A DISSERTATION ON

"BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES *BY CANNABIS SATIVA* AND EVALUATION OF THEIR CYTOTOXIC EFFECT ON CERVICAL CANCER HELA CELLS"

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTERS OF SCIENCE
IN BIOTECHNOLOGY

BY

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M.Sc. BIOTECHNOLOGY (IVth SEMESTER)

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. SADAF SIDDIQUE** student of M.Sc. Biotechnology, (IV semester), Integral University has completed her four months dissertation work entitled ***"Biogenic synthesis of silver nanoparticles by Cannabis sativa and evaluation of their cytotoxic effect on cervical cancer HeLa cells"*** successfully. She has completed this work from the Department of Biosciences, Integral University, under the supervision of **DR. IRFAN AHMAD ANSARI**. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

Dr. Snober S.Mir

Head Of Department

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. SADAF SIDDIQUE** during the months February – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the script of thethesis has been written by the candidate herself. The thesis entitled is ***"Biogenic synthesis of silver nanoparticles by Cannabis sativa and evaluation of their cytotoxic effect on cervical cancer HeLa cells"*** therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow.

SUPERVISOR

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SADAF SIDDIQUE

M.Sc. Biotechnology

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ABBREVIATIONS

%	Percentage
M	Molar solution
mM	milli Molar
CNTs	Carbon nano tubes
QDs	Quantum dots
DLS	Dynamic Light scattering
OD	Optical density
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
UV-Vis	Ultraviolet visible spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-ray Diffraction
Nm	Nano meter
MIC	Minimum Inhibitory Concentration
AgNPs	Silver nanoparticles
NPs	Nanoparticles
CNB	*Cannabis sativa*

INTRODUCTION

INTRODUCTION

Over the last three decades, research and development in the field of nanotechnology has increased. At the same time, the use of nanoparticles in drug development reduces the use of additional components in the formulation to protect therapeutics from degradation and increase circulation time. The unique physicochemical properties and nano scale effects have sparked interest in nanoparticles as drug delivery systems for the treatment of diseases such as cancer, cardiovascular diseases, pathogenic infections, and diabetes. Despite the increased interest in developing nanoparticles, not as many have been approved for therapeutic use (Wang *et al*., 2017). Nanoparticles with dimensions of 20-100 nm are gaining great recognition due to their amazing properties (de Kozak *et al* ., 2004). In recent years, noble metal nanoparticles have gained great importance in the discipline of life sciences due to their wide range of applications. Metal nanoparticles are produced by various chemical and physical processes. But such processes can use toxic chemicals and harm the environment.

Recent advances in nanoscience and nanotechnology have radically changed the way we diagnose, treat and prevent various diseases in all aspects of human life. Presently, different metallic nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver (Dubchak *et al* ., 2010). Nanoparticles are being used for diverse purposes, from medical treatments, using in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes. Silver nanoparticles (AgNPs) are one of the most important and fascinating nanomaterials among several metallic nanoparticles involved in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, especially in nanomedicine. Although several noble metals have been used for different purposes, AgNPs have focused on potential applications in cancer diagnosis and therapy.

Silver nanoparticles (AgNPs) are increasingly used in various fields including medical, food, healthcare, consumer and industrial purposes due to their unique physical and chemical properties. These include optical, electrical and thermal, high electrical conductivity and biological properties (Liu, *et al* ., 2016a). Due to their special properties,

they have been used for various applications, including as antibacterial agents, in industrial, household and healthcare products, consumer products, coatings for medical devices, optical sensors and cosmetics, in the pharmaceutical and food industries, in diagnostics, orthopedics, drug delivery, as anticancer agents and ultimately have enhanced the tumoricidal effects of anticancer agents (Gurunathan, *et al*.,2015). Silver nanoparticles have been shown to be the most effective against bacteria, viruses and other eukaryotic microorganisms due to their good antimicrobial activity. They are undoubtedly the most widely used nanomaterials and are therefore used as antimicrobial agents, in the textile industry, for water treatment, as sunscreen lotions, etc. Studies have already reported the successful biosynthesis of silver nanoparticles by plants such as Azadirachta indica, Capsicum annuum and Carica papaya (Jha & Prasad, 2010).

Using whole plant parts for nanoparticle synthesis offers exciting and potential advantages with a large investigative capacity. Silver is one of the most commonly used metals due to its inhibitory effect on bacteria and microorganisms by producing reactive oxygen species and thereby damaging cell organelles. Silver nanoparticles are considered a new and likely replacement for standard antibiotics, as these pathogenic bacteria exhibit increased multidrug resistance properties. The biosynthesis of silver nanoparticles using the abundant and sustainable plant resources is suitable to meet the great demand for biocompatible and eco-friendly synthesized nanoparticles, especially for applications in biomedical and environmental fields.

The biological synthesis of AgNPs is an alternative to overcome the toxicity problems generally associated with the synthesis of metal nanoparticles synthesized by various chemical and physical methods. Therefore, an alternative to this problem is the biological production of AgNPs. Recently, the biological or green synthesis approach of nanoparticles has gained more focus than the physical and chemical production processes because this approach is non-toxic, clean and environmentally friendly. Green synthesis of nanoparticles involves the production of nanoparticles by designing and advancing the production process using renewable materials, non-hazardous and non-toxic solvents and well-disposed reaction media. Therefore, the green synthesis of nanoparticles is emerging as a leading area of nanotechnology; whereas using biological

agents such as microorganisms, plant biomass or plant extract to synthesize nanoparticles might be a better option than chemical and physical methods in an environmentally friendly way. AgNPs have found applicability in various research fields as they possess catalytic properties and antimicrobial potential, antibacterial, anticancer, and many different researches have considered AgNPs as a precursor reagent for the fabrication of biomaterial synthesis with different applications AgNPs possess new and improved properties based on their morphology, distribution and size (Chouhan & Guleria, 2020).

Cannabis sativa L. is a small genus belonging to the order Urticales and the family Cannabaceae or Cannabinaceae. It is considered one of the oldest plants and its association with humans has existed since the discovery of agriculture in the Old World, probably ten thousand years ago. It is commonly called bhang, but in different parts of the world and in India it is known by many other names (e.g. Bhang, Ganja, Charas, Bhanga, Hursini, Kinnab, Darkte-bang, Darakhte-Kinnab, Hashish, Kif, Maryoane, Pot, Acapulco, Gold, Marijuana, Marijuana, Dagga, Vijaya, Subjee and Sidhee etc). In English is it commonly known as hemp. It was also mentioned as an important medicinal plant in our ancient and it is still used in both the Ayurvedic and Unani systems of indigenous medicine. Plants of the genus *Cannabis* are found in the northern hemisphere and produce more than 400 known secondary metabolites; more than 60 of these are cannabinoid compounds. The genus has two main subspecies: *C. sativa* and *Cannabis indica. C. sativa* is the tallest with pale green, thin leaves and is one of the fastest growing plants. It is very popular for industrial use due to its low but variable lignin content and enrichment with bast fibers. This makes the plant fibers suitable for the production of textiles, paper, rope, biofuels, biodegradable plastics, insulation, paint and animal feed. *C. sativa* plants have higher psychoactive tetrahydrocannabinol (THC) levels compared to *C. indica*. In recent decades, the plant has been increasingly used for medicinal treatments against various diseases such as inflammation, cancer, obesity, osteoporosis, multiple sclerosis, vomiting, epilepsy, pain, glaucoma, anorexia, etc. To treat various neurodegenerative diseases (such as Tourette's syndrome, Huntington's disease, Alzheimer's disease and Parkinson's disease). Cannabinoids, the active components of *Cannabis sativa*, mimic the effects of endogenous cannabinoids (endocannabinoids) and

activate specific cannabinoid receptors, specifically CB1, which is predominantly found in the central nervous system, and CB2, which is predominantly found in cells involved in immune function. Delta-9-tetrahydrocannabinol, the main bioactive cannabinoid in the plant, is available as a prescription drug approved for the treatment of chemotherapy-induced nausea and vomiting associated with cancer and anorexia associated with AIDS Wasting Syndrome. Cannabinoid also useful in the management of cancer-related pain, potentially synergistically with opioid analgesics. Cannabinoids have been shown to be beneficial in the treatment of HIV-related peripheral neuropathy, suggesting they should be explored in patients with other neuropathic symptoms. Cannabinoids have a favorable drug safety profile, but their medicinal uses are mainly limited by their psychoactive effects and limited bioavailability. *Cannabis sativa* (hemp) is a source of various biologically active compounds, for instance, cannabinoids, terpenes and phenolic compounds, which exhibit antibacterial, antifungal, anti-inflammatory and anticancer properties. With the purpose of expanding the auxiliary application of *C. sativa* in the field of bio-nanotechnology, we explored the plant for green and efficient synthesis of gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), and zinc nanoparticles ZnNPs) . In addition, cannabinoids exhibit anticancer effects in multiple models by suppressing cancer cell proliferation, migration and/or invasion and tumor angiogenesis. In addition to cannabinoids, cannabis contains several other compounds that have also been shown to have antitumor effects

Cancer is a major public health problem worldwide. Global demographics predict rising cancer incidence over the next few decades, with more than 20 million new cancer cases expected annually through 2025. According to GLOBOCAN data, 14.1 million new cases and 8.2 million deaths from cancer were estimated in 2012. Cancer of the female breast, colon, prostate and lung are the most commonly diagnosed cancers in Europe. Lung cancer remains the leading cause of cancer incidence and mortality worldwide (Zugazagoitia *et al*., 2016). Cancer is a complex, multifactorial disease that has the characteristic feature of uncontrolled growth and spread of abnormal cells caused by multiple factors, including a combination of genetic, external, internal and environmental factors, and is treated by various treatments, including chemotherapy, hormone therapy, surgery, radiation, immunotherapy and targeted therapy (Society, 2014). Therefore, the

challenge is to identify effective, inexpensive, and sensitive lead molecules that exhibit cell-directed specificity and increase sensitivity. Recently, AgNPs have received much interest due to their therapeutic applications in cancer as anticancer agents, in diagnostics, and in probing. Considering the literature, in this review we focused on recent developments in the synthesis, characterization, properties, and bio applications, mainly on the antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and antiangiogenic properties of AgNPs in a single platform. We emphasize the mechanism of anticancer activity, therapeutic approaches, and the challenges and limitations of nanoparticles in cancer therapy.

Due to excellent potential properties of physical, chemical, and biological, AgNPs are known to exhibit antibacterial, antiviral, anti-inflammatory, anticancer, and antiangiogenic activity. AgNPs are able to modulate the PGP activity and able to enhance chemotherapeutic efficacy in multidrug resistant cancer cells, and also it seems to be potential combinational partners with the anticancer drug. Furthermore, the mechanism of toxicity of apoptosis induced by AgNPs is well established in a variety of cell lines such as human lung cancer cells, ovarian, and breast cancer. Therefore, AgNPs are the best, suitable, and alternative nanoparticles working with any anticancer drug. AgNPs are known to exhibit anticancer activity and DNA topoisomerase I inhibitor such as CPT analogs exhibit toxic effects against cancer cells by inducing the activation of apoptotic caspases and formation of ROS and ultimately increases anticancer activity. To explore the possibility of the combination effect of CPT and AgNPs in HeLa cells, initially, the cells were treated with various concentrations of CPT and AgNPs; the results depicted that both CPT and AgNPs induce cell death dose-dependently. The combination of CPT and AgNPs significantly inhibits cell proliferation and increases cytotoxicity and apoptosis by increasing ROS generation and leakage of LDH and also altering mitochondrial membrane potential and activation of caspase 9, 6, and 3 (Yuan *et al* ., 2018).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Nanobiotechnology

Nanotechnology has achieved status as one of the pivotal research efforts of the early 21st century as scientists take advantage of the unique properties of atomic and molecular assemblies built at the nanometer scale. Our ability to manipulate the physical, chemical and biological properties of these particles offers researchers the opportunity to rationally design and use nanoparticles for drug delivery, imaging contrast agents and diagnostic purposes (ullah *et al*., 2017a). While biotechnology deals with metabolic and other physiological processes of biological subjects including microorganisms, in combination with nanotechnology, nanobiotechnology can play a vital role in developing and implementing many useful tools in the study of life. Although the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, therapy, and drug delivery vehicles, bio-nanotechnology research is still in its infancy (Niemeyer & Mirkin, 2004). Nanobiotechnology is the application of nanotechnology in biological fields. Nanotechnology is a multidisciplinary field that currently recruits approach, technology and facility available in conventional as well as advanced avenues of engineering, physics, chemistry and biology (Fakruddin *et al*., 2012a). The prefix ‘nano’ is referred to as a Greek prefix meaning ‘dwarf’ or something very small and depicts one thousand millionths of a meter (10−9 m). We should distinguish between nanoscience and nanotechnology. Nanoscience is the study of structures and molecules on the scales of nanometers ranging between 1 and 100 nm, and the technology that utilizes it in practical applications such as devices, etc. is called nanotechnology. Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to transform the theory of nanoscience into useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale nanotechnology (Bayda *et al*., 2019)

The National Nanotechnology Initiative (NNI) in the United States defines nanotechnology as a science, engineering, and technology performed at the nano-scale (1 to 100 nm), where unique phenomena enable novel applications in a variety of fields,

from chemistry, physics, and Biology, medicine, technology and electronics. This definition suggests the existence of two conditions for nanotechnology. The first is a matter of scale: nanotechnology deals with the exploitation of structures by controlling their shape and size at the nanometer scale. The second problem has to do with novelty: nanotechnology has to deal with small things in such a way that some properties due to the nano-scale are exploited (Allhoff, 2007).

Recently, the British Standards Institution (Jeevanandam *et al*., 2018a) proposed the following definitions for the scientific terms that have been used:

- Nano-scale: Approximately 1 to 1000 nm size range.
- Nanoscience: The science and study of matter at the nano-scale that deals with understanding their size and structure-dependent properties and compares the emergence of individual atoms or molecules or bulk material related differences.
- Nanotechnology: Manipulation and control of matter on the nano-scale dimension by using scientific knowledge of various industrial and biomedical applications.
- Nanomaterial: Material with any internal or external structures on the nano-scale dimension.
- Nano-object: Material that possesses one or more peripheral nano-scale dimensions.
- Nanoparticle: Nano-object with three external nano-scale dimensions. The terms nanorod or nanoplate are employed, instead of nanoparticle (NP) when the longest and the shortest axes lengths of a nano-object are different.
- Nanofiber: When two similar exterior nano-scale dimensions and a third larger dimension are present in a nanomaterial, it is referred to as nanofiber.
- Nanocomposite: Multiphase structure with at least one phase on the nano-scale dimension.
- Nanostructure: Composition of interconnected constituent parts in the nano-scale region.
- Nanostructured materials: Materials containing internal or surface nanostructure.

The field of nanoparticles (NPs) is one of the pathways to nanotechnology associated with nano-scale materials with very small particle sizes ranging from 1 to 100 nm. NPs

exhibit distinctive properties due to their extremely small size and high surface area-to-volume ratio, which is attributed to the significant differences in properties from their bulk counterparts (Singh *et al*., 2011).

Advantages of nanobiotechnology

The pathophysiological conditions and anatomical changes of diseased or inflamed tissues can potentially trigger many scopes for the development of various targeted nanotechnology products (Fakruddin *et al*., 2012b).

1. Drug targeting can be achieved by taking advantage of the distinct pathophysiological features of diseased tissues;
2. Various nanoproducts can be accumulated at higher concentrations than normal drugs);
3. increased vascular permeability coupled with an impaired lymphatic drainage in tumors improve the effect of the nano-systems in the tumors or inflamed tissues through better transmission and retention;
4. Nano-systems have capacity of selective localization in inflamed tissues.
5. Nanoparticles can be effectively used to deliver/transport relevant drugs to the brain overcoming the presence of blood–brain barrier (meninges);
6. Drug loading onto nanoparticles modifies cell and tissue distribution and leads to a more selective delivery of biologically active compounds to enhance drug efficacy and reduces drug toxicity.

Nanoparticles

Nanoparticles are materials with overall dimensions in the nano-scale, i.e. below 100 nm. In recent years, these materials have become important players in modern medicine, with applications ranging from contrast agents in medical imaging to carriers for gene delivery into single cells. Nanoparticles have a number of properties that distinguish them from bulk materials simply because of their size, such as chemical reactivity, energy absorption, and biological mobility (de Kozak *et al* ., 2004). Nanoparticles are also referred to as zero-dimensional nanomaterials. This definition stems from the fact that all

of their dimensions are at the nano-scale, in contrast to one-dimensional nanomaterials, which have one dimension larger than the nano-scale (such as nanowires and nanotubes), and two-dimensional nanomaterials, which have two dimensions larger than the nano-scale (like self-assembled monolayer films) (Khan *et al*., 2019).

The word nano derives from nanos, which is a Greek word meaning extremely dwarf. Different types of nanoparticles based on morphology and physical/chemical properties are carbon-based nanoparticles, polymer nanoparticles, metal nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, lipid-based nanoparticles, etc. (Jeevanandam *et al*., 2018b).

NPs themselves are not simple molecules and therefore consist of three layers, i.e. (a) the surface layer, which can be functionalized with a variety of small molecules, metal ions, surfactants, and polymers. (b) The shell layer, which is chemically distinct from the core in all aspects, and (c) The core, which is essentially the central part of the NP and usually refers to the NP itself (Shin *et al* ., 2016). Owing to such exceptional characteristics, these materials got immense interest of researchers in multidisciplinary fields. Scanning electron microscopy (SEM) and transmittance electron microscope (TEM) images of mesoporous and nonporous methacrylate-functionalized silica (MA-SiO_2). Mesoporous imparts additional characteristics in NPs. The NPs can be employed for drug delivery, chemical and biological sensing, gas sensing, CO_2 capturing (Ganesh *et al* ., 2017) etc.

Types of nanoparticles

NPs are broadly classified into different categories based on their morphology, size, and chemical properties. Based on physical and chemical properties, some of the well-known classes of NPs are given as follows.

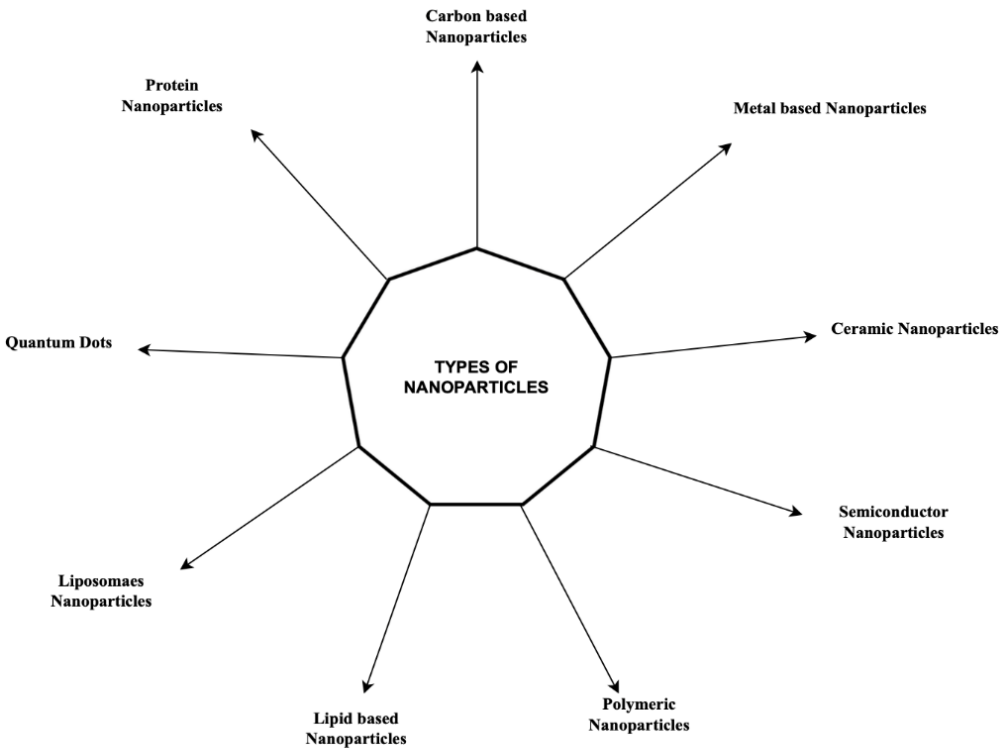

Figure 1: Types of Nanoparticles

Carbon based nanoparticles

Fullerenes and carbon nanotubes (CNTs) represent two major classes of carbon-based NPs. Fullerenes contain nanomaterials composed of spherical hollow cages like allotropic forms of carbon. They have attracted remarkable commercial interest due to their electrical conductivity, high strength, structure, electron affinity, and versatility (Ibrahim, 2013). These materials have ordered pentagonal and hexagonal carbon units, with each carbon being sp2 hybridized. CNTs are elongated, tubular structures with a diameter of 1-2 nm (Aqel *et al* ., 2012). These can be predicted to be metallic or semiconducting depending on their diameter telicity. These structurally resemble sheets of graphite rolling on themselves. The rolled sheets can be single-, double- or multi-walled and are therefore referred to as single-wall (SWNTs), double-wall (DWNTs) or multi-wall carbon nanotubes (MWNTs). They are widely synthesized by the deposition of carbon precursors, particularly an atomic carbon, which are vaporized from graphite onto metal particles by laser or by electric arcs. Recently, they have been synthesized by chemical vapor

deposition (CVD) (Saeed & Khan, 2016). Due to their unique physical, chemical and mechanical properties, these materials are used not only in pure form but also in nanocomposites for many commercial applications such as fillers, efficient gas adsorbents for environmental remediation and as a support medium for various inorganic and organic catalysts (Dreaden *et al*., 2012).

Metal based nanoparticles

Metal-based nanoparticles are the most popular inorganic nanoparticles and represent a promising solution against resistance to traditional antibiotics. Not only do they use mechanisms of action that are completely different from those described for traditional antibiotics, and they show activity against bacteria that are already developing resistance but they also target multiple biomolecules that affect the development of resistant strains. Metal-based nanoparticles can be characterized by numerous techniques. These methods provide valuable information about their morphology, physicochemical and electrical properties, which are crucial for their in vivo activity. The most relevant properties of nanoparticles include aspects such as size, shape, roughness and surface energy (Wang *et al*., 2017). Metal NPs consist exclusively of the metal precursors. Owing to the known properties of localized surface plasmon resonance (LSPR), these NPs possess unique optoelectrical properties.

Ceramic nanoparticles

Ceramic NPs are inorganic nonmetallic solids that are synthesized by heating and then cooling. They come in amorphous, polycrystalline, dense, porous or hollow. Therefore, these NPs are receiving much attention from researchers due to their use in applications such as catalysis, photocatalysis, photodegradation of dyes, and imaging applications. They are able to transport molecules such as proteins, enzymes or drugs without swelling or affecting their porosity due to external influences of pH or temperature. The components most commonly used in ceramic nanoparticles are silica and aluminum. However, the core of these nanoparticles is not limited to these two materials; in fact, they can consist of a combination of metallic and non-metallic materials (Singh *et al*., 2016).

The controlled release of drugs is one of the most exploited areas in terms of ceramic nanoparticle application in biomedicine. In this field, the dose and size are important. Also, some features that make nanoparticles a potential tool in controlling drug delivery are high stability, high load capacity, easily incorporation into hydrophobic and hydrophilic systems, and different routes of administration (oral, inhalation, etc.). In addition, a variety of organic groups which may be functionalized on its surfaces allow for a directed effect (Ali *et al*., 2017).

Semiconductor nanoparticles

Semiconductor materials possess properties intermediate between metals and nonmetals and thus found diverse applications in the literature due to this property. Semiconductor NPs have large band gaps and therefore showed a significant change in their properties with band gap tuning. Therefore, they are very important materials in photocatalysis, photo optics and electronic devices (Hisatomi *et al*., 2014). For example, various semiconductor NPs have proven exceptionally efficient in water splitting applications due to their appropriate band gap and band edge positions (Mansha *et al* ., 2017).

Polymeric nanoparticles

These are normally organic based NPs and in literature a special term polymer nanoparticle (PNP) collective used for it. They are mostly nanospheres or nano capsular shaped. The former are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface. In the latter case the solid mass is encapsulated within the particle completely. The PNPs are readily functionalize and thus find bundles of applications in literature (Abd Ellah & Abouelmagd, 2017).

lipid based nanoparticles

These NPs contain lipid moieties and effectively using in many biomedical applications. Generally, a lipid NP is characteristically spherical with diameter ranging from 10 and

1000 nm. Like polymeric NPs, lipid NPs possess a solid core made of lipid and a matrix contain soluble lipophilic molecules. Surfactants or emulsifiers stabilized the external core of these NPs. Lipid nanotechnology is a special field, which focus the designing and synthesis of lipid NPs for various applications like drug carriers and delivery and RNA release in cancer therapy (Gujrati *et al*., 2014).

Liposomes nanoparticles

Liposomes are vesicles synthesized through the hydration of dry phospholipids. They can be produced in different structures, compositions, sizes and flexibility with a large number of lipid molecules and further surface modifications. One of the key advantages of liposomes is their ability to fuse with the cell membrane and release their contents into the cytoplasm, making them suitable smart carrier systems for targeted delivery. The simplest liposome consists of a lipid bilayer surrounding a hollow core 50-1000 nm in diameter. The therapeutic molecules can be loaded into this hollow core for delivery (Emilian Leucuta, 2010). Depending on the number of bilayers, they are classified into three basic types: multilamellar, small unilamellar, and large unilamellar. The neutrally or positively charged small liposomes have a longer circulation time compared to large, unmodified liposomes. In addition, surface modifications can be obtained by coating them with either a functionalized polymer or PEG chains that improve targeting and increase their circulation time in biological systems. The liposomes are being studied for a variety of therapeutic applications such as: B. cancer diagnostics and therapy, vaccines, brain-directed drug delivery and antimicrobial therapy (Sercombe *et al*., 2015).

Quantum dots

Quantum dots (QDs) are tiny particles or nanocrystals of a semiconducting material with diameters in the range of 2-10 nm. These particles consist of a semiconductor inorganic core such as CdSe and an aqueous organic coated shell such as ZnS. QDs produce distinctive fluorescence colors that are partly the result of unusually high surface-to-volume ratios for such particles. The core structure of QDs determines the color emitted, while the outer aqueous shell can be used for conjugation of biomolecules such as

peptides, protein, or DNA (Daraee *et al*., 2016). QDs can also carry a cap, which improves their solubility in aqueous buffers. Due to their narrow emission, bright fluorescence, and high photo-stability, QDs can be used for tracking therapeutic agents within the cells/tissues. Although the medical use of QDs is still debated, their surfaces for versatile bioconjugation, adaptable photophysical properties for multiplexed detection, and superior stability for longer investigation periods make them a superior candidate than other fluorescence agents (Kim *et al*., 2018).

Protein nanoparticles

Viruses are very efficient and natural carrier systems for transferring their genetic material, which is encapsulated by the capsid proteins. Virus-Like Particles (VLP), a type of protein nanoparticles, are defined as nanocarrier systems that have a morphologically similar virus-isolated structure but do not contain the viral genetic material. Additionally, caged proteins (CP) are defined as self-assembled protein nanostructures that are morphologically similar to, but not virus-derived from, viruses. The VLPs and CPs are attractive nanocarrier systems for cancer vaccine development as they can induce antigen-specific immune responses against cancer cells (Yetisgin *et al* ., 2020). In addition, there are protein nanoparticles produced by the self-assembly of protein polymers isolated from proteins of animal or plant origin, such as collagen, gelatin, silk, albumin, elastin, and soy. Through genetic engineering, protein polymers themselves are assembled into functional drug delivery vehicles with the advantages of polymer-based nanoparticles. Abraxane is an FDA-approved protein nanoparticle drug that allows albumin to deliver paclitaxel. On the other hand, an HIV vaccine made from VLPs led to key developments that accelerated research into protein nanoparticles for clinical use (Neek *et al*., 2019).

Characterization of nanoparticles

Nanoparticles and based formulations have occupied several important application areas that have direct relevance to our routine. Nanoparticle applications range from pharmaceuticals and drug delivery to the development of efficient solar cells. For their

applications, it is crucial to characterize these nanoparticles in terms of multiple physical, chemical, and biological properties. Lists of various essential properties that require adequate characterization. We have several state-of the art tools at our disposal to characterize nanoparticles with regard to the properties mentioned above. These tools provided detailed information on the physical and chemical properties of nanoparticles.

Important physicochemical properties of nanoparticles

Purity, Reactivity and Hydrophobicity, particle shape, size and distribution Surface chemistries, stability, dispersion, swelling, agglomeration and aggregation. The characterization of nanoparticles for biomedical applications, especially for in vivo delivery, is of crucial importance. A plethora of characterization techniques have been approved, but achieving a full characterization profile of nanoparticles is still a challenging task. Important bottlenecks in such characterizations are summarized. An important reason for an incorrect or insufficiently characterized formulation of nanoparticles is the time-dependent fluctuations in their chemical and physical properties.

Dynamic light scattering

Dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering, is used to determine the nanoparticle size in the colloidal suspension polymer solution. DLS measures the hydrodynamic size of particles through the mechanism of light scattering from a laser passing through a colloidal solution and analyzes the modulation of the intensity of the scattered light as a function of time (Lim *et al.,* 2013). The Brownian motion of particles correlates with their hydrodynamic diameter. The smaller the particle, the faster it diffuses than a larger one, and the DLS instrument generates a correlation function mathematically related to particle size and its time-dependent light scattering capacity. DLS has been used to measure the particle size of dispersing colloidal samples to study the stability of formulations and to detect the presence of aggregation or agglomeration. DLS is the ultimate tool to determine and measure the state of agglomeration of nanoparticles. The bead size of microemulsions can also be measured using the DLS technique (Raval *et al* ., 2018). By comparing DLS and transmission electron microscopy (TEM) image data, the aggregation state of

nanoparticles can be easily determined. The working principle of the DLS is to shield the elastic scattering intensity of the light from the Brownian motion of the sample. The particle size can be obtained from the motion dependent autocorrelation function of Einstein's equation (Stetefeld *et al.,* 2016).

Electron Microscopy

Electron microscopy (Scanning EM/Tunneling EM) can be used to reveal the details of the nanoparticle shape and surface. However, the demanding and laborious sample preparation approaches associated with biological samples (nucleic acid-coated particles) constitute the main limitation of their use. Particles coated with biomolecules result in poor quality electron micrographs. Sample preparation causes desiccation and shrinkage of the sample. Drying samples tend to aggregate, introducing additional artifacts. In addition, longer analysis times and the inability to calculate dispersity present another set of challenges (Kumar & Dixit, 2017).

Transmission Electron Microscopy (TEM)

Transmission electron microscopy is undoubtedly one of the maximum critical nanoparticle characterization strategies. TEM employs a centered electron beam on a skinny (generally much less than two hundred nm) pattern to produce micrographs of nano-scale materials with excessive lateral spatial resolution (Samsonov *et al.,* 2019). Current electron microscopes can gain resolutions down to zero.05–0.1 nm by way of decreasing photo distortion via aberration correctors, for this reason offering high-decision snap shots with atomic resolution (Bhattacharjee, 2016). Thanks to this high spatial resolution and selectivity, TEM permits the investigation of size, form, and crystal shape at the single-particle stage. Once a representative institution of snap shots of the nanoparticle sample is received, the character length of ≈1000 randomly decided on nanoparticles need to be measured to gain implying data for length distribution determination. This seasonedcedure may be performed either manually (inherently tormented by human mistakes, bias, and subjectivity) or the use of automated particle analysis techniques. Although TEM enables visible inspection of single particles with

nanometer resolution, the entire workflow of pattern guidance, dimension, and evaluation may be extremely hard work in depth (Murthy *et al*., 2015).

Scanning Electron Microscope (SEM)

Scanning electron microscope permits imaging the pattern floor with the aid of detecting secondary electrons emitted from the sample upon interaction with the impinging electron beam (Goldstein *et al*., 2018). Current electron microscopes can obtain resolutions right down to 0.5–0.1 nm by means of lowering photo distortion via aberration correctors, therefore imparting excessive-resolution photos with atomic resolution. TEM additionally permits reading the crystalline structure of decided on microscopic regions of crystalline materials through spatially confining and focusing the impinging beam and detecting the ensuing electron diffraction sample. SEM usually calls for conductive substrates for high-resolution imaging and nonconductive samples may be lined with a thin (5–10 nanometer) metal movie before being analyzed. This change of size and surface shape of nonconductive nanoparticles because of sample coaching must be accounted for while interpreting SEM micrographs (LUO *et al*., 2013).

X-ray diffraction

X-ray diffraction is now a common method for the take a look at of crystal structures and atomic spacing. X-ray diffraction is primarily based on constructive interference of monochromatic X-rays and a crystalline pattern. These X-rays are generated via a cathode ray tube, filtered to supply monochromatic radiation, collimated to concentrate, and directed closer to the pattern (Bunaciu *et al* ., 2015). X-ray diffraction (XRD) is a substances characterization approach that is broadly used for all kinds of strong particles along with nanoparticles of all sizes. XRD is used for characterization of nano powders of any sizes, and the found adjustments in positions of diffraction peaks are used to make conclusions on how crystal shape and mobile parameters changes with the alternate in nanoparticles size and shape. It ought to be referred to, however, that the traditionally used XRD theory turned into advanced for fairly big debris with big quantity of diffracting planes and for which floor results on XRD are negligibly small (Vorontsov & Tsybulya, 2018).

UV-visible spectroscopy

UV-Vis spectroscopy is one of the most popular characterization techniques to determine particle formation and its properties. Furthermore, the surface plasmon resonance spectrum of nanoparticles is known to be affected by size, shape, interparticle interactions, free electron density, and the surrounding medium, (Ghosh & Pal, 2007) suggesting that it is an efficient tool to monitor electron injection and aggregation is about NPs. UV/Vis spectroscopy is the only method that used to study the kinetics of swelling and deswelling of polymer microgels and hybrid microgels having small particle size. UV/Vis spectroscopy is commonly used to characterize hybrid microgels loaded with plasmon nanoparticles and to study their application. It has been reported as an effective tool to study optical properties of plasmonic nanoparticles loaded into polymer microgels. The catalytic activity of nanoparticles can also be probed by UV/Vis spectroscopy. UV Visible spectroscopy is commonly used to confirm the synthesis and stability of metal NPs/colloidal particles. The synthesis is confirmed based on the absorbance of the samples at a wavelength of 230 to 800 nm, and NPs ranging from 1 to 100 nm can be used for the analysis (Naseem *et al*., 2018).

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is considered a powerful and simple technique. It plays a compelling role in biological systems to measure concentration of chemicals, surface chemistry, functional group and atomic arrangement of biological NP samples. In NP synthesis, FTIR can analyze whether the biomolecules are involved in the synthesis or not. It also shows which biomolecules are present in the sample. The FTIR measurement depends on the vibration of molecular bonds positioned at different frequencies and the nature of the bonds. The outstanding features of FTIR are high sensitivity, high-cube corner interferometer, adjustable workspaces, and hyperspectral imaging (Jeyaraj *et al*., 2019).

Zeta-Potential

The zeta-potential is the potential measured at the slipping plane of a particle under an electrical field. It reflects the potential difference between the electric double layer (EDL)

of electrophoretic mobile particles and the layer of dispersant around them (aqueous or organic environment) at the slipping plane. The EDL surface of a particle in solution develops instantaneously and is formed of two layers. The inner layer, the so-called Stern layer, is composed of opposite charged particles tightly coupled to the core of the central particle. The second and outermost layer is a diffusive layer consisting of both opposite and same charged ions/molecules. When an electrical field is applied to the sample, the particles move to the opposite electrode. Within the diffuse layer there is a hypothetical plane that acts as the interface between the moving particles and the layer of the surrounding dispersant while in the electrical field. This plane is the characteristic slipping/shear plane and zeta potential is the potential at this particle-fluid interface (Bhattacharjee, 2016). The zeta-potential is measured by the electrophoretic mobility of charged particles under an applied electric field. The electrophoretic mobility (μe) of the particles is calculated by Henry's equation (Kaszuba *et al*., 2010),

$$\mu e = 2\varepsilon r\varepsilon 0\zeta f(Ka)\ 3\eta \quad (1)$$

where εr is the relative permittivity/dielectric constant, ε0 is the permittivity of vacuum, ζ is the zeta-potential value, f(Ka) is the Henry's or Helmholtz-Smoluchowski function, and η is the viscosity at the experimental temperature. Depending on the solvent where the particles are dispersed, the value of f(Ka) is assumed to be 1 or 1.5, for organic medium or aqueous medium, respectively (Domingues *et al*., 2008).

Silver Nanoparticles

Metallic oxides represent a popular zone of research because of their notable electronic, optical and physiochemical attributes (Williams *et al*., 2018). Hence, metal oxide NPs are frequently fabricated and investigated for various applications in sensors, electronics, catalysis, energy storage devices, magnetic resonance imaging, drug delivery and medicine. Their nano medicinal uses have gained tremendous popularity and considerable research has been performed on the nano medicinal applications of metal oxide NPs. The properties of the nano-scale materials are usually different from their bulky counterparts. The nano size and surface area to volume ratio of materials imparts distinctive biological properties and therefore, actively studied for use in different

applications (Ezhilarasi *et al*., 2016). Green synthesis is an environmentally friendly approach in order to synthesize nanoparticles which might concrete the way for researchers throughout the world to find out the capability of different herbs (Hameed *et al*., 2019). Silver nanoparticles (AgNPs) are one of the most important and fascinating nanomaterials among several metallic nanoparticles involved in biomedical applications. AgNPs play an important role in nanoscience and nanotechnology, especially in nanomedicine. Although several noble metals have been used for different purposes, AgNPs have focused on potential applications in cancer diagnosis and therapy (Abbasi *et al*., 2019).

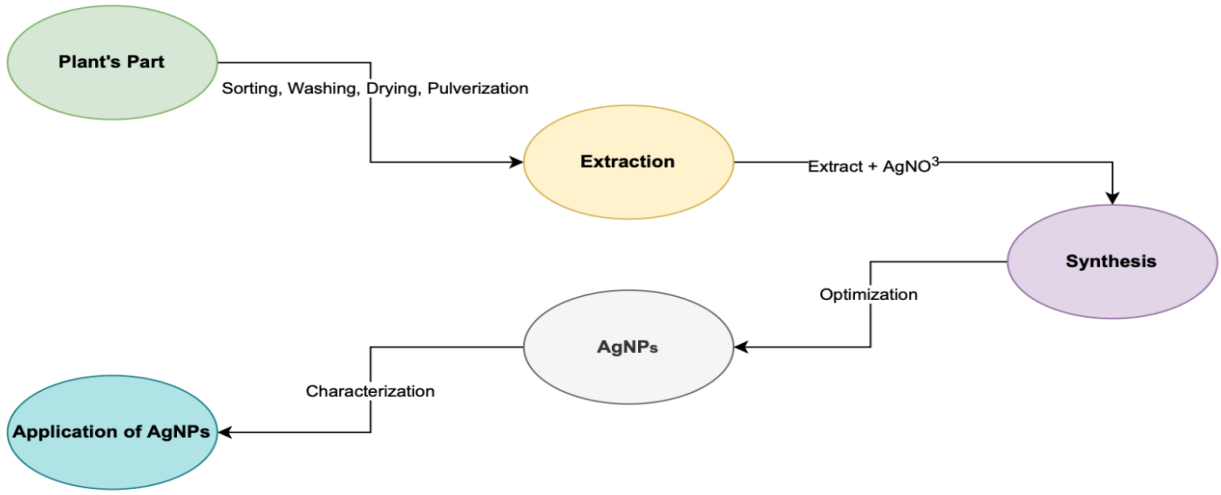

Figure 2: Synthesis and characterization of AgNPs

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties. Due to their uniquely properties, they have been used for various applications, including as antibacterial agents, in industrial, household and healthcare products, in consumer products, coatings of medical devices, optical sensors and cosmetics, in the pharmaceutical industry food industry, in diagnostics, orthopedics, drug delivery, as anti-cancer agents and have ultimately enhanced the tumor-killing effects of anti-cancer drugs. Recently, AgNPs have been widely used in many textiles, keyboards, wound dressings, and biomedical devices. Nano-sized metallic particles are unique and can significantly alter physical, chemical, and biological

properties due to their surface-to-volume ratio; Therefore, these nanoparticles have been used for various purposes (Chernousova & Epple, 2013). In order to meet the requirements of AgNPs, various synthetic methods have been adopted. In general, conventional physical and chemical methods seem to be very expensive and dangerous. Interestingly, biologically produced AgNPs show high yield, solubility, and high stability (Liu, *et al* ., 2016a). Among several methods for synthesizing AgNPs, biological methods appear to be simple, rapid, nontoxic, reliable, and environmentally friendly approaches that can generate well-defined size and morphology under optimized conditions for translational research. Finally, an environmentally friendly approach to the synthesis of AgNPs is promising.

Synthesis Techniques of Ag Nanoparticles

Au–NPs are considered to be synthesized from several parts of herbal plants like leaves, bark, stem roots etc. (Sharma *et al*., 2009). Several chemical and physical means are suggested for the synthesis of Au–NPs including laser/vapor deposition, epitaxy, thermal evaporation, sol-gel, sono-chemical, pyrolysis, electrodeposition, solvothermal and hydrothermal, etc. (Taylor *et al*., 2013). Despite their high efficacy, these methods have certain disadvantages, such as the physical methods are often energy intensive, while chemical routes generate toxic waste [Chemical synthesis is not preferred due to the use of noxious chemicals (Iqbal *et al*., 2019). However, green synthesis of Au–NPs has no such disadvantages, and therefore considered as a more acceptable process. A paradigm shift has been observed in the utilization of economical and green resources for the fabrication of nanoparticles (Khalil *et al*., 2018). Au-NPs, also called gold colloids, have engrossed attraction for fabrication of quick sensing increasing attention due to their exclusive properties in devices use in biomedical field as diagnostic tools in multi-disciplinary research areas (Zhang *et al.*, 2007).

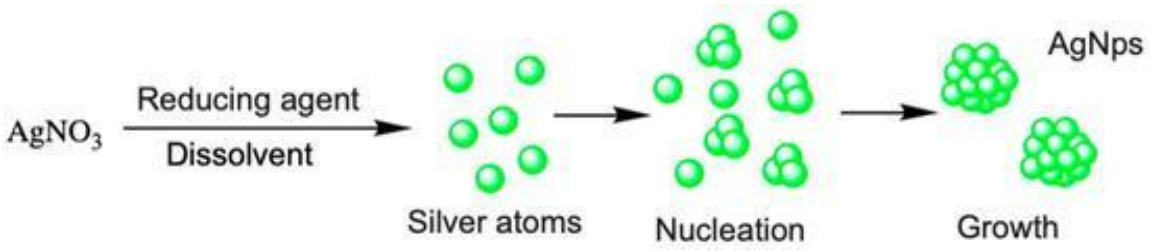

Figure 3: Process for the synthesis of AgNPs

Physical Method

In general, the synthesis of nanoparticles has been accomplished using three different approaches, including physical, chemical, and biological methods. In physical methods, nanoparticles are prepared by evaporation-condensation in a tube furnace at atmospheric pressure (Mittal *et al*., 2013a). Traditional physical methods such as spark discharge and pyrolysis have been used for the synthesis of AgNP. The advantages of physical methods are speed, radiation as a reducing agent, and no use of hazardous chemicals, but the disadvantages are low yield and high energy consumption, solvent contamination, and lack of uniform distribution (Tien *et al*., 2008).

Chemical Method

Chemical methods use water or organic solvents to produce the silver nanoparticles (Elsupikhe *et al*., 2015). This process usually employs three main components, such as metal precursors, reducing agents, and stabilizing/capping agents. Basically, the reduction of silver salts involves two steps (1) nucleation; and (2) subsequent growth. In general, silver nanomaterials can be obtained by two methods classified as “top-down” and “bottom-up” (Tao *et al* ., 2006). The top-down process is the mechanical comminution of bulk metals with subsequent stabilization using colloidal protective agents (Deepak *et al*., 2011). The bottom-up methods include chemical reduction, electrochemical methods and sono-decomposition. The main advantage of chemical methods is high yield in contrast to physical methods which have low yield. The methods mentioned above are extremely expensive. Apart from these disadvantages, the particles produced do not have the expected purity since their surfaces have been sedimented with chemicals. Chemical methods make use of techniques such as cryo-chemical synthesis, laser ablation,

lithography, electrochemical reduction, laser irradiation, sono-decomposition thermal decomposition and chemical reduction. The advantages of chemically synthesizing nanoparticles are ease of preparation, low cost, and high yield; However, the use of chemical reducing agents is harmful to living organisms.

Biological Method

To overcome the shortcomings of chemical methods, biological methods have emerged as viable options. Recently, the biologically mediated synthesis of nanoparticles has been shown to be a simple, inexpensive, reliable, and environmentally friendly approach, and much attention has been devoted to the production of well-defined size AgNPs in high yield using different biological systems, including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids as an alternative method to chemical methods, not only for AgNPs but also for the synthesis of several other nanoparticles, like gold and graphene (Liu, *et al* ., 2016b).

The biosorption of metals by gram-negative and gram-positive bacteria provided evidence for the synthesis of nanoparticles before this biological method flourished; however, the synthesized nanomaterials were aggregates and not nanoparticles (Ganaie *et al*., 2015). Several studies reported the synthesis of AgNPs using environmentally friendly, inexpensive, and biocompatible methods without using toxic chemicals in biological methods. In this green chemistry approach, several bacteria including *Pseudomonas stutzeri* AG259, *Lactobacillus strains, Bacillus licheniformis*; *Escherichia coli* (E. coli) (Liu *et al*., 2016b), *Brevibacterium casei*, fungi including *Fusarium oxysporum, Ganoderma neo-japonicum Imazeki*, plant extracts such as *Allophylus cobbe, Artemisia princeps and Typha angustifolia* used. In addition to these, several biomolecules such as biopolymers (Leung *et al*., 2010), starch, fibrinolytic enzymes and amino acids (Kumar *et al*., 2014) were used. The biological synthesis of nanoparticles depends on three factors including (a) the solvent; (b) the reducing agent; and (c) the non-toxic material. The main advantage of biological methods is the availability of amino acids, proteins, or secondary metabolites present in the synthesis process, the elimination of the additional step required to prevent particle aggregation, and the use of biological molecules for the synthesis of AgNPs is environmentally friendly and non-toxic. Biological

methods seem to provide controlled particle size and shape, which is an important factor for various biomedical applications (Shankar & Rhim, 2015). Using bacterial proteins or plant extracts as reducing agents, we can control the shape, size and monodispersity of the nanoparticles. The other advantages of biological methods are the availability of a large number of biological resources, reduced time requirements, high density, stability and the easy solubility of prepared nanoparticles in water (Gurunathan *et al*., 2014b).

The biological activity of AgNPs depends on the morphology and structure of AgNPs, which is controlled by the size and shape of the particles (Thakkar *et al.,* 2010). In terms of size and shape, smaller and truncated triangular nanoparticles appear to be more effective and have superior properties. Although many studies have successfully synthesized AgNPs with different shapes and size ranges, they still have certain limitations. To achieve control over morphology and structure, excess strong reducing agents such as sodium borohydride (NaBH4) were used for the synthesis of monodisperse and uniformly sized silver colloids (Pal *et al.,* 2007). Compared to chemical methods, biological methods allow easier control over the shape, size and distribution of the produced nanoparticles by optimizing the synthesis methods including the number of precursors, temperature, pH and the amount of reducing and stabilizing factors (Liu*, et al*., 2016b).

Properties of AgNPs

Physical and chemical properties of AgNPs, including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/encapsulation, agglomeration, dissolution rate, particle reactivity in solution, ion release efficiency, cell type, and finally type of reducing agents used for synthesis are crucial factors for the determination of cytotoxicity. Some Previous studies supported the assertion that smaller size particles could cause more toxicity than larger, because they have larger surface area. Shape is also important in determining toxicity (Sriram *et al* ., 2012). For example, in the biomedical field, different types of nanostructures have been used, including nanocubes, nanoplates, nanorods, spherical nanoparticles, flower-like ones and so on. AgNP toxicity mainly depends on the availability of chemical and/or biological coatings on

the nanoparticle surface (Rycenga *et al.*, 2011). AgNP surface charges could determine the toxicity effect in cells. For example, the positive surface charge of these NPs makes them more suitable, allowing them to stay in the bloodstream for longer periods compared to negatively charged NPs (Suresh *et al*., 2012), which is a major route for anticancer drug delivery.

Because of their unique properties, AgNPs have been widely used in household utensils, healthcare industry and food storage, as well as environmental and biomedical applications. Several review articles and book chapters have been devoted to different areas of application of AgNPs. Here we are interested in highlighting the applications of AgNPs in various biological and biomedical applications, such as B. antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer and anti-angiogenic. Here we've looked specifically at previously published landmark articles and end with recent updates. A schematic diagram depicting various applications of AgNPs is given in figure 4.

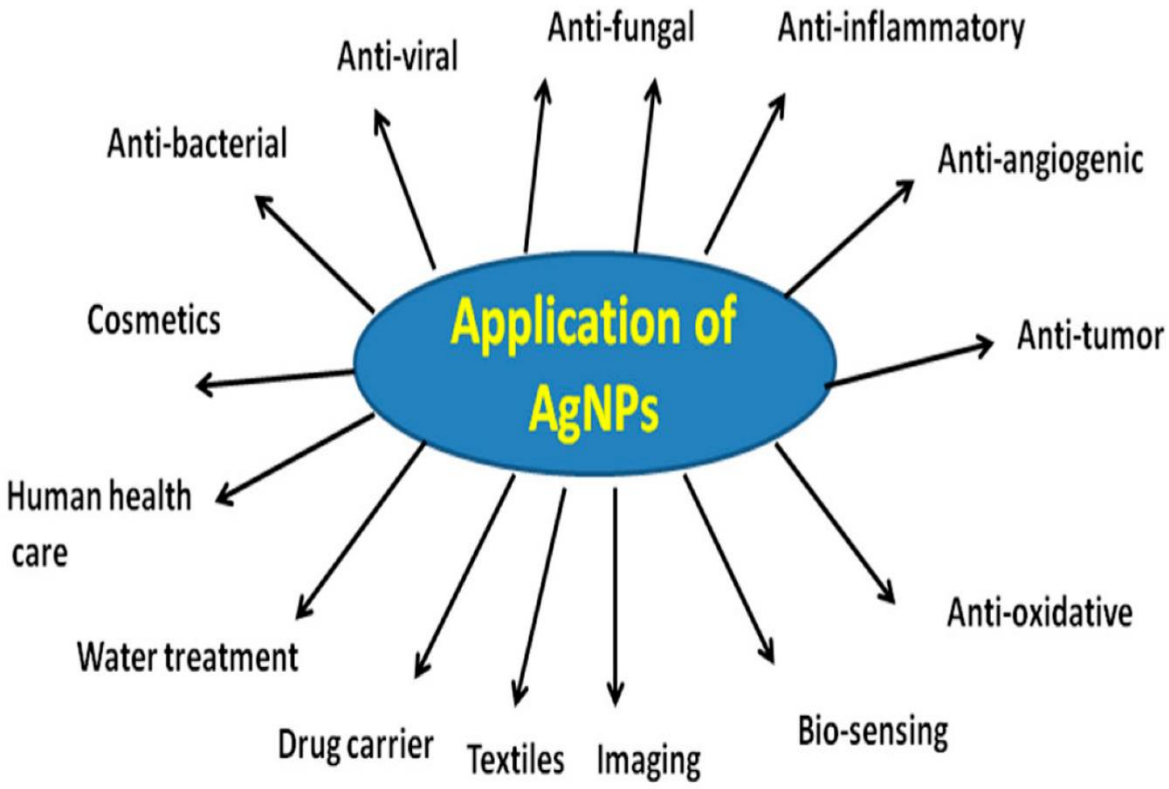

Figure 4: Various applications of AgNPs

Synthesis by bacteria

AgNPs appear to be alternative antibacterial agents to antibiotics and have the ability to overcome bacterial resistance to antibiotics. Therefore, it is necessary to develop AgNPs as antibacterial agents. Among the several promising nanomaterials, AgNPs appear to be potential antibacterial agents because of their high surface area-to-volume ratios and crystallographic surface structure. Bacteria have been studied in the synthesis of silver NPs. It was reported that highly stable silver NPs (40 nm) could be synthesized by bioreduction of aqueous silver ions with a culture supernatant of the nonpathogenic bacterium *Bacillus licheniformis*. Furthermore, well-dispersed silver nanocrystals (50 nm) were synthesized using the bacterium *B. licheniformis*. Saifuddin and co-workers (Saifuddin *et al*., 2009) reported a new combinatorial synthetic approach to the formation of silver NPs by using a combination of *B. subtilis* culture supernatant and microwave irradiation in water. They reported the extracellular biosynthesis of monodisperse Ag NPs (5–50 nm) using supernatants from *B. subtilis*, but to increase the reaction rate and reduce aggregation of the NPs produced, they used microwave radiation, which is responsible for uniform heating the NPs and could support the digestive maturation of particles without aggregation. Another study reported the rapid biosynthesis of metallic silver NPs using aqueous Ag+ ion reduction by culture supernatants of *Klebsiella pneumoniae, E- coli*, and *Enterobacter cloacae*. The synthesis process was quite fast and silver NPs were formed within 5 minutes after silver ions came into contact with the cell filtrate. It appears that nitroreductase enzymes could be responsible for the bioreduction of silver ions (Shahverdi *et al*., 2007).

Synthesis by Fungus

Fungal infections are more common in immunocompromised patients, and overcoming fungal-mediated diseases is a lengthy process due to the limited number of antifungal drugs currently available. Therefore, there is an inevitable and urgent need for the development of antifungal agents, which should be biocompatible, non-toxic and environmentally friendly. Silver NPs (5–50 nm) could be synthesized extracellularly using Fusarium oxysporum without evidence of particle flocculation even one month after the reaction. The long-term stability of the nanoparticle solution could be due to the

stabilization of the silver particles by proteins. The morphology of the NPs was highly variable, with generally spherical and occasionally triangular shapes observed in the micrographs. Silver NPs have been reported to interact strongly with proteins including cytochrome c (Cc). This protein could be self-assembled on a citrate-reduced silver colloid surface (Shahverdi *et al*., 2007). Ingle et al., (Ingle *et al.,* 2008) demonstrated the potential ability of Fusarium acuminatum Ell and Eve (USM-3793) Cell extracts in the biosynthesis of silver NPs. The NPs produced within 15–20 min was spherical with a broad size distribution ranging from 5–40 nm with an average diameter of 13 nm. A nitrate-dependent reductase enzyme could act as the reducing agent.

Enzyme mediated synthesis

The enzyme-mediated synthesis of nanoparticles is one of the most recent advances in nanotechnology. In most cases, active enzymes catalyze the formation of nanoparticles, while under certain conditions enzymes are denatured to release amino acids that act as reducing and stabilizing agents in the synthesis of nanoparticles. Sometimes the enzyme itself can act as a reducing and capping agent in the formation of nanoparticles. Pure -amylase has been used in the synthesis of gold nanoparticles (AuNPs) where the catalytic activity of the enzyme is still retained in the AuNP-amylase complex. Other microbial enzymes relevant for nanoparticle synthesis are laccase, ligninase, cellulase, nitrate reductase and sulfite reductase. Recently, we reported the synthesis of AgNPs using crude keratinase produced by a feather-degrading Bacillus safensis LAU 13 isolated from a feather dump, and extracellular laccase from Lentinus edodes. Therefore, there is an increasing use of enzymes in the synthesis of nanoparticles in the green way (Adelere, *et al.,* 2016).

Synthesis from Plant

In synthesizing AgNPs from plants to promote large-scale production of AgNPs, which is a remedy for the high demand for AgNPs, plant leaf is more preferable than whole plant in synthesizing extracellular AgNPs. Plant extract possesses various active biomolecules such as phenolic acids, sugars, terpenoids, alkaloids, polyphenols and proteins, which play a crucial role in this biological reduction and stabilization of silver ions (Castro *et al*.,

2011). The Size and shape of AgNPs depend on the following parameters: amount of plant extract used, silver concentration, temperature, reaction time and pH of the reaction. The process of synthesizing AgNPs includes collecting and identifying parts of plants (seeds, fruits, leaves, rhizomes, roots, barks, pulps, stems, pods and milky sap), cleaning parts of plants by sorting and washing, drying parts of plants at Room temperature to prevent evaporation of volatile biomolecules, pulverization of dried plant parts to aid rapid extraction, extraction with water or other solvents at minimum temperature. Applying minimal heat to the mixture with continuous stirring until a color change is observed; finally, the solution is filtered and incubated (Akintelu *et al*., 2020). The report showed that the biosynthesis of AgNPs in the silver salt solution could be completed within minutes at a temperature of 25 °C; however, the types of secondary metabolites present in the plant extract are of great importance. The concentration of the extract, the concentration of the silver salt, the temperature, the pH value and the contact time are also important (Mittal *et al*., 2013b).

Cannabis sativa

Cannabis sativa L. (Cannabis), a member of the Cannabaceae family, is one of the oldest domesticated crops in the world (Long *et al*., 2017). It is believed to have originated in Central Asia, from where its cultivation quickly spread throughout Asia and Europe. Nowadays, legal and illegal cannabis cultivation takes place worldwide ((Van Bakel *et al* ., 2011). *Cannabis sativa* has a rich history of medicinal use, dating back to ancient times. The first report of its medicinal use comes from the Middle East and Asia in the 6th century BC. Its introduction into Western medicine came much later, in the early 19th century. This species has been indicated for the treatment of pain, glaucoma, nausea, depression and neuralgia. The therapeutic value of Phyto cannabinoids has also been used for symptom management of HIV/AIDS and treatment of multiple sclerosis (ElSohly *et al*., 2017). *Cannabis sativa* is known in many cultures for its medicinal potential. Its complexity contributes to the historical use of various parts of the plant in ethnomedicine and pharmacotherapy. *C. sativa* has been used to treat rheumatism, epilepsy, asthma, skin burns, pain, treatment of sexually transmitted diseases, difficulties during child labor,

postpartum hemorrhage, and gastrointestinal activities. However, the use of *C. sativa* is still limited and illegal in most countries.

Nomenclature

Botanical Nomenclature

The taxonomic classification of *Cannabis sativa* is as shown below:

Kingdom:	Plantae (plants)
Subkingdom:	Trophobiont (vascular plants)
Super division	Spermatophyta (seed plants)
Division:	Magnoliophyte (flowering plants)
Class:	Magnoliopsida (dicotyledons)
Subclass:	Hamamelidid
Order:	Urticales
Family:	Cannabaceae
Genus:	*Cannabis*
Species:	*sativa*
Taxonomic authority abbreviation:	L

Origin and botanical description of C. sativa

The genus name *Cannabis* means cane-like, while Sativa means seeded, meaning the plant is propagated from the seed and not the roots. It is believed to have originated in Asia and is widespread in Africa. Central and Southeast Asia are the potential natural origins for the domestication of the *Cannabis* genus, and it is known by various common names in various languages (hemp, marijuana, cannabis sativa, ganja, bhang, and al-bhango). Taxonomically, Linnaeus, a Swedish botanist, was the first to coin the name *Cannabis sativa*. Other botanists stated that there are different types of *cannabis* based on their size, shape and resin content (breeding and selection). This review specifically discusses *C. sativa*. The *cannabis* phenotype (its observable traits or properties, such as its leaf shape and flower color) is based on two main factors: its genetic code (genotype) and external environmental factors. The roots are branched and about 3060 cm deep (Farag and Kayser, 2017). The inflorescence of *cannabis* consists of multiple flower

heads found on long petioles of each leaf axil. The leaves, bracts, and stems of the plant are rich in trichrome, which is a diverse group of structures containing the secondary metabolites (phyto-cannabinoids and terpenoids) responsible for defenses, plant interactions, and distinctive odor. *Cannabis sativa* is a dioecious plant that belongs to the Cannabaceae family and it originates from Central and Eastern Asia (Alexander et al ., 2009). It is widely distributed in countries including Morocco, South Africa, United States of America, Brazil, India, and parts of Europe. *Cannabis sativa* grows annually in tropical and warm regions around the world. Different ethnic groups around the world use *Cannabis sativa* for smoking, preparing concoctions to treat diseases, and for various cultural purposes. According to (Thafeni et al., 2012), it is composed of chemical constituents including cannabinoids, nitrogenous compounds, flavonoid glycosides, steroids, terpenes, hydrocarbons, non-cannabinoid phenols, vitamins, amino acids, proteins, sugars and other related compounds. Cannabinoids are a family of naturally occurring compounds highly abundant in *Cannabis sativa* plant. Screening of *Cannabis sativa* has led to isolation of at least 66 types of cannabinoid compounds. These compounds are almost structurally similar or possess identical pharmacological activities and offer various potential applications including the ability to inhibit cell growth, proliferation and inflammation. One such compound is cannabidiol (CBD), which is among the top three most widely studied compounds, following delta-9-tetrahydrocannabinol (Δ9 -THC). It has been found to be effective against a variety of disorders including neurodegerative disorders, autoimmune diseases, and cancer. In a research study conducted by (Shrivastava et al., 2011), it was found that CBD inhibited cell proliferation and induces apoptosis in a series of human breast cancer cell lines including MCF-10A, MDA-MB231, MCF-7, SK-BR- 3, and ZR-7-1 and further studies found it to possess similar characteristics in PC-3 prostate cancer cell line. However, to allow us to further our studies in clinical trials a range of cancers in vitro should be tested to give us a clear mechanism before we can proceed. *Cannabis sativa* in particular cannabidiol, we propose it plays important role in helping the body fight cancer through inhibition of pain and cell growth. Therefore, the aim of this study was to evaluate the cytotoxic and anti-proliferative properties of *Cannabis sativa* and its isolate, cannabidiol in cervical cancer cell lines (Sharma *et al.*, 2014).

Anticancer Activity of AgNPs

In our lifetime, 1 in 3 people has the possibility of developing cancer. Although many chemotherapy drugs are currently used in various cancers, the side effects are enormous, and in particular, the administration of chemotherapy drugs by intravenous infusion is a lengthy process. It is therefore essential to develop technologies to avoid systemic side effects. At this point, many researchers are interested in developing nanomaterials as an alternative tool to create formulations that can specifically target tumor cells. Several research laboratories have used different cell lines to study the possibility of finding a new molecule to fight cancer. Here we have summarized the work of various laboratories reporting anticancer activities using both in vitro and in vivo model systems. Gopinath *et al* .2008 investigated the molecular mechanism of AgNPs and found that programmed cell death was concentration-dependent under conditions. Further, they observed a synergistic effect on apoptosis using uracil phosphoribosyl transferase (UPRT)-expressing cells and non-UPRT-expressing cells in the presence of fluorouracil (5-FU). In these experimental conditions, they observed that AgNPs not only induce apoptosis but also sensitize cancer cells. The anticancer property of starch-coated AgNPs was studied in normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251). AgNPs induced alterations in cell morphology, decreased cell viability and metabolic activity, and increased oxidative stress leading to mitochondrial damage and increased production of reactive oxygen species (ROS), ending with DNA damage. Among these two cell types, U251 cells showed more sensitivity than IMR-90. The same group also demonstrated that the cellular uptake of AgNPs occurred mainly through endocytosis. AgNP-treated cells exhibited various abnormalities, including upregulation of metallothionein, downregulation of major actin binding protein, filamin, and mitotic arrest . The morphology analysis of cancer cells suggests that biologically synthesized AgNPs could induce cell death very significantly. Jun *et al* 2016 elegantly prepared multifunctional silver-embedded magnetic nanoparticles, in which the first type consist of silver-embedded magnetic NPs with a magnetic core of average size 18 nm and another type consist of a thick silica shell with silver having an average size of 16 nm; the resulting silica-encapsulated magnetic NPs (M-SERS dots) produce strong surface-enhanced Raman scattering (SERS) signals and have magnetic properties, and these two

significant properties were used for targeting breast-cancer cells (SKBR3) and floating leukemia cells (SP2/O) (Liu *et al*., 2016b).

Recently, the anticancer property of bacterial (B-AgNPs) and fungal extract-produced AgNPs (F-AgNPs) was demonstrated in human breast cancer MDA-MB-231 cells. Both biologically produced AgNPs exhibited significant cytotoxicity. Among these two AgNPs, fungal extract-derived AgNPs had a stronger effect than B-AgNPs, which is due to the type of reducing agents used for the synthesis of AgNPs. Similarly, AgNPs derived from Escherichia fergusoni showed dose-dependent cytotoxicity against MCF-7 cells. Plant extract-mediated synthesis of AgNPs showed more pronounced toxic effect in human lung carcinoma cells (A549) than non-cancer cells like human lung cells, indicating that AgNPs could target cell-specific toxicity, which could be the lower level of pH in the cancer cells (Shen *et al*., 2016).

Therapies to treat cancer have evolved in response to human needs, but resistance to therapy is a public health issue (Arnold *et al*., 2015). Nanotechnology has long attempted to combat this burden by improving the pharmacokinetics and pharmacodynamics of chemotherapy drugs that target solid tumors. For this, drug encapsulation has been studied and tested in vivo, with the first molecules being approved by the FDA in the mid-1990s, namely Doxil and DaunoXome. Both therapeutics consist of liposomes with encapsulated active substances, doxorubicin (DOX) or a mixture of anthracycline and daunorubicin (Allen and Cullis, 2013). Cancer drugs face multiple challenges, necessitating the development of new drugs according to the nature of the target: solid tumors or circulating cancer cells (Pearce *et al*., 2012). n solid tumors, bypassing the mononuclear phagocyte system (MPS) and staying in the tumor tissue is essential for the drug to be effective, while in circulating cancer cells, the drug needs to be internalized to ensure its effect at the target site (Jain*, et al*., 2015). Considering the current scenario, various strategies have been pursued to overcome these limitations. Metal nanoparticles with gold or silver cores have been tested and have shown natural anticancer activity against tumors and cancer cells either in vitro or in vivo. Following the improvements in nanoparticle development, combinations of copper and chitosan have also been tested, with observable anticancer activity and less toxic effects (Solairaj *et al*., 2017). Here, DLS

was used not only as a pure characterization technique, but as a tool to identify metal structures with higher colloidal stability and better size distribution (Shmarakov *et al*., 2017). Di Francesco *et al*., using nonionic surfactant vesicles (NSVs) loaded with DOX, developed nano-systems with different ratios of Tween21/Tween80 that promote a pH-responsive approach with anticancer properties (Di Francesco *et al*., 2017). These NSVs showed fusogenic behavior and increased targeting efficiency, which translated into higher anticancer activity. As already mentioned, nanoparticles with direct activity can also be used as drug carriers. Designed Silica NPs with encapsulation tetrazole, a cyclic/aromatic molecule with antimicrobial, antifungal and anticancer activity (Zakerzadeh *et al*., 2017).

Drug delivery

Chemotherapy drugs are more effective at suppressing all uncontrolled and rapidly growing cancer cells. At the same time, they can also influence the growth of healthy cells. Damage to normal cells by chemotherapy leads to painful side effects. The model with synergistic combinations of the drug with nanoparticles has excellent potential to overcome this effects. Drug delivery based on nanotechnology has gained enormous potential in human medicine with a wide range of applications. There is ongoing research to improve versatility in tissue targeting, access to deep molecular targets, and sustained drug release. This manuscript proposes a protocol and strategy developed to minimize secondary infection in cancer therapy and maximize treatment efficiency. Paclitaxel is a widely used drug used to treat various cancer therapies to eradicate solid cancerous tumors. Silver nanoparticles are chosen as the carrier because they are highly antibacterial compared to other metals in the following order: silver (Ag)>mercury (Hg)>copper (Cu)>cadmium (Cd)>chromium (Cr)>lead (Pb)) > Cobalt (Co) > Gold (Au) > Zinc (Zn) > Iron (Fe) > Manganese (Mn) > Molybdenum (Mo) > Tin (Sn) and therefore exert a strong bactericidal effect in killing a variety of bacteria out (Chavan *et al*., 2021).

In advances, NPs are also used in the delivery of heat therapy, indicating infrared light absorption, radiofrequency ablation, and magnetically induced heating. The use of radiolabeled NPs labeled with radionuclides and fluorescent NPs, such as B. NPs doped with organic dyes, quantum dots, and multifunction NPs that can be conjugated with

multiple functional molecules that can promote a new diagnostic tool in cancer therapy. Nanotechnology has enabled drug delivery approaches to normally reduce the systemic distribution and associated side effects typically seen with conventional chemotherapeutic molecules. Recent research has developed a range of NPs such as metal, semiconductor, and polymer particles that can be used as imaging probes, diagnostics, and as delivery vehicles in cancer therapy. NPs play an important role in cancer diagnosis. The particles like organic dyes, doped polymers, liposomes and quantum dots are used in cancer diagnosis. Multifunctional NPs have the ability to simultaneously carry therapeutic agents, squares to represent contrast agents, diamonds, and targeting entities (circle) that can be used as anticancer agents. Drug-loaded NPs can also be used in the treatment of cancer in animal models. Multifunctional micelles have been developed for in vitro targeting of cancer cells, distribution imaging and anti-cancer delivery. Doxorubicin (DOX) drugs released from micelles have potent effects on the viability of the human hepatic carcinoma (Hep G2) cell line. In addition to DOX drugs, loaded NPs have greater anti-cancer activity in HER-2 over the expressing human breast adenocarcinoma cell line (SK-BR-3) (Shah *et al*., 2004). Among the inorganic nanomaterials, silica or mesoporous silica materials can be used as potential delivery vehicles and imaging probes due to their effective biocompatibility and easy surface fictionalization. NPs play an important role in targeting the drug to the malignant tumor cells by reducing the systemic toxicity of the anticancer drugs. Rapamycin-loaded polymeric (poly lactide-co-glycolide) (PLGA) NPs conjugated with antibodies against the epidermal growth factor receptor (EGFR) enabling efficient and targeted delivery of anticancer drugs.

OBJECTIVE

Objective

I. To biosynthesize silver nanoparticles by using *Cannabis sativa* leaf extract.

II. To characterize silver nanoparticles by using UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.

III. To check the potency of silver nanoparticles against cervical cancer HeLa cells.

MATERIAL AND METHOD

Material and Methods

HI Media, India; Merck and Sigma-Aldrich Co. (St. Louis, MO, USA) provided all the chemicals required for the study.

Plant Collection and extract preparation:

Cannabis sativa plant was kind gifted from Dr. Maqbool Ahmad Khan, Deputy Director, CCRUM, Kursi Road, Basaha Lucknow. Healthy green leaves were collected rinsed properly with the distill water to remove all the dust and unwanted visible impurities. The leaves were crushed with the help of pestle mortar and tris buffer was added in it. Take some ice cubes in the polypropylene moulded tray and place pestle & mortar in it with plant extract and leave it for some time. Then again crush the extract and filter it with the help of Whatsman filter paper in the centrifuge tube and then the tubes were placed in centrifuge at 6000 rpm at 4°C for 10 min. Then remove the pellet from the extract and take the supernatant in another centrifuge tube. Extract is stored in refrigerator for future purposes.

***Cannabis sativa* mediated synthesis of silver nanoparticles**

In vitro synthesis of AgNPs was performed by 3 ml of the prepared plant extract was taken in 20 ml of centrifuge tube and 3mM of silver nitrate salt was added to the plant extract and volume makeup with water. Keep the reaction tube in incubator at about 37°C . After 120 h (5 days) the different period the sample was ejected and analysed on a bio spectrum-Kinetic spectrophotometer using a quartz cuvette having the path length of 1 cm to a affirm the synthesis of *cannabis sativa* encapsulated silver nanoparticle subsequently, the solution was filtered using a syringe with a filter having the pore size of 2 micrometer, the unbound proteins and phytochemicals were expelled using ethanol treatment for 30 minutes and utilized further characterization.

Characterization of silver nanoparticles

The transformation of silver salt into silver nanoparticles was investigated by using the shimadzu UV-1601 dual beam spectrometer. This measurement has a special resolution of one nanometer (200 nm to 800 nm). The technique is done on the basis of reducing

metal salts to synthesize silver nanoparticles result in colour change. Particle size analyser (Zetasizer Nano-ZS, Model ZEN3600, Malvern Instrument Ltd., Malvern, UK) was used to analyse the mean particle size of AgNPs. The diluted sample (0.5% w/v) was sonicated for 1 min. and taken in a low volume disposable sizing cuvette of 1.5 mL. The mean particle size was the average of triplicate measurement for a single sample. The zeta potential measures the colloidal stability of nanoparticles in a solution, as previously described, that metal nanoparticles carry charge for capping agents, Zeta potential may also be used to assess the shielding or exposure of charged groups, as well as the concentration distribution of nanoparticles (Mishra et al., 2022).

Cell Culture

The human cervical cell line (HeLa cell) was purchased from National Centre for Cell Science (NCCS), Pune, India. The aforementioned *in-vitro* cytotoxic potential analysis of CNB-extract and CNB-AgNPs was performed on HeLa cells using MTT assay. The cells were cultured in MEM medium, supplemented with 10% FBS and 1% antibiotics containing 10,000 units/ml of penicillin, 10 mg/ml of streptomycin, and 25 µg/ml of amphotericin B in a humidified atmosphere containing 5% CO_2 at temperature 37°C. All the cell stocks were maintained in 25 cm^2 tissue culture flasks.

Assessment of cytotoxicity

To assess the cytotoxic effect of CNB-extract and CNB-AgNPs, HeLa cells were placed in 96-well plate with density of 5×10^3 cells per well and incubated in a humidified incubator with 5% CO_2 at 37°C for 24 h. Further the cells were treated with CNB-extract, AgNPs different concentrations in triplicates, and incubated for 24 h. After incubation, the media was discarded and 10µL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-tetrazolium bromide] (5 mg/mL in PBS) was added to each well. The plates were further incubated for 2 h in a CO_2 incubator. The resulting formazan crystals were solubilized in 100µL of DMSO. The extent of MTT reduction was measured spectrophotometrically at 595 nm using a Bio-Rad Elisa reader , and the cell survival was expressed as percentage over the vehicle. Experiments were conducted in triplicate. Cytotoxicity was expressed as the concentration of compound inhibiting cell growth by 50%(IC50). The IC50 values were

determined with GraphPad Prism5 computer program. Percentage cell viability was calculated as follows:

$$\% \text{ Cell viability} = \frac{¼ \text{ Absorbance of treated cells} - \text{Absorbance of blank}}{\text{Absorbance of untreated cells} - \text{Absorbance of blank} \times 10038}$$

Measurement of cytomorphological changes in HeLa cells

HeLa cells were pre-treated with different concentrations (100-400µg/ml) of each, CNB-Extract, CNB-AgNPs incubated for 24 h at 37°C in an atmosphere 5% CO_2. Post-incubation, the morphological changes in HeLa cells occurred in the all the treated groups were examined using an inverted phase contrast microscope (FLoid imaging station).

RESULT AND DISSCUSSION

Result and Discussion

Cannabis sativa Extract Mediated synthesis of AgNPs (CNB-AgNPs)

This study used *Cannabis sativa* leaf extract as a reducing sugar and capping agent, whereas 3Mm silver nitrate ($AgNO_3$) served as the silver precursor. The synthesis of CNB-AgNPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites, which combine to convert $AgNO_3$ was convert AgNPs. The creation of CNB-AgNPs was confirmed visually by a shift in the color of the extract from green to dark brown, indicating silver reduction.

Characterization of CNB-AgNPs

Due to Surface Plasmon resonance, an unusual phenomenon is seen in noble metal nanoparticles (SPR). This imbues the nanoparticles surface with the character of powerful electromagnetic fields, resulting in scattering and absorption. Thus, the formation of CNB-AgNPs was confirmed here in using UV-vis spectra (Figure: 5,6 and 7). The absorption peak was observed 456nm, which corresponds to the SPR band of the CNB-AgNPs.

The phyto-constituents in *Cannabis sativa* leaf extract reduced the silver salt ($AgNO_3$) into AgNPs and encapsulated the silver nanoparticle preventing the nanoparticles from the aggregating and providing stability to the CNB-AgNPs. The change in colour from light yellow to Ruby red indicated the successful synthesis of CNB-AgNPs, and the result of SPR band confirm that at 430 and however there was no discernible peek for *Cannabis sativa* leaf extract. The technique of dynamic light extracting (DLS) was used to determine the average particle size and provide of the particle size distribution of CNB-AgNPs had an average particle size of 70 d.nm and as shown in figure. Furthermore, the Zeta potential of the prepared CNB-AgNPs was observed at the room temperature, to be a -12.7 mV, indicating the significantly high stability of the nanoparticles. When the aqueous dispersion of AgNPs was observed at room temperature no clumping or accumulation was observed. This was most likely due to the silver and a particles electrostatic repulsive effects. The nanoparticles are prevented from colliding because of this repulsion.

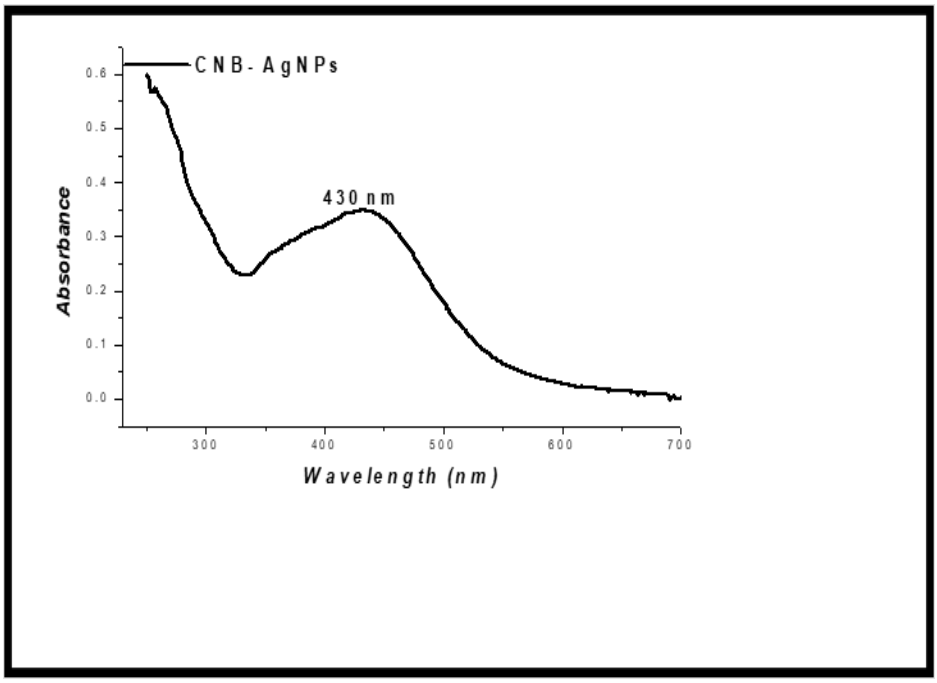

Figure 5: Characterisation of CNB-AgNPs under UV-Visible spectra (430 nm).

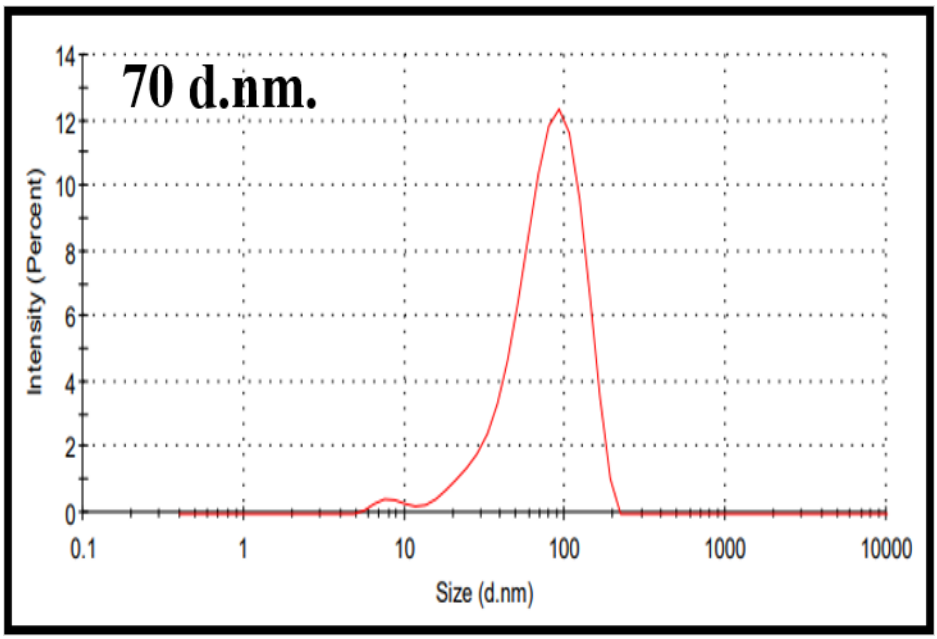

Figure 6: DLS profile of CNB-AgNPs showing size of 70 d.nm.

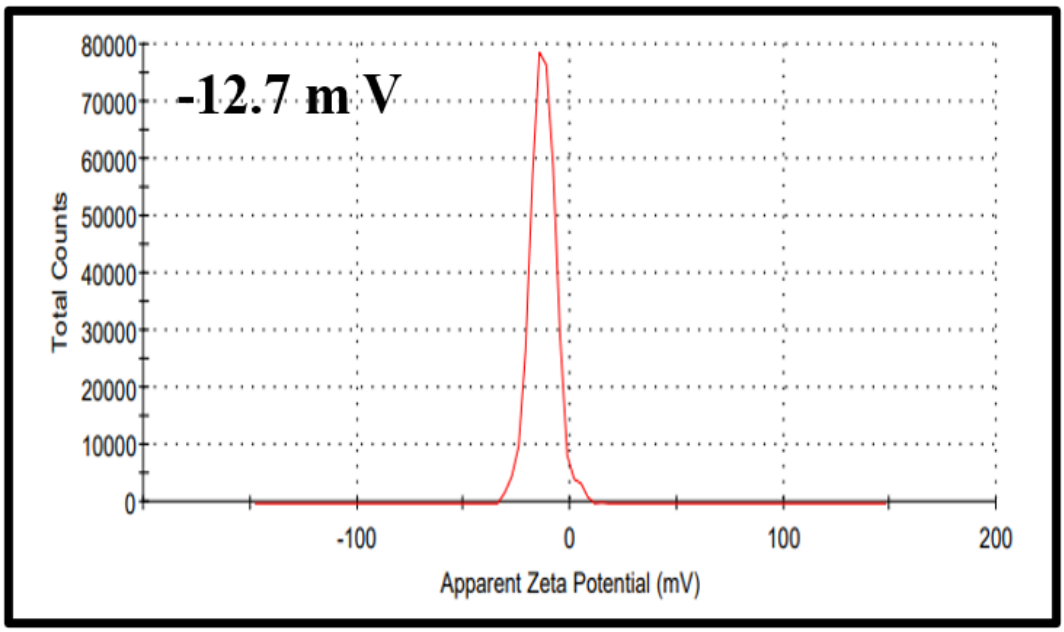

Figure 7: Zeta potential of CNB-AgNPs confirmed the stability at -12.7 mV.

Determination of cytomorphological changes in the HeLa cells

Morphological analysis of the CNB- extract and CNB-AgNPs, treated HeLa cells was performed using a phase contrast microscope. A dose dependent change in the cell morphology was observed in HeLa cells after treatment with CNB- extract (100, 200, 300, 400 µg/ml) and CNB-AgNPs, (5, 10, 20, 40 µg/ml) concentrations for 24 h. In the presence of different doses CNB- extract and CNB-AgNPs, HeLa cells showed round morphology with small shrinkage and nuclear condensation. A proportion of the cells revealed swelling, cell membrane lysis and disintegration of organelles, suggesting cytotoxicity in HeLa cell. These morphological changes in Cervical cancer cells were more evident with the increase in the dose in AgNPs. In contrast, well spread flattened morphology was observed in untreated control cells.

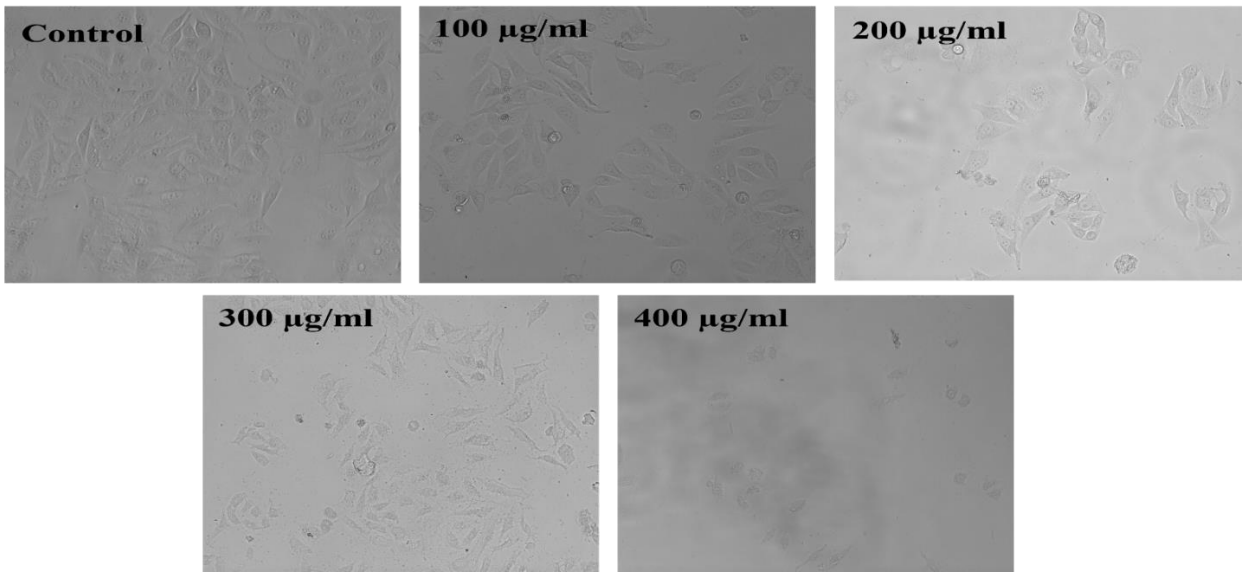

Figure 8: The phase contrast microscopy of HeLa cell treated with either vehicle control or different concentration of *Cannabis sativa* (100, 200, 300, 400 µg/ml) for 24 h in a time and dose dependent manner. Images shown are representative of three independent experim (Shrivastava et al., 2011) ent (Scale bar: 100µm; Magnification: 20X).

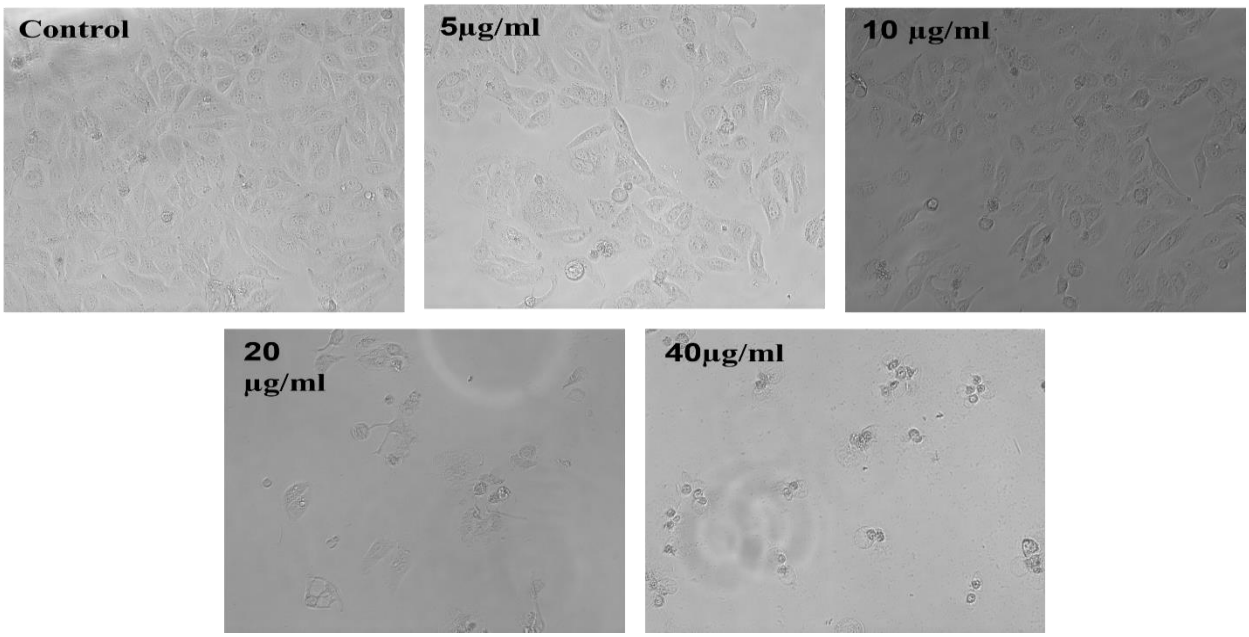

Figure 9: The phase contrast microscopy of HeLa cell treated with either vehicle control or different doses/concentration of CNB-AgNPs (5, 10, 20, 40 µg/ml) for 24 h in a time and dose dependent manner. Images shown are representative of three independent experiment (Scale bar: 100µm; Magnification: 20X).

In vitro cytotoxicity of CNB- extract and CNB-AgNPs,

To evaluate the sensitivity of cervical cancer cells to these drugs, HeLa cells were treated with different doses of CNB- extract and CNB-AgNPs, for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, AgNPs at a concentration of 187.3±1.12 µg/ml reduced growth of HeLa cells by 50%, while inhibition of 50% viability of HeLa was observed at 3.531±1.33 µg/ml, CNB-AgNPs, respectively. AgNPs, were found to be more cytotoxic for Cervical cancer cells in comparison to pure extract and the effect was observed to be dose-and time-dependent.

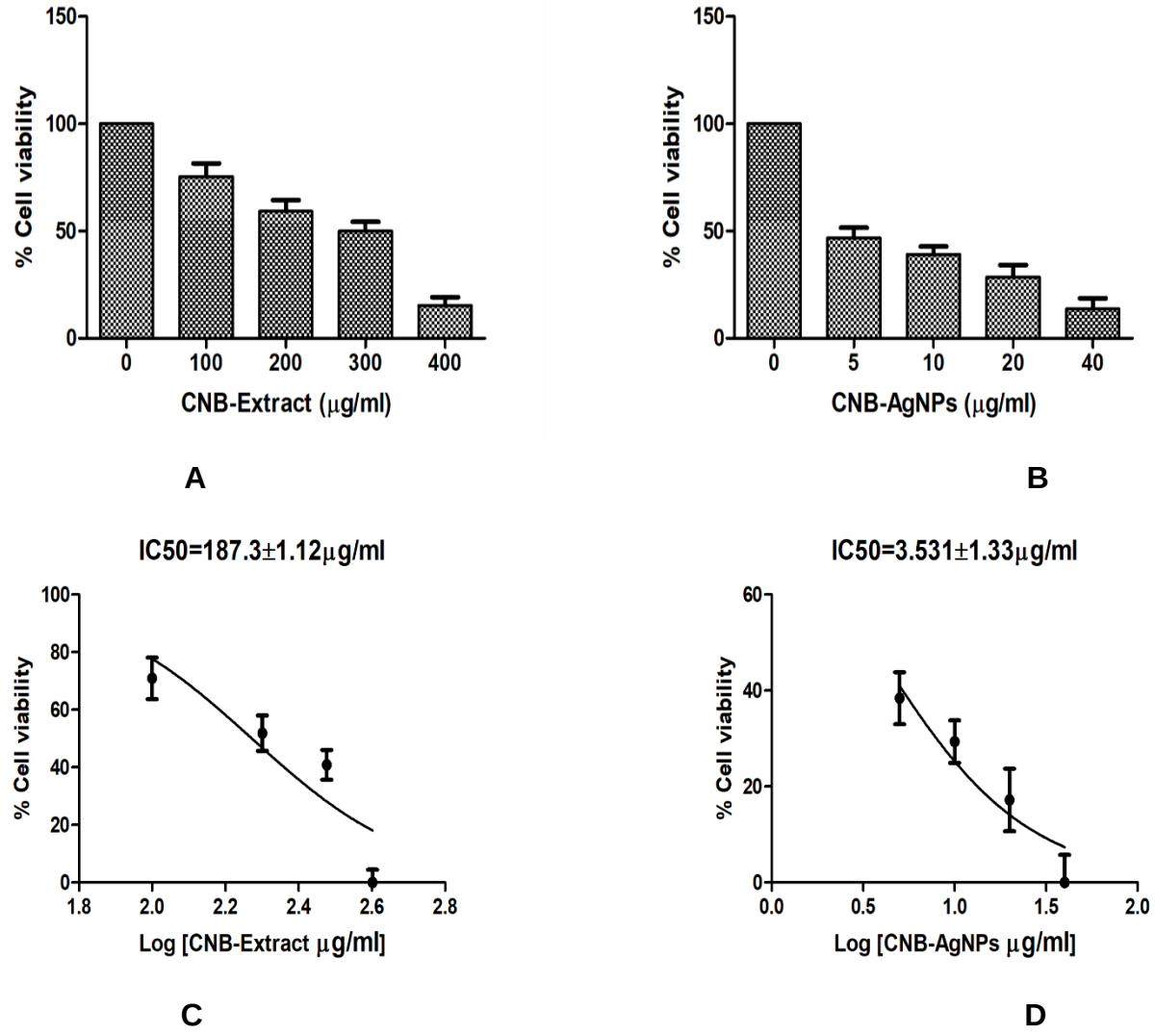

Figure 10: (A) Percent cell viability of HeLa cells treated with different doses of *Cannabis sativa*

(100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) assessed by MTT Assay 24h. (C) Graph showed that *Cannabis sativa* exhibited IC50 value 187.3µM at 24 h, against HeLa cervical cancer cell.

(B) Percent cell viability of HeLa cells treated with different doses of CNB-AgNPs (5,10,20,40µg/ml) assessed by MTT Assay 24h. (D) Graph showed that CNB-AgNPs exhibited IC50 value 3.531±µg/ml at 24 h, against HeLa cervical cancer cell. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

Conclusion and future Perspectives

In this study we showed a Leaf extract mediated green synthesis of silver nanoparticles from *Cannabis sativa* plant and their characterization, anticancer property analysis. This study investigates an efficient and sustainable route of AgNP preparation from 3mM aqueous AgNO3 using leaf extracts of *Cannabis sativa* plants. The Ag NPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS) and Zeta potential.

This study addressed synthesis, characterization, and bio-applications of silver nanoparticles, with special emphasis on anticancer and also therapeutic approaches for cancer using AgNPs. Recently, both academic and industrial research has explored the possibility of using AgNPs as a next-generation anticancer therapeutic agent, due to the conventional side effects of chemo- and radiation therapy. Although AgNPs play an important role in clinical research, several factors need to be considered, including the source of raw materials, the method of production, stability, bio-distribution, controlled release, accumulation, cell-specific targeting, and finally toxicological issues to human beings. The development of AgNPs as anti-angiogenic molecules is one of the most interesting approaches for cancer treatment and other angiogenesis-related diseases; it can overcome poor delivery and the problem of drug resistance.

Although AgNPs have been focused on therapeutic purposes, further research is inevitable in animal models to confirm the mechanisms and to gain a comprehensive picture of biocompatibility vs. toxicity of AgNPs. Finally, if we succeed in all these studies, it would help the researchers of the nanoscience and nanotechnology community to develop safer, biocompatible, efficient cancer or anti-angiogenic agents containing AgNPs. Eventually, to ensure the biosafety of the use of AgNPs in humans, studies dealing with biocompatibility of AgNPs and their interaction with cells and tissues are inevitable. Finally, the great concern is that the developing nanotechnology-based therapy should be better than available technologies, and it should overcome the limitations of existing treatment techniques. Finally, it has to provide a safe, reliable, and viable treatment of diseases with high accuracy in a patient-friendly manner.

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