A DISSERTATION ON

"GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES BY *CANNABIS SATIVA* AND ASSESSMENT OF THEIR CYTOTOXIC EFFICACY ON CERVICAL CANCER HELA CELLS."

IN PARTIAL FULFILMENT
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BY

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TO WHOM IT MAY CONCERN

This is to certify that Mr. MOHAMMED MAISRA student of M.Sc. Biotechnology, (IV semester), Integral University has completed his four months dissertation work entitled **"Green synthesis of zinc oxide nanoparticles by Cannabis sativa and assessment of their cytotoxic efficacy on cervical cancer hela cells"** successfully. He 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 his M.Sc. degree.

I wish her good luck and a bright future.

Dr. Snober S.Mir
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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Mr. MOHAMMED MAISRA** during the months February – June, 2022 reported in the present thesis was under my guidance and supervision. The results reported by his are genuine and the script of the thesis has been written by the candidate himself. The thesis entitled is **"Green synthesis of zinc oxide nanoparticles by Cannabis sativa and assessment of their cytotoxic efficacy on cervical cancer Hela cells"** therefore, being forwarded for the acceptance in partialfulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow.

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MOHAMMED MAISRA

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
ZnONPs	Zinc oxide nanoparticles
NPs	Nanoparticles
CNB	*Cannabis sativa*

INTRODUCTION

INTRODUCTION

Nanotechnology, the manipulation of matter on atomic and molecular scales, is a relatively new branch of science. The idea was first discussed by world-renowned physicist and Nobel laureate Richard Feynman in 1959(Appenzeller, 1991). The application of nanotechnology to medicine, commonly referred to as nanomedicine, involves the use of nanoscale materials for preventive, therapeutic and diagnostic purposes (L. Zhang *et al*., 2008). There are emerging novel approaches in which nanotechnology can enhance current treatment as well as advance new therapeutic strategies, such as gene therapy and immunotherapy. Moreover, some nanomaterials have therapeutic effects by themselves. Nanotechnology can also play a major role in preventive strategies for developing vaccines and microbic. The major function of nanotechnology is said to be the synthesis of nanoparticles, mainly relying on the three methodologies such as physical, chemical, biological methods. of these methodologies biological synthesis plays major role when compared with the two other methodologies (Nie *et al*., 2007). Biologicallymediated synthesis is further classified into eco-friendly synthesis, which is comprised of plants and plant sources with the corresponding advantages of simplification and lower cost (KN & Kim, 2007). Metal oxide nanoparticles have various significant application possibilities, such as anti-microbial, cell line studies and dye degradation properties(Oskam, 2006).

Over the last decade, novel synthesis approaches/methods for nanomaterials (such as metal nanoparticles, quantum dots (QDs).carbon nanotubes (CNTs), graphene, and their composites) have been an interesting area in nanoscience and technology *(*Su *et al*., 2014). Nanostructured metal oxides have already been extensively studied for their promising use in technology. This has resulted in development of numerous reproducible procedures for the synthesis of nanoparticles with desired characteristics like size, shape, morphology, defects in the crystal structure, mono dispersity providing a rich background for research relevant to antibacterial applications. Characterization of these nanoparticles can be helpful in modifying and tuning their antibacterial and cytotoxic effects. The synthesis technique employed is functional in determining the biological characteristics of a given nanoparticle. As potential novel antibacterial agents,

metal oxide nanoparticles like Fe_3O_4, TiO_2, CuO and ZnO are being thoroughly investigated. Their relatively low toxicity against human cells(J. Zhang *et al*., 2008) low cost , size-dependent effective inhibition against a wide range of bacteria, ability to prevent biofilm formation and even eliminate spores make them suitable for application as anti-bacterial agents in the fabric , skincare products, biomedical and food-additive industries (Ai *et al*., 2011). Due to the increased demand for various metallic and non-metallic nanoparticles over the past two decades, a wide range of physical and chemical techniques have been developed to produce nanoparticles of different sizes, shapes, and compositions. Traditionally, nanoparticles have been synthesized and stabilized via physical and chemical techniques. The physical approach includes techniques such as laser ablation , lithography and high-energy irradiation (Ahmad & Senapati, 2003). While the chemical approach uses techniques such as: chemical reduction, electrochemistry, and photochemical reduction. In recent years, biological synthesis has emerged as an attractive alternative to traditional synthesis methods for producing nanoparticles. Biosynthesis involves using an environment-friendly green chemistry based approach that employs unicellular and multicellular biological entities such as actinomycetes, bacteria , fungi , plants , viruses, and yeast (Smijs & Pavel, 2011). Zinc oxide nanoparticles (ZnONPs), as one of the most important metal oxide nanoparticles, are popularly employed in various fields due to their peculiar physical and chemical properties (Newman *et al.,* 2009). Due to the strong UV absorption properties of ZnO, they are increasingly used in personal care products, such as cosmetics and sunscreen. In addition, ZnONPs have superior antibacterial, antimicrobial, and excellent UV-blocking properties. It is generally known that zinc as an essential tr

ace element extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis (Rasmussen *et al*., 2010). Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, nano-ZnO is commonly used as a food additive. Moreover, ZnO is graded as a “GRAS” (generally recognized as safe) substance by the US Food and Drug Administration (FDA).With these properties, ZnONPs have received more attention in biomedical applications.

Compared with other metal oxide NPs, ZnONPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging (Lellouche *et al.,* 2009). Biofilms are the complex communities of microorganisms attached to any biological or non-biological surface that remain enclosed in self-produced hydrated polymeric matrix (Iconaru *et al.*, 2013). The ability of nanomaterials for biofilm disruption has been reported. For example, Simona and Prodan et al investigated the effect of glycerol iron oxide nanoparticles for biofilm inhibition produced by *Pseudomonas aeroginosa*. Among nanosized metal oxides, zinc oxide (ZnO) has gained much more attention due to its interesting properties such as high surface to volume ratio, low cost and long-term environmental stability (Sirelkhatim et al., 2015). According to Sirelkhatim et al. and Dhillo *et al*., it is already reported by several studies that ZnO nanoparticles are non-toxic to human cells and toxic to bacterial cells. Toxicity studies showed that DNA in human cells do not get damaged by zinc ions. This fact made ZnO nanoparticles biocompatible to human cells (Y. Zhang *et al*., 2013). Recently, nanomaterial-based nanomedicine, with high biocompatibility, easily surface functionalization, cancer targeting and drug delivery capacity, has demonstrated the potential to overcome these side effects. Zn^{2+} is an essential nutrient for adults, and ZnO nanomaterials are considered to be safe in vivo. Taking into account these advantages, ZnONPs can be selected as biocompatible and biodegradable nanoplatforms and can also be explored for cancer treatment (Z.-Y. Zhang & Xiong, 2015).

Zinc oxide nanoparticles (ZnONPs) had been synthesized by a green method using aqueous extract of *Cannabis sativa* leaves. A facile, eco-friendly, time saving and beneficial procedure for the preparation of metal nanoparticles had been developed. UV-visible spectroscopy, DLS and zeta potential were used to characterize the ZnONPs composition, their shape, size and crystallinity. Ultraviolet-visible spectra showed the absorbance band between 300 and 400 nm, which indicated the synthesis of stable ZnO nanoparticles.

REVIEW OF LITERATURE

Nanotechnology

The term ‘nanotechnology’ first came to wide public prominence in a 1986 book by K. Eric Drexler entitled ‘Engines of creation: the coming era of nanotechnology’ (Drexler Eric, 1986). The concept was based on an idea first propounded by Nobel laureate Richard Feynman in a presentation he gave in 1959 entitled ‘There's Plenty of Room at the Bottom’. The first proposed applications of nanotechnology involved chemical synthesis by the application of nanoscales machines, and information storage at the atomic level. Since then, nanotechnology has been applied in diverse fields such as waste water treatment , textile industry , high performance batteries , biology and medicine (Fontanarosa and Bauchner, 2015) In medical applications, nanotechnology has led to significant improvements in cancer therapy , diagnostic imaging of diseases , tissue engineering and most importantly in drug and gene delivery systems (Karimi *et al*., 2016) Today, the application of nanotechnology in biomedical sciences, and healthcare as a whole, has come to be called ‘nanomedicine’ and is considered a hot growth area of nanotechnology (Sayes *et al*., 2017) Over the past few decades, the US FDA has approved commercialization of 100 nanomedicine applications and products. This shows that nanotechnology is playing an immense role in today's biomedical science. Consequently, the US federal authorities have provided more than $1.4 billion funding for the National Nanotechnology Initiative, which confirms the importance of nanotechnology. Nanotechnology has attracted a huge attention around the world. According to a recent report by Forbes, nanotechnology is one of the fifth biggest growth technologies to watch over the coming decade. As a result of this growing interest, a thorough review of the currently approved nanomedicines seems to be timely (Iyer *et al*., 2015) Although about 100 nanomedicines have been approved by the FDA , there has not been sufficient description of the current market trends in this field. Herein, the most recent nanomedicine agents and the market trend along with the major challenges of conventional drug-delivery systems (DDSs) are addressed. Nanotechnology plays a significant role in the field of medicine and drug delivery, mainly due to the major limitations and problems that affected conventional pharmaceutical agents, and older formulations and delivery systems. In comparison with conventional

DDSs, drug administration using nanoparticles (NPs) has several advantages, which are listed below:

- NPs are much smaller than the basic material unit of conventionally formulated drugs. Attaching small-molecule therapeutic agents to these small nanocarriers will form overall nanodrugs (Etheridge et al., 2013)
- Nanoformulation of drugs is one strategy to deliver pharmaceutical agents more precisely to the targeted tissue and reduce the overall dose and potentially toxic side effects.
- The enhanced permeability and retention (EPR) effect can allow passive targeting and accumulation of nanosized drugs at malignant tumors and other pathological sites.
- Nanosized formulations, in comparison with conventional microsized formulations, lead to an increased active concentration and bioavailability.
- NPs demonstrate better safety and efficacy.
- Nanodrugs can be far cheaper than conventional therapies.
- Drug release can occur at a constant rate over the desired timescale (Bosetti et al., 2013)

On the other hand, there are various challenges on the way of using NPs in DDSs. One of the most important goals in pharmaceutical researches, is the synthesis or discovery of new chemical entities with minimum side effects and maximum clinical benefits **.**

One of the key applications of nanotechnology is in medicine, especially for the treatment of cancer. Nanotechnology has made a strong impact on medical imaging and diagnostics. In maging, advances in nanotechnology have resulted in the clinical translation of iron oxide NPs as MRI contrast agents. The most extensively studied agent is the lymphotropic superparamagnetic iron oxide NP ferumoxtran-10, which can be used to detect sub centimeter lymph node metastases. Nanotechnology has also been applied in x-ray generation. Carbon nanotubes (CNTs) have been used as electron emitters in x-ray imaging (Panda *et al*., 2012) The potential benefits of

nanotechnology in agricultural sector have created a great interest, as it can enhance agricultural productivity with low input of cost and energy. Importantly, nanotechnology by virtue of nanoparticles, has offered enormous potential applications in agriculture sector that include nano fertilizer, nano pesticide, nano herbicide, nano sensor, and smart delivery systems for controlled release of agrochemicals. To date, several studies have addressed how nanotechnological approach is benefiting the agricultural sector in number of ways. For examples: pesticides encapsulation in nanoparticles for their sustained release; nanoparticles mediated delivery of genetic material for crop improvement; carbon nanotube assisted seed germination of rain-fed crops; nano fertilizer for enhanced crop nutrition & crop productivity; nano pesticide for plant disease management; nano herbicide for weed elimination and nano sensors for detection and forecast of pathogens and soil monitoring. Nevertheless, nanotechnology renders precise capability to revolutionize the agricultural sector but at the same time it is also important to note that its concrete contribution to agriculture is still uncertain and at its nascent stage. In response to this, we highlight the future directions for improved Agri-nanotechnology researches with special emphasis on; (i) optimization of the safe use of nanoparticles at permissible level for agricultural benefits by modulating the fate, behavior, bioavailability and toxicity determining factors(ii)advancement in experimental design and, (iii) incorporation of biosynthesized nanoparticles and assessing their relative advantages over nanoparticles from non-biological sources. The field of RNA nanotechnology. has advanced rapidly over the past decade.RNA's versatility in structure and function, propensity for bottom-up self-assembly, defined size and structure, favorable in vivo attributes, and large potential as a therapeutic modality make it an attractive candidate as a biomaterial for nanoparticle drug delivery. RNA's ability to adopt complex quaternary structures to base stack, to form canonical Watson−Crick (A−T, G−C) and noncanonical (G−U wobble, sheared G−A pair, G−A amino pair, A−U reverse Hogsten) base pairing leads to a variety of natural structural motifs(Briggs & Knecht, 2012) RNA nanotechnology uses such properties to construct nanoparticles for use in nanomedicine and bio nanotechnology applications.

Nanoparticles

Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100nm, that can drastically modify their physio-chemical properties compared to the bulk material. It is worth noting that nanoparticles can be made from a fully variety of bulk materials and that they can explicate their actions depending on both the chemical composition and on the size and/or shape of the particles (Schröfel *et al*., 2014) .

Depending on the origin, a further distinction is made between three types of NSPs:

1- Natural- Natural nanoparticles have existed from the beginning of the earth' history and still occur in the environment (volcanic dust, lunar dust, mineral composites, etc.)

2- Incidental- Incidental nanoparticles, also defined as waste or anthropogenic particles, take place as the result of manmade industrial processes (diesel exhaust, coal combustion, welding fumes, etc.).

3- Engineered-Engineered nanomaterials can be grouped into four type: 1 - carbon based materials, usually including fullerene, single walled carbon nanotube (SWCNT) and multiwalled carbon nanotubes (MWCNT); 2 - metal based materials such as quantum dots, nanogold, nanozinc, nano aluminum, and nanoscales metal oxides like TiO2, ZnO and Al2O3; 3 - dendrimers which are nano-sized polymers built from branched units, capable of being tailored to perform specific chemical function; 4 - composites which combine nanoparticles with other nanoparticles or with larger bulk-type materials and present different morphologies such as spheres, tubes, rods and prisms .

Nanoparticles can have amorphous or crystalline form, and their surfaces can act as carriers for liquid droplets or gases. To some degree, nanoparticulate matter should be considered a distinct state of matter, in addition to the solid, liquid, gaseous, and plasma states, due to its distinct properties large surface area and quantum size effects. Nanoparticles exhibit novel properties which depend on their size, shape and morphology which enable them to interact with plants, animals and microbes (P. Singh *et al*., 2016). The prefix "nano," derived from the Greek "nanos," signifying "dwarf," is

becoming increasingly common in scientific literature. The nano scale is the place where the properties of most common things are determined just above the scale of an atom. Nano scale objects have at least one dimension (height, length, depth) that measures between 1 and 999 nanometers (1-999 nm) . The brief explanation of pharmaceutical nano system is as follows: Pharmaceutical nanotechnology is divided in two basic types of nano tools viz. nano materials and nano devices. These materials can be sub classified into nano crystalline and nano structured materials. Nano structure consists of nano particles, dendrimers, micelles, drug conjugates, metallic nano particles etc.

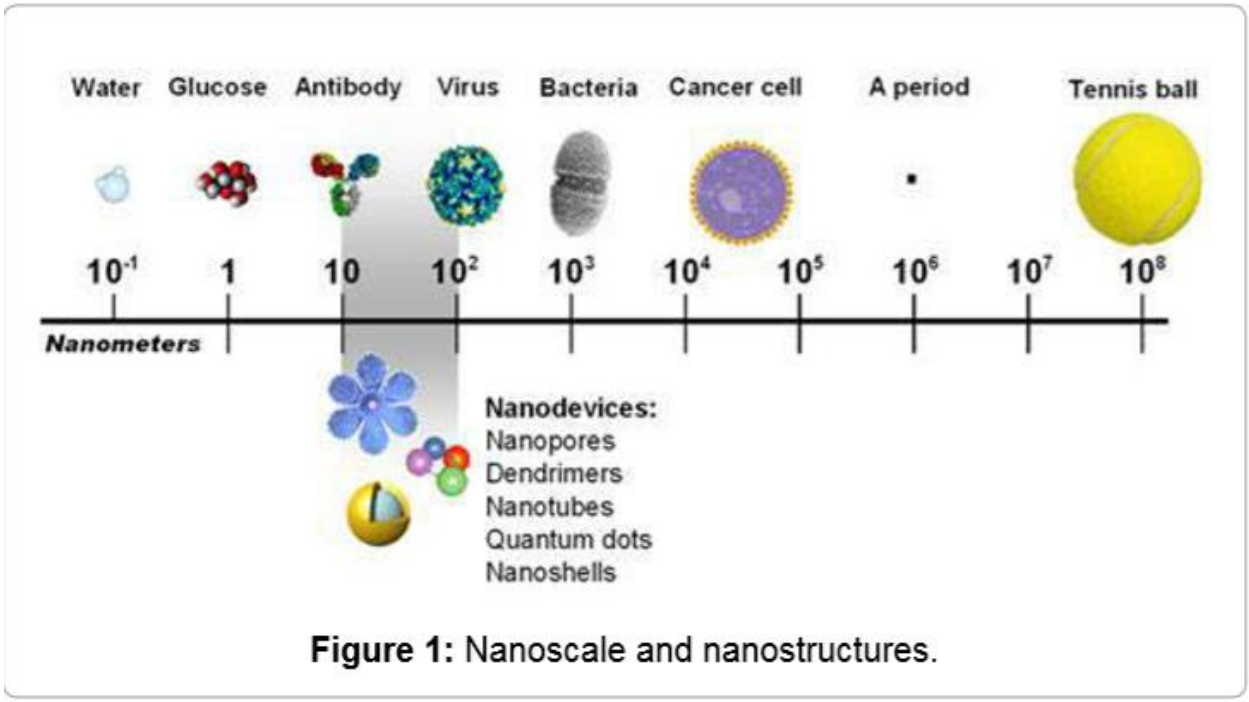

Figure 1: Nanoscale and nanostructures.

FIGURE-1: Nanoscale and nanostructure

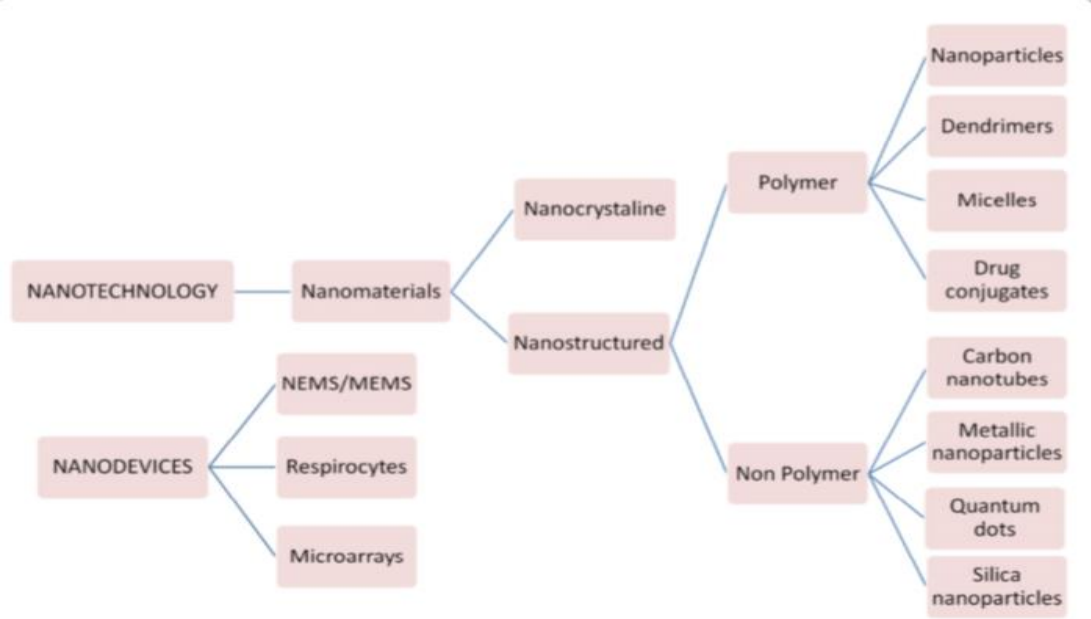

Figure 2: Schematic diagram of various types of pharmaceutical nano systems.

FIGURE-2: Schematic diagram of various types of pharmaceutical nano systems

Types of nanoparticles

Carbon nanotubes- Carbon nano tubes are carbon based tubular structures that are discovered in 1991(H. Singh *et al*., 2018) These structures are arranged in fashion like a graphite sheet rolled up into a cylinder and capped at one or both ends by a buckyball. These are hexagonal networks of carbon atoms having diameter of one nanometer and length from 1 to 100 nm. These carbon networks are arranged layer of graphite rolled up into a cylinder. There are two carbons based configuration that have received much attention recently: single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs).

Quantam dots (QDs) –Quantum dots are nanocrystals of semiconducting materials measuring around 2–10 nm, consisting of a semiconductor inorganic core (CdSe), an aqueous organic coated shell (e.g., ZnS) to improve optical properties, and can be made to fluorescence when stimulated by light. Quantum dots bear a cap which enables them in improving their solubility in aqueous buffers. They are neither atomic

nor bulk semiconductors. Core of the quantum dots determines the color emitted and outer aqueous shell is available for conjugation with biomolecules. Biomolecular conjugation of the quantum dots can be modified according to target various biomarkers (Chen *et al*., 2008)Their properties originate from their physical size, which ranges from 2 to 10 nm in radius. As far as the its applications are concerned QDs covers medical areas as a diagnostic as well as therapeutic tool for invitro and invivo detection and analysis of biomolecules, immunoassays, DNA hybridization, diagnostic tools (magnetic resonance imaging, MRI), time graded fluorescence imaging of tissue, development of non-viral vectors for gene therapy, labeling of cells, as therapeutic tools for cancer treatment and transport vehicles for DNA, protein, drugs or cells.

Paramagnetic Nanoparticle

Magnetic nanoparticles are a class of particulate materials of less than 100 nm size that can be manipulated under the magnetic field. These particles are composed magnetic elements such as cobalt, nickel, iron and their respective oxides such as magnetite, maghemite, cobalt ferrite and chromium dioxide. The classification of these particles is based on their magnetic susceptibility which is defined as ratio of induced magnetization to the applied field. Paramagnetic nanoparticles have a greater magnetic susceptibility than conventional contrast agents. They are investigated for both diagnostic and therapeutic purposes. For diagnostic purpose paramagnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. Targeting with paramagnetic nanoparticles enables identification of specific organs and tissues (El-Sayed *et al*., 2005)

Dendrimers- Dendrimers are a unique class of polymers, are hyper branched, tree-like structures, whose size and shape can be precisely controlled and have compartmentalized chemical polymer. Dendrimers are fabricated from monomers using either convergent or divergent step growth polymerization. Size of these regular branching polymeric nanostructures is dependent on the number of branching which can be controlled. These nanostructures arise several branches from the core in shape of a spherical structure by means of polymerization, resulting in formation of cavities within the dendrimer molecule which can be used for drug transport. Free ends of

dendrimer can be utilized for conjugation or attachment to another molecule. These end groups that can be tailored according to requirements. Such interconnecting networks transport the attached molecules at desirable site and give dendrimers various functional applications (Gao *et al*., 2005) These well-defined nanostructures are equipped with surface functionalization capability, monodispersity of size, and stability properties that make them attractive drug carrier candidate Dendrimers covers various distinct applications mainly are solubilization, gene therapy, dendrimer based drug delivery, immunoassay and MRI contrast agent.

Metallic Nanoparticles- Currently these nanoparticles are emerging as good delivery carrier for drug and biosensor. For the synthesis of metallic nanoparticles diverse metals have been explored though silver and gold nanoparticles are of prime importance for biomedical use. Surface functionalization on these nanoparticles can easily been done and various ligands have been decorated onto the surface. Variety of ligands such as sugars, peptide, protein and DNA has been linked to nanoparticles Metallic nanoparticles have been used for active delivery of bioactive, drug discovery, bioassays, detection, imaging and many other applications due to surface functionalization ability, as an alternative to quantum dots.

6-Liposomes- Liposomes are lipid-based vesicles that are extensively explored and most developed nanocarriers for novel and targeted drug delivery. These have been extensively explored and most developed nano carriers for novel and targeted drug delivery due to their small size, these are 50-200 nm in size. When dry phospholipids are hydrated, closed vesicles are formed. Liposomes are biocompatible, versatile and have good entrapment efficiency. It finds application as long circulatory and in passive and active delivery of gene, protein and peptide. These vesicles are synthesized by hydration of dry phospholipids. Depending upon on their size and number of bilayers vesicles are classified into three basic types:

Multilamellar vesicles -These vesicles consist of several lipid bilayers separated from one another by aqueous spaces. These entities are heterogeneous in size, often ranging from a few hundreds to thousands of nanometers in diameter.

Small unilamellar vesicles- Small unilamellar vesicles consist of a single bilayer surrounding the entrapped aqueous space having size range less than 100 nm.

Large unilamellar vesicles-These vesicles consist of a single bilayer surrounding the entrapped aqueous space having diameters larger than 100 nm (Khan *et al*., 2021)

Zinc Oxide Nanoparticles:

Zinc oxide is an inorganic compound with the molecular formula ZnO. It appears as a white powder and is nearly insoluble in water. The powder ZnO is widely used as an additive in numerous materials and products including ceramics, glass, cement, rubber (e.g., car tyres), lubricants, paints, ointments, adhesives, plastics, sealants, pigments, foods (source of Zn nutrient), batteries, ferrites, and fire retardants. In the Earth crust ZnO is present as zincite mineral but mostly ZnO used for commercial purposes is produced synthetically. ZnO is often called II-VI semiconductor in materials science because zinc and oxygen belong to the 2nd and 6th groups of the periodic table. ZnO semiconductor has several unique properties such as good transparency, high electron mobility, wide band gap, and strong room temperature luminescence. These properties account for its applications in transparent electrodes in liquid crystal display and in energy-saving or heat-protecting windows and other electronic applications. Nowadays the unique properties of nano materials have motivated the researchers to develop many simpler and inexpensive techniques to produce nanostructures of technologically important materials. Several metal oxide nanoparticles are produced with possible future applications. Among them zinc oxide is considered to be one of the best exploited at nano dimensions. The wide band gap and large excitonic binding energy have made zinc oxide important both for scientific and industrial applications. It is generally known that zinc as an essential trace element extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis. Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, nano-ZnO is commonly used as a food additive. Moreover, ZnO is graded as a “GRAS” (generally recognized

as safe) substance by the US Food and Drug Administration (FDA) (Sahoo *et al*., 2007) With these properties, ZnONPs have received more attention in biomedical applications. Compared with other metal oxide NPs, ZnONPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bio imaging.

Synthesis Techniques of ZnO Nanoparticles

Zinc oxide nanoparticles can be synthesized using either conventional or non-conventional methods. While physical, chemical, and biological (green) synthesis techniques are included among the conventional methods, microfluidic reactor-based synthesis is introduced as a non-

Conventional Method

Both top-down and bottom-up approaches can be used to synthesize nanomaterials. The top-down approach involves physically slicing or cutting bulk materials into nano-sized materials conventional method.(Krupiński *et al*., 2016) The bottom-up approach, on the other hand, uses atoms and molecules to build nanostructures through chemical or biological synthesis, or controlled deposition and growth . Biological synthesis, which is otherwise referred to as “green synthesis,” is desirable due to the simplest, most efficient, reproducible, and ecologically responsible option. However, the mechanism of green synthesis is not yet fully understood.

Physical Method

Physical methods include arc plasma, thermal evaporation, physical vapor deposition, ultrasonic irradiation, and laser ablation. These processes are chemically pure and technically simple, which makes them ideal for carrying out industrial processes at high production rates (Chang, 2013) Arc plasma, which is based on electrical arc discharge synthesis, is one of the most commonly used physical methods for converting bulk materials into nanomaterials via condensation and evaporation. Peng et al. described a plasma method that involved reacting Zn powder with oxygen gas at 0.5–50 L/min to

produce wool-like ZnO nanorods. Using thermal evaporation, ZnO thin films and nano/micro ZnO rods were synthesized via deposition on substrates. Fouad et al. reported the synthesis of highly oriented needle-like ZnO crystals (thickness: 10–80 nm) that showed the photocatalytic decomposition of azo-reactive dye at a deposition and oxygen temperature of 350–650 °C for 10 to 30 min. Zhang et al. also reported the synthesis of nano/micro ZnO rods using simple thermal evaporation at 650–850 °C for 60 to 120 min. Zinc oxide nanowires on Al_2O_3 substrate that were fabricated via physical vapor deposition between 450 and 600 °C of low growth temperature presented with a high-quality structure and crystallinity (Thareja & Shukla, 2007). Using ultrasonic irradiation for 75 to 270 min, Yadav et al. nonchemically synthesized histidine (capping agent)-based ZnONPs with a tunable band gap. Thareja et al. used pulsed a laser ablation technique with an Nd: YAG laser (10 Hz, 130 mJ/pulse, 5 ns/pulse duration) to produce a colloidal suspension of ZnONPs.

Chemical Method

Chemical methods include microemulsion, sol–gel, precipitation, hydrothermal, solvothermal, and chemical vapor deposition. Wet chemical synthesis, which is based on the physical states of the solid and liquid phases, is the most commonly used method for producing NPs (Oliveira *et al*., 2003) During industrial-scale wet chemical synthesis, capping agents/stabilizers are used extensively in spite of their toxicity to control particle size and to prevent the agglomeration. Triethylamine (TEA), oleic acid, thioglycerol, and polyethylene glycol are representative capping agents/stabilizers although they have immunogenic and apoptotic/necrotic potential. In a microemulsion, stabilizers are used to generate thermodynamically stable fluid droplets from immiscible phases of hydrocarbon and water. Fricke et al. reported a method for mini-emulsion-based ZnONPs synthesis using TEA to control the size (<200 nm) and shape (hexagonal wurtzite crystal) of ZnONPs. Using sol–gel synthesis, Valdez et al. created dodecylamine (DDA)-capped ZnO nano crystals with a low surface density of DDA (25%) due to the hydroxide groups (protons) on the surface of the ZnONPs. This precipitation technique involves a reaction initiated using a source of zinc and alkali

(sodium hydroxide, potassium hydroxide, ammonium, or urea) to promote aggregation. The precipitates of ZnONPs are then collected by filtration or centrifugation. Oliveira et al. described the controlled precipitation of ZnONPs from zinc nitrate and zinc sulfate with sodium hydroxide. Demir et al. reported the precipitation of ZnO nanocrystals using the acid-catalyzed esterification of zinc acetate in a mixture of l-pentanol and m-xylene. Hydrothermal and solvo thermal techniques involve the material synthesis under heated aqueous and non-aqueous conditions, respectively (Raja *et al*., 2018) The parameters of the hydrothermal and solvo thermal techniques used for the synthesis affect the structure, morphology, composition, and assembly of the resulting ZnONPs.

Biological Methods

Biological methods are promising alternatives to physical and chemical synthesis methods because they are eco-friendly (Saravanan *et al*., 2017) Microorganisms (bacteria, fungi, yeast, algae, and phage), DNA, proteins, and plant extracts have been studied extensively for the biological synthesis of ZnONPs .Zinc oxide NPs can be synthesized in appropriate microorganisms using various enzymes and biochemical pathways. Bacteria including *Bacillus megaterium* NCIM2326, *Halomonaselongata* IBRC-M 10214, *Sphingobacterium thalpophilum*, and *Staphylococcus aureus* have been used to synthesize ZnONPs (10–95 nm; rod/cubic, multiform, triangle, acicular) for antimicrobial agents. Fungal species including *Aspergillus niger* and *Candida albicans*, can also synthesize ZnONPs. Zinc oxide NPs synthesized from fungi had spherical to quasi-spherical shapes of 61 nm and 25 nm, respectively. These NPs were used for antimicrobial applications and steroidal pyrazoline synthesis. *Pichia kudriavzevii* and *Pichia fermentans* JA2 as yeast systems can also synthesize ZnONPs. In yeast, hexagonal wurtzite and smooth/elongated ZnONPs (10–61 nm) were produced and these NPs were used for antimicrobial applications. In algae, *Chlamydomonas reinhardtii* and *Sargassum muticum* were used to synthesize ZnONPs. These algal species produced nanorods/nanoflowers (55–80 nm from HR-SEM; 21 nm from XRD) and hexagonal wurtzite NPs (30–57 nm from FE-SEM; 42 nm from XRD). A phage-directed system of M13 bacteriophage exposing ZnO-binding peptides on pIII or pVIII phage coat protein produced photo luminescent wurtzite ZnONPs(Sun *et al*., 2016)

The DNA, amino acids, and proteins can also be used for the ZnONPs synthesis. Li et al. used DNA to guide the synthesis of ZnONPs chains and to control their growth. L-alanine, gelatin, and egg albumin were used for ZnONPs synthesis. Plant extracts are attractive for use in the biological synthesis of metal oxide NPs due to the presence of components such as flavonoids, terpenoids, and polysaccharides (Ambika & Sundrarajan, 2015)

Synthesis by fungus

The biological method was applied with the help of using the biologically active products of bacteria, fungi, plants, and yeasts represent the excellent sources for the production of nanoparticles. Mostly fungi were chosen instead of bacteria because of their tolerance, better metal bioaccumulation ability, and high binding capacity. There are wide applications of fungi as they produce huge enzymes, ease in the scale-up process, economic viability, and ease in handling the biomass (Bhunia *et al*., 2016). Moreover, the fungi exhibited the ability to secrete a large number of extracellular redox proteins and enzymes. As such, this contributed to the reduction of the metal ions into NPs in larger amounts, which is suitable for the large-scale production. The higher amount of protein secreted in the medium by the fungi acted as capping protein that further bound and encapsulated the NPs surface and conferred to the stability. For instance, Raliya and demonstrated the synthesis of ZnONPs by using *Aspergillus fumigates* TFR-8 that resulted in the formation of NPs with the average diameter size of 3.8 nm and high mono dispersity particles (uniformly distributed) without any agglomeration. Moreover, the authors suggested that the protein secreted by the fungi was bound and encapsulated the spherical NPs and prevented the NPs from agglomerate. Subsequently, the stability of NPs was examined for 125 days by measuring the size using particle size analyzer. The results demonstrated that NPs were stable until day 90 and the size increased thereafter due to the agglomeration. This concludes that protein could act as a capping agent to stabilize the NPs up to 90 days. Therefore, the use of fungi for the synthesis of NPs is favorable as the fungi are efficient in secreting of extracellular enzymes and protein.

Synthesis by bacteria

Numerous microbes have been exploited to synthesis ZnONPs in which bacteria are preferred due to the ease of handling and genetic manipulative attributes compared to other eukaryotic micro-organisms. The reproducible bacteria such as lactic acid bacteria (LAB) have attracted increased attention in bacteria mediated synthesis of NPs due to their non-pathogenic properties and high production of various enzymes. Moreover, LAB also recognized as the health beneficial bacteria, which are abundant in the food products (Raliya & Tarafdar, 2013) Furthermore, the LAB are facultative anaerobic bacteria that are known to have negative electro kinetic potential. This causes LAB to be easily attracted to the metal ions for the NPs synthesis under both oxidizing and reducing condition.

Synthesis by yeast

Similar to fungi, yeast has been proven to synthesize metallic NPs due to their higher tolerance to the toxic metal. A study conducted by Moghaddam et al., (Velusamy et al., 2016) demonstrated that a new isolated *Pichia kudriavzevii* yeast strain was able to synthesize ZnO NPs with10–61 nm of the size range of NPs produced. The formation of NPs was reported to depend on the reaction duration, which was found to play an important role in the size, shape and distribution of ZnO NPs.The microbes mediated synthesis of ZnO NPs seems to be eco-friendly and safe as it does not involve the use of any toxic and hazardous chemical in the synthesis process. In addition, the biologically active compounds secreted by the microbes were acted as a reducingandcappingagent.Thus, this approach is more advantageous than the conventional methods. Furthermore, fungal mediated synthesis seems to be a promising candidate for the synthesis as it produces more biologically active compounds than the other microbes. Nevertheless, in term of the cell's growth activity, the bacteria are promising compared to the other alternatives. Moreover, the mechanisms of biological synthesis of ZnONPs among the microbes are different and are not fully understood yet, hence, further investigation is needed.

Synthesis by plant

The synthesis of nanoparticles by using plant extracts is beneficial in comparison with microorganisms because it eradicates the difficult process of preserving cell cultures and it can be effectively scaled up for synthesis of nanoparticles at large scale.Zinc oxide nanoparticles (ZnONPs) had been synthesised by a green method using aqueous extract of *Cannabis sativa* leaves. A facile, eco-friendly, time saving and beneficial procedure for the preparation of metal nanoparticles had been developed.Common name of *Cannabis sativa* plant is Hemp, Marijuana and belongs to Cannabidaceae family. *Cannabis sativa* is an annual herbaceous flowering plant (JiangH, 2006). The presence of certain important phytochemicals, i.e.,cannabinoids, tetrahydrocannabinol (THC),terpenes, flavonoids, nitrogenous compounds, glycoproteins, amino acids and simple alcohols were reported in this plant. These phytochemicals play important role in the formation, biological reduction and stabilization of metal NPs. Furthermore, phenolic compounds like flavonoids, tannins and phenolic acids are considered to be involved in antioxidant or reducing activities of medicinal plants. Due to redox activities of the phenolic compounds, they act as singlet oxygen quenchers, reducing agents, hydrogen donators and as metal chelating agents (Yen & Hsieh, 1998) .ZnO NPs have attracted great attention because of their superior optical properties. Visual color change dark brown to pale yellow represents the preliminary test for synthesis of ZnONPs. The optical density of sample was measured through UV-Vis spectrophotometry to observe the reduction of zinc ions. The basic absorption peak was appeared in the wavelength range of 380 nm confirmed.

Characterization of ZnONPs

Characterization tools are necessary to identify the properties of engineered nanomaterials. ZnO NPs include XRD, SEM, TEM, and BET analysis.

X -Ray differaction (XRD)

X-ray diffraction is a well-established technique for analyzing the size, shape, and crystal structures of inorganic, carbon-based, or complex crystalline materials. It offers

high spatial resolution at the atomic scale, but it is limited to crystalline materials (Rossi *et al*., 2014)

Scanning electron microscopy (SEM)

Scanning electron microscopy is a high-resolution method for estimating size, size distribution, shape, aggregation, dispersion (cryo-SEM), and crystallinity (electron backscattering detection) (Khalaj *et al*., 2016). It may be used to analyze inorganic, organic, carbon-based, biological, and complex materials. Scanning electron microscopy is limited to the analysis of conductive or coated materials under non-physiological conditions. Various ZnO NP shapes have been reported from SEM analyses, including spheres and rods.

Transmission electron microscopy (TEM)

Transmission electron microscopy measures size and size distribution and confirms the nanomaterial shapes with higher resolution compared to SEM. Aggregation, dispersion (environmental TEM), and crystal structure can also be determined by TEM. It can be used to visualize inorganic, organic, carbon-based, biological, and complex materials as spherical and equiaxial particles, tubes, flakes, rods, or fibers. The TEM technique is extensively used to determine the size, size distribution, and morphology of ZnO NPs based on the stabilizer (glycerol)-to-zinc source ratios during the synthesis. TEM to demonstrate the nucleation and growth of ZnO NPs.(Lee & Hyeon, 2012)

Brunauerемmett teller (BET)

Emmett-Teller analysis provides the specific surface area and porosity of spherical and equiaxial particles of inorganic, carbon-based, and complex materials. BET cannot distinguish between particles and nonparticulate porous materials. This technique is limited to the analysis of volatile compound-free materials.

Fourier transform infrared (FTIR)

Fourier transform infrared spectroscopy (FTIR) can provide fundamental information on the molecular structure of organic and inorganic components, and is one of the most

versatile analytical techniques for the non-destructive, chemical characterization of geological samples, such as coal, shale, fluid and melt inclusions, silicate glass, minerals, and microfossils .(Chowdhury, 2016)

Dynamic light scattering (DLS)

DLS also called photon-correlation spectroscopy, which is a widely popular and highly adaptable analytical method applied in different fields of life and material sciences, as well as in industrial quality control processes. Dynamic light scattering (DLS) has become an indispensable technique of size determination for diverse materials: from sub-micron particles to protein formulations (D'Mello *et al*., 2017). DLS is non-invasive, requires little sample preparation as well as small sample amounts. The quality of a measurement depends on instrument hardware, as well on the properties of the sample, such as size, contrast, concentration, and the presence of impurities.

Zeta potential

The zeta potential (ZP) is a function of the surface charge which develops when any material is placed in a liquid. Colloidal particles dispersed in a solution are electrically charged due to their ionic characteristics and dipolar attributes. Each particle dispersed in a solution is surrounded by oppositely charged ions called the fixed layer. Outside the fixed layer, there are varying compositions of ions of opposite polarities, forming a cloud- like area. This area is called the fixed layer. Outside the fixed layer, there are varying compositions of ions of opposite polarities, forming a cloud-like area. This area is called the diffuse double layer, and the whole area is electrically neutral. When a voltage is applied to the solution in which particles are dispersed, particles are attracted to the electrode of the opposite polarity, accompanied by the fixed layer and part of the diffuse double layer, or internal side of the" sliding surface". Zeta potential is considered to be the electric potential of this inner area including the conceptual "sliding surface". As these electric potential approaches zero particles tend to aggregate. The ZP is used to predict and control dispersion stability.

UV-VIS Spectroscopy

Absorption spectroscopy in the visible region has long been an important tool to the analyst.Color transition arise due to molecular and structural changes in the ability to absorb light in the visible region of the electromagnetic spectrum. Appearance of color arises from the property of the colored material to absorb selectively within the visible region of the electromagnetic spectrum. Absorption of energy leads to a transition of electron from ground state to excited state.

Applications of ZnONPS

- **Biomedical applications**

  Zinc oxide nanoparticles have been studied for biomedical applications because they have shown anticancer, antidiabetic, antimicrobial, anti-inflammatory, and wound healing activities. They have also been used in imaging agents and biosensors. Various three-dimensional structures of ZnONPs and aggregates affect biomedical activity by modulating the surface characteristics of the hierarchically porous architectures (Jiang *et al*., 2018).

- **Medicinal uses of ZnONPs**

  ZnONPs play some potential role in CNS and perhaps during development processes of diseases through mediating neuronal excitability or even release of neurotransmitters. Some studies have indicated that ZnONPs affected functions of different cells or tissues, biocompatibility, and neural tissue engineering (Rasmussen *et al*., 2010) but little information is present about the influence on CNS and CNS related diseases.

- **Role of ZnONPs in agriculture**

  Agriculture is backbone of third world economics but unfortunately now, the agriculture sector is facing various global challenges like climate changes, urbanization, sustainable use of resources, and environmental issues such as runoff, accumulation of pesticides and fertilizers; human population is increasing

day by day and food demand is growing rapidly and estimated population increase in world from current level of 6 billion to 9 billion by 2050 is expected. So, we must adopt efficient techniques to make agriculture more sustainable.

Nanotechnology has a dominant position in transforming agriculture and food production. Nanotechnology has a great potential to modify conventional agricultural practices. Most of the agrochemicals applied to the crops are lost and do not reach the target site due to several factors including leaching, drifting, hydrolysis, photolysis, and microbial degradation. Nanoparticles and nano capsules provide an efficient means to distribute pesticides and fertilizers in a controlled fashion with high site specificity thus reducing collateral damage. Zinc oxide nanoparticles have potential to boost the yield and growth of food crops. Peanut seeds were treated with different concentrations of zinc oxide nanoparticles. Zinc oxide nanoscale treatment (25 nm mean particle size) at 1000 ppm concentration was used which promoted seed germination, seedling vigor, and plant growth and these zinc oxide nanoparticles also proved to be effective in increasing stem and root growth in peanuts (Prasad *et al*., 2012).

Anticancer activity of zinc oxide nanoparticles

A ZnO nanoparticles, as a wide band-gap semiconductor, can readily absorb UV rays. Owing to this property, ZnO nanoparticles have a wide range of application, from electronic devices, cosmetics and facial products to bio- medical application. ZnO nanoparticles are now being widely researched for their ant cancerous properties. Zinc is a silver-grey-colored transition metal with oxidation state +2, having five stable isotopes. It is the most important and abundant trace element in the body after iron. The total body zinc content has been estimated to be 30 mM (2g). It is required in the human diet in trace quantities, which is approximately 15 mg Zn per day (King & Turnlund, 1989). It is present in all body tissues and fluid. Zinc plays a major role in the immune system, affecting a number of aspects of humoral and cellular immunity. Zinc has a role in regulating a large number of enzymes present in the body, which later participate in synthesis and degradation of carbohydrates, lipids, proteins and nucleic acid, as well as in metabolism of other micronutrients, thus playing a crucial role in maintaining proper body condition and homeostasis.Zinc is the co-factor of over 300 mammalian enzymes

and plays a vital role in host defence against the initiation and progression of cancer. The tumor suppressor p53 gene and caspase enzyme help to check cells regularly and prevent them from becoming cancerous. If a cell shows any kind of malignancy, a DNA repair mechanism is activated to repair the altered DNA. If this mechanism fails to repair the DNA, then the cell undergoes ‘programmed cell death’, known as apoptosis, to prevent the altered cell from dividing, which may later develop in the cancerous cell. In one way or another, zinc is involved in all these processes of protecting cells against cancer.

The basic mechanism behind the cytotoxicity of ZnO NPs is the intracellular release of dissolved zinc ions, followed by ROS induction. This event causes zinc-mediated protein activity disequilibrium and oxidative stress, eventually killing the cell. Soluble extracellular zinc shows very little cytotoxicity. Recent research shows that extracellular soluble zinc, when exposed to cell culture and media, forms poorly soluble amorphous zinc-carbonate phosphate precipitates (phosphate from media). This precipitate is supposed to protect the cell from the cytotoxicity of zinc (Turney *et al*., 2012). On the other hand, with the release of soluble zinc ions inside the cell, a cascade of pathways interrelated to each other takes place, which is responsible for the cytotoxic response of the ZnO nanoparticles.

ROS, a reactive species of molecular oxygen, is produced inside the cell during various cellular processes, including mitochondrial respiration, inflammatory response, microsome activity, peroxisome activity, etc. It acts as a biomolecule and plays an important role in cell signaling and homeostasis. Exogenously, ROS is induced in response to various stimuli including nanomaterials (Manke *et al*., 2013). ROS is induced by ZnO NPs in two ways. One is due to the pro inflammatory response of the cell against nanoparticles and the other is due to the characteristic surface property of ZnO NPs that makes them a redox reaction system producing ROS. The ability to induce oxidative stress through ROS generation by ZnO nanoparticles is due to its semiconductor properties. Holes (h+) act as a powerful oxidant, breaking water molecules into hydrogen and hydroxyl ions. Similarly, electrons act as a powerful reducer, reacting with adsorbed and dissolved oxygen molecules, generating

superoxide radical anions (O2 −). These superoxide radical anions further react with hydrogen ions, producing HO2 − radicals, which further react to create H_2O_2. All these radicals are a highly reactive oxygen species known as ROS, which acts as a strong oxidizing agent. Accumulation of these species in great amounts leads to a misbalance in the oxidative reductive homeostasis of the cell, leading to oxidative stress, which is very harmful to the cell and eventually causes cell death. Thus, holes and electrons in ZnO nanoparticles act as a redox reaction system, producing a reactive oxygen species and thus increasing oxidative stress in the cell(Rasmussen *et al*., 2010)With elevated levels of ROS and oxidative stress, ZnO NPs show a deleterious effect on the lipid, protein and nucleic acid of the cell. Elevated ROS can cause membrane damage through lipid peroxidation and protein denaturation, resulting in cell death by necrosis and DNA damage, resulting in cell death by apoptosis. These DNA breakages and crosslinks damage DNA, leading to the activation of a mitochondrial apoptotic pathway, eventually causing cell death by apoptosis (Ng *et al*., 2011). Apoptosis, a programmed cell death, is believed to be the major mechanism of cell death in this cytotoxic response of ZnONPs.

OBJECTIVE

OBJECTIVES

I. Green synthesis of zinc oxide nanoparticles by using *Cannabis sativa* leaf extract.

II. Characterization of zinc oxidenanoparticles byUV-Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential.

III. Assessment of the efficacy of zinc oxide nanoparticles against cervical cancer HeLa cells.

MATERIAL AND METHOD

MATERIAL AND METHODS

Material and Methods

HiMedia, 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 kindly gifted from Dr. Maqbool Ahmad Khan Deputy Director of CCRMU, Kursi Road, Basaha, Lucknow (226026)

Healthy green leaves were collected rinsed properly with the still 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 molded 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 Whatman 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 Zinc oxide nanoparticles

In vitro synthesis of ZnONPs was performed by 3 ml of the prepared plant extract was taken in 20 ml of centrifuge tube and 3mM of zinc sulphate salt was added to the plant extract and volume makeup with water. Keep the reaction tube in incubator at about 37°C. After 48hthe different period the sample was ejected and analyzed on a bio spectrum-Kinetic spectrophotometer using a quartz cuvette having the path length of 1 cm to confirm the synthesis of *Cannabis sativa* encapsulated zinc 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 Zinc oxide nanoparticles

The transformation of zinc sulphate into zinc oxide 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 zinc oxide nanoparticles result in color change. Particle size analyser (Zetasizer Nano-ZS, Model ZEN3600, Malvern Instrument Ltd., Malvern, UK) was used to analyze the mean particle size of ZnONPs. 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-ZnONPs 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.

Measurement of morphological changes in HeLa cells

HeLa cells were pre-treated with different concentrations of each, CNB- Extract, CNB-ZnONPs 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, Thermofisher, USA).

Assessment of cytotoxicity

To assess the cytotoxic effect of CNB-extract and CNB-ZnONPs, HeLa cells were placed in 96-well plate with density of 1×10^4 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, ZnONPs 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:

$$\% \text{ Cell viability} = \frac{¼ \text{ Absorbance of treated cells} - \text{Absorbance of blank}}{\text{Absorbance of untreated cells} - \text{Absorbance of blank} \times 10028}$$

RESULT AND DISSCUSSION

Result and discussion

Cannabis sativa-Mediated synthesis of ZnONPs (CNB-ZnONPs)

This study used *Cannabis sativa* leaf extract as a reducing and capping agent, whereas 3mM zinc sulphate ($ZnSO_4$) served as the zinc precursor. The synthesis of CNB-ZnONPs is considered to be induced by the aqueous extracts reducing enzymes and capping agents, such as secondary metabolites. The creation of CNB-ZnONPs was confirmed visually by a shift in the color of the extract from green to colorless, indicating zinc reduction.

Characterization of CNB-ZnONPs

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.The Phyto constitution in *Cannabis sativa* leaf extract reduced the zinc sulphate ($ZnSO_4$) into ZnONPs and encapsulated the zinc oxide nanoparticle preventing the nanoparticles from the aggregating and providing stability to the CNB-ZnONPs. The change in color from light green to colorless indicated the successful synthesis of CNB-ZnONPs and the result of SPR band confirm that at 378 and however there was no discernible peek for cannabis sativa peel 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-ZnO had an average particle size of 62.54 d nmas shown in figure. Furthermore, the Zeta potential of the prepared CNB-ZnONPs was observed at the room temperature, to be a -18.7mV, indicating the significantly high stability of the nanoparticles. When the aqueous dispersion of ZnONPs was observed at room temperature no clumping or accumulation was observed. This was most likely due to the zinc and a particle electrostatic repulsive effect. The nanoparticles are prevented from colliding because of this repulsion.

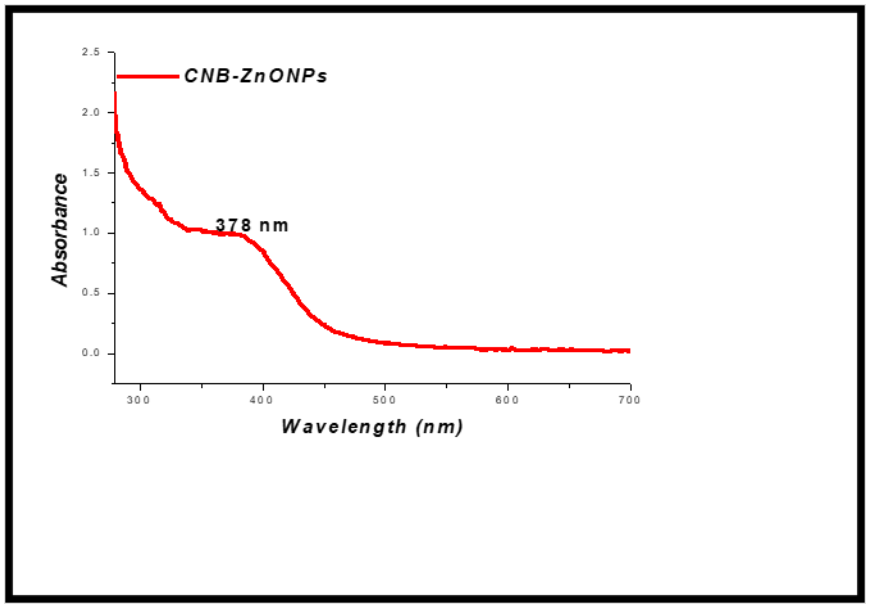

FIGURE-3:-. Characterisation of ZnONPs under UV-Visible spectra (378 nm)

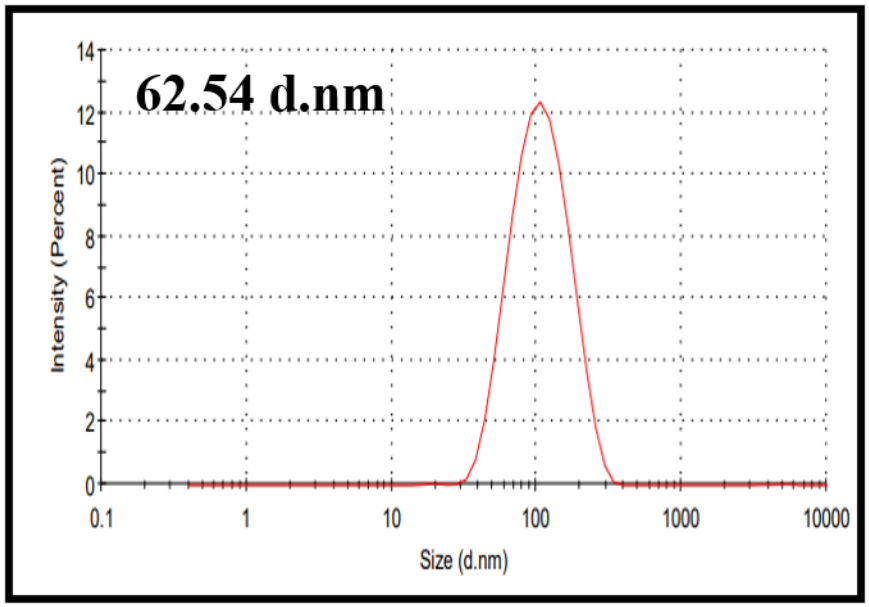

FIGURE-4 : DLS profile of ZnONPs showing size of 62.54 d.nm.

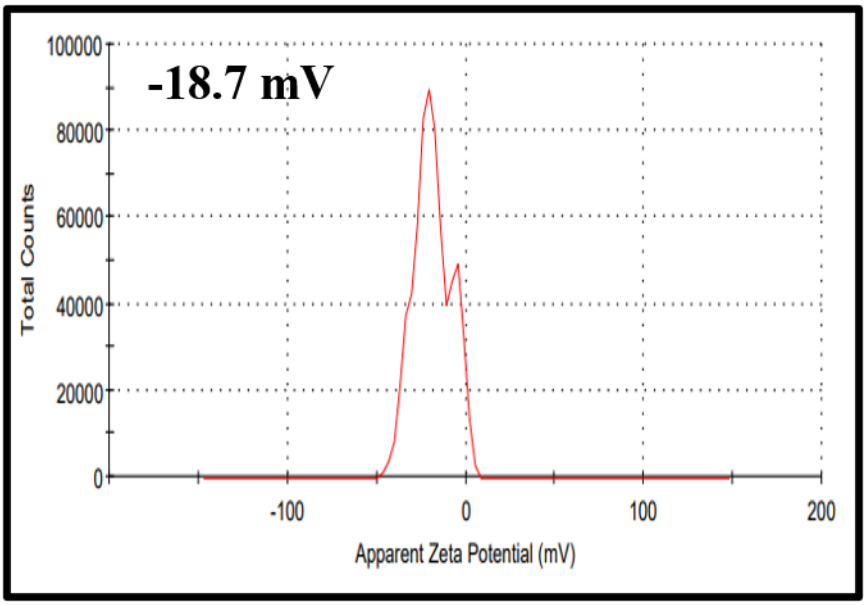

FIGURE -5: Zeta potential of ZnONPs confirmed the stability at -18.7 mV

Determination of morphological alteration in the HeLa cells

Morphological analysis of the CNB- extract and CNB-ZnONPs, 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 (100µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) and CNB-ZnONPs, (5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml) concentrations for 24 h. In the presence of different doses CNB- extract and CNB-ZnONPs 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 ZnONPs. In contrast, well spread flattened morphology was observed in untreated control cells.

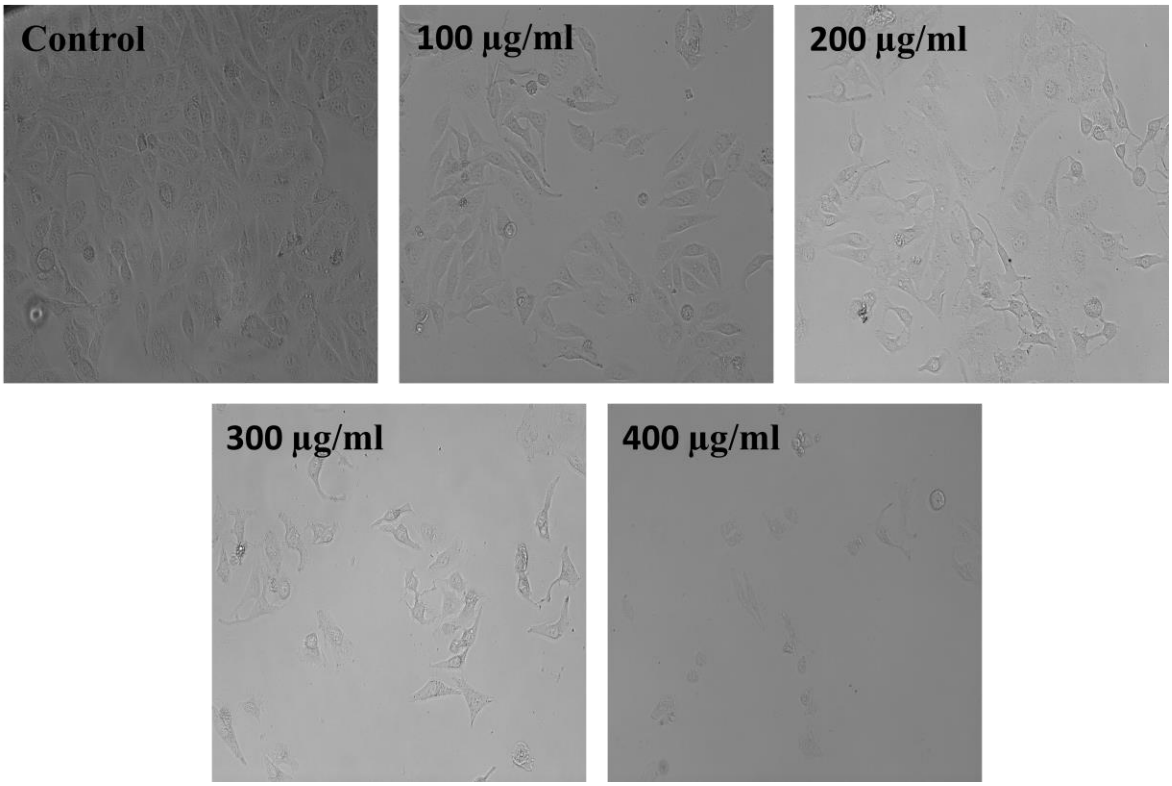

FIGURE-6: - The phase contrast microscopy of HeLa ell treated with either vehicle control or different concentrations (100µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml) of CNB-extract for 24hours in a time and dose-dependent manner .Images shown are representative of three independent experiments (Scale bar:100µm; Magnification: 20X)

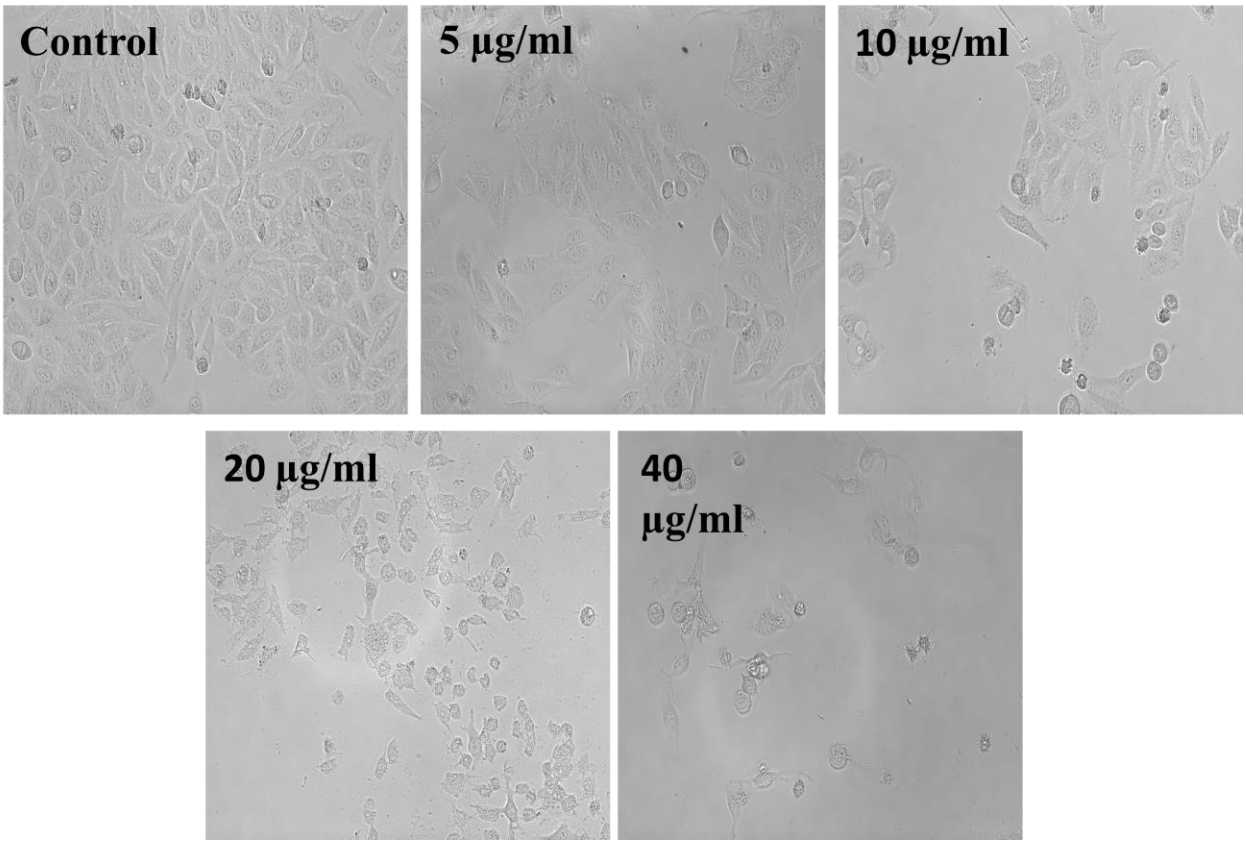

FIGURE-7: - The phase contrast microscopy of HeLa ell treated with either vehicle control or different concentrations (5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml)of CNB-ZnONPs for 24hours in a time and dose dependent manner. . Images are shown are representative of three independent experiments (Scale bar:100µm; Magnification:20X)

In vitro cytotoxicity of CNB- extract and CNB-ZnONPs

To evaluate the sensitivity of lung cancer cells to these drugs, HeLa cells were treated with different doses of CNB- extract and CNB-ZnONPs for 24 h followed by MTT assay. Our results showed that, after 24 h of treatment, ZnONPs at concentration of IC50=150.7±1.14µg/ml reduced growth of HeLa cells by 50%, while inhibition of 50% viability of HeLa was observed at concentration ofIC50=7.833±1.11µg/ml CNB-ZnONPs, respectively. ZnONPs, 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.

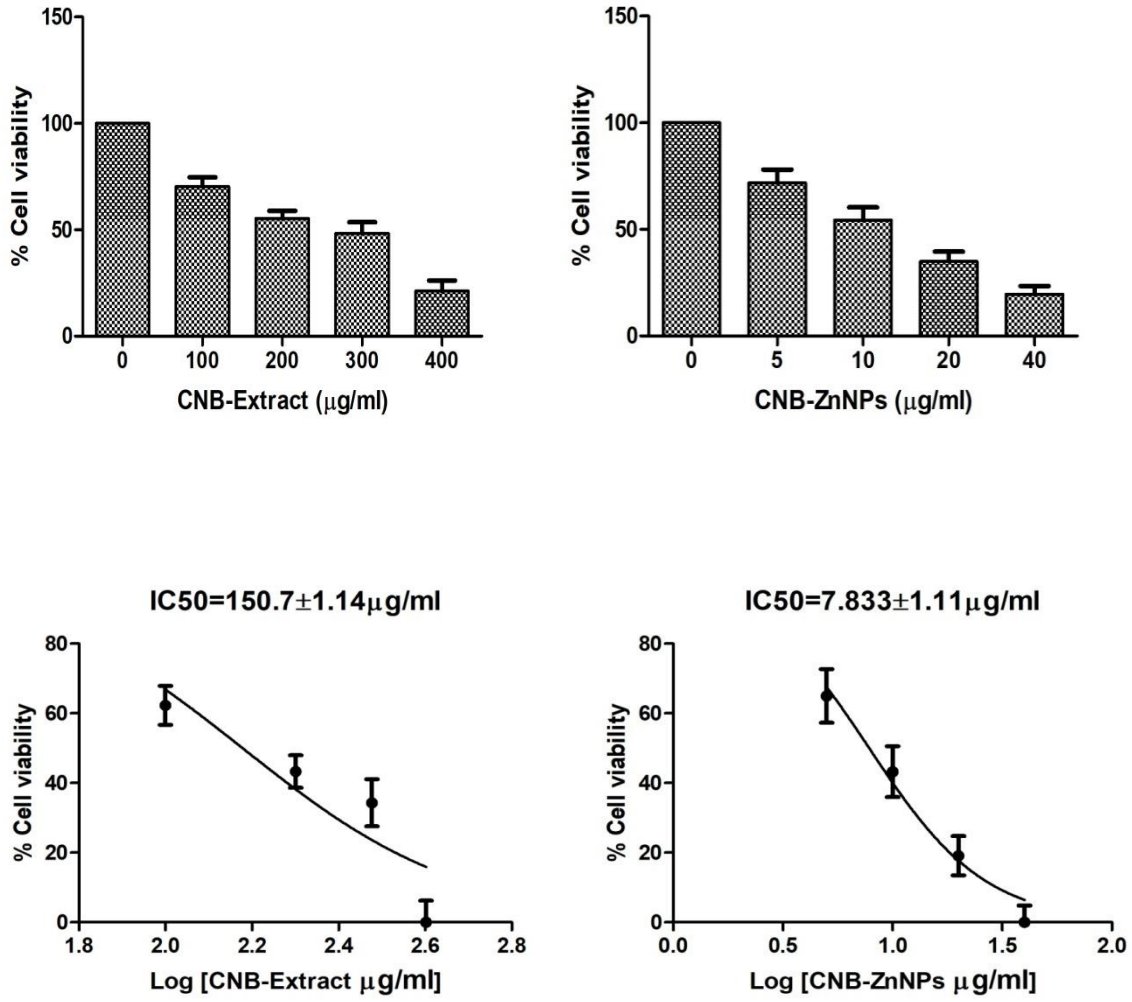

FIG-9: Percent cell viability of HeLa cells with different concentration of Cannabis sativa (100-400µg/ml) assessed by MTT Assay 24 h. Graph showed that Cannabis sativa exhibited IC50 value 150.7±1.17µg/ml and 7.833±1.11µg/ml at 24 h, against HeLa cervical cancer cells. The result represented are the mean ±SEM of three independent experiment performed in triplicate.

CONCLUSION

CONCLUSION

In this study we showed a Leaf extract mediated green synthesis of zinc nanoparticles from *Cannabis sativa*plant and their characterization, anticancer and antioxidant property analysis. This study investigates an efficient and sustainable route of ZnONP preparation from 3mM aqueous $ZnSO_4$ using leaf extracts of *Cannabis sativa*plants. The ZnONPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS) and Zeta potential.

This study comprehensively addressed synthesis, characterization, and bio-applications of zinc nanoparticles, with special emphasis on anticancer and antioxidant activity and also therapeutic approaches for cancer using ZnONPs. Recently, both academic and industrial research has explored the possibility of using ZnONPs as a next-generation anticancer therapeutic agent, due to the conventional side effects of chemo- and radiation therapy. Although ZnONPs 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 ZnONPs 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 ZnONPs 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 ZnONPs. 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 ZnONPs. Eventually, to ensure the biosafety of the use of ZnONPs in humans, studies dealing with biocompatibility of ZnONPs 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.

REFERENCE

REFERENCES

Ai J., Biazar E., Jafarpour M., Montazeri M., Majdi A., Aminifard S., Zafari M., Akbari H.R., Rad H.G. Nanotoxicology and nanoparticle safety in biomedical designs. Int. J. Nanomed. 2011;6:1117–1127.

Ambika S., Sundrarajan M. Green biosynthesis of ZnO nanoparticles using Vitex negundo L. extract: Spectroscopic investigation of interaction between ZnO nanoparticles and human serum albumin. J. Photochem. Photobiol. B. 2015;149 doi: 10.1016/j.jphotobiol.2015.05.004.

Andreescu S., Ornatska M., Erlichman J. S., Estevez A., Leiter J. C. *Fine Particles in Medicine and Pharmacy*. Boston, MA: Springer; 2012. Biomedical applications of metal oxide nanoparticles; pp. 57–100.

Appenzeller T. The man who dared to think small. Science. 1991;254:1300.

Bhunia A., Kamilya T., Saha S. Optical and Structural Properties of Protein Capped ZnO Nanoparticles and Its Antimicrobial Activity. J. Adv. Biol. Biotechnol. 2016;10:1–9. doi: 10.9734/JABB/2016/29626.

Bosetti R, Marneffe W, Vereeck L. Assessing the need for quality-adjusted cost–effectiveness studies of nanotechnological cancer therapies. *Nanomedicine*. 2013;8(3):487–497.

Briggs BD, Knecht MR. Nanotechnology meets biology: peptide-based methods for the fabrication of functional materials. *J. Phys. Chem. Lett*. 2012;3(3):405–418.

Chang Dr I. Plasma synthesis of metal nanopowders. Adv. Powder Metall. Prop. Process. Appl. 2013:69–85. doi: 10.1533/9780857098900.1.69.

Chen P. C., Mwakwari S. C., Oyelere A. K. Gold nanoparticles: from nanomedicine to nanosensing. *Nanotechnology, Science and Applications*. 2008;Volume 1:45–66. doi: 102147/nsa.s3707.

Chowdhury EH. Nanotherapeutics: From Laboratory to Clinic. Boca Raton, FL: CRC Press; 2016.

D'Mello SR, Cruz CN, Chen ML, Kapoor M, Lee SL, Tyner KM 2017. The evolving landscape of drug products containing nanomaterials in the United States. Nat Nanotechnol 12(6):523–529.

El-Sayed I. H., Huang X., El-Sayed M. A. Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Letters*. 2005;5(5):829–834. doi: 10.1021/nl050074e.

Eric DK. *Engines of Creation. The Coming Era of Nanotechnology*. Doubleday; NY, USA: 1986.

Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomed. Nanotechnol. Biol. Med.* 2013;9(1):1–14.

G.C. Yen and C.L. Hsieh, J. Agric. Food. Chem 46, 3952 (1998). doi:10.1021/jf9800458.

Gao H., Shi W., Freund L. B. From the cover: mechanics of receptor-mediated endocytosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102(27):9469–9474. doi: 10.1073/pnas.0503879102.

H.E. Jiang, X. Li, Y.X. Zhao, D.K. Ferguson, F. Hueber, S. Bera, Y.F. Wang, I.C. Zhao, C.J. Li and C.S. Li, J. Ethnopharmacol 108, 414 (2006). doi:10.1016/j.jep.2006.05.034.

Iyer R, Hsia CC, Nguyen KT. Nano-therapeutics for the lung: state-of-the-art and future perspectives. *Curr. Pharm. Des.* 2015;21(36):5233–5244.

Jiang J., Pi J., Cai J. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. Bioinorg. Chem. Appl. 2018;2018:18. doi: 10.1155/2018/1062562.

Karimi M, Mirshekari H, Aliakbari M, Sahandi-Zangabad P, Hamblin MR. Smart mesoporous silica nanoparticles for controlled-release drug delivery. *Nanotechnol. Rev.* 2016;5(2):195–207.

Khalaj MJ, Ahmadi H, Lesankhosh R, Khalaj G. Study of physical and mechanical properties of polypropylene nanocomposites for food packaging application: Nano-clay modified with iron nanoparticles. Trends Food Sci Tech. 2016;51:41–48.

Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. NanomedNanotechnolBiol Med. 2007;3:95–101. doi: 10.1016/j.nano.2006.12.001.

King J, Turnlund J. Human Zinc Requirements. In: Mills C, editor. Zinc in Human Biology. ILSI Human Nutrition Reviews: Springer London; 1989. p. 335-50.

Krupiński P., Kornowicz A., Sokołowski K., Cieślak A.M., Lewiński J. Applying Mechanochemistry for Bottom-Up Synthesis and Host–Guest Surface Modification of Semiconducting Nanocrystals: A Case of Water-Soluble β-Cyclodextrin-Coated Zinc Oxide. Chem. Eur. J. 2016;22:7817–7823. doi: 10.1002/chem.201600182.

Langer R, Weissleder R. Scientific discovery and the future of medicine. *JAMA*. 2015;313:135–136.

Lee N, Hyeon T. 2012. Designed synthesis of uniformly sized iron oxide nanoparticles for efficient magnetic resonance imaging contrast agents. Chem. Soc. Rev. 41, 2575–2589.

Lellouche J., Kahana E., Elias S., Gedanken A., Banin E. Antibiofilm activity of nanosized magnesium fluoride. Biomaterials. 2009;30:5969–5978.

Manke A, Wang L, Rojanasakul Y. Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxici- ty. BioMed Research International. 2013;2013:15.

Newman M. D., Stotland M., Ellis J. I. The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. *Journal of the American Academy of Dermatology*. 2009;61(4):685–692. doi: 10.1016/j.jaad.2009.02.051.

Ng KW, Khoo SP, Heng BC, Setyawati MI, Tan EC, Zhao X, et al. The role of the tumor suppressor p53 pathway in the cellular DNA damage response to zinc oxide nanoparticles. Biomaterials. 2011;32(32): 8218-25.

Nie S, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. Annu Rev Biomed Eng. 2007;9:257–288.

Oliveira A.P.A., Hochepied J.-F., Grillon F., Berger M.-H. Controlled Precipitation of Zinc Oxide Particles at Room Temperature. Chem. Mater. 2003;15:3202–3207. doi: 10.1021/cm0213725.

Oskam G. Metal oxide nanoparticles: synthesis, characterization and application. J Sol–gel Sci Technol. 2006;37:161–164. doi: 10.1007/s10971-005-6621-2.

Panda V., Ashar H., Srinath S. Antioxidant and hepatoprotective effect of *Garcinia indica* fruit rind in ethanol induced hepatic damage in rodents. Interdiscip. Toxicol. 2012;5:207–213.

Raja A., Ashokkumar S., PavithraMarthandam R., Jayachandiran J., Khatiwada C.P., Kaviyarasu K., Ganapathi Raman R., Swaminathan M. Eco-friendly preparation

of zinc oxide nanoparticles using Tabernaemontanadivaricata and its photocatalytic and antimicrobial activity. J. Photochem. Photobiol. B. 2018;181:53–58. doi: 10.1016/j.jphotobiol.2018.02.011.

Raliya Ramesh, Tarafdar J. C. ZnO Nanoparticle Biosynthesis and Its Effect on Phosphorous-Mobilizing Enzyme Secretion and Gum Contents in Clusterbean (Cyamopsistetragonoloba L.) Agricultural Research. 2013;2(1):48–57. doi: 10.1007/s40003-012-0049-z.

Rasmussen J. W., Martinez E., Louka P., Wingett D. G. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opinion on Drug Delivery*. 2010;7(9):1063–1077. doi: 10.1517/17425247.2010.502560.

Rossi M., Cubadda F., Dini L., Terranova M. L., Aureli F., Sorbo A., et al. (2014). Scientific basis of nanotechnology, implications for the food sector and future trends. Trends Food Sci. Technol. 40 127–148. 10.1016/j.tifs.2014.09.004

Sahoo S., Maiti M., Ganguly A., George J. J., Bhowmick A. K. Effect of zinc oxide nanoparticles as cure activator on the properties of natural rubber and nitrile rubber. *Journal of Applied Polymer Science*. 2007;105(4):2407–2415. doi: 10.1002/app.26296.

Saravanan A., Huang B., Kathiravan D., Prasannan A. Natural Biowaste-Cocoons Derived Granular Activated Carbon-Coated ZnO Nanorods: A Simple Route to Synthesis Core-Shell Structure and Their Highly Enhanced UV and Hydrogen Sensing Properties. ACS Appl. Mater. Interfaces. 2017:9. doi: 10.1021/acsami.7b11051.

Sayes CM, Aquino GV, Hickey AJ. Nanomaterial drug products: manufacturing and analytical perspectives. *AAPS J.* 2017;19(1):18–25.

Schrofel A., Kratosova G., Safarik I., Safarikova M., Raska I., Shor L.M. Applications of biosynthesized metallic nanoparticles—A review. ActaBiomater. 2014;10:4023–4042. doi: 10.1016/j.actbio.2014.05.022.

Simona L.I., Prodan A.M., Coustumer P.L., Predoi D. Synthesis and antibacterial and antibiofilm activity of iron oxide glycerol nanoparticles obtained by co-precipitation method. J. Chem. 2013;Volume:6. (Article ID 412079)

Singh H., Du J., Singh P., Yi T.H. Ecofriendly synthesis of silver and gold nanoparticles by *Euphrasia officinalis* leaf extract and its biomedical applications. Artif. Cells Nanomed. Biotechnol. 2018;46:1163–1170. doi: 10.1080/21691401.2017.1362417.

Singh P., Kim Y.J., Wang C., Mathiyalagan R., El-AgamyFarh M., Yang D.C. Biogenic silver and gold nanoparticles synthesized using red ginseng root extract, and their applications. Artif. Cells Nanomed. Biotechnol. 2016;44:811–816. doi: 10.3109/21691401.2015.1008514.

Sirelkhatim A., Mahmud S., Seeni A., Kaus N.M., Ann L.C., Bakhori S.M., Hasan H., Mohamad D. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. J. Nano-Micro Lett. 2015;7(3):219–242.

Thareja R.K., Shukla S. Synthesis and characterization of zinc oxide nanoparticles by laser ablation of zinc in liquid. Appl. Surf. Sci. 2007;253:8889–8895. doi: 10.1016/j.apsusc.2007.04.088.

Velusamy P, Kumar GV, Jeyanthi V, Das J, Pachaiappan R. Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application. Toxicol Res. 2016;32:95–102. doi: 10.5487/TR.2016.32.2.095.

Żelechowska K., Karczewska-Golec J., Karczewski J., Łoś M., Kłonkowski A.M., Węgrzyn G., Golec P. Phage-Directed Synthesis of Photoluminescent Zinc Oxide Nanoparticles under Benign Conditions. Bioconjug. Chem. 2016;27:1999–2006. doi: 10.1021/acs.bioconjchem.6b00196.

Zhang G., Wang D.J. Fabrication of heterogeneous binary arrays of nanoparticles via colloidal lithography. J. Am. Chem. Soc. 2008;130:5616–5617. doi: 10.1021/ja710771j.

Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC. Nanoparticles in medicine: Therapeutic applications and developments. Clin

Zhang Z. Y., Xiong H. M. Photoluminescent ZnO nanoparticles and their biological applications. *Materials*. 2015;8(6):31013127.doi:10.3390/ma8063101.