# *Chapter VI*

**CHAPTER – VI**

# CELL VIABILITY ANALYSIS OF PURE & DOPED COBALT OXIDE NANOPARTICLES

## 6.1 Introduction

Nanomaterials are special in view of their many characteristics and the most important one is its dimension. Nanostructures were 10,000 times lower than width of human hair [1]. They are potential materials for various practical applications [2]. Nanobiomedicine with its advances is widely used in numerous fields of nanomedicine fields [3]. In recent days, nanobiomedicine has given rise to numerous developments in chemotherapy, hyperthermia & radiotherapy that cause clinical variations [4-8]. Arrival of nanostructures which can cause merging of therapeutics & diagnostics can change cancer therapeutics & control [9]. Among the entire area of nano biomedical applications, concentrated and targeted nanoparticles occupy an important position in view of their specific physical and chemical parameters [10]. The nanocarriers facilitate focused transport of drugs and show lower cytotoxicity on another cell that renders them highly beneficial. One of fundamental reasons of the boom in efficacy of the nana platforms is the ability to protect medicines from degeneration which reduces unwanted toxicity towards normal tissues [11].

Nanomedicine is the department of drugs which applies information & tools of nanotechnology to prevent and remedy for disorder [12]. Nanomedicine entails usage of nano substances, along with biocompatible nanoparticles & nanorobots, for diagnosis, delivery, sensing/actuation purposes in residing creature. Encapsulation of biomolecules via those strategies allows us to keep away from the degradation of the free protein and permits the prolonged and steady launch of a protein drug in steady time [13]. Nanomaterials by their targeting skills & advanced efficacy, becomes highly significant in current cancer remedy & beginning to overshadow conventional cancer treatments along with chemotherapy radiation & surgical treatment [14].

Nanotechnology had given direct straight entry to cancerous cells selectively by elevated drug localization & cellular uptake [15]. Nanoparticles are designed to identify cancerous cells providing selective & precise medicinal transport warding off interplay

with the healthful cells [16]. The nanoparticles play powerful free radical scavengers & will do anti-inflammatory activities [17]. Those nanoparticle antioxidants also can supply chance for counteract pathogenicity of microbes & their biofilm creation. The promotions in nano research give modern research tools, materials, structures & alertness in nanomedicine [18].

Cancer nanotechnology is a department of nanotechnology associated with the utility of nanomaterials (collectively with nanoparticles for tumour imaging or drug delivery) and nanotechnology (which includes nanoparticle primarily based theranostics) procedures for the prognosis and treatment of cancer [19]. Fig 6.1 shows the stepwise conversion of normal cells to malignant tumor through initiation, promotion and progression.

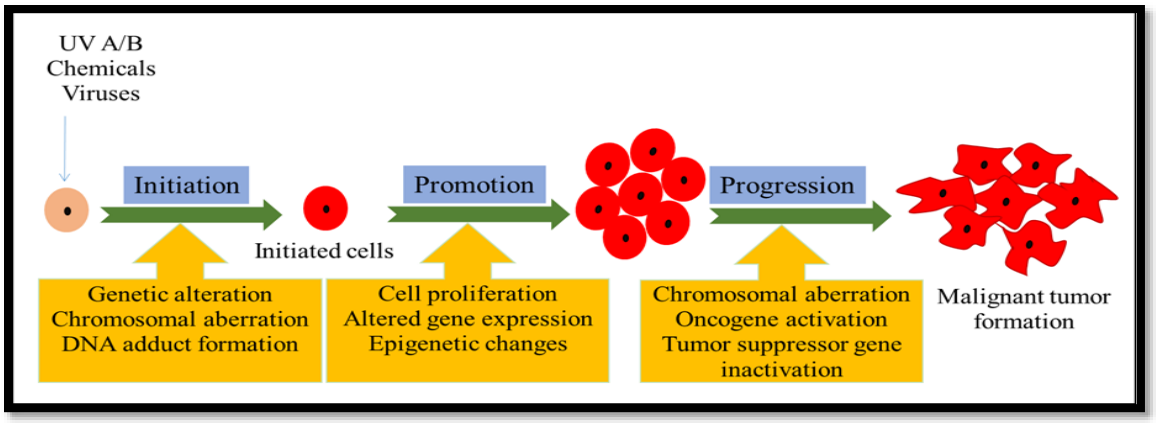


**Figure: 6.1. Stepwise conversion of normal cells to malignant tumor through initiation, promotion and progression step.**

The primary benefit of nanoparticles is the high biodistribution in the organism as compared with other drug shipping structures. Nanoparticles made from natural and artificial polymers (biodegradable and non-biodegradable) have acquired more attention because they can be custom designed for focused delivery of medication, enhanced bioavailability, and offer a managed release of drugs from a single dose, via adaptation of the system [20]. Massive surface region additionally had huge accordance for drugs & small molecules such as ligands or antibodies, targeted & regulated launch functions [21]. Nanocarrier systems adsorbs a medicine, a healing gene molecule on their surface & additionally joins selected pointing molecule to floor of nanocarrier & bound to cellular surface specific receptor by targeting molecule [22].

In the biomedical applications, nanoparticles are used for antimicrobial applications, biosensing, imaging, and drug delivery [23]. Engineered nanomaterials (nanoparticles) have the potential to transport therapeutics to unique sites of a sickness, accordingly decreasing the off-track toxicity of many drugs [24]. This is specifically genuine in the use of chemotherapeutics wherein astray reaction causes critical effects in most cancer patients. Due to the fact that all types of cells have precise locations, nanotechnology may be used to "realize" cells of interest [25]. This allows associated pills and therapeutics to reach the diseased tissues, averting healthy cells. Nanoparticles are a promising treatment choice for cancers that are immune to common treatment options [26].

In a single treatment the cells absorb nanoparticles, after which infrared light is used to warm up the debris to kill the cells [27]. This new era promises to provide better, higher targeted answers to cancer treatment destroying most cancers tumors with minimal harm to healthy tissues and organs. Nanobiomedicine, utility of nanoparticles & devices for meeting scientific issues had facilitated amazing progress in the diagnosis, remedy, and monitoring of many critical ailments, which include most cancers, cardiovascular and neurological issues, HIV/AIDS and diabetes [28]. The intensity of this impact of nanoparticles on cancer cells depends not only on the endocytosis but also on the buildup and residing time of the nanoparticles inside cells [29].

Primary factors that arise afterwards the cellular demise are lack of membrane integrity that allowed chemical compounds to enter/leave cell freely. Cytotoxicity assays detect presence of dead cells through the study of outflux of the specific proteins/ the influx of chemicals (DNA dye) [30]. For powerful medicine to the tumor, it's essential for drug to pass through tumor vessel walls & interstitial vicinity [31]. Nanoparticles with capacity to overcome those difficulties that play an important role in tumor remedy. Nanocarriers enhance intracellular penetration & improve absorption of medicine to specific tissues of tumors [32]. Nanoparticles offer sustained release of medicine and notably increase their efficacy. Microparticles with diameters greater than 1 μm cannot be manage through intravascular routes. Nanomaterials were tiny enough to enter inside intracapillary area [33]. After intravenous management, nanoparticles follow pathways similar to other foreign substances and pathogens [34].

With the developing concern in nanomaterial studies, toxicity of nanoparticle is a developing critical problem in nanotechnology [35]. The usage of nanomaterials for medical programs, together with drug & gene delivery, biosensors, cancer treatment & diagnostic equipment, has been extensively examined in last years [36]. By progressed formation & utilizing of nanoparticles in numerous aress, unintentional detrimental affect of nanoparticle is a growing & developing issues academically & socially [37-40].

Numerous challenges were occurred because of shortage of standardized protocols. To enhance experimental situations of nanoparticle cytotoxicity studies, extreme attention are required for dependable and practical information [41]. The cell type has to be decided on as a means of introducing path & specific organ of nanoparticles. Further, nanoparticle dosage should replicate a sensible quantity of nanoparticles & shipped in dissolved form to check precise size & shape-based impact. Cytotoxicity assay is vital to pick out best method which would decrease the toxicity of interest with true advantageous/disadvantageous misunderstand of toxicity end result [42].

Various studies on the basis of diverse features together with shape, size, surface chemistry, chemical composition, surface activity & solubility [43-47]. Having a greater rigorous assessment of toxicity, it is necessary to identify the functionality of nanoparticles which may be penetrating the skin [48]. In view of the developing nanoparticles, its effect on the body, standardized techniques are analyzed for evaluating nanoparticle toxicity [49].

Nanomedicine enables medical experts to apply nanotechnology to dispose boundaries and barriers in therapy, diagnosis and prevention of diseases [50]. Nanomedicine makes use of nanotechnology based on manipulating molecules, atoms and particles [51]. With the aid of nanomedicine utility, scientists have overcome difficulties in treating incurable ailments and done away with useless surgeries [52]. Developing nanomedicine allows access to less expensive methods and enables expansion of research in medical science. Economic effect of developing nanomedicine is also very important [53].

### 6.2 Main Branches of Nanomedicine

Nanotechnology furnished new techniques in three branches of medicine as follows [54]:

- Diagnostic Branch
- Drug delivery Branch
- Tissue Engineering Branch

#### 6.2.1 Diagnostic Branch

The technologies of spectral coding are anticipated to offer new possibilities for diagnostics in the medical field. For measuring biologically varied colored optic coding can be attained by substituting quantum spots in specific sizes into microbeads of polymer. Similarly, nanomachines of DNA may be hired as bimolecular sensors [55]. Technology of imaging in medical is promoted through operation of nanodevices. Imaging at the level of molecule facilitates accurate diagnosis [56].

#### 6.2.2 Drug Delivery Branch

Engineering technology presents methods to transport drugs to deliver in decided fractions at the stage of therapy inside the body. There are some barriers to provide medicines with the best performance. Unavoidably, the body suffers from facet results of drug during treatment in cases like most cancers. Quantity of carried drug and the target region in the body are managed in drug delivery as a smart system [57].

#### 6.2.3 Tissue Engineering Branch

With the aid of tissue engineering, nanotechnology is capable of renewing damaged tissues. The tissue engineering makes use of artificial production of prompted cell through nanoscale elements of suitable size & shape. As an instance, bones development is possible on some sorts of carbon nanotubes. Therapies like transplants of limbs or artificial implantation can be substituted by using tissue engineering. This approach of nanotechnology can be further enhanced [58].

### 6.3 Applications of Nanomedicine

Nanomedicines are widely categorized with the aid of 3 most important sections primarily based upon their application fields [59]. These are nanodiagnostics, nanopharmaceuticals and regenerative medicines.

Nanodiagnostics are efficient sensing devices in both vitro & vivo conditions. Specialized molecular characteristics of sensors in nanodomain were utilized for detection and estimations of biologically useful molecules at very low concentrations. Physicochemical properties of metal nanoparticles like plasmonics, fluorescence, functional molecules are all applied for molecularly complex diagnosis [60]. In addition, the polymeric nanoparticle properties like affinity chemistry, surface charge, tribology in organic fluids, nir response, flocculation and tissue homing are remarkably beneficial in prognosis [61]. Nanodiagnostics have supplied speedy, efficient and patient friendly alternatives in diagnostics. Size miniaturization, surface functionalization and biocompatibility are a number of the important traits in case of tissue implantable gadgets [62].

Regenerative medicine pertains to disease-modifying therapeutics that can allow tissue regeneration in-situ. Nanoparticles for genetic molecular delivery are examples of regenerative nanomedicines. The NIH USA has defined regenerative medicine as “the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects” [63]. Biocompatibility properties of diverse nano-structured polymers and metals produce structures like nanofibres, porous scaffolds, nanowires, nanoguides etc. with various surface morphologies, nanospheres, dendrimers, nano-composites and other macromolecule architectures [64 - 67].

The most intensively experimented field is nanopharmaceuticals. Many related terminologies have been proposed for engineered nanomedicines [68]. Nanopharmaceuticals are particularly those which are all the more useful as formulation pharmaceuticals. Nanotoxicology & nanomedicine focus on phenomena, mechanisms & engineering at nano-bio interface. [69]. Nanoscale drug transport gadgets have extended sizable scope in the technology and generation of clinical chemistry, disease biology and bio-molecular interactions [70].

Cells exhibit a complex structure and several molecular interactions are compartmentalized by using intracellular membranes and organelles [71]. Cell environment additionally offers some possibilities for delivery modulations by the usage of exogenous molecules [72]. Target equipment was developed to intervene in unique cellular objectives for beneficial impact in pharmaco-therapeutics [73]. Nanotechnology provides superior methods of nanomedicine to prevent, to diagnose and to treat illnesses [74, 75].

### 6.4. Advantages of nanomedicine for cancer imaging and therapy:

The conventional strategies for most cancer therapy involve surgical treatment, chemotherapy and radiation treatment. Recently, with the improvement of modern technology, exclusive remedy strategies have been studied which includes angiogenesis inhibitors therapy, biological remedy, photodynamic therapy, hyperthermia therapy and gene therapy [76]. Regardless of persevering with advances in most cancer prognosis and treatment in current years, there is no observable change in most cancer associated death rates because of challenges in first level identification and the near concurrence of numerous anatomical systems important for appropriate motor, cognitive, reflexive & extra features [77].Hence, further advances are demanded. Such advances encompass progressed early screening and evaluation, as well as remedy regimens which can be more selectively taken up by the study of tumor cells and characteristic decreased off-target toxicity [78, 79].

By National institutes of health in United States of America, the implementation of nanotechnology to sickness remedy, prognosis, tracking and to the control of organic structures is currently known as ‘‘nanomedicine’’ [80, 81]. Because of their nanosize, nanoparticles can drift without difficulty through blood capillaries and enter the target cancer cells. In addition, they provide a recuperation reaction inside a tumor. New imaging agents, new diagnostic chips and new focused remedies can come collectively and broaden from nanotechnology to facilitate a form of unique remedy for cancers wherein early and precise detection ends in speedy initiation of remedy [82, 83].

Nanoplatform based delivery structures provide more than one advantage over traditional drug shipping systems. The dimensions of nanocarriers will modulated to govern loading/releasing of encapsulated/covalently related medicine imaging additives [84].

The potential for delivering imaging or therapeutic agents precisely for tumors at essential quantities are important element for efficiency of most cancer examination & treatments [85]. The delivery systems were proven to accelerate broad constancy form of healing agents like small hydrophobic molecules, peptides, and oligo nucleotides [86].

### 6.5 Nanomedicine and nanotechnology

Nanomedicine has been defined by the European Science Foundation on application terms- "Nanomedicine uses nano-sized tools for the diagnosis, prevention and treatment of disease and to gain increased understanding of the complex underlying pathophysiology of disease and the ultimate goal is to improve the quality of life" [87].

Science and technology encompass but does no longer limit to the disease analysis, bio-imaging, bio-molecular evaluation, drug delivery and nano therapeutics. Nanotechnology (Greek nano means dwarf) is defined by NNI as "understanding and control of matter at a dimension roughly 1 to 100 nm, where unique phenomena enable novel applications" [88]. A more confining definition of nanotechnology can be proposed as, "the design, characterization, manufacturing, and application of devices and systems through managed manipulation of magnitude and shape at a nanometer scale (atomic, molecular, macromolecular scale) that produces gadgets and structures with a minimum one novel or superior feature or belongings" [89].

### 6.6 Nanotechnology and drug delivery

Drug shipping, as an automatic tool, is capable to assist increase of robust association with supplying pills if essential. As a utility of nanotechnology, drug shipping structures supply certain form of capsules at the promised time and critical doses [90]. The traditional drug possesses challenges like low loading capacity and non-targeted delivery of the drugs. Furthermore, the use of costly polymers to load and release drugs are not economically feasible. Therefore, nanoparticles may be used as efficient nanocarrier materials to load and launch drugs. For instance, nanoparticles can be used as tumor destroying hyperthermia dealers which can be injected into the tumor after which they are activated to produce warmth and ruin tumour cells either by magnetic fields, X-rays or light. Interaction of ligands on floor of nanomaterials is aimed to tumour cells. When nanomaterials bound with receptors, then fastly go through receptor-mediated

endocytosis/phagocytosis with the aid of cells, ensuing cell internalization of encapsulated drug. The most essential implementation of nanoparticles in most cancer remedies is usage of nanoparticle mediated drug delivery of anticancerous drugs [91].

The idea of focused drug shipping was first proven by Paul Ehrlich and termed as "magic bullet" [92]. The principal techniques for targeted drug shipping are to (i) supply effective molecules receptor specially, (ii) boom the efficiency of present drugs, (iii) decrease detrimental results, (iv) permits fast access molecules in therapeutics, (v) creates another ways of management and (vi) acquire site precise activity [93].

Particular interface era and generation became applicable to the nanomedicines drug delivery traits [94]. Engineered nanomaterials provide a variety of applications for advantages in bio-clinical interfaces. These consist of i) improving drug solubility, ii) facilitated drug permeability, iii) tissue self- repairing, iv) molecular level diagnostics, v) imaging cell based abnormalities, vi) theragnostics for pin point remedy and several others [95].

### 6.7 Trends in nanomedicines

The size, shape, charge, soft matter chemistry & surface modulations effects of nanomaterials play an important role [96].

#### 6.7.1 Size and shape

Nanotechnology absolutely revolves across the idea on knowledge and manipulation of materials at the nanoscale level. Larger size nanoparticles inhale effortlessly by way of phagocytosis/macropinocytosis whereas tiny nanoparticles were commonly serves via endocytosis [97]. Shape also had specific act in cell internalization of nanoparticles. Nanorod had been shown to be internalized more abruptly than the round particles which usually comply with a couple of internalization pathways regardless of their size [98]. The foremost nanotechnology targeted on under 100 nm dimension; but, for tumor delivery, size varies often between 100-200 nm because of enhanced permeability & retention (EPR) outcomes [99].

Multifarious research moreover recommended that an optimal nanoparticle size in the region of 10-250 nm is powerful in anticancer chemotherapy [100]. Metallic nanoparticles additionally exhibited size established cytotoxicity. Consequently, control

of particle size is a manner of tuning drug release rates [101]. On the other hand, drug launch from the spherical nanocarriers have become more important than the non-spherical form particles [102].

### 6.7.2 Effect of materials chemistry

Nanomaterials chemical architecture & characteristics control the delivery and performance of the active elements. In polymer drug conjugates, polymer molecular weight, structure, polydispersity and toxicity are the key properties which must be taken into consideration to formulate a drug delivery system [103,104]. A fast release of drug to tumor site was pronounced in case of pH sensitive poly (β-amino ester) polymer [105]. In addition, certain materials responded because of temperature adjustments in conjunction with nanoparticles made from hydrochlorinated poly (isoprene). The tension of the nanoparticles decreased via heating above its glass transition temperature ($T_g$) [106,107]. Material flexibility is another vital parameter for the nano delivery systems of liposomes [108]. Particles having higher grade of ability possess much binding capability [109]. Moreover, chemical properties show essential position in delivery of nanotherapeutics [110].

### 6.7.3 Effect of charge

Surface charge is vital for the dissociated particles to discriminate & constant for longer storage. For imparting electrical stabilization to nanoparticles, surfactants or stabilizers and ions are used in the processing of nanoparticles [111]. Other than the stableness, surface charge additionally performs a critical function in the interplay with the organic systems. Cationic nanoparticles will interact highly with the biological gadget as many cellular components are negatively charged [112]. Particle surface charge moreover had a vital role in drug loading for enhancement of nanotherapeutics [113]. Particle charge is utilized to form stimuli responsive drug delivery systems. The surface charge also affects the opsonization of the nanoparticles in the motion [114].

### 6.7.4 Surface Characteristics

Surface features of nanoparticles had strong effect on the storage stableness, biological interplay, drugs adsorption and versatility to ligands attachment. Conjugation

of numerous targeting ligands to the nanoparticle surface is a very different way to interact inside the preferred site on specific delivery of nanoparticles [115].

### 6.7.5 Chemical agglomeration

Aside from size, form & surface features of nanoparticles, particle agglomeration is another contributor which alters the behaviour of the nanoparticles [116]. Biomolecular agglomerations like albumin interactions outcomes are other important factors in shaping nanobiomedicines efficacy [117].

## 6.8 Nanomedicines synthesis and therapeutic applications

Synthesis of nanomedicines is based totally on two common methods namely top-down & bottom-up [118]. In top-down technique, the substances were steadily decreased to nanoscale level through physical & chemical methods. The second procedure entails formation of nanomaterials through reaction of atoms and molecules in the best synthetic surrounding. A number of strategies analyzed for the preparation of nanoparticles specifically rely upon chemical composition, size & shape of prepared nanoparticles.

Physicochemical properties of drug, which include solubility, chemical compatibility and chemical stability, are very essential parameters for appropriate approach for nanoparticle preparation [119]. Suitable selection of polymeric substances and stabilizers affects drug loading, drug release pattern and nanoparticles stability [120].

Inorganic nanoparticles can be exactly tailored for the required crystal phase, size, shape, composition and surface traits [121]. Recently, inorganic nanoparticles had massive functionality as medicine transport vectors because of it’s particular physicochemical properties which encompass vast surface area in keeping with unit volume, optical & magnetic distinctiveness & capability to functionalized by a big range of ligands to improve affinity towards target molecules [122]. Specific sorts of inorganic nanoparticles are presently being studied for their essential applications such as imaging for diagnostic features, cell tracking, photothermal therapy (PTT), and drug delivery [123].

Nanomedicines are reported to conquer the majority of drawbacks faced in traditional treatment plans which include low bioavailability, insufficient intention specificity & associated toxic effects [124]. Initial evaluations of nanoparticles in therapy

were as a provider of drug molecules for enhanced transport & therapeutic results [125]. Currently, numerous inorganic nanoparticles had been found to be effective in drug delivery applications which encompass silicon nanocarriers [126]. Numerous metallic nanoparticles like quantum dots, gold nanoparticles & iron oxide nanoparticles had also been exploited as drug delivery carriers [127].

Some other therapeutic applications of nanomedicines are based on photodynamic therapy which pertains to systems activated by means of light. Comparable agents have been introduced in the treatment of most cancers, dermatological disorders and microbial infections [128]. Nanoparticles are similarly explored in photothermal therapy in which nanoparticle itself acts as medication [129]. Further, magnetic nanoparticles under an external magnetic field are used for focusing on a particular diseased location [130]. Anyway, nanotoxicity is an important challenge by inorganic nanocarriers which contain metallic atoms [131].

### 6.9 Targetable nanomedicine approaches

Targeting of drugs though effective is difficult to achieve. It is important to consider the favored period to elicit pharmacological action [132]. Various targeting tactics are reported for nanomedicines, which can similarly be categorized as passive and active targeting approaches [133, 134].

#### 6.9.1 Passive targeting approaches

Passive targeting approach absolutely relies upon pathophysiological, anatomical and other associated opportunities accessible in the diseased condition [135]. The inflamed or infected tissue indicates an increased vascular permeability on the side of impaired lymphatic drainage of the tumors, which permits developed permeability & retention (EPR) effect of nanosystems in tumor tissues [136, 137]. Different types of active targeting approaches are explored to improve therapeutic efficacy of drugs [138 - 140].

#### 6.9.2 Active targeting approaches

Active targeting in most instances relies on particular alteration of drug carrier nanosystems by “active” agent attaining specific affinity for spotting & interacting by particular cell, tissue or organ in body [141]. Active targeting is also achieved by

conjugation of the nanoparticles surface with active agents or ligands such as sugars, folic acid, peptides & particularly engineered antibodies [142, 143].

### 6.10 Trends in nanomedicine targeting

Nanomedicines should control the required drug concentrations for a sustained time period [144,145]. Magnetic nanoparticles loaded by therapeutic agents could purposefully be delivered to site of action by usage of localized magnetic field gradients and recovered after treatment [146-148]. Regardless of many other trends, nanomedicines are overtly accepted against infectious diseases and cancer conditions [149].

### 6.11 Nanomedicines in cancer chemotherapy

Chemotherapy of most cancers causes drastic effects due to poisonous manifestation of drugs and lack of tissue selectivity. The toxic nature once in a while rivals the effect of the chemotherapy and in the long run lessens their healing efficacy [150]. Nanomaterials are superb oncologic drug shipping vectors, amassing at better concentrations in cancer tissue by EPR effect [151,152]. The medical relevance of EPR is increasingly applied in cancer chemotherapy. Targeting to particular locations can be furthermore improved with the conjugation of unique ligands to the nanoparticle surface [153,154].

### 6.12 Experimentation of cytotoxicity

#### 6.12.1 Aim

The in vivo cytotoxicity of the given drug on DLA or EAC cells can be studied through trypan blue exclusion approach. The drug injures cells & makes holes in the membrane via that trypan blue arrives. Broken cells were coloured blue with the aid of trypan blue stain. The technique by which live cells were eliminated from colouring is defined as dye exclusion method.

#### 6.12.2 In Vivo Characterization

Interactions of nanomaterials with organic organisms by deriving cells from animals were examined in the Invivo characterization [155]. Using animals in vivo studies overcomes a number of drawbacks of in vitro studies. Researchers could rapidly determine toxicity, safety & efficacy of drug candidate in a complex model [156]. It is utilized & confirmed to be much perfect in sending the drug, lowering cancerous size

& generating much lower cytotoxicity [157,158]. Toxicity checking out in animals gives unfavorable results because of exposure to an agent and increased dose-reaction relationships that allow assessment of responses at other exposures [159]. Cytotoxicity of nanoparticles are lower than the medication they will be bringing & they are effective [160].

Animal models were vital in cancer research for obvious practical and moral concerns related with human experimentation [161]. Usually, rodents are utilized due to the fact that they are small, breed without difficulty, may be genetically modified as an alternative without difficulty, and are generally inexpensive. The mice and human beings are at least 95% identical in the genomic stage [162]. Animal models are seemed to be essential for explaining biochemical & physiologic approaches of cancer treatment in living creatures [163,164].The stepwise cancer development from simple to harmful is proven in rodents & humans [165,166]. It is notable that mice can be used to study harmful cancers exhibiting a couple of genetic changes in a brief time (6-18 months), but in humans may additionally take a few years to attain life-threatening stage [167,168]. With the aid of cautiously technical situations, in vitro toxicity research by properly determined nanomaterials could mask in vivo system & performs as reliable technique to research poisonous effects [169-171]. Animal models would stay completely precise supply of in vivo data & bridged among in vitro research & patients [172,173].

### 6.12.3 The purpose of trypan blue

Trypan blue is a stain utilized to count living cells via naming lifeless cells completely. As live cells had a complete membrane, it can't pierce cell membrane of live cells & arrive cytoplasm [174]. In non living cells, trypan blue passes by way of the porous cell membrane & reach cytoplasm. Beneath light microscopy evaluation, blue colour for dead cells because it would quench fluorescence on cell membrane [175-177]. Thus, whilst trypan blue is applied, fluorescence determined via flow cytometry is from cells having internalized fluorescent/ cell auto fluorescence. Quantity of trypan blue solution utilized typically 0.4% (w/w) [178]. Regardless of goal of a test in cell biology, cell counting & viability evaluation are continuously achieved [179].

The trypan blue assay technique is considerably used for cell viability evaluation. For estimating cytotoxicity dead cells soak up trypan blue to cytoplasm due to lack of membrane selectivity, but live cells remain unstained [180]. Relative number of dead & live cells is studied through the use of optical microscopy by way of counting the stained (dead) & unstained (live) cells via the utilization of a Neubauer chamber [181]. This traditional trypan blue exclusion assay, while utilized for big wide form of samples, could provide low-precision consequences due to prolonged run time and intensive microscopic examination required [182,183].

The calculation of viability & decrement of cancer culture population were essential parameters for calculating efficiency of treatment method [184]. The assessment of cell population density, total amount of living cells inside culture & cell viability, proportion of residing cells within sample, is significant at some stage of biological research [185]. Also, the merged utility of TB by a hemocytometer is considered as standard technique to calculate cell population density [186, 187]. The reliability of the technique used to evaluate those parameters is important in this assessment [188]. Different kind of strategies (e. G. Alamar Blue and MMT assay) & systems (e.g. Bio-Rad TC20™ Automated Cell Counter, Chemo Metec Nucleo Counter, Beckman Coulter Vi-CELL™ XR Cell Viability Analyzer) can be utilized to examine the cell viability [189 - 192]. Lastly, cell viability is calculated as percentage of healthy cells in sample [193]. Anyway, Trypan Blue (TB) dye exclusion assay, first technique proposed in literature, is considered standard cell viability measurement method and is broadly utilized approach [194-196].

### 6.13. Cell viability analysis

Diagnostic techniques which are primarily nanotechnology based efficient tools for real time, reliable & affordable cancers diagnosis & detection [197]. Nanotechnology promotes chemotherapy and decreases its detrimental outcomes through the usage of guiding medicines to selectively target cancer cells [198]. Nanotechnology can be used for shipping of medicines, genes, and proteins into the tumor tissue; therefore, it decreases toxicity of anticancer agents for normal tissues [199,200].

Cell viability evaluation is done to get the facts of bioavailability of the substances within the cells [201-203]. Percentage of viable cells is calculated as below [204]:

### 6.13.1 Materials required

1. DLA (Dalton's lymphoma ascites) bearing mice
2. Phosphate buffered saline (PBS)
   - NaCI- 4g
   - Na2HP04 - 0.72 g
   - KH2P04 - 0.1 g
   - KCI - 0.1 g
   - D.H2O - 500 ml
3. Trypan blue 1% in saline
4. Hemocytometer

### 6.13.2 Procedure

1. Cells have been aspirated from peritoneal cavity of tumor bearing mice
2. Cells have been washed three instances use of PBS
3. Viability of cells is checked by the use of trypan blue; cell viability need to be above 98%
4. Different dilutions of $10^1$, $10^2$ and $10^3$ were made
5. Number of cells in $10^3$ dilution was counted using Hemocytometer &cell number adjusted to1 x $10^7$ cell/ml
6. The experiment was once carried out by means of incubating distinctive attention of the drug with 1 x $10^6$ cells
7. The last extent of the assay combination used to be made up to 1ml by the use of PBS & was incubated at $3^0$C for about three hours
8. 100µl of trypan blue was once introduced after incubation & range of dead cells were counted by Hemocytometer proven in Fig 6.2 [205].

Cell viability evaluation is a beneficial device in diverse experimental tactics, which include those for tumor susceptibility, microbiological resistance, and spontaneous cell death after submission to various experimental situations [206]. It has been recognized that cell membrane integrity is a simple criterion for discriminating dead from live cells [207]. The cell viability was quantified by the trypan blue exclusion approach which calculates in vivo cytotoxicity as below [208].

% cytotoxicity = [No. of dead cell/ (No. of live cell + No. of dead cell)] x 100

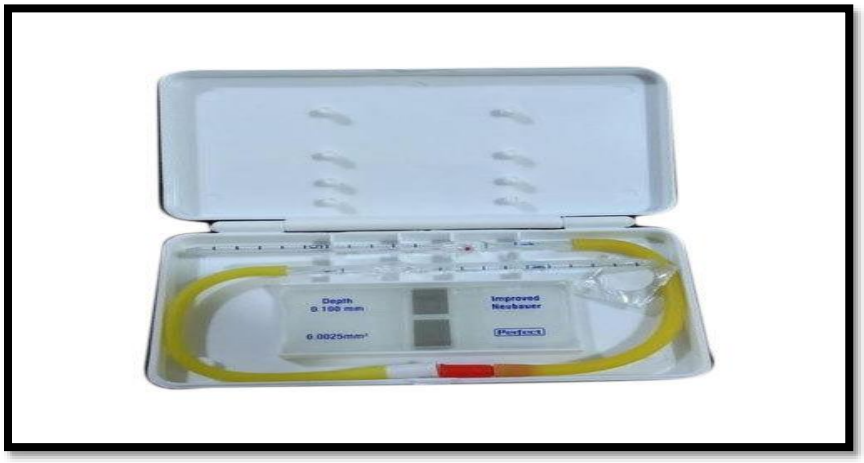


**Figure 6.2. Hemocytometer**

### 6.14 Cytotoxicity study of $Co_3O_4$ nanoparticles

Cytotoxicity is referred as the capacity of chemical substances to wreck living cells. Generally, it is the aspect of being toxic to cells due to chemical stimuli, reveal to another cells, physical/environmental situations (radiation exposure, temperature or pressure extremes), etc. [209]. Chemical toxicity could happen in various approaches. However, hypothesized may be widely labeled to major categories: destroying of particular biomolecular targets or pathways and generally destroying of cell machines which cause cellular stress & cytotoxicity [210].

Cell disruptive methods encompass protein, DNA, or lipid reactivity, physicochemical damaging of proteins or membranes/procedures which include apoptosis, oxidative stress response, mitochondrial disruption, endoplasmic reticulum (ER) stress, microtubule disruption, heat shock response [211]. Medicating cells by cytotoxic compound could bring about various cell fates. Through the use of cytotoxic compound, healthy living

cells could triggered to go through necrosis (accidental cell death) or apoptosis (programmed cell death) [212]. While apoptotic cellular death is slow, in order, & genetically regulated, cells can also undergo necrosis, however, hastily loss membrane integrity & kill due to cellular lysis [213]. The cells can hinder active development and division (reduce in cellular viability).

Cytostasis is unique division of cytotoxicity, in which cells stay alive but cannot develop & divide [214]. Cell death is not only the driving force for that event. Cytotoxicity can navigated through means of physicochemical elements, along with protein denaturation/reactivity that may depend on cell free & cell-based assays [215]. Lower affinity non covalent binding to receptors & enzymes takes place at higher concentrations [216].

Magnetic nanoparticles have remarkable capacity for application in catalysts as well as in nanobiomedicine, as assessment development mediators for magnetic resonance imaging and drug shipping [217]. Similar traits for cobalt nanoparticles enhanced severe challenges about safety [218].

From the present study, $Co_3O_4$ nanoparticles are characterized to understand their suitability for biological applications. Electron microscopy is applied to consider shape, size & aggregation. It is significant for understanding the cytotoxicity and evaluating the cytotoxic level of the pure $Co_3O_4$ nanoparticles. For examining their toxicity to human cells, the cellular viability [219] is calculated.

$Co_3O_4$ nanoparticles introduced as concentration & time dependent impairment of cellular viability, even though cobalt ions are highly toxic [220]. Cobalt makes a fast induction of ROS furnished in manner of $Co_3O_4$ nanoparticles, better than ions. $Co_3O_4$ nanoparticles are capable to go through cell fastly and remain enclosed in vesicles within cytoplasm. It had been seemed additionally within cell nuclei, having much lower regularly. Quantity of cobalt ions launched via $Co_3O_4$ nanoparticles became lower & not reached powerful concentrations. As soon as they enter the cells, $Co_3O_4$ nanoparticles may dissolve with greater efficiency, act as a reservoir increases toxicity. The nanoparticle bound to plasma membrane & cellular uptake is a vital requirement for nanoparticles to apply toxicity [221].

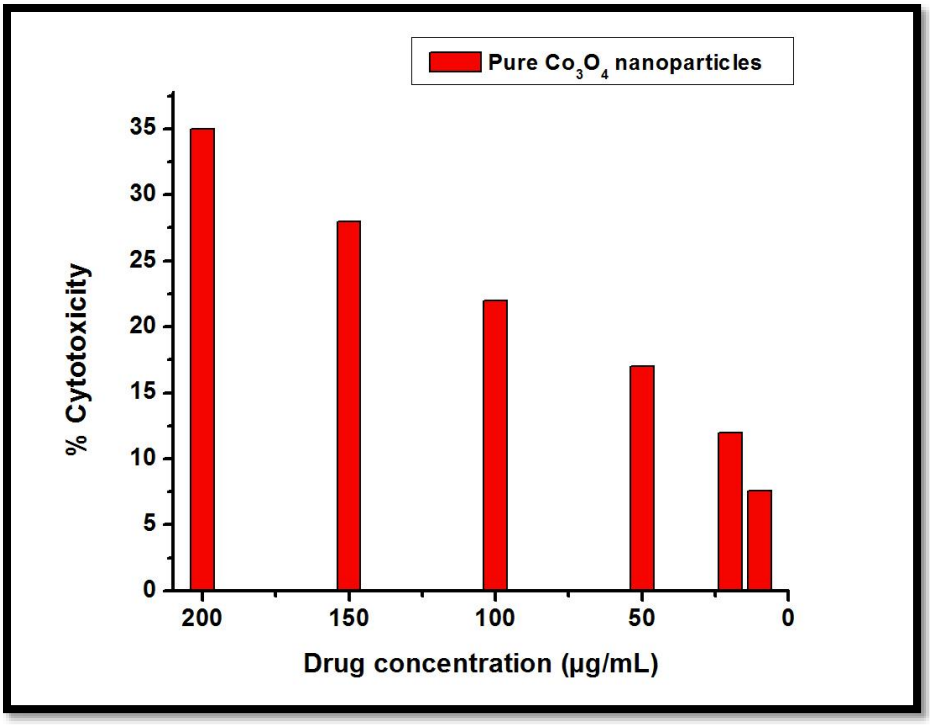


**Figure 6.3. Percentage Cytotoxicity for different concentrations of pure $Co_3O_4$ nanoparticles**

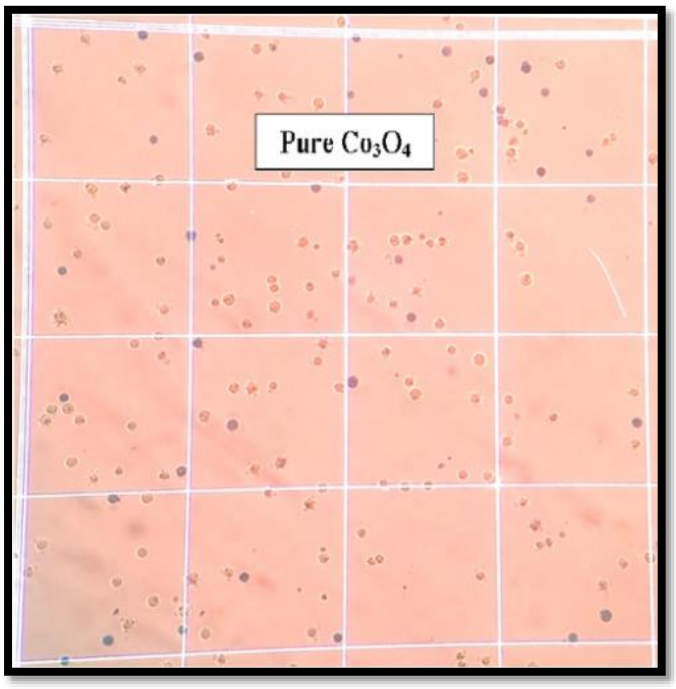


**Figure 6.4. The confocal micrograph for 200µg concentrations of pure $Co_3O_4$ nanoparticles**

It is utilized in numerous nanobiomedical applications like hyperthermic treatment & gene therapy [222]. The most probable pathway by which $Co_3O_4$ nanoparticles enter the cells involves endocytosis of nanoparticle agglomerates.

$Co_3O_4$ nanoparticles are specifically inserted & deposited in the cytoplasmatic vesicles which incorporate nanoparticle agglomeration [223]. This could relevant because adsorption of proteins inside culture medium at $Co_3O_4$ nanoparticle surface might provide its cellular uptake; in vivo & in vitro research on fabricated nanoparticles determined closed bridge among ROS production, oxidative stress & nanotoxicity [224].

$Co_3O_4$ nanoparticles have been able to result in dose-established boom for ROS production. This can assigned to distinct sensitivity of cell lines. Cell membrane gives a notable hindrance of more ions & found that cobalt, with ionic shape, is taken through cells having less efficiacy [225]. The cells are exposed to cobalt for very brief time duration, revealing that cobalt causes faster inspiration of ROS, when distributed inside the form of $Co_3O_4$ nanoparticles than cobalt ions. It may relate to fast & huge intake of nanoparticles through cells.

**Table 6.1. Representation of percentage cytotoxicity for different drug concentrations of pure $Co_3O_4$ nanoparticles**

| Drug Concentration (µg/ml) | Percentage Cytotoxicity of $Co_3O_4$ nanoparticles |
|---|---|
| 10 | 7.6±0.9 |
| 20 | 12±1.3 |
| 50 | 17±1.3 |
| 100 | 22±1.8 |
| 150 | 28±1.7 |
| 200 | 35±1.8 |

In this investigation, the toxicity of $Co_3O_4$ nanoparticles was measured by trypan blue exclusion technique and is given in the Table 6.1. The results in the graphical representation exhibit the cell lines at measured doses (10 µg/ml, 20 µg/ml, 50 µg/ml,

100 µg/ml, 150 µg/ml, 200 µg/ml) of pure $Co_3O_4$ nanoparticles (Fig 6.3). The cytotoxicity values were found to be7%, 12%, 17%, 22% and 35% respectively. The drug concentration of 10 µg/ml & 200 µg/ml, attain lower & higher toxicity levels of 7% and 35% cytotoxicity respectively for $Co_3O_4$ nanoparticles. The results concluded that percentage Cell viability decreases by increase in concentration of sample.

It is concluded that $Co_3O_4$ nanoparticles are conveniently taken up through cells. Their toxicity is a demerit for a few applications, which includes diagnosis through MRI. The toxicity can be modified by way of an appropriate covering by tailored features. Inversely, nanoparticle toxicity is utilized in different applications, including cancer treatment

### 6.15. Cytotoxicity study of Zn doped $Co_3O_4$ nanoparticles

Zinc, an essential trace element of human body & cofactor of greater than 300 mammalian enzymes, takes essential act in vital cellular procedures such as oxidative stress, DNA replication, DNA repair, cell cycle development & apoptosis. ZnO nanoparticles, with their precise features which include easy, biocompatibility, high selectivity & advantageous cell viability can be efficient anticancer agents. Consequently, change in zinc levels in cancers cells causes harmful impact [226].

ZnO nanoparticles have a more potent cytotoxic impact that makes them highly reactive in invitro & invivo surroundings. Cytotoxic action dwindles with increasing Zn concentration. Cell viability is affected noticably in samples further impact falls at greater doping ranges; cells exposed to ZnO nanoparticles makes much quantity of ROS, that additionally reduces while Zn concentration is enhanced [227]. Zinc oxide nanoparticles have many healing outcomes; they have antibacterial, anticancer, immune modulatory, sunscreen and antioxidant effects, or they can be utilized as an adjuvant remedy to chemotherapeutic drugs to relieve their toxic consequences [228]. This drug delivery to cancer cells & minimum consequences on another ordinary cells results in the decrease in side effects that attain massive effect on transferring towards proper route in cancer theranostics [229]. The decrease in the cytotoxic results indicates the opportunity by utilizing Zn doping for enhancing cytocompatibility of ZnO nanomaterials, though having properties like tiny particle size & massive surface area, which are usually associated with high cytotoxic results [230].

While utilizing ZnO nanoparticles its primary significance to make sure of their biocompatibility whilst retaining preferred functional properties. Studies have shown that high zinc concentration in cells instigates toxic effects [231]. Protein activity disequilibrium & oxidative stress by reactive oxygen species (ROS) can be the possible reaction responsible for cytotoxicity [232]. Precised localization of ZnO nanoparticles in the direction of tumour cells because of EPR effect, electrostatic interplay & specific cytotoxicity because of accelerated ROS can particularly target & destroy cancer cells, thus providing an efficient anticancer vehicles [233].

In this study, the toxicity of Zn doped $Co_3O_4$ nanoparticles was found utilizing trypan blue exclusion method and is described in the Table 6.2. Figure 6.5 revealed that for cell lines at measured doses (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) the cytotoxicity values of 3%, 5% and 10% Zn doped $Co_3O_4$ nanoparticles were between 5-15%, 6-22%, 6-35% respectively. 10 µg/ml & 200 µg/ml drug concentration shows lower & higher toxicity levels of 5% & 15% cytotoxicity respectively for 3% of Zn doped $Co_3O_4$ nanoparticles. The cell line micrographs for 200µg concentrations of 3%, 5% & 10% Zn doped $Co_3O_4$ nanoparticles were shown in Figure 6.6.

**Table 6.2.Representation of percentage cytotoxicity for different drug concentrations of 3%, 5% and 10% Zn doped $Co_3O_4$ nanoparticles**

| Drug Concentration (µg/ml) | Percentage Cytotoxicity of Zn doped $Co_3O_4$ nanoparticles | | |
|---|---|---|---|
| | 3% Zn | 5% Zn | 10% Zn |
| 10 | 5.48±0.48 | 6.12±0.5 | 6.72±0.53 |
| 20 | 6.54±0.48 | 7.18±0.5 | 8.76±1.93 |
| 50 | 7.47±0.36 | 10.05±1.6 | 12.1±1.94 |
| 100 | 11.1±1.23 | 16.02±1.8 | 16.8±1.38 |
| 150 | 13.2±1.13 | 19.02±1.8 | 26.8±1.18 |
| 200 | 15.9±1.26 | 22.2±1.11 | 35.15±1.4 |

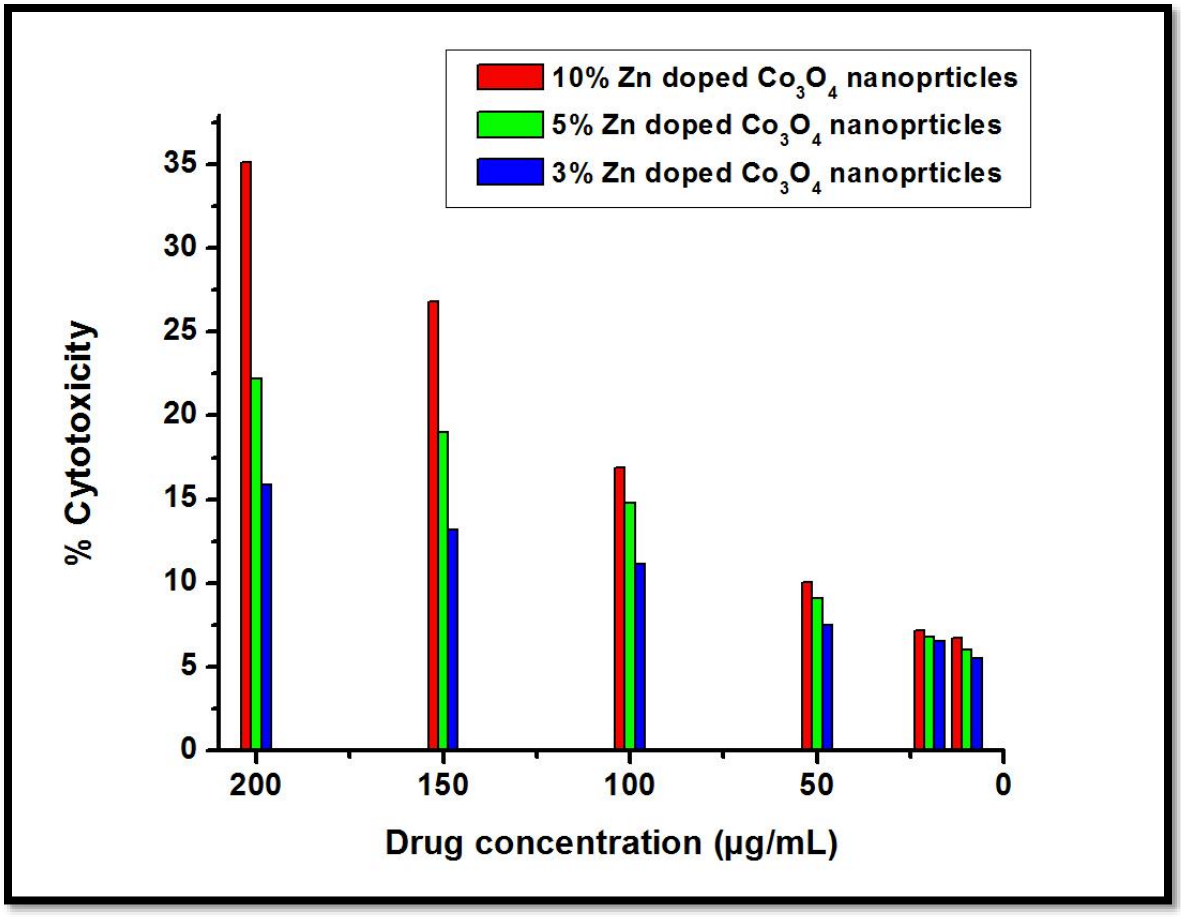


**Figure 6.5. Percentage Cytotoxicity for different drug concentrations of 3%, 5% and 10% Zn doped $Co_3O_4$ nanoparticles**

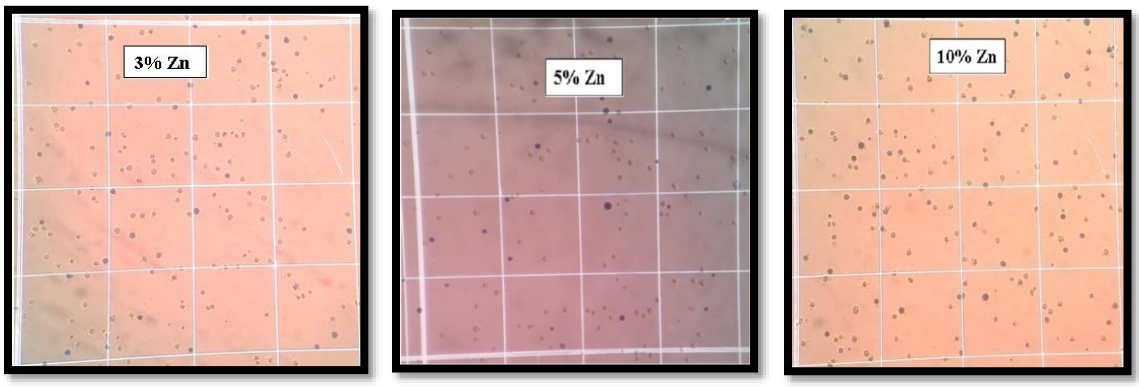


**Figure 6.6. The confocal micrographs for 200µg concentrations of 3%, 5% and 10% Zn doped $Co_3O_4$ nanoparticles**

The principal technique to locate most cancers cells is based on binding of nanoparticle probes conjugated by moieties (protein, short peptides, antibodies, oligonucleotide aptamers) on cancer cells & those getting into cells & detecting the genetic content [234]. A crucial benefit of supplying nanoparticles for most cancer examination tests laid on huge surface area to volume ratio in relation to substances [235]. Because of this feature, nanomaterial surface may be thickly blanketed by antibodies, tiny molecules, peptides, aptamers & distinctive moieties. Those moieties bound & apprehend unique cancer molecules. With the aid of offering several binding ligands to cancers cells, multivalent outcomes may executed that could develop selectivity & sensitivity of an assay [236]. ZnO nanoparticles have a flexible surface chemistry that could effortlessly be developed to save aggregation and improve colloidal stability [237] or to achieve new characteristics as drug delivery systems (DDS). ZnO nanoparticles may additionally bring about higher intake of chemotherapeutic drugs by lowest toxicity to normal cells.

### 6.16. Cytotoxicity study of Fe doped $Co_3O_4$ nanoparticles

The iron oxide nanoparticles have captured substantial interest because of their notable paramagnetic features and usage in various biomedical applications [238]. The cytotoxicity of iron oxide nanoparticles is because of production of oxygen free radicals converted to hydrogen peroxide to reactive hydroxyl radicals [239]. Those free radicals plays a couple of routes such as depolymerization of polysaccharides, breaking of DNA strands, inactivation of enzymes & peroxidation of membrane lipids that finally resulted in the death of the bacteria [240].

**Table 6.3. Representation of percentage cytotoxicity for different drug concentrations of 3%, 5% and 10% Fe doped $Co_3O_4$ nanoparticles**

| Drug Concentration (µg/ml) | Percentage Cytotoxicity of Fe doped $Co_3O_4$ nanoparticles | | |
|---|---|---|---|
| | 3% Fe | 5% Fe | 10% Fe |
| 10 | 4.55±0.42 | 5.7±0.5 | 6.73±1.37 |
| 20 | 4.79±0.71 | 6.4±0.5 | 7.54±0.88 |
| 50 | 5.25±1.56 | 8.18±0.8 | 9.27±1.06 |
| 100 | 7.47±0.93 | 11±1 | 11.7±1.42 |
| 150 | 9.47±0.93 | 11.4±1 | 13.7±1.42 |
| 200 | 12.2±2.04 | 16.1±1.5 | 16.6±1.67 |

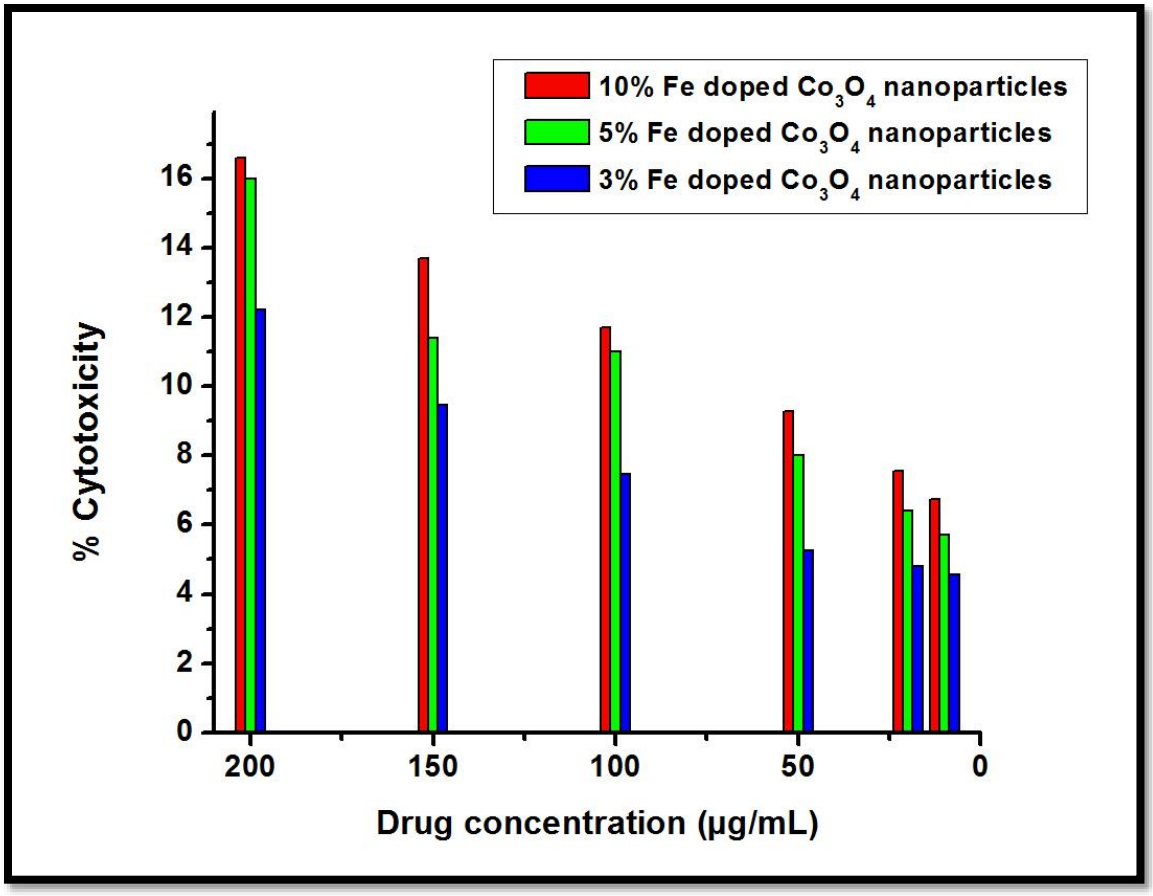


**Figure 6.7. Percentage Cytotoxicity for different drug concentrations of 3%, 5% and 10% Fe doped $Co_3O_4$ nanoparticles**

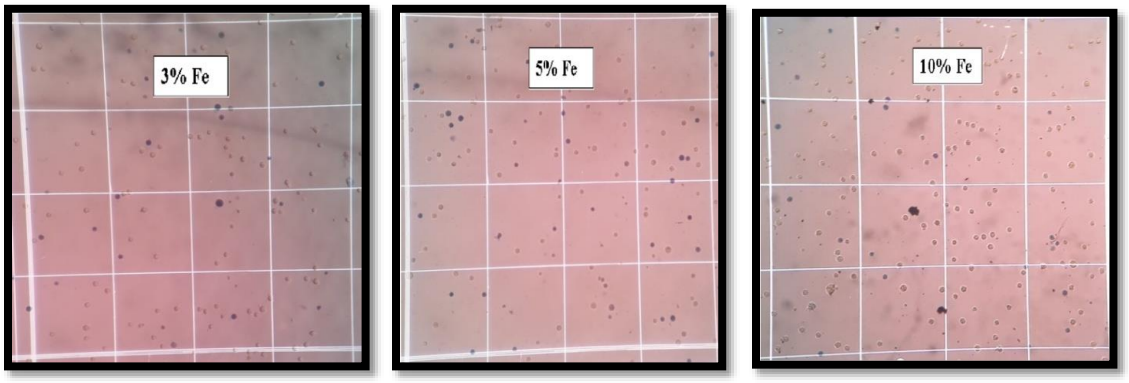


**Figure 6.8. The confocal micrographs for 200µg concentrations of 3%, 5% and 10% Fe doped $Co_3O_4$ nanoparticles**

Further to facilitating targeted drug delivery, the precept benefit of fast sedimentation of drug-particle complex to target place appreciably decreases time & dose of the vector [241]. Thus nanoparticles spear cells, skip via membrane and supply target drug, was efficaciously used for preserving a higher rate of cellular viability [242]. The drugs possibly bound stronger to nanoparticles by positively charged surface. As soon as the drug nanoparticle complexes attain target organs, drug molecules may be launched infront of anions. Magnetic particles to behave as effective carriers for pharmaceutical agents, surface of particles need to first be changed to permit attachment of target molecules [243].

The increase of the negative surface charge increases electrostatic repulsion among nanocomposite & negatively charged bacterial cells thereby restricting their interaction [244]. Relatively, $Fe_3O_4$ nanoparticles have a huge negative surface charge and can therefore overcome the electrostatic barrier, facilitating the cellular nanoparticle interplay, consequently leading to decreased toxicity [245]. Reduce in particle size & enhancing in the negative surface charge lessens interplay of the nanocomposite, and this is considered within the elevated ROS manufacturing in bacterial cells [246]. One prime element for iron oxide nanoparticle viability is the generation of hydroxyl radicals through Fenton reaction [247, 248].

In this work, the toxicity of Fe doped $Co_3O_4$ nanoparticles was examined through trypan blue exclusion method was tabulated in Table 6.3. The graph in Figure 6.7, shows that the cytotoxicity values at measured doses (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) of 3%, 5% and 10% Fe doped $Co_3O_4$ nanoparticles were only between 4–12%, 5- 16% and6- 16% respectively. 10 µg/ml & 200 µg/ml drug concentration have lower & higher toxicity levels of 4% & 12% cytotoxicity respectively for 3% Fe doped $Co_3O_4$ nanoparticles. The confocal micrographs of cell lines for 200µg concentrations of 3%, 5% & 10% Fe doped $Co_3O_4$ nanoparticles were shown in Figure 6.8.

### 6.17. Cytotoxicity study of Cu doped $Co_3O_4$ nanoparticles

Metallic nanoparticles like gold, cobalt, silver, cerium and others have been substantially examined for their anticancer characteristics [249-252]. Copper nanoparticles have turned out to be more effective because of their cost effectiveness, more desirable

cytotoxic efficiency in opposition to cancer cells of lower dosage & larger constancy duration in comparison to Au & Ag nanoparticles. There are numerous varieties of copper-derived nanoparticles having proven anticancer activity which include CuI, CuO, $CuCO_3$, Cu $(PO_4)_2$, $Cu_2O$, CuS [253- 256]. Copper nanoparticles, copper-derived nanoparticles may be effortlessly confined to non-toxic, bioavailable and biodegradable nano-carriers to specified governing for precise cellular targets [257]. In comparison to silver & gold, cytotoxic activity of copper was much less examined [258].

Chemically synthesized copper nanoparticles are processed to promote nanoformulations of much inflexible shapes & sizes through moderating reaction temperature, time, pH & concentration of metallic salt [259]. In most of the ingredients, copper is currently confined to macromolecules rather than metallic ions [260]. Recently recommended dietary allowance (RDA) reviews allow everyday copper uptake of 900 μg for adults, 340 μg/day for children for first 3 years of age, 440 μg/day for age from 4-8 years, 700 μg/day for ages from 9-13 years & 890 μg/day for ages from 14-18 years [261]. Furthermore, RDA copper values of 1000 μg/day & 1300 μg/day have been advocated at pregnancy & lactation respectively.

Quantitative tissue assessment acts a vast role inside the layout of targeted processes for nanoparticle-based therapy. It has also been determined that copper nanoparticles provide an adequate dissolution level in acidic environment, however lesser than copper ions [262]. Those findings imply prevailing interdependence of organic system and physicochemical fetures of copper nanoparticles in addition affects invivo toxic manner. They demonstrated that nanoparticles could provide direct antitumor effects or indirect hyperthermic anticancer activity, in vitro & in vivo. Furthermore, nanoparticles could perform as conventional anticancer drug, decreasing aspect of side effects & preferred dosage [263].

New studies endeavor to permit the improvement of novel forms of metallic nanoparticles, collectively with copper nanoparticles, with enhanced & selective anticancer activity, improved biocompatibility, biodistribution & less toxicity for normal tissues [264]. Copper nanoparticles doesnot substantially examined, specifically invivo. The multifunctional characteristics & traits offer copper nanoparticles as perfect

nanomaterials for theranostic applications as evaluation agents/nuclear tracers for several diagnostic & imaging strategies, photothermal tumour cell damaging and managed application in drug release [265].

The promising anticancer ability of nanoparticulate metal types of gold, silver & copper is increasingly analyzed [266]. Copper-derived nano-therapeutics is gaining importance because of the cost-effectiveness of copper & extensively studied anticancer capability of copper-based nanoparticles which include copper oxide nano-formulations [267]. The healing efficiency of nano-scale molecules lies highly on improved reactive surface area, in comparison to traditional tiny molecule drugs & pharmaceuticals [268]. Here, we provide analytical assessment of therapeutic applications of copper nanoparticles as better anticancer agents.

Copper nanoparticles represent a specific group of metallic nanoparticles produced through modern strategies and with excellent physicochemical, organic and mechanical features [269, 270]. Furthermore, researchers established copper & copper-based nanoparticles display greater toxicity towards numerous microorganisms, hence turning into a powerful antimicrobial and antifungal agent [271- 274]. Copper is affordable in comparison to gold & silver directs much attention in research on the usage of copper nanoparticles as therapeutics. Their nano dimensions & surface-to-volume ratios assist their efficacy as medicinal, pharmaceutical & therapeutic agents [275, 276]. Furthermore, copper nanoparticles may implemented as DNA cleavage agents & influence anticancer therapeutics for binding ability & refinable surface features by combination with numerous bio-molecules involves enzymes & proteins [277- 281].

Copper nanoparticles performed as powerful drug shipping nano formulations & molecular doping systems working as moderators of tumour cell growth [282, 283]. Even though there are various clinical reports on the anticancer potency of copper oxide nanoparticles [284 - 288] constrained research outcomes propagated on antitumor efficiency of copper nanoparticles [289, 290], specifically because of their flexible and effortlessly oxidized form at ambient temperature situations. These results inferred a comparable cytotoxic description of the processed CuO nanoparticles to all the cancer

cell lines, confirming their effectiveness [291, 292] and the capability of copper nanoparticles to choose particular cancer cells detaching peripheral healthy cells unaffected [293].

**Table 6.4. Representation of percentage cytotoxicity for different drug concentrations of 3%, 5% and 10% Cu doped $Co_3O_4$ nanoparticles**

| Drug Concentration (µg/ml) | Percentage Cytotoxicity of Cu doped $Co_3O_4$ nanoparticles | | |
|---|---|---|---|
| | 3% Cu | 5% Cu | 10% Cu |
| 10 | 4.17 ±0.1 | 4.78±0.52 | 5.6 ±0.4 |
| 20 | 5.61±0.8 | 6.14±1.34 | 6.5±0.9 |
| 50 | 6.7±1.3 | 9.5±1.52 | 11.22±2.1 |
| 100 | 7.7±0.7 | 14.03±1.41 | 14.05±1.4 |
| 150 | 9.7±0.7 | 16.03±1.41 | 18.05±1.4 |
| 200 | 11±1.2 | 22.1±0.75 | 24.56±2 |

In this study, the toxicity of Cu doped $Co_3O_4$ nanoparticles was analyzed through trypan blue exclusion method and is shown in Table.6.4. The results shown in the Fig 6.9 indicate that the cytotoxicity of cell lines at measured dosages (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) of 3%, 5% and 10% Cu doped $Co_3O_4$ nanoparticles, was between 5-11%**,** 4-22% and 4-24% respectively. 10 µg/ml & 200 µg/ml drug concentration has lower & higher toxicity of 5% and 11% cytotoxicity respectively for 3% Cu doped $Co_3O_4$ nanoparticles. Figure. 6.10 shows the confocal cell line micrographs for 200µg concentrations of 3%, 5% & 10% Cu doped $Co_3O_4$ nanoparticles.

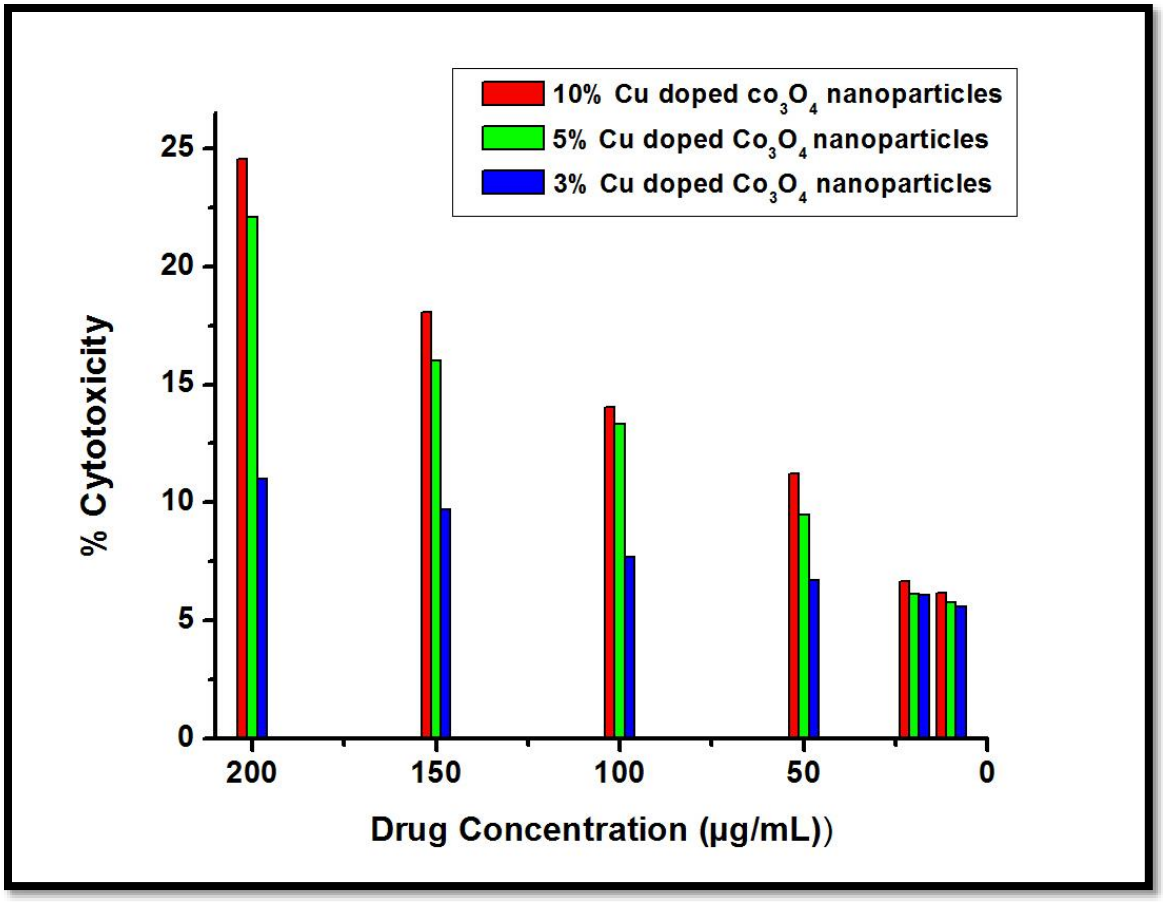


**Figure 6.9. Percentage Cytotoxicity for different drug concentrations of 3%, 5% and 10% Cu doped $Co_3O_4$ nanoparticles**

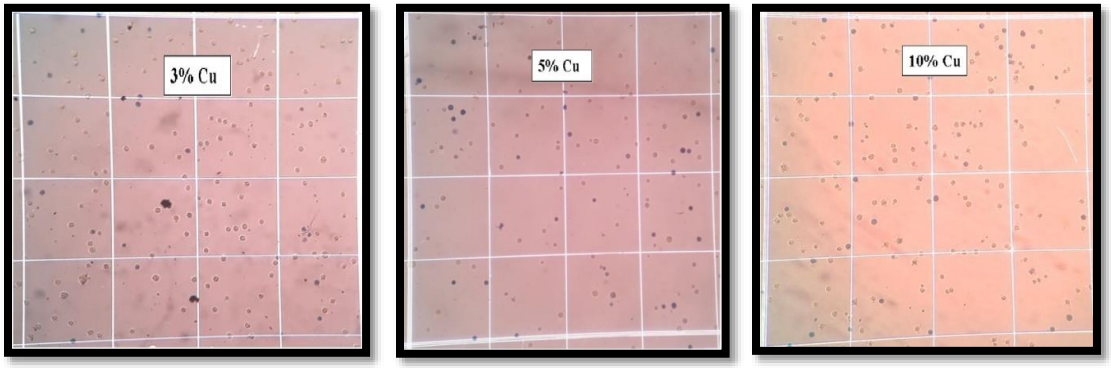


**Figure 6.10. The confocal micrographs for 200µg concentrations of 3%, 5% and 10% Cu doped $Co_3O_4$ nanoparticles**

### 6.18. Cytotoxicity study of Ni doped $Co_3O_4$ nanoparticles

Nickel is a micronutrient important for thorough functioning of the human body, because it accelerates hormonal activity and is concerned with lipid metabolism. This metal makes its route through the human body via the respiration tract, digestive system and skin. Nickel nanoparticles have been used for established functions in diverse fields along with electronics, magnetism, electricity technology and biomedicines [294- 296].

**Table 6.5. Representation of percentage cytotoxicity for different drug concentrations of 3%, 5% and 10% Ni doped $Co_3O_4$ nanoparticles**

| Drug Concentration (µg/ml) | Percentage Cytotoxicity of Ni doped $Co_3O_4$ nanoparticles | | |
|---|---|---|---|
| | 3% Ni | 5% Ni | 10% Ni |
| 10 | 5.36 ±0.8 | 5.61±0.6 | 6.02 ±1.16 |
| 20 | 6.63±0.3 | 8.29±1.2 | 9.06±1.13 |
| 50 | 7.90±0.8 | 12.7±1.6 | 14.8±2.56 |
| 100 | 10.29±1.2 | 19.3±1.4 | 19.6±2.25 |
| 150 | 11.19±1.2 | 21.3±1.4 | 24.6±2.25 |
| 200 | 12.26±1.8 | 24.2±1.5 | 28.1±1.42 |

Because of their higher reactivity, simplicity & eco-friendly features are utilized to catalyze diverse organic reactions inclusive of chemo selective oxidative coupling of thiols, reduction of aldehydes and ketones, hydrogenation of olefins, synthesis of stilbenes from alcohol via Wittig-type olefination, and $\alpha$-alkylation of methyl ketone [297- 301]. Additionally, they catalyze some inorganic reactions such as decomposition of ammonia [302]. They also feature in production of carbon nanotubes [303]. In addition, they are used in environmental applications for the adsorption of dangerous dyes & inorganic pollutants & hence act as a critical function in maintaining neatness of the environment [304].

Because of their antibacterial & anti-inflammatory functions, were widely utilized in area of nanobiomedicine [305]. They exhibit cytotoxicity toward tumour cells proved from deformation of morphology of those cells afterwards medication by NiO

nanoparticles [306]. Biocompatibility of NiO nanoparticles capping by biomolecules inclusive of glucose was tremendously accelerated & they utilized as biosensors & heat non mediators for cancer hyperthermia [307].

In current years need for the synthesis of magnetic nanoparticles of Fe, Co & Ni is enhancing. Because of their advanced magnetic characteristics & abilities, they find use in various fields inclusive of catalysis, memory storage devices & sensors. In medication area, they may be utilized for magnetically regulated drug delivery, magnetic resonance imaging & hyperthermia treatment of cancer cells [308 - 310]. The metallic salts such as nitrates, chlorides, oxides & sulphates had excessive reduction capacity because of attaching metal with chloride, oxide & sulphide elements & their desire to donate electrons [311, 312]. Due to those factors, the electronic density of conjugative salts of metal will accelerate. Hence, metals in ionic form could effortlessly got separated from their anionic part & get converted to the stable form [313]. In higher rate of reaction, the system of nucleation dominates over growth & vice versa [314, 315]. Size of nanoparticles will additionally increment when their growth is anisotropic. But, dimensions of anisotropically developing nanomaterials may be managed with aid of adjusting the experimental situations such as pH, ratio of metallic ion & reducing agent, irradiation time, their strength & reaction time [316].

In this analysis, the toxicity of Ni doped $Co_3O_4$ nanoparticles was found by trypan blue exclusion method and is shown in Table 6.5. These results are graphically represented in Fig. 6.11 that shows that cytotoxicity of cell lines at measured doses (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml) of 3%, 5% and 10% Ni doped $Co_3O_4$nanoparticles, ranged between 6-12%, 5-24%, 5-28% respectively. 10 µg/ml and 200 µg/ml drug concentration has the lower & higher toxicity levels of 6% & 12% cytotoxicity are examined respectively for 3% Ni doped $Co_3O_4$ nanoparticles. In Fig.6.12, confocal micrographs for 200µg concentrations of 3%, 5% & 10% Ni doped $Co_3O_4$ nanoparticles are shown.

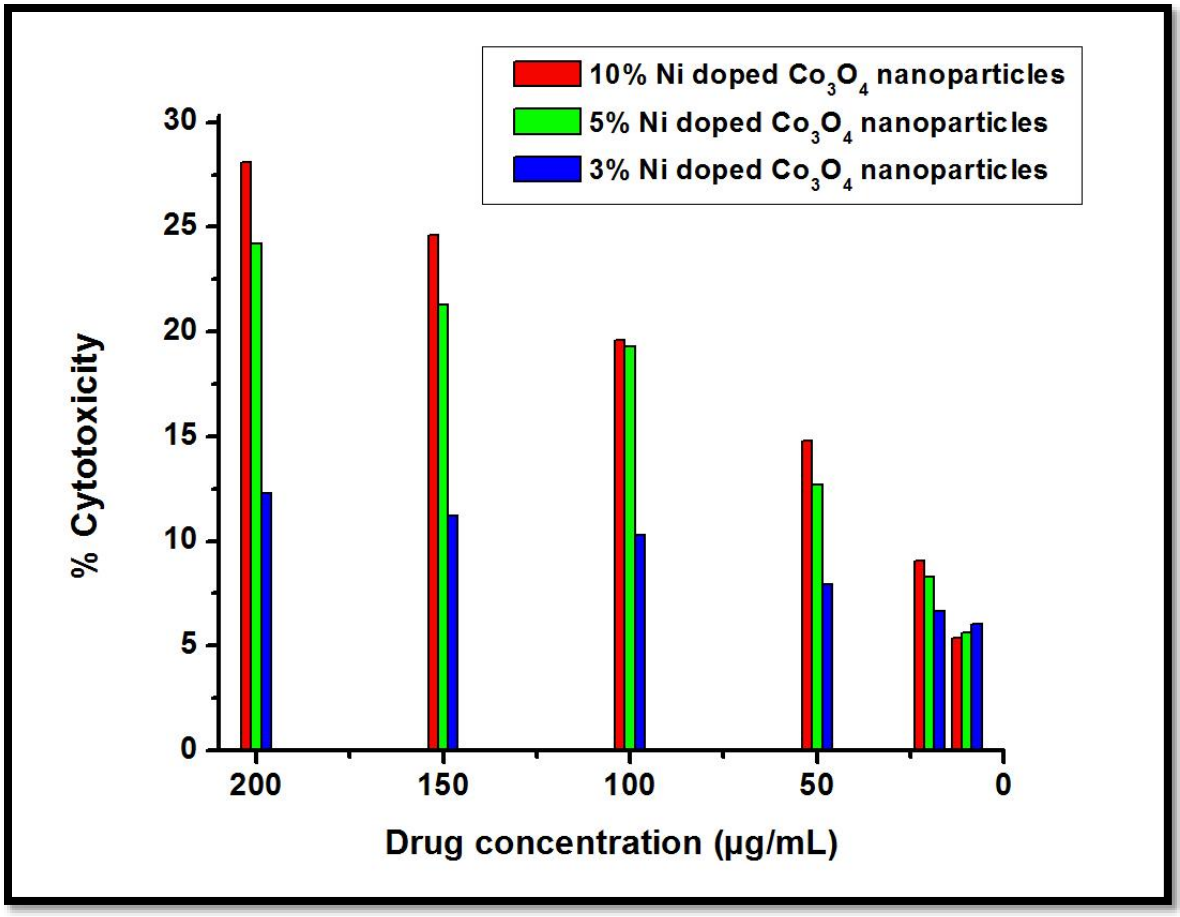


**Figure 6.11. Percentage Cytotoxicity for different drug concentrations of 3%, 5% and 10% Ni doped $Co_3O_4$ nanoparticles**

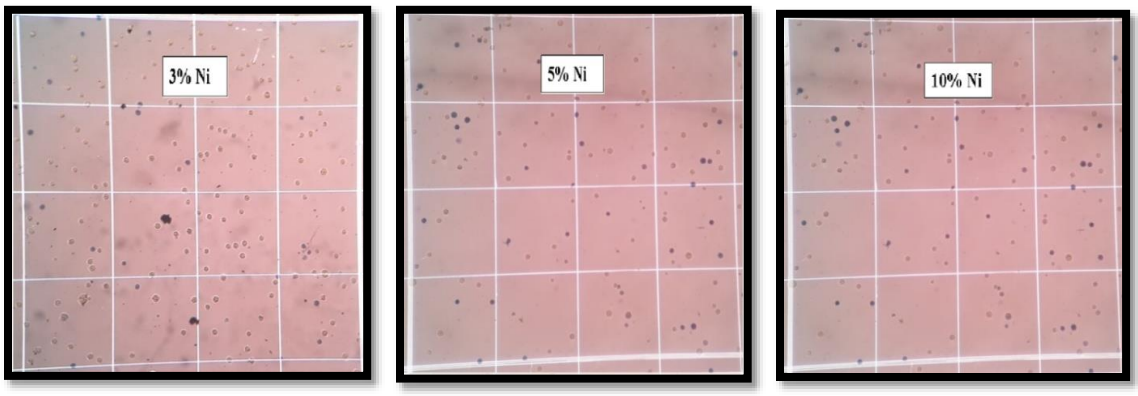


**Figure 6.12. The confocal micrographs for 200µg concentrations of 3%, 5% and 10% Ni doped $Co_3O_4$ nanoparticles**

The cell viability of nanoparticles is a characteristic of chemical composition, shape, concentration, photoactivation & size of nanoparticles [317, 318]. Mechanism of cytotoxic pastime is defined as follows: with the aid of UV & visible light, activation of NiO takes place that triggers the creation of electron-hole pairs [319]. With useful resource of hydrolysis & redox reactions hydrogen peroxide is formed that enters into cell membrane & destroy the microorganism. An ideal anticancer drug or nanoparticle must not distort the normal human tissues [320]. We discovered that the cytotoxic effects of NiO nanoparticles are non-significant. Consequently, NiO nanoparticles are utilized in biomedical applications & in the treatment of cancer, conceivably without affecting the stability of the normal cells [321].

### 6.19. Summary of the results

Nanoparticles are identified as foreign substances by biological systems. Regardless of several therapeutic applications, a few nanoparticles exhibit usual to severe risky or toxicological outcomes [322]. The toxic impact of nanoparticles is predicated by extented type of host-guest interactions in addition to the dose administered [323]. In a few instances nanoparticles can also diffuse freely in biological systems & overcrowd diverse tissues, cells, and sub cellular booths which induced toxicity over the years [324]. Excess accumulation of inorganic nanoparticles ends in the production of inflammatory cytokines, fibrosis, carcinogenesis and lung toxicity, cytoskeleton defects and genetic disorders [325]. Excess manufacturing of ROS occurs due to uncontrolled launch of ions resulting from matrix degradation of nanoparticles [326].

Additionally, nanoparticles' size, biodegraded merchandise, charge, reactivity & defects exist in the nanoparticles surface additionally contribute to ROS era on interplay by biological systems [327]. Previous research confirmed that the direct interaction of nanoparticles using DNA may introduce oxidative stress that induces ROS & RNS (reactive nitrogen species) [328]. This condition similarly promotes breakage of DNA strand, DNA fragmentation and suppression of DNA functions which includes replication & transcription & DNA mutations which finally leads to malignant transformations [329].

Moreover, nanoparticles were stated to indirectly make apoptosis through triggering of oxidative stress and genotoxicity [330]. The affiliation of proteins by nanoparticles could cause making of protein corona which may modify characteristics of

nanoparticles, capabilities of protein & ultimately affect the cellular signaling pathways [331]. To triumph over toxic nature of nanoparticles several techniques have been adopted like change of surface chemistry, size, shape and composition [332].

The present work explained recent method for preparing cobalt oxide nanoparticles & it was found that, its efficiency enhanced on addition of suitable dopants. This study used the trypan blue exclusion method to investigate the cytotoxicity of pure & doped cobalt oxide nanoparticles. Anticancer drugs normally had a lower medical value due to their nonspecific cytotoxicity. Utilization of nanosystems to deliver medication towards cancer cells decreases toxicity. Thus nanoparticles have good potential for use in cancer treatment.

According to our findings, the cytotoxicity of Zn, Fe, Cu and Ni doped $Co_3O_4$ nanoparticles was lower than that of pure $Co_3O_4$ nanoparticles. While magnetic targeting is beneficial in various situations, as another option for effective treatment of various illnesses by future research. When using cobalt oxide nanoparticles, at a concentration of 10 µg/ml the lowest cytotoxicity was reported. On malignant cell lines, when compared to other dopants the synthesized Fe doped $Co_3O_4$ nanoparticles have been reported to show decreased toxicity below 200 µg/ml. It is also clear that it is a biocompatible nanomaterial. The cytotoxicity of nanoparticles is clearly dependent on the concentration value. Fe doped $Co_3O_4$ nanoparticles can be used safely in industrial, commercial, & nanomedicinal applications, if dosage rates were regulated according to way of exposure. The utilization of Fe doped $Co_3O_4$ nanoparticles as a simple medication for elimination of tumour stem cells is evidently demonstrated in this research. As a result, in the near future, by all inventions of new materials, improved designs, & optimization researchers predicted the cancer overcoming era will emerge.

## 6.20. Conclusion

Pharmaceutical and biotechnology organizations are undergoing huge pressure to supply higher grade products to the public while sustaining the byproducts profitably. Recently much importance is given to efficient technologies based on miniaturization and nanotechnology that permit rapid drug discovery and drug development. Polymeric, liposomal and nanocrystal formulations are the important candidates among all the

currently accepted nanomedicine systems. Several pharmaceutical agencies have efficaciously followed nanoparticles formed technologies to discover creative and alternative techniques to triumph over the drug delivery barriers. The main intention is to enhance the therapeutic efficacy of the drug. Commercialization of nanomedicines confronted tremendous challenging situations. A remarkable one is the assessment of novel nanomaterials with regard to safety and toxicity. The present study is an effort in this direction.

## References


[1] Mahmoud Nasrollahzadeh, Mohaddeseh Sajjadi, et. al, Interface Sci & Tech. 28 (2019) 1-27.

[2] Nikalje AP, Med chem., 5 (2015) 081-089.

[3] Singh Deepika, Dilnawaz Fahima, Sahoo Sanjeeb Kumar, Nanomedicine. 15 (2020) 111-114.

[4] Beik J, Khademi S, Attaran N, et al, Curr Med Chem. 24 (2017) 4399-4416.

[5] Sanna V, Pala N, Sechi M, Int J Nanomed. 9 (2014) 467-483.

[6] Beik J, Abed Z, Ghoreishi FS, et al, J. Control Release.235 (2016) 205-221.

[7] Her S, Jaffray DA, Allen C, Adv Drug Deliv Rev. 109 (2017) 84-101.

[8] Beik J, Abed Z, Ghadimi-Daresajini A, et al, J Therm Biol. 62 (2016) 84-89.

[9] Gavas et al. Nanoscale Research Letters (2021) 16:173

[10] Zahin N, Anwar R, Tewari D, et al, Environ Sci Pollut Res. 27 (2020) 19151-19168.

[11] Cheng Z, Li M, Dey R, Chen Y, Journal of Hematology & Oncology, 14 (2021) 1-27.

[12] Tinkle S, McNeil S E, Mühlebach S, et al, Ann. N. Y. Acad. Sci. 1313 (2014) 35-56.

[13] Pena Juarez M, Guadarrama Escobar O R, et. al, J. of Pharma. Innovation. (2021)1-14.

[14] Gunjan Bisht, Sagar Rayamajhi, Nanobiomedicine. 3 (2016) 1-9.

[15] Kumar Bishwajit Sutradhar, Md. Lutful Amin, Nanotechnology. (2014) 1-12.

[16] Baharara Javad, et.al, Annals of Tropical Medicine & Public Health. 10 (2017) 1265-1270.

[17] Ibrahim Khalil, Wageeh A. Yehye, Abeer A. Alhadi, et. al, Antioxidants. 9 (2020) 24.

[18] Linlin Wang, Chen Hu, Longquan Shao, Int J Nanomedicine. 12 (2017) 1227-1249.

[19] Ye Zhang, Ting Liu, et.al, Journal of Hematology & Oncology. 12 (2019) 137.

[20] Syed A.A. Rizvi, Ayman M. Saleh, Saudi Pharm J. 26 (2018) 64-70.

[21] Wilczewska A.Z, Car H, et.al, Pharmacological reports. 64 (2012) 1020-1037.

[22] Deng Y, Zhang X, Shen H, He Q, Wu Z, et.al, Front. Bioeng. Biotechnol. 7 (2020) 489.

[23] Sonia Maraya, Tiquia Arashiro, Debora F Rodrigues, In book: Extremophiles: Applications in Nanotechnology. (2016) 163-193.

[24] Domenico Lombardo, Mikhail A. Kiselev, et.al, Journal of Nanomaterials. (2019) 26 pages.

[25] Gil Gonçalves, Mercedes Vila, et.al, Advanced Healthcare Materials. 2 (2013) 1072-1090.

[26] Phuong Tran, et.al, Journal of Pharmaceutical Investigation. 50 (2020) 261-270.

[27] Ivan H.El Sayed, Xiaohua Huang, Mostafa A.El Sayed, Cancer Letters. 239 (2006) 129-135.

[28] Simona Mura Patrick Couvreur, Advanced Drug Delivery Reviews. 64 (2012) 1394-1416.

[29] GuidoCrisponi, Valeria M.Nurchi, et.al, Antimicrobial Nanoarchitectonics. (2017) 511-546

[30] Maqusood Ahamed, Hisham Alhadlaq,et al, Nanomaterials. 10 (2020) 1675.

[31] Mark W. Dewhirst, Timothy W. Secomb, Nature Reviews Cancer. 17 (2017) 738-750.

[32] Mauro Ferrari, Nature Reviews Cancer. 5 (2005) 161–171.

[33] Anil Kumar, Fei Chen, Anbu Mozhi, Xu Zhang, et al, Nanoscale. 5 (2013) 8307-8325.

[34] Priya Prakash Karmali, Dmitri Simberg, Expert Opinion on Drug Delivery. 8 (2011).

[35] Bokyung Kong, Ji Hyun Seog, Lauren M Graham, Sang Bok Lee, 6 (2011) 929-941.

[36] Dasari Shareena T.P, McShan D, Dasmahapatra A.K, et al, Nano-Micro Lett. 10, 53 (2018).

[37] Lewinski N, Colvin V, Drezek R, Small. 4 (2008) 26-49.

[38] Nel A, Xia T, Madler L, Li N, Science. 311 (2006) 622-627.

[39] Oberdoster G, Oberdoster E, Oberdoster, J. Nanotoxicology.11 (2005) 823-839.

[40] Subcommittee on Nanoscale Science, Engineering, and Technology, Committee on Technology, National Science and Technology Council: National Nanotechnology Initiative: Strategy for Nanotechnology Related Environmental, Health, and Safety Research. (2008) 1-102.

[41] Royal Society and Royal Academy of Engineering: Nanoscience and Nanotechnologies: Opportunities and Uncertainties. (2005) 1-127.

[42] Michael Aschner, Sandra Ceccatelli, Pamela J. Lein, ALTEX. 34 (2017) 49-74.

[43] Chithrani BD, Ghazani AA, Chan WCW, Nano. Lett. 6 (2006) 662-668.

[44] Murphy CJ, Gole AM, Stone JW, et al. Acc. Chem. Res. 41 (2008) 1721-1730.

[45] Hussain SM, Hess KL, Gearhart JM, et.al, Toxicol. In vitro 19 (2005) 975-983.

[46] Oberdorster G, Stone V, Donaldson K, Nanotoxicology. 1 (2007) 2–25.

[47] Nan A, Bai X, Son SJ, Lee SB, Ghandehari H, Nano. Lett. 8 (2008) 2150-2154.

[48] Stone V, Johnston H, Schins RPF Crit. Rev. Toxicol. 39 (2009) 613-626.

[49] Dhawan A, Sharma V, Anal. Bioanal. Chem. 398 (2010) 589-605.

[50] Hakho Lee, Tae-Hyun Shin, et.al, Chem. Rev. 115 (2015) 10690-10724.

[51] Syed Abeer, Future Medicine: Nanomedicine. 25 (2012) 187.

[52] Jingxin Zhang, Weiyue Shi, Qiang Ma, Haixin Cui, Liang Zhang, Coatings 11(2021) 430.

[53] Lenoir, Herron, J Nanomedine Biotherapeutic Discov. 5 (2015) 5.

[54] Houria Boulaiz, Pablo J. Alvarez, Antonia Aranega, Int J Mol Sci. 12 (2011) 3303-3321.

[55] Beissenhirtz M. K, & Willner I, Organic & biomolecular chemistry, 4 (2006) 3392-3401.

[56] Wang C, Fan W, Zhang Z, Wen Y, et.al, Advanced Materials. 31 (2019) 1904329.

[57] Patra, J.K, Das G, Fraceto L.F, et al, J Nanobiotechnol. 16 (2018) 71.

[58] Agata Przekora, Cells. 9(2020) 1622.

[59] Chugh Heerak, Sood Damini, et al, Artificial Cells, Nanomed. & Biotech (2018) 1-11.

[60] Sima Singh, Arshid Numan, Stefano Cinti, Anal. Chem. 94 (2022) 26-40.

[61] BaljinderKaur, et.al, Biosensors & Bioelectronics, 197 (2022) 113805.

[62] Robert Herbert, et.al, Advanced Healthcare Materials. 10 (2021) 2100158.

[63] Zachary L McCormick, MD Michael Hooten, Pain Medicine. 20 (2019) 1459-1460.

[64] Raucci, Maria Grazia, et.al, Luigi Advanced Healthcare Materials (2020) 2000349.

[65] Chiara Attanasio, et.al, Tissue Engineering Part B: Reviews. 22 (2016).

[66] Rebekah M. Samsonraj, et.al, Stem Cells Translational Medicine. 6 (2017) 2173-2185.

[67] NaghmehAbbasi, et.al, Journal of Science: Advanced Materials and Devices. 5 (2020) 1-9.

[68] Lucia Salvioni, Maria Antonietta Rizzuto, et.al, Cancers. 11 (2019) 1855.

[69] Bondarenko O, Mortimer M, Kahru A, et. al, Nano Today, 39 (2021) 101184.

[70] X. Yang, M. Yang, B. Pang, M. Vara, Y. Xia, Chem. Rev.115 (2015) 10410-10488.

[71] OmarTorres, QuesadaJohanna, E.Mayrhofer, et.al, Cellular Signalling. 37 (2017) 1-11.

[72] Ergul Belge Kurutas, Nutrition Journal. 15 (2015) 71.

[73] Vishwas, Sukriti, Gulati, Monica, et al, Current Neuropharmacology. 19 (2021) 957-989.

[74] Syed Abeer, JIMSA. 25 (2012) 3.

[75] K.K. Jain, Med Princ Pract. 17 (2008) 89-101.

[76] Manuel Arruebo, Nuria Vilaboa, Africa Gonzalez, Fernandez, Cancers. 3 (2011) 3279-3330.

[77] Siegel R.L., Miller K.D, Jemal A, CA: A cancer journal for clinicians, 66 (2016) 7-30.

[78] Paul D. Brown, Stephanie Pugh, Nadia N. Laack, Neuro Oncology. 15 (2013) 1429-1437.

[79] Vincent J.Venditto, Francis C.SzokaJr, Advanced Drug Delivery Reviews.65 (2013) 80-88.

[80] Janet L.Markman, et.al, Advanced Drug Delivery Reviews. 65 (2013) 1866-1879.

[81] Andreas Wicki, Dominik Witzigmann, et.al, J. of Controlled Release, 200 (2015) 138-157.

[82] LauraSaenzdel Burgo, et.al, Nanomedicine: Nanotec. Bio & Medicine. 10 (2014) 905-919.

[83] Sonali, Matte Kasi Viswanadh, et.al, Nanotheranostics. 2 (2018) 70-86.

[84] Twan Lammers, Silvio Aime, et.al, Acc. Chem. Res. 44 (2011) 1029-1038.

[85] Mahaveer Swaroop Bhojani, et.al, Mol. Pharmaceutics. 7 (2010) 1921-1929.

[86] Jeremy D. Heidel, Mark E. Davis, Pharmaceutical Research. 28 (2011) 187-199.

[87] Rizzolio, Flavio, Current Medicinal Chemistry. 25 (2018) 4190-4191.

[88] Samer Bayda, Muhammad Adeel, Flavio Rizzolio, Molecules. 25 (2019) 112.

[89] Jaison Jeevanandam, et.al, J Nanotechnol. 9 (2018) 1050-1074.

[90] Kewal K. Jain, Part of the Methods in Molecular Biology book series. 437 (2008).

[91] Asiri Ediriwickrema, W. Mark Saltzman, ACS Biomater. Sci. Eng. 1 (2015) 64-78.

[92] Ashagrachew Tewabe, et.al, J Multidiscip Healthc. 14 (2021) 1711-1724.

[93] Suphiya Parveen, et.al, Nanomedicine: Nanotec, Bio & Med. 8 (2012) 147-166.

[94] Anas Ahmad, Farheen Khan, et.al, J. Med. Chem. 62 (2019) 10475-10496.

[95] Swain Suryakanta, et.al, Current Drug Delivery. 13 (2016) 1290-1302.

[96] Sara Soares, Joao Sousa, Alberto Pais, Carla Vitorino, Front. Chem. 6 (2018) 360.

[97] Nuri Oh, Ji Ho Park, Int J Nanomedicine. 9 (2014) 51-63.

[98] Mitchell M.J, Billingsley M.M, Haley R.M, et al, Nat Rev Drug Discov. 20 (2021) 101-124.

[99] Blanco E, Shen H, Ferrari M, Nat Biotechnol. 33 (2015) 941-951.

[100] Peng Zhang, Jing Ma, Qianqian Zhang, et al, J. Med. Chem. 62 (2019) 7857-7873.

[101] R. Singh, J.W. Lillard Jr, Experimental and Molecular Pathology. 86 (2009) 215-223.

[102] Zhao Y, Wang Y, Ran F, et al, Sci Rep. 7 (2017) 4131.

[103] Fabiana Canal, et al, Curr Opin Biotechnol. 22 (2011) 894-900.

[104] Pradeep K Dhal, et al, Adv Drug Deliv Rev. 61(2009) 1121-30.

[105] Mengxue Zhou, et al, Nanomaterials (Basel). 8 (2018) 952.

[106] Raja Azhar Ashraaf Khan, et.al, Soft Matter. 17 (2021) 8095-8104

[107] Sun SB, Liu P, Shao FM, Miao QL, Int J Clin Exp Med. 8 (2015) 19670-19681.

[108] Akbarzadeh A, Rezaei Sadabady R, Davaran S, et al, Nanoscale Res Lett. 8 (2013) 102.

[109] Zhang J, Tang H, Liu Z, Chen B. Int J Nanomedicine. 12 (2017) 8483-8493.

[110] Pamela T. Wong, Seok Ki Choi, Chemical Reviews. 115 (2015) 3388-3432.

[111] Omid Naderi, et.al, Journal of Molecular Liquids. 273 (2019) 645-652.

[112] Robin Augustine, et.al, Materials Today Communications. 25 (2020) 101692.

[113] Peng Wen, Yan Wen, Min Hua Zong, et.al, Food Chem. 65 (2017) 9161-9179.

[114] Elvin Blanco, Haifa Shen, Mauro Ferrari, Nat Biotechnol. 33 (2015) 941-951.

[115] Gabriel De Crozals, Romaric Bonnet, et.al, Nanotoday. 11 (2016) 393-536.

[116] Annalisa Cardellini, et al, J. Phys.: Condens. Matter. 28 (2016) 483003.

[117] Saptarshi SR, Duschl A, Lopata AL, J Nanobiotechnology. 11 (2013) 26.

[118] Soares, et al, Frontiers in Chemistry. 6 (2018) 360.

[119] Pegi Ahlin Grabnar, Julijana Kristl, Journal of Microencapsulation. 28 (2011) 323-335.

[120] Byung Kook, et.al, Advanced Drug Delivery Reviews. 107 (2016) 176-191.

[121] Jutaek Nam, Nayoun Won, et al, Advanced Drug Delivery Reviews. 65 (2013) 622-648.

[122] Rania Dghaim, et.al, Journal of Environmental and Public Health. (2015) 6.

[123] Mohammad Afsar Khan, et.al, European J. of Pharm. Sciences. 164 (2021) 105892.

[124] Anna Ulldemolins, Joaquin Seras Franzoso, et al, Cancer Drug Resist. 4 (2021) 44-68.

[125] Agnieszka Z.Wilczewska, et.al, Pharmacological Reports. 64 (2012) 1020-1037.

[126] Domenico Lombardo, et.al, Journal of Nanomaterials. (2019) 26.

[127] Rahman Mahfoozur, et al, Current Drug Discovery Technologies. 9 (2012) 319-329.

[128] Diogo Silva Pellosi, et.al, Expert Opinion on Drug Delivery. (2017) 1-12.

[129] Hyung Shik Kim, Dong Yun Lee, Polymers. 10 (2018) 961.

[130] Rainer Tietze, et al, Biochem & Biophysical Research Communi. 468 (2015) 463-470.

[131] Md. Arshad Ali, Temoor Ahmed, Nanomaterials. 10 (2020) 1146.

[132] Zongmin Zhao, Anvay Ukidve, Jayoung Kim, Samir Mitragotri, cell. 181 (2020) 151-167.

[133] Elvin Blanco, Haifa Shen, Mauro Ferrari, Nat Biotechnol. 33 (2015) 941-951.

[134] Mohamed F. Attia, et.al, journal of Pharmacy and pharmacology. 71 (2019) 1185-1198.

[135] Vasir Jaspreet K, Reddy Maram K, et.al, Current Nanoscience. 1 (2005) 47-64.

[136] Wiebke Ulbrich, Alf Lamprecht, J R Soc Interface. 7 (2010) 55-66.

[137] Dong Huang, Lingna Sun, Yanzuo Chen, J Pers Med. 11(2021) 124.

[138] Yu B, Tai HC, Xue W, Lee LJ, Lee RJ. Mol Membr Biol. 27 (2010) 286-298.

[139] Rubel Chakravarty, Hao Hong, Weibo Cai, Mol. Pharmaceutics. 11 (2014) 3777-3797.

[140] Wen H, Jung H, Li X, AAPS J. 17 (2015) 1327-1340.

[141] Glassman P. M, Myerson J W, et. al, Advanced drug delivery reviews, 157 (2020) 96-117.

[142] Zaki, Tirelli, Expert Opin. Drug Deliv. 7 (2010) 895- 913.

[143] Vinod Kumar Khanna, Pharmacology. (2012) 9.

[144] Moghimipour E Rezaei, et al, Journal of microencapsulation. 35 (2018) 548-558.

[145] Kimia Kardani, et.al, Expert Opinion on Drug Delivery. 16 (2019) 1227-1258.

[146] Joan Estelrich, Elvira Escribano, et.al, Int. J. Mol. Sci. 16 (2015) 8070-8101.

[147] Dobrenkov K, Cheung N-KV, Niederhuber JE, Armitage JO, Kastan MB, Doroshow JH, Tepper JE, editors. Abeloff's clinical oncology. 6th ed. Philadelphia: Sanders (2020) 486-99.

[148] Jolanta B Zawilska, Jakub Wojcieszak, et.al, Pharmacological Reports. 65 (2013) 1-14.

[149] Singh L, Kruger HG, Ther Adv Infect Dis. 4 (2017) 105-131.

[150] Wang X, Zhang H, Chen X, et.al, Cancer Drug Resist. 2 (2019) 141-160.

[151] Minakshi Prasad, et al, Biomedicine & Pharmacotherapy. 97 (2018) 1521-1537.

[152] SarbariAcharya, Sanjeeb K.Sahoo, Advanced Drug Delivery Reviews. 63 (2011) 170-183.

[153] D. Friedman, et.al, Current Pharmaceutical Design. 19 (2013) 6315-6329.

[154] Qi Yao Wei, Yan Ming Xu, Andy T. Y. Lau, Cancers. 12 (2020) 2783.

[155] Akhlesh K. Jain, Suresh Thareja, Artificial Cells. 47 (2019) 524-539.

[156] Lorian V. Antimicrob Agents Chemother. 32(1988) 1600-1601.

[157] Gillette JR. In: Benet L.Z., et.al, (eds) Pharmacokinetics. Springer, Boston, MA. (1984).

[158] Miwa T, Kanda M, Umeda S, et al, In Vivo. 33 (2019) 1785-1792.

[159] Parasuraman S, J Pharmacol Pharmacother. 2 (2011) 74-79.

[160] Jeevanandam J, et. al, J Nanotechnol. 9 (2018) 1050-1074.

[161] Marion de Jong, Theodosia Maina, Journal of Nuclear Medicine. 51 (2010) 501-504.

[162] Pennacchio LA, Mamm Genome. 14 (2003) 429-436.

[163] Sausville EA, Newell DR. Eur J Cancer. 40 (2004) 783-784.

[164] Teicher BA. Mol Cancer Ther. 5 (2006) 2435-2443.

[165] Sausville EA, Burger AM, Cancer Res. 66 (2006) 3351-3354.

[166] Wall RJ, Shani M. Theriogenology. 69 (2008) 2-9.

[167] Mather S, Bioconjug Chem. 20 (2009) 631-643.

[168] Lyons SK, J Pathol. 205 (2005) 194-205.

[169] Aravalli RN, Golzarian J, Cressman ENK. Eur Radiol. 19 (2009) 1049-1053.

[170] Koo V, Hamilton PW, Williamson K, Cell Oncol. 28 (2006) 127-139.

[171] Maina T, Nock BA, Zhang H, et al. J Nucl Med. 46 (2005) 823-830.

[172] Greve JM, Les AS, et al. Am J Physiol Heart Circ Physiol. 291 (2006) H1700-H1708.

[173] Perel P, Roberts I, Sena E, et al, Br Med J. 334 (2007) 197-200.

[174] Tran SL, Puhar A, Ngo Camus M, Ramarao N. PLoS One. 6 (2011) e22876.

[175] Chan LL, Wilkinson AR, Paradis BD, Lai N, J Fluoresc. 22 (2012) 1301-1311.

[176] Chen SF, Lu WF, Wen ZY, Li Q, Chen JH, Pharmazie. 67 (2012) 781-788.

[177] Altiok N, Ersoz M, Koyuturk M. Oncol Lett. 2 (2011) 1281-1285.

[178] Puoci F, Morelli C, Cirillo G, et al, Anticancer Res. 32 (2012) 2843-2847.

[179] Kroemer G, Galluzzi L, et al, Cell Death Differ. 16 (2009) 3-11.

[180] Tennant JR. Transplantation. 2 (1964) 685-694.

[181] Song K, Li W, et al, Appl Biochem Biotechnol. 167 (2012) 2381-2387.

[182] Zanatta G, Steffens D, et.al, Braz J Med Biol Res. 45 (2012) 125-130.

[183] Kim JS, Nam MH, An SS, Lim CS, Hur DS, et al, J Clin Lab Anal. 25 (2011) 90-94.

[184] Zanoni M, Piccinini F, Arienti C, Zamagni A, Santi S, et al, Sci Rep. 6 (2016) 19103.

[185] Stoddart MJ. Clifton: Humana Press; 2011.

[186] McMahon TA, Rohr JR, EcoHealth. 11 (2014) 164-167.

[187] Canning DR, Cunningham RL, In Vitro Cell Dev Biol Anim. 51 (2015) 507-514.

[188] Santo VE, Rebelo SP, Estrada MF, et. al, Biotechnol J. 12 (2017) 1600649.

[189] Cesarz Z, Tamama K. Stem Cells Int. (2016) 9176357.

[190] Kawaguchi N, Machida M, Hatta K, et.al, Biochem Res Int. (2013) 730874.

[191] Louis KS, Siegel AC, Methods Mol Biol. 740 (2011) 7-12.

[192] Piccinini F, Tesei A, et.al, Comput Methods Prog Biomed. 117 (2014) 448-463.

[193] Hannan A, Kang JY, et al, In vitro Cell Dev Biol Anim. 48 (2012) 535-44.

[194] Pappenheimer AM, J Exp Med. 25 (1917) 633-650.

[195] Chan LLY, Kuksin D, Laverty DJ, Saldi S, Qiu J, Cytotechnology. 67 (2015) 461-473.

[196] Pamphilon D, Selogie E, Mckenna D, et al, Cytotherapy. 15 (2013) 255-62.

[197] Ye Zhang, Maoyu Li, et.al, Journal of Hematology & Oncology. 12 (2019) 137.

[198] Mukhtar M, Bilal M, Rahdar A, et al, Chemosensors. 8 (2020) 117.

[199] Yao Y, Zhou Y, Liu L, Xu Y, Chen Q, et al, Front. Mol. Biosci. 7 (2020) 193.

[200] Colone M, Calcabrini A, Stringaro A, Molecules. 25(2020) 4560.

[201] Patra JK, Das G, Fraceto LF, et al, J Nanobiotechnology. 16 (2018) 71.

[202] Mahmoud Nasrollahzadeh S, et. al, Interface Science and Technology. 28 (2019) 1-27.

[203] Choi G, Eom S, Vinu A, Choy J.H, The Chemical Record. 18 (2018) 1033-1053.

[204] Milad Ashrafizadeh, Ali Zarrabi, et al, ACS Comb. Sci. 22 (2020) 669-700.

[205] Ajith TA, Janardhanan KK, Journal of ethnopharmacology. 84 (2003) 157-162.

[206] B.A. Avelar, FreitasV.G, et al, Braz J Med Biol Res. 47 (2014).

[207] Bowman AM, Nesin OM, Pakhomova ON, et al, J Membrane Biol. 236 (2010) 15-26.

[208] Paul Karl Horan, John W.Kappler, Journal of Immunological Methods. 18 (1977) 309-316.

[209] Judson R, Houck K, Martin M, et al, Toxicological Sciences. 152 (2016) 323-339.

[210] Li W, Zhou J, Xu Y. Biomedical Reports. 3 (2015) 617-620.

[211] Richard Judson, Keith Houck, et al, Toxicological Sciences. 152 (2016) 323-339.

[212] Tang D, Kang R, Berghe TV, et al, Cell Res. 29 (2019) 347-364.

[213] Tomris Ozben, Journal of Pharmaceutical Sciences. 96 (2007) 2181-2196.

[214] M.Kuznetso, J.Clairambault, V.Volpert, Physics of Life Reviews. 39 (2021) 1-48.

[215] Richard Judson, Keith Houck, et al, Toxicological Sciences. 152 (2016) 323-339.

[216] Pichler WJ, Hausmann O, Int Arch Allergy Immunol. 171 (2016) 166-179.

[217] ElenaPapis, FedericaRossi, et al, Toxicology Letters. 189 (2009) 253-259.

[218] Sengul AB, Asmatulu E, Environ Chem Lett. 18 (2020) 1659-1683.

[219] Katrien Luyts, Dorota Napierska, et.al, Environ. Sci. Processes Impacts. 15 (2013) 23-38.

[220] Faisal M, Saquib Q, Alatar AA, Al Khedhairy AA, et al, Biol Res. 18 (2016) 20.

[221] Blanco E, Shen H, Ferrari M, Nat Biotechnol. 33 (2015) 941-951.

[222] Pucci C, Martinelli C, Ciofani G, Ecancermedicalscience. 13 (2019) 961.

[223] De Jong WH, Borm PJ, Int J Nanomedicine. 3 (2008) 133-149.

[224] Abdal Dayem A, Hossain MK, Lee SB, et al, Int J Mol Sci.18 (2017) 120.

[225] M. Mahmoudi, A Simchi, et.al, J. Colloid. Interface. Sci. 336 (2009) 510-518.

[226] Kim YJ, Perumalsamy H, et al. Int J Nanomedicine. 9 (2019) 8195-8208.

[227] Wiesmann N, Kluenker M, et al. J Trace Elem Med Biol. 51 (2019) 226-234.

[228] Akhtar MJ, Ahamed M, Kumar S, et al. Int J Nanomedicine. 7 (2012) 845-57.

[229] Ng CT, Yong LQ, Hande MP, et al. Int J Nanomedicine. 28 (2017) 11621-1637.

[230] Aryal S, Bisht G. Aryal S, et al, Biomedicines. 5 (2017) 38.

[231] Kudzin MH, Giełdowska M, Krol P, et al. Materials (Basel). 15 (2022) 2746.

[232] Zadeh FA, Bokov DO, Salahdin OD, et al, Rend Lincei Sci Fis Nat. 21 (2022) 1-7.

[233] Kashyap VK, Peasah Darkwah G, et al. Pharmaceutics. 14 (2022) 611.

[234] Aljabali AAA, Obeid MA, Bakshi HA, et al. Molecules. 27 (2022) 1827.

[235] Alghamdi MD, Nazreen S, Ali NM, et al, Nanomaterials (Basel). 12 (2022) 664.

[236] Gowda R, Jones NR, Banerjee S, Robertson GP, J Nanomed Nanotechnol. 4 (2013).

[237] Wang R, Billone PS, Mullett WM, Journal of Nanomaterials. 12 (2013).

[238] N.D. Thorat, O.M. Lemine, et.al, Phys. Chem. Chem. Phys. 33 (2016) 941-951,

[239] N.D. Thorat, O.M. Lemine, et.al, Phys. Chem. Chem. Phys. 18 (2016) 21331-21339.

[240] Bhushan M, Kumar Y, Periyasamy L, et al, Appl Nanosci. 8 (2018) 137-153.

[241] Imran ShairMohammad, et.al, Int. Journal of Pharmaceutics. 562 (2019) 187-202.

[242] Mian Wang, Yuanfeng Xin, Hao Cao, et.al, Biomater. Sci. 9 (2021) 1088-1103.

[243] McBain SC, Yiu HH, Dobson J, Int J Nanomedicine. 3 (2008) 169-180.

[244] Lin Mei, Chunlei Lin, et.al, ACS Appl. Nano Mater. 2 (2019) 2902-2908.

[245] R.Abhinayaa, G.Jeevitha, et.al, Colloids & Surfaces B: Biointerfaces 169 (2018) 395-403.

[246] A. Kumar, A. K. Pandey, S. S. Singh, Free Radic. Biol. Med. 51 (2011) 1872-1881.

[247] H. Kettiger, G. Quebatte, B. Perrone, BBA. 9 (2016) 2163-2170.

[248] T. B Das, S. K Dash, D. Mandal, ARAB. J. CHEM. 10 (2017) 862-876.

[250] Geetha R, Ashokkumar T, Tamilselvan S, et al, Cancer Nanotechnol. 4 (2013) 91-98.

[251] Khan S, Ansari AA, Khan AA, et al, J. Biol. Inorg. Chem. 20 (2015) 1319-1326.

[252] Sreekanth TVM, Pandurangan M, Kim DH, Lee YR. J. Clust. Sci. 27 (2015) 671-681.

[253] Pesic M, Podolski RA, Stojkovic S, Matovic B, et al, Chem. Biol. Interact. 5 (2015) 85-93.

[254] Laha D, Pramanik A, Maity J, et al. Biochim. Biophys. Acta 1840 (2014) 1-9.

[255] Dipranjan L, Debalina B, Arindam P, Panchanan P, et al, Toxicol. Res. 1 (2012) 131- 136.

[256] Pramanik A, Laha D, Dash SK, Roy S, et al, Toxicol. Res. 4 (2015) 1604-1612.

[257] Nagajyothia PC, Muthuramanb P, et.al, Arab. J. Chem. 10 (2017) 215-225.

[258] Wang Y, Zi XY, Su J, Zhang HX, et al, Int. J. Nanomedicine. 7 (2012) 2641-2652.

[259] Guo Y, Zhang J, Yang L, et al, Chem. Commun. (Camb) 46 (2010) 3493-3495.

[260] Din MI, Arshad F, Hussain Z, Mukhtar M. Nanoscale Res. Lett. 12 (2017) 638.

[261] Bhattacharya PT, Misra SR, Hussain M, Scientifica (Cairo). (2016) 5464373.

[262] Zhou M, Tian M, Li C, Bioconjugate Chem. 27 (2016) 1188-1199.

[263] Imali A. Mudunkotuwa, et.al, Environ. Sci. Technol. 46 (2012) 7001-7010.

[264] EdgarPerez Herrero, et.al, European J. of Pharm. & Biopharmaceutics. 93 (2015) 52-79.

[265] Halevas EG, Pantazaki AA, NanomedNanotechnol J. 2 (2018) 119.

[266] Skeeters SS, Rosu AC, et al, ACS Appl Mater Interfaces. 12 (2020) 50203-50211.

[267] Wang L, Hu C, Shao L, Int. J. Nanomedicine. 12 (2017) 1227-1249.

[268] Giannousi K, Pantazaki A, Dendrinou Samara C, Grumezescu AM. (editors). Chapter No 23. (published by Elsevier). Subject: Nanostructures for Antimicrobial Therapy (2017) 515-529.

[269] Giannousi K, Lafazanis K, et.al, J. Inorg. Biochem. 133 (2014) 24-32.

[270] Castro L, Blazquez ML, et.al, Rev. Adv. Sci. Eng. 3 (2014) 199-216.

[271] Dahoumane SA, Jeffryes C, et.al, Bioengineering (Basel). 4 (2017) 14.

[272] Theivasanthi T, Alagar M, Int. J. Phys. Sci. 6 (2011) 3662-3671.

[273] Giannousi K, Sarafidis G, Mourdikoudis S, et.al, Inorg. Chem. 53 (2014) 9657-9666.

[274] Giannousi K, Hatzivassiliou E, et al, J. Inorg. Biochem. 164 (2016) 82-90.

[275] Antonoglou O, Giannousi K, et al, J Inorg Biochem. 177 (2017) 159-170.

[276] Patil MP, Ngabire D, Thi HHP, Kim MD, Kim GD, J Clust Sci. 28 (2017) 119-132.

[277] Zharov VP, Kim JW, Curiel DT, Everts M, Nanomedicine. 1 (2005) 326-345.

[278] Fang J, Yu L, Gao P, Cai Y, Wei Y, Anal. Biochem. 399 (2010) 262-267.

[279] Thanh NTK, Green LAW, Nano Today. 5 (2010) 213-230.

[280] Wang P, Wang X, Wang L, et al, Sci Technol Adv Mater. 16 (2015) 034610.

[281] Ann FF, Zhang XH, Theranostics. 7 (2017) 3667-3689.

[282] Pissuwan D, Niidome T, Cortie MB, J. Control. Release. 149 (2011) 65-71.

[283] Jahangirian H, Lemraski EG, et.al, Int J Nanomedicine. 12 (2017) 2957-2978.

[284] Liu Y, Miyoshi H, Nakamura M, Int J Cancer. 120 (2007) 2527-2537.

[285] Wang Z, Li N, Zhao J, White J C, Qu P, et al, Chem Res Toxicol. 25 (2012) 1512-1521.

[286] Siddiqui MA, Alhadlaq HA, e al, PLoS ONE. 8 (2013) e69534.

[287] Sun T, Yan Y, Zhao Y, Guo F, Jiang C, PLoS ONE. 7 (2012) e43442.

[288] Wang Y, Yang F, Zhang HX, Zi XY, Pan XH, et al, Cell Death Dis. 4 (2013) e783.

[289] Laha D, Pramanik A, Maity J, et al, Biochim Biophys Acta. 1840 (2014) 1-9.

[290] Chena Z, Menga H, Xing G, Chena C, Zhaoa Y, et al, Toxicol Lett. 163 (2006) 109-120.

[291] Ahmad MZ, Akhter S, Jain GK, et al, Expert Opin Drug Deliv. 7 (2010) 927-942.

[292] Studera AM, Limbach LK, Duc LV, et al, Toxicol. Lett. 197 (2010) 169-174.

[293] Midander K, Cronholm P, Karlsson HL, Elihn K, Moller L, et al, Small. 5 (2009) 389-399.

[294] Chakraborty R, Basu T, Nanotechnology. 28 (2017) 105101.

[295] T Hyeon, Chemical Communications. 9 (2003) 927-934.

[296] R Karmhag, T Tesfamichael, et.al, Solar Energy. 68 (2000) 329-333.

[297] A A Mariam, M Kashif, et al, Digest J of Nanomat. & Biostructures. 9 (2014) 1007-1019.

[298] A Saxena, A Kumar, S Mozumdar, J of Molecular Catal. A: Chemical. 269 (2007) 35-40.

[299] F Alonso, P Riente, M Yus, Tetrahedron. 64 (2008) 1847-1852.

[300] A Dhakshinamoorthy, K Pitchumani, Tetrahedron Letters. 49 (2008) 1818-1823.

[301] F Alonso, P Riente, M Yus, European J. Organic Chemistry. 34 (2009) 6034-6042.

[302] F Alonso, P Riente, M Yus, European J. Organic Chemistry. 29 (2008) 4908-4914.

[303] X K Li, W J.Ji, J Zhao, S J Wang, CT Au, Journal of Catalysis. 236 (2005) 181-189.

[304] Y Li, B Zhang, X Xie, J Liu, Y Xu, W Shen, Journal of Catalysis. 238 (2006) 412-424.

[305] C J Pandian, R Palanivel, et.al, Chinese J. of Chem. Eng. 23 (2015) 1307-1315.

[306] G Angajala, S Radhakrishnan, Inflammation and Cell Signaling. 1 (2014).

[307] M Chen, Y Zhang, B Huang, et al, Journal of Nanomaterials. Article ID 623497 (2013) 6.

[308] M Vaseem, N Tripathy, G Khang, Y B Hahn, RSC Advances. 3 (2013) 9698-9704.

[309] H Rui, R Xing, Z Xu, Y Hou, S Goo, S Sun, Advanced Materials. 22 (2010) 2729-2742.

[310] I Brigger, C Dubernet, P Couvreur, Advanced Drug Delivery Reviews. 54 (2002) 631-651.

[311] A K Gupta, M Gupta, Biomaterials. 26 (2005) 11565-1573.

[312] S Sudhasree, A Shakila Banu, et.al, Toxicol. & Environ. Chem. 96 (2014) 743-754.

[313] Muhammad Imran Din, Aneela Rani, Int. J. of Anal. Chem. Article ID 3512145 (2016) 14.

[314] N L Holy, Chem. Rev. 74 (1974) 243-277.

[315] B Viswanath, Paromita Kundu, et.al, J. Phys. Chem. C. 113 (2009) 16866-16883.

[316] Mattias Törnquist, Thomas C. T, et.al, Chem. Commun. 54 (2018) 8667-8684.

[317] Haohong Duan, Dingsheng Wang, Yadong Li, Chem. Soc. Rev. 44 (2015) 5778-5792.

[318] Moustafa M G Fouda, et.al, Synthetic Metals. 269 (2020) 116553.

[319] S Sudhasree, A Shakila Banu, et.al, Toxico & Environmental Chem. 96 (2014) 743-754.

[320] Imran Din M, Rani A, Int J Anal Chem. (2016) 3512145.

[321] Lingyun Zhou, Tian Qiu, et.al, Adv. Healthcare Mater. (2018) 1800670.

[322] Shahanavaj Khan, et al, Journal of Trace Elements in Med and Biology. 52 (2019) 12-17.

[323] Wided Najahi Missaoui, et.al, Int. J. Mol. Sci. 22 (2021) 385.

[324] Guocan Yu, Xiaoyuan Chen, Theranostics. 9 (2019) 3041-3074.

[325] Gunjan Bisht, Sagar Rayamajhi, Nanobiomedicine. (2016) 3-9.

[326] Ata Ur Rehman, SadiaNazir, et al, Journal of Molecular Liquids. 321 (2021) 114455.

[327] Zhonglei He, Qian Xu, Ben Newland, et al, J. Mater. Chem. B. 9 (2021) 6326-6346.

[328] Wendelin J. Stark, Angew, Chem. Int. Ed. 50 (2011) 1242-1258.

[329] Sarkar Abhijit, et.al, Journal of Nanoscience and Nanotechnology. 14 (2014) 730-743.

[330] Elizabeth M. Black, Simona Giunta, Genes. 9 (2018) 615.

[331] MH Kathawala, et al, Materials and Design. 88 (2015) 41-50.

[332] Rong Cai, Chunying Chen, Adv. Mater. (2018) 1805740.

[333] VickiStone, BerndNowack, et al, Sci. of The Total Environment. 408 (2010) 1745-1754.