2015). The disadvantage of this method was, it requires strong and weak chemical reducing agents as well as capping agents like sodium borohydride, sodium citrate and alcohols. These agents are generally highly toxic, flammable, cannot be easily disposed (Ingale and Chaudhari, 2013).

2.2.3 Biological Methods

The disadvantages of physical and chemical methods can be overcome by biological methods. Green plant and microorganisms have a remarkable ability to synthesize nanoparticles. Biological synthesis of nanoparticles was proven to be eco-friendly and economical (Khan *et al.*, 2018; Sharma *et al.*, 2009). Extracts obtained from bio-organisms act as reducing and capping agents in nanoparticles synthesis. Several microorganisms extract have been utilized to grow silver nanoparticles intracellularly or extracellularly. The phytoconstituents present in plant parts interact in the synthesis of nanoparticles (Jadhav *et al.*, 2015; Lokina *et al.*, 2015). The synthesis of nanoparticles was influenced by the nature of the plant species, reaction medium, pH, temperature and reaction time (Quester *et al.*, 2013). The waste product of food like peel (Konwarh *et al.*, 2011) and seeds (Aromal and Philip, 2012) are also used for nano synthesis.

Figure 2

Biological method of nanoparticle synthesis



(Singh *et al.*, 2012)

2.3 CHARACTERIZATION OF NANOPARTICLES

A number of different techniques are used to characterize the size, shape, intensity and stability of nanoparticles.

2.3.1 Size

The size of the nanomaterial ranges from 1-100 nm. The nano-sized particles have lot more biomedical applications than the large material due to their surface volume ratio which enable particle uptake and receptor interactions (Skuland *et al.*, 2014). Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) are the two most effective techniques to determine the size of the synthesized nanoparticles. The size can also be determined by Dynamic light scattering (DLS). Size distribution can be studied by (Shang *et al.*, 2014) Atomic force microscopy (AFM).

2.3.2 Shape

The shape determines the physical characteristics of nanoparticles. SEM and TEM are commonly used to determine the shape of nanoparticles. SEM defines the morphology of the nanoparticles (Etape *et al.*, 2018). Whereas, TEM was used to determine the structural interaction and chemical characterization of nanoparticles (Lin *et al.*, 2014).

Other than these techniques, Atomic Force Microscopy (AFM), High Resolution Transmission Electron Transmission Microscopy (HRTEM) and Scanning Tunneling Microscopy (STM) are used to study the detailed structural nature of the nanomaterial (Linkov *et al.*, 2013).

2.3.3 Distribution

The distribution of the nanoparticle is based on pH, ionic strength of the solution, size and surface chemistry of the nanomaterials. Generally, nanoparticles have the tendency to aggregate and agglomerate each other. Nanoparticles synthesized from protein source usually agglomerate (Sauer *et al.*, 2015). The degree of aggregation and agglomeration is important to study, as it is important to understand the biological responses of the cell exposed to nanomaterial. The dispersity of nanoparticles at low level can be measured by DLS (Meulendijks *et al.*, 2018). Field flow fractionation (FFF) is

also used to measure the aggregation and agglomeration of nanoparticles (Baalousha and Lead, 2015).

2.3.4 Functional Groups

The FTIR technique is used to determine the chemical composition of biological samples, inorganic materials, organic chemicals and minerals. In the plant mediated synthesis of nanoparticles the functional groups present in the active phytoconstituents are responsible for nanoparticle formation (Suresh *et al.*, 2018). FTIR identifies the molecules involved in capping and responsible for efficient stabilization of nanoparticles (Devaraj *et al.*, 2013). The functional groups present in the phytoconstituents influence the size, stability and morphology of nanoparticle formation (Makarov *et al.*, 2014).

2.3.5 Surface Charge

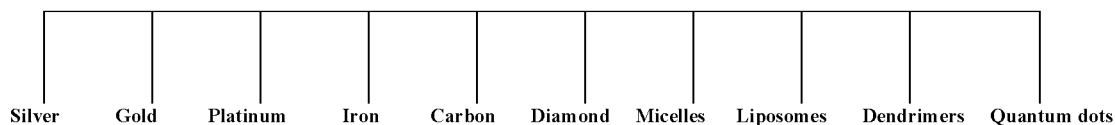
Zeta potential also known as the electrokinetic potential is used to quantify the “effective” electric charge on the surface of nanoparticle and determines the stability of colloidal nanoparticles (Bhattacharjee, 2016). The surface charge also used to determine the potential of receptor binding, aggregation of nanoparticle and physiological barrier penetration (Shaban *et al.*, 2018). The net charge on the surface of nanoparticle was screened by increased concentration of ions of opposite charge. Particles with positive zeta potential will bind to negatively charged surfaces and vice versa. The magnitude of the zeta potential provides information regarding the particle stability, higher the magnitude potentials exhibits increased electrostatic repulsion and therefore increased stability (Bonde, 2011).

2.3.6 Composition and Nature of Nanoparticles

The composition and nature of the particles was determined using X-ray diffraction analysis. It utilizes multiple beams of X-ray to create a three dimensional picture which depends on the density of electrons. The purpose is to identify the high degree certainty, composition of the molecules on an atomic scale. The most important application of X-ray powder diffraction, it is widely used for the identification of unknown crystalline materials (Biao *et al.*, 2018). Determination of unknown solids is used in the studies of geology, environmental science, material science, engineering and biology (Das *et al.*, 2010).

2.4 TYPES OF NANOPARTICLES

The different types of nanoparticles are shown below.



2.4.1 Silver Nanoparticles

Silver nanoparticles have drawn attention among scientists and researchers due to its versatility in different areas of research like medicine, physics, engineering and chemistry. In biomedical fields silver nanoparticles are widely used in diagnosis, drug delivery, molecular imaging, anti-tumor, wound healing, anti-inflammatory, antiproliferative and for the development of medical devices (Abdel- Fattah and Ali, 2018; Babu *et al.*, 2013; Santos *et al.*, 2014; Deb *et al.*, 2015; Patel *et al.*, 2015). Nanosilver based products are commercially available in the market around the world. Silver nanoparticles have commercially increased the rate of nanotechnology several fold, due to its increase value in industries (Tran *et al.*, 2013).

2.4.2 Gold Nanoparticles

Gold nanoparticles have distinct characteristic features which distinguishes from other metal nanoparticles. Gold nanoparticles are biocompatible, non-toxic and they can penetrate into the tumor through its vasculature (Aljabali *et al.*, 2018). They are fabricated with various peptides and antibodies and used in drug delivery systems (Spirou *et al.*, 2015). Gold nanoparticles synthesized from maple leaf and pine needle are used in hypothermal and microwave based tumor therapy (Krishnaswamy and Orsat; 2015).

2.4.3 Platinum Nanoparticles

Platinum nanoparticles have gained attention among researchers due to its properties like nano-catalysts, electrical conductivity, optics and nonlinear optics (Stepanov *et al.*, 2014; Gama-Lara *et al.*, 2018). Platinum based drugs are used in various treatments like coronary artery diseases, neuromodulation device and catheters. They induces apoptosis in human cervical cancer cells (Alshtawi *et al.*, 2015). Due to its better catalytic property

they are used in electrochemical sensor for the detection of ascorbic acid (Maouche *et al.*, 2015).

2.4.4 Iron Nanoparticles

Iron nanoparticles are widely used in the treatment or remediation of chlorinated organic compounds, arsenic, nitroaromatic compounds, heavy metals, cationic and anionic dyes pollutants present in the waste water and soil (Fu *et al.*, 2014; Tosco *et al.*, 2014). These nanoparticles effectively detoxify the effluents of dyeing industry, textiles, printing, paper, leather and cosmetics consisting of toxic compounds, which cannot be easily degraded, by the higher reaction rate in degrading the compounds (Nairat *et al.*, 2015). Iron nanoparticles are used as magnetic biosensors, local heat sources for cancer treatment, drug carriers, agents for magnetic resonance imaging, diagnostic assays, gene delivery systems and in gene therapy (Gutierrez *et al.*, 2014; Majidi *et al.*, 2015). The iron oxide nanoparticles designed in combination with silica and polymers have been extensively used in the theranostic applications, providing fluorescence imaging as well as drug delivery vehicle (Garcia *et al.*, 2018; Ling *et al.*, 2015).

2.4.5 Carbon

For the removal of organic and inorganic pollutants in water treatment carbon nanoparticles are extensively used due to its important properties like high adsorption, thermal stability and low cost (Bora and Dutta, 2014). Carbon nanoparticles are widely used as therapeutic and diagnostic agent for permanent bone and dental implanted prosthetics. Carbon nanotubes can perform as intracellular transporters by transporting protein, peptides, immunogenic molecules, DNA and drugs targeted to gene and target site due to their non-toxic and high fluorescence qualities (Pardo *et al.*, 2018; Heister *et al.*, 2013).

2.4.6 Diamond

Diamond nanoparticles possess anticancer activity. The surface of diamond nanoparticles possess functional hydroxyl and carboxyl groups which facilitate the anchoring of biomolecules. Diamond nanoparticles have the capacity to conjugate with anticancer drugs, nucleic acid, antibodies and peptide used for biosensing, drug delivery and other applications in biomedical field (Briones *et al.*, 2015). Diamond nanoparticles

are well known for its antimicrobial activity. Diamond nanoparticles conjugated with mint camphor showed high bactericidal activity against gram-positive and gram-negative bacteria (Turcheniuk *et al.*, 2014). Lapina *et al.* (2013) reported that diamond nanoparticles conjugated with folic acid inhibits cell proliferation in HeLa cells. They also have antiviral activity, by blocking the viral entry into the cell (Khanal *et al.*, 2013).

2.4.7 Micelles

Micelles are core-shell where the inner core is hydrophobic composed of amphiphilic diblock or triblock polymers possess space for lipophilic drugs to soluble, surrounded by hydrophilic polymer. They play an important role in targeted drug delivery system, deliver hydrophobic drugs, increased stability, less toxicity and controlled drug release (Ahmad *et al.*, 2014). To overcome the disadvantages of conventional therapy, micelles are of great importance in cancer therapy (Cabral and Kataoka, 2014).

2.4.8 Liposomes

Liposomes are synthesized from phospholipids, composed of lipid bilayers which are spherical in shape. The size ranges from 2.5nm and 25nm. They can act as both drug carriers and immunological adjuvants. Liposomes encapsulated with drugs are delivered to the target cells by endocytosis or by cell fusion (Riaz *et al.*, 2018; Ghalandarlaki *et al.*, 2014). Vaccines administered in the form of liposomes were prepared against hepatitis, influenza virus and lung carcinoma. Liposomes can also act as gene delivery vehicles (Kraft *et al.*, 2014). Liposome encapsulated with curcumin induced apoptosis in osteosarcoma and breast cancer cell lines both *in vitro* and *in vivo* (Dhule *et al.*, 2012).

2.4.9 Dendrimers

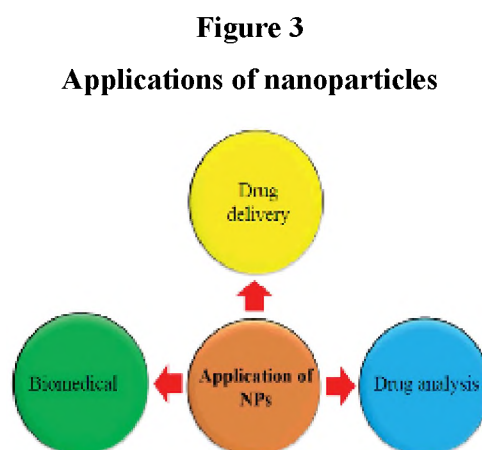
Dendrimers are high branching units, globular, nano-sized particles within 100nm. They play an important role in biomedical field as a drug carrier. Based on the functional groups and their applications in drug and gene delivery they are of different types namely chiral dendrimers, peptide dendrimers, hybrid dendrimers, core shell dendrimers, glycodendrimers and liquid crystalline dendrimers (Kesharwani *et al.*, 2014). Dendrimers are also used as an imaging agent (Sk and Kojima, 2015) for diagnostics purpose.

2.4.10 Quantum dots

Quantum dots are composed of semiconductors, which possess unique properties like tunable light emission, broad absorption spectra with simultaneous excitations of multiple fluorescence colours (Holtkemper, 2018). Quantum dots are used in drug discovery, protein tracking, disease detection, biosensing, immunohistochemical detection and selective site targets of intracellular and extracellular nature (Kairdolf *et al.*, 2013).

2.5 BIOMEDICAL POTENTIAL OF GREEN SYNTHESIZED NANOPARTICLES

Magnetic nanoparticles have gained vast interest in the field of biomedicine due to its wide applications such as molecular imaging, photothermal therapy, magnetic hyperthermia and as drug delivery vehicles. The multimode nature (Stafford *et al.*, 2018) of these nanoparticles can act as both therapeutic and diagnostic tools (theranostic agents). Silver nanoparticles are used commonly for biomedical applications such as antimicrobial coatings, wound dressings and biomedical devices (Satyavani *et al.*, 2011).



2.5.1 Biocompatibility of Nanoparticles

Biocompatibility first drew the attention of researchers between 1940 and 1980 in the context of medical implants and their interaction within the body. Recently the concept of biocompatibility was more pronounced and formally defined as **“The ability of a material to perform with an appropriate host response in a specific situation”**. The three dogma which play important role are that a material has to perform its intended

functions and not merely be presented in the tissue. The induced reaction has to be proper for the intended application and the nature of the reaction to a particular material and its suitability may be different from one context to another (Naahidi *et al.*, 2013).

In 2010, Kohane and Langer explained biocompatibility in the context of drug delivery and defined biocompatibility as **“an expression of the benignity of the relation between a material and its biological environment”**. However, some researchers have expanded the definition of biocompatibility by denoting acceptable functionality of a biomaterial. High degree of biocompatibility is achieved when a material interacts with the body without inducing unacceptable toxic, immunogenic, thrombogenic, and carcinogenic responses (Dobrovolskaia and McNeil, 2007, Mel *et al.*, 2012).

When nanoparticles interact with the body, a variety of responses may occur. These include alterations in the immune system or interaction with blood. These reactions vary significantly with nanoparticle composition (Engin and Hayes, 2018). For example, gold nanostructures may interact differently with the body when compared to polymeric particles. For this reason, nanoparticles have to be evaluated individually or “on a case-by-case basis” to understand their effect on the body in a better way (Kononenko *et al.*, 2015).

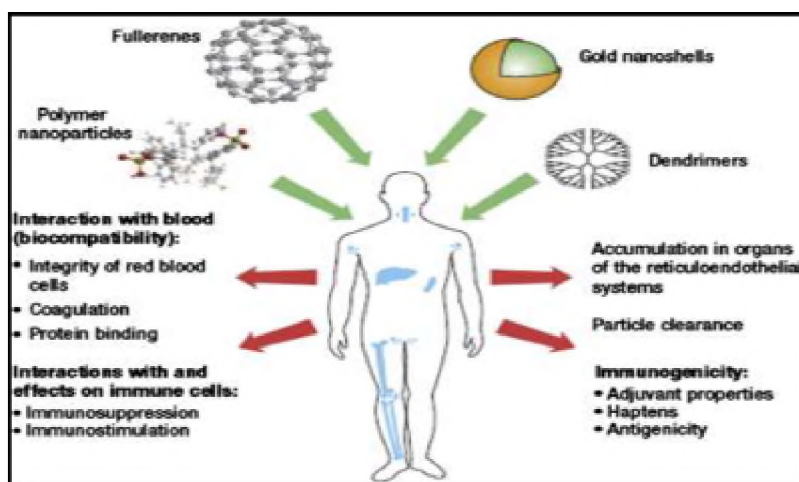
To establish a safe nanotechnology it was necessary to study the non genotoxic nature of the nanomaterials. Several genotoxicity assays were carried out under *in vitro* condition. *In vitro* cytotoxicity studies of nanoparticles using different cell lines, with different nanomaterials are increasingly studied. The techniques that can be used to assess toxicity of nanomaterials include:

- (1) *In vitro* assays for cell viability/proliferation using MTT, LDH assay and mechanistic assays [ROS generation, apoptosis, necrosis and DNA damaging potential] using ROS assay
- (2) Microscopic evaluation of intracellular localization using SEM-EDS, TEM
- (3) Gene expression analysis, high-throughput systems
- (4) *In vitro* hemolysis and
- (5) Genotoxicity

The biocompatibility study of nanoparticles were carried out in pulmonary, erythrocytes and endothelial cells for cardiovascular disease, ovarian cancer, in animals spleen injury, lung inflammation, mouse embryonic fibroblasts, human monocytes, human spermatozoa, murine glioma cells and ocular use (Vadlapudi *et al.*, 2014).

Figure 4

Biocompatibility of nanoparticles



(Naahidi *et al.*, 2013)

2.5.2 Drug Releasing Profile of Nanoparticles

Nanotechnology has been utilized in medical field for therapeutic drug delivery system and the development of treatments for a variety of diseases and disorders. So, there are very significant advances in these disciplines. In the early 1970s, controlled drug delivery systems aimed to deliver drugs at predetermined rates and predefined periods of time, have drawn increasing attention (Qiu and Park, 2001).

On the other hand, drug delivery is an emerging field focused on targeting drugs or genes to a desirable group of cells. The ultimate goal of this targeted delivery is to transport proper amounts of drugs to the desirable sites such as tumors and diseased tissues. While minimizing unwanted side effects of the drugs on other tissues (Tran *et al.*, 2009).

Recently numerous proteomic and drug design profile have emerged to target various cellular processes, creating a demand for the development of intelligent drug

delivery systems that can respond directly to pathophysiological conditions. Micro and nano scale intelligent systems can maximize the efficacy of therapeutic treatments in numerous ways because they have the ability to rapidly detect and respond to disease states directly at the target site, sparing physiologically healthy cells and tissues and thereby improving a patient's quality of life. This new class are designed to perform various functions like detection and release of therapeutic agents for the treatment of diseased conditions. Stimuli responsive biomaterials are very promising carriers for the development of advanced intelligent therapeutics (Moore and Peppas, 2009).

Polymeric nanoparticles are biodegradable/ biostable and copolymers. The drug molecules can be entrapped or encapsulated within the particle, physically adsorbed on the surface or chemically linked to the surface of the particle (Parveen *et al.*, 2012).

2.5.3 Nanoparticles as Free Radical Scavengers

Rapid synthesis of metallic nanoparticles with appropriate morphology and sizes have drawn the attention of researchers due to its evocative therapeutic applications such as antioxidant, antibacterial, anticancer, larvicidal, catalytic and wound healing activities (Fridhouse and Lalitha, 2015). The *in vitro* antioxidant activity of the nanoparticles was determined by their efficiency to scavenge free radicals. The silver nanoparticles produced from the aqueous extract of *A. marschalliana* showed a potent *in vitro* antioxidant activity by scavenging 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay (Salehi *et al.*, 2016). Nagaich *et al.* (2016) reported that the hydrogels loaded silver nanoparticles synthesized from the flavonoids of apple extract exhibited high radical scavenging activity by DPPH assay with the per cent radical inhibition of 75.16 per cent \pm 0.04. The gold and silver nanoparticles synthesized from the aqueous extract of *Solanum torvum* fruit served as strong radical quenching by effectively scavenging hydroxyl, superoxide, nitric oxide and DPPH radicals (Ramamurthy *et al.*, 2013).

2.5.4 Nanoparticles as Antioxidants

Antioxidants are substances that inhibit oxidative damage by scavenging free radicals and protect the human body. Imbalance between antioxidants and free radicals leads to oxidative stress which results in cellular damage (Ghasemzadeh *et al.*, 2012). Our body naturally produces free radicals as a by- product when cells utilize oxygen, the

produced free radicals has the tendency to cause damage. Antioxidants has the capacity to scavenge free radicals and protects the humans against heart disease, muscular degeneration, diabetes mellitus, cancer and aging are all contributed by oxidative damage. Nanoparticles as antioxidants counteract the oxygen levels and ROS production (Elswaifi *et al.*, 2009). Sriramulu and Sumathi (2017) reported that the silver nanoparticles synthesized from mushroom explored potent *in vitro* antioxidant, anti-inflammatory and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Direct delivery of antioxidant enzymes to cells is highly impossible. To overcome these difficulties design of nanoparticles to deliver enzymes is important for therapeutic purposes. Lin *et al.* (2016) resulted that mesoporus silica nanoparticles fused with transactivator of transcription (TAT) peptide simultaneously delivered two upstream antioxidant enzymes such as SOD and GPx which exerted synergistic effect to cell by scavenging ROS.

2.5.5 Nanoparticles as Anti Lipid Peroxidative Agent

Lipid peroxidation (LPO) is an autocatalytic process causes cell death. The reaction is initiated by the decomposition of lipid peroxides which produce reactive carbonyl compounds. The by-products of lipid peroxidation are MDA and 4-hydroxy nonenal (HNE) involved in cataractogenesis, mainly due to its cross linking ability. A free radical attack on cell membrane PUFA initiates the autocatalytic process which generates large amount of toxic radical products which initiates tumor initiation and promotion of colorectal cancer (Bhagat *et al.*, 2011). Green synthesized phytonanoparticles acts as a good antilipid peroxidative agent. Pathak *et al.*(2015) reported that nanocarriers formed by the assembly of two oppositively charged lipid and polymer for curcumin acted as an antilipid peroxidative agent determined by *in vitro* TBARS assay the encapsulated had a great potential as functional food ingredient than the un encapsulated form.

2.5.6 Nanoparticles as Apoptotic Agents

Nanomedicine has revolutionized this modern era, by designing personalized drugs for cancer treatments by providing a clear-cut solution faced by the current

systemic therapeutic drugs. Combinations of drugs and nanoparticles increases the synergetic approach which provides greater therapeutic effects than does single drug treatment (Mignani *et al.*, 2015). Nanoparticles combined with different anticancer drugs is an advanced therapeutic strategy. Nanoparticle mediated targeted drug delivery systems permits the drug to accumulate in tumors, by either active or passive targeting and increases the intracellular drug concentration and substantially enhances the cytotoxic effect of various antitumor agents (Cho *et al.*, 2008). Plant kingdom produces a vast naturally occurring secondary metabolites, nano-medicine enhances the anticancer activities of the plant derived drugs by converting them to nano form. Nanoparticles synthesized by plant mediated source typically enhances the apoptotic activity by its targeted controlled release of the drug (Green well and Rahman, 2015). The biosynthesized silver nanoparticles provides a better therapeutic frame work as apoptotic agent against a cluster of cells in the initial stages of cancer by overwhelming the biological barriers (Shargh *et al.*, 2016). Biogenic silver nanoparticles became a potential cancer therapeutic, diagnostic and apoptotic agent in the current era (Khan *et al.*, 2017).

2.5.7 Nanoparticles in DNA Fragmentation

Apoptotic cell death is characterized by certain morphological and biochemical changes which distinguish from other forms of cell death. DNA fragmentation is an important feature of apoptosis (Elshawy *et al.*, 2016). Caspase-Activated DNase (CAD) is the enzyme responsible for DNA fragmentation. This enzyme cleaves DNA at inter nucleosomal linker sites between nucleosomes. Degradation of nuclear DNA into nucleosomal units and formation of DNA fragments is one of the hallmarks of apoptotic cell death (Wlodkowic *et al.*, 2011). A conventional agarose gel electrophoresis was commonly used to analyze fragmented nuclei in cells (Matassov *et al.*, 2004). Biogenic silver nanoparticles showed effective cytotoxicity and antiproliferative effect than chemically synthesized silver nanoparticles. The induction of apoptosis by DNA fragmentation was found to be highly effective in the synthesized silver nanoparticles against K562 leukemia cell line (Datkhile *et al.*, 2017).

2.5.8 Nanoparticles in Cell Cycle Regulation

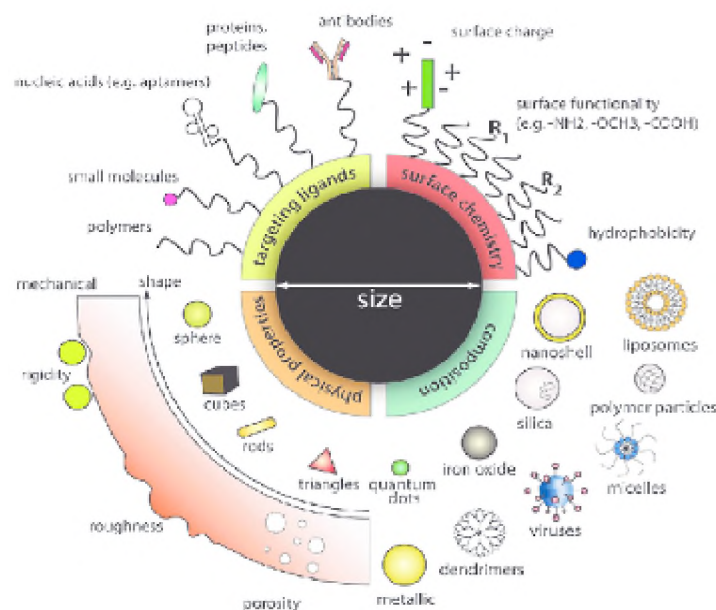
Cell cycle analysis was studied by flow cytometry measurement. Flow cytometry is a laser based instrument used in cell counting, cell sorting, biomarker detection and protein engineering. In flow cytometer the cells are suspended in a stream of fluid which passes through an electronic detection apparatus (Basiji *et al.*, 2007). There are four widely used methods to analyze cell cycle by flow cytometry. The first two methods are based on analysis of cellular DNA content followed by staining the cells with either propidium iodide (PI) or 4', 6'-diamidino-2-phenylindole (DAPI) based on univariate analysis. These methods expose the distribution of cells in three phases of the cell cycle (G_1 vs S vs G_2/M) which enables to detect apoptotic cells with fractional DNA content. The third method is based on analysis of DNA content and proteins associated with proliferation. This method enables to distinguish cells, in different phases of cell cycle to identify mitotic cells or intracellular expression of proteins to the cell cycle position. The fourth method depends on the detection of 5'-bromo-2'-deoxyuridine (BrdU) labelled on the DNA replicating cells (Romar *et al.*, 2016; Pozarowski and Darzynkiewicz, 2004).

2.5.9 Nanoparticles as Antitumorigenic Agents

One of the important challenge in the field of Oncology was to find a technology for the controlled targeted release of the drug and eradicate tumor cells by sparing normal cells (Lee *et al.*, 2012). In this context, biodegradable nanometer sized particles gained great interests among pharmaceuticals due to its novel structural and physical properties for targeted delivery of anticancer agents and imaging contrast agents (Zhu *et al.*, 2015). Nanocarriers are used to overcome the side effects associated with conventional antitumor drugs such as their non-specificity, burst release of the drug and damaging the normal cells. Moreover, nanocarriers improve the bioavailability and increase the therapeutic efficiency of the antitumor drugs at the target site by preferential accumulation (Din *et al.*, 2017). Nanoparticles synthesized from medicinal plants targeting biological pathways has tremendous advantages due to its higher efficacy and fewer side effects compared to commercial cancer drugs (Visweswara Rao *et al.*, 2016).

Figure 5

Design of nanoparticle based on drug delivery platform

Chou *et al.*, 2011

2.6 PLANT SELECTED FOR THE PRESENT STUDY

Traditionally many plants have been used for curing various diseases, among which the leaves of *Volkameria inermis* have been used in the present study.

2.6.1 *Volkameria inermis* (L.)

Volkameria inermis (L.) have been used traditionally in the treatment of scrofulous and venereal infections. The fresh leaf juice was used externally for treating skin diseases. The roots are boiled with oil and used in rheumatic affections (Snafi, 2016). *Volkameria inermis* is mainly found in India, Nepal, Bangladesh, Sri Lanka, Southeast Asia and Mediterranean. It was introduced in all tropical and inter tropical countries of the world. Commonly it was known as Seaside clerodendrum, Wild jasmine, and Sorcerers bush in Tamil it was known as Sangam (Mokini *et al.*, 2013).

Nomenclature of *Volkameria inermis*

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclass	: Asteridae
Order	: Lamiales
Family	: Verbenaceae
Genus	: <i>Clerodendrum</i>
Species	: <i>Volkameria inermis</i>

The experimental procedures adapted for the research were presented in the succeeding chapter.