NANOMEDICINE AND NANOTECHNOLOGY JOURNAL Open Access

SCIENTIFIC LITERATURE

REVIEW ARTICLE

Copper Nanoparticles as Therapeutic Anticancer Agents

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ARTICLE INFO

Article history:
Received: 18 July 2018
Accepted: 05 September 2018
Published: 10 September 2018

Keywords:
Copper;
Nanoparticles;
Nano-formulations;
Therapeutic;
Anticancer agents

Citation this article: Halevas EG, Pantazaki AA. Copper Nanoparticles as Therapeutic Anticancer Agents. NanomedNanotechnol J. 2018; 2(1):119.

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ABSTRACT

Bio-nanotechnology exploits physicochemical approaches and biological principles in order to produce specifically functionalized nano-sized particles. Nanoparticles may be very effective molecules in the development of novel therapeutic approaches against several diseases including cancer. Consequently, synthesis of metallic nanoparticles for the improvement of therapeutic index and drug delivery applications is coming up as an effective strategy in the conventional therapeutic anticancer research. In recent years, the auspicious anticancer potential of nano-particulate metallic forms of gold, silver, and copper is progressively being established. As a result, the development of copper-derived nano-therapeutics is challenging due to the cost-effectiveness of copper, and the already extensively studied anticancer potential of copper-based nanoparticles such as copper oxide nano-formulations. However, limited investigations have been conducted on the anticancer efficacy of metallic copper nanoparticles. Herein, we present an analytical overview of the therapeutic applications of copper nanoparticles as efficient anticancer agents.

Introduction

Nano-biotechnology, and more specifically the synthetic procedures of metallic nanoparticles (NPs) along with their biological applications have attracted increasing scientific interest due to their distinctive chemical and physical properties [1], synthetic feasibility, low production cost [1] and, additionally, because of their significantly valuable applicability in the fields of medicine and pharmaceutical science [3-6] Until today, several physicochemical approaches have been developed on the synthesis of diversified types of metallic NPs. Various research fields such as experimental medicine, drug design, drug delivery, electrical and electronics engineering, electrochemical sensor and biosensor development, agricultural science and biochemistry are delving into the development and further implementation of novel metallic NPs [7-9].

Worldwide, cancer constitutes one of the most common health issues. Nowadays, novel cancer treatment approaches include utilization of nanomaterials along with their unique biochemical properties. The therapeutic efficacy of nano-scale molecules relies heavily on their enhanced reactive surface area, compared to conventional small-molecule drugs and pharmaceuticals. Until recently, the anticancer potential of nano-particulate

forms of metals including gold, silver, and copper has progressively being documented [10-13]. However, the low cost of copper compared to that of gold and silver, results in the enhanced development of copper-based NPs as therapeutics. Copper NPs constitute a particular group of metallic NPs produced via contemporary technologies and with advantageous physicochemical, biological and mechanical properties [14,15]. Moreover, it has been proved that copper and copper-based NPs exhibit enhanced toxicity against various microorganisms, thus becoming an effective antimicrobial and antifungal agent [16-22].

Synthesis of copper NPs involves short and simple experimental procedures and cost-effective reaction conditions with high production yields compared to other types of metal NPs [23,24]. Their wide range of nano-dimensions and surface-to-volume ratios abet their potency as medicinal, pharmaceutical, and therapeutic agents [25,26]. Moreover, copper NPs can be utilized as DNA-cleavage agents and potent anticancer therapeutics due to their binding capacity and modifiable surface properties through conjugation with various bio-molecules including enzymes and proteins [27-30]. Copper NPs can also function as effective drug delivery nano-formulations and molecular doping systems operating as controllers of cancer cell growth [31-33].

However, the toxic profile of the implemented chemicals and the environmentally harmful physicochemical methods applied during the production of copper NPs [34,35] urges the need for the development of eco-friendlier methods of synthesis [36], maintaining the desired morphology, size, and stability combined with improved pharmaceutical and medicinal properties [37-40]. Eco-friendly synthesized bio-mediated nanoparticles [41] can be used as an effective nano-drug against several types of cancer disease [42,43].

Although there are several scientific reports on the anticancer potential of copper oxide NPs [44-48], limited research results have been disseminated on the antitumor potency of metallic copper NPs [49,50], mainly due to their unstable and easily oxidized form at ambient temperature conditions. Herein, we present a concise overview of the therapeutic applications of copper NPs as anticancer agents.

Synthesis and characterization techniques of metallic NPs

1. Physical and chemical synthetic methods of copper NPs

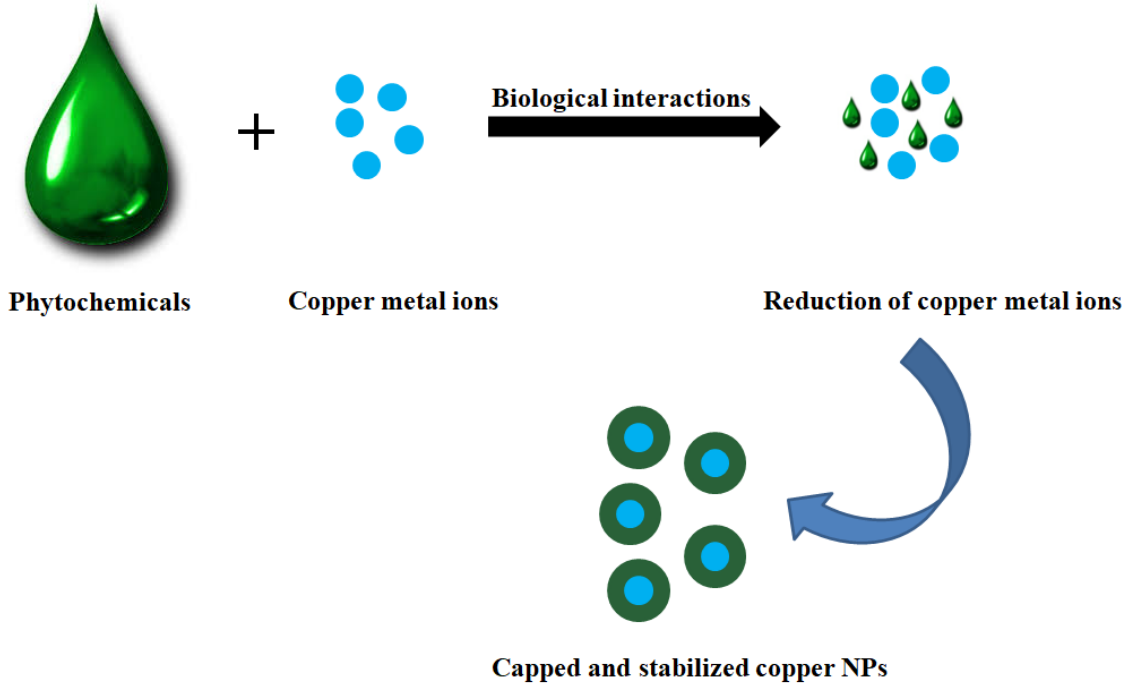
Various physical and chemical methods have been applied for the synthesis of metallic NPs including copper NPs such as: microwave-assisted processes and sol-gel procedures [51], co-precipitation [52], pulsed wire discharge [53],laser ablation [54],high-energy irradiation [55], vacuum-vapor deposition [56], mechanical milling [57], lithography [58], electrochemistry and photochemical reduction, [59-63], hydrothermal reaction [64], micro-emulsion [65], electrospray synthesis [66], and chemical reduction [67].

2. Biosynthetic methods or "green"-synthesized copper NPs

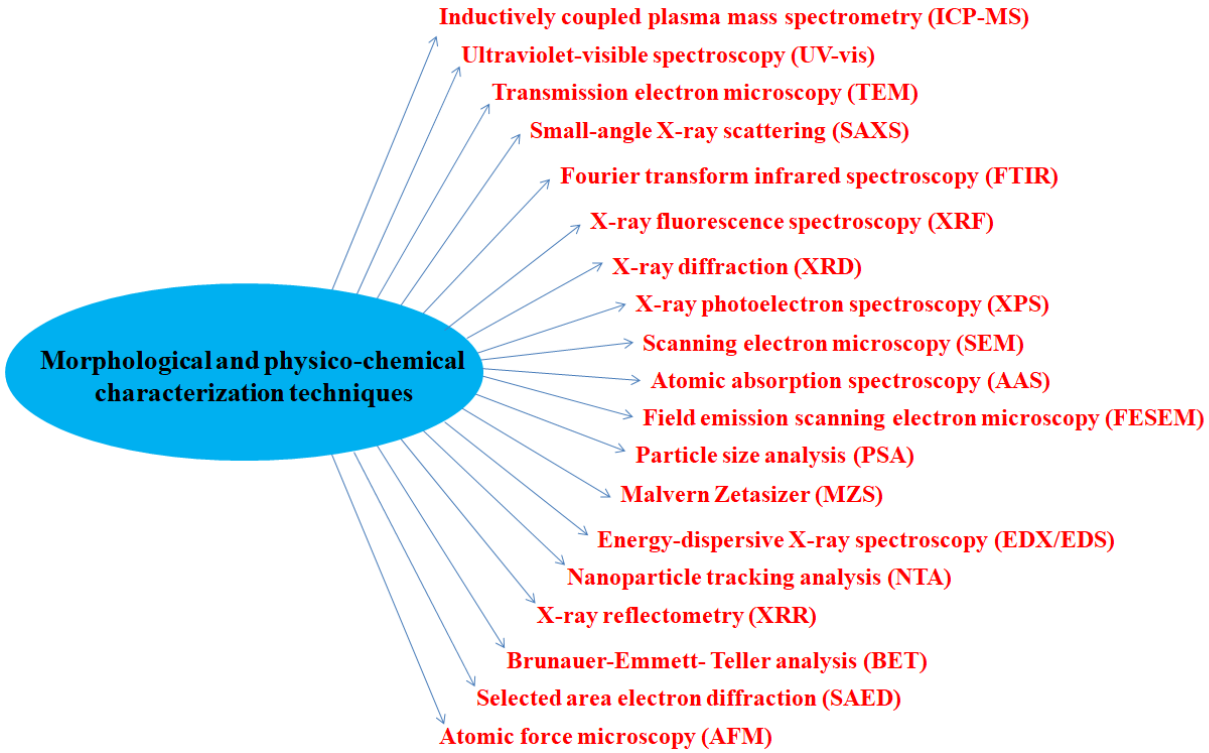
The biosynthetic procedures of NPs are based on green chemistry methods employing different biological systems including plants [68,69], fungus [40,70-72], actinomycetes [73,74] yeast [75-77], bacteria [78-82], and viruses [83,84].

"Green"-synthesized copper NPs are produced through the utilization of phytochemicals in order to generate nano-formulations of more flexible shapes and sizes by controlling either the reaction temperature, time, and pH and, additionally, the concentrations of the employed plant extract and/or metal salt [67]. The successful reduction of the copper ion and the subsequent formation of the pursued NPs, which is an instant phenomenon, are confirmed through the observed color change of the reaction mixture. Phytochemicals also operate as the stabilizing agents of the produced NPs, as depicted in Scheme 1 [67].

"Bionanofactories", such as macroalgae, can synthesize highly stable and functional metallic NPs capable of eliminating cell maintenance [85]. Recently, gold NPs were synthesized through the utilization of algae extracts including *Sargassum wightii* [86], *Laminaria japonica* [87], *Turbinaria conoides* [88], and *Stoechospermum marginatum* [89]. In general,



Scheme 1: Reduction of copper ions and stabilization-capping of the produced copper NPs.



Scheme 2: Morphological and physicochemical characterization techniques of metallic NPs.

macroalgae (seaweeds) constitute a significant source of biomedical substances and compounds with exceptional antibacterial [90], antifouling [91], and anticoagulant activity [92].

3. Characterization techniques

Several techniques are employed for the physicochemical and morphological characterization of the produced NPs as depicted in Scheme 2. A detailed overview of the determined characteristics and attributes of each morphological and physicochemical characterization technique is presented in Table 1.

The most widely used analytical methods for the detection of copper in aqueous media and/or biological fluids are Atomic Absorption Spectrometry (AAS) with flame detection or stabilized temperature platform graphite furnace, inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectroscopy, and X-ray fluorescence spectrometry [93-97]. One of the main applications of the specific analytical techniques is the determination of main and trace element concentration in different human fluids and tissues [98,99]. It has been proved that increased copper concentrations in serum results in tumor genesis, malignant proliferation and recurrence in several types of human cancers [98-100].

4 Dietary copper intake and therapeutic copper dosing

Copper is considered an essential nutrient. In the majority of foods, copper is presently bound to macromolecules rather than as a free metal ion [101]. Recent recommended dietary allowance (RDA) reports established a daily copper intake of 900 μg for adults, 340 μg/day for children for the first 3 years of age, 440 μg/day for ages between 4-8 years, 700 μg/day for ages between 9-13 years, and 890 μg/day for ages between 14-18 years [102]. Moreover, RDA copper values of 1000 μg/day and 1300 μg/day are recommended during pregnancy and lactation, respectively. However, no sufficient RDA data have been established for infants. Reports on the copper concentration in human milk implied that copper intakes of 200 μg/day and 220 μg/day were sufficient for the first 6 months and for the second 6 months of life, respectively. The estimated daily average amounts of required copper are 12.5 $\mu g \cdot kg^{-1}$ of body weight for adults and 50 $\mu g \cdot kg^{-1}$ of body weight for infants, respectively [103]. The established tolerable copper intake level from foods and supplements for adults (including breastfeeding age) is 10 mg/day, 1 mg/day for children of age 1-3 years, 3 mg/day for children of age 4-8 years, 5 mg/day for children of age 9-13 years, and 8 mg/day for pregnancy age and ages between 14 and 18 years [102]. For 19-year-old men and women the copper RDA is 900 mcg/day, and for pregnancy and breastfeeding age the copper RDA is 1000 mcg/day and 1300 mcg/day for women of all ages, respectively [104]. The therapeutic copper dose levels that have been scientifically studied are [105-109]:

Orally:

- For copper deficiency: daily doses up to 0.1 $mg \cdot kg^{-1}$ of $CuSO_4$.
- For osteoporosis: daily mixed doses of 2.5 mg copper in combination with 1000 mg calcium, 15 mg zinc, and 5 mg manganese.

Intravenously:

- For severe copper deficiency: intravenous provision of copper (2-4 mg/day). For mild to moderate copper deficiency: intravenous provision of copper (3-8 mg/day).

Anticancer Activity of Copper NPs

1. Anticancer activity of "green"-synthesized copper NPs

Recent studies on the anticancer potential and cytotoxic activity of "green" synthesized copper NPs from *Sargassum polycystum* brown seaweed (Figure 1), utilizing copper sulfate ($CuSO_4$) as the precursor, indicated that NP concentrations of 100 $\mu g \cdot ml^{-1}$ can efficiently provoke inhibition of the growth of MCF-7 breast cancer cells at percentages higher than 93% and with an IC_{50} value of 61.25 $\mu g \cdot ml^{-1}$ [110].

Studies on the biosynthesis of copper NPs utilizing plants including *Nerium oleander* [111], and *Magnolia Kobus* [112], have previously been reported. The findings of recent studies on the biosynthetic procedure of copper NPs utilizing the *E. prostrate* aqueous leaf extract as a

reducing reagent; suggested a promising copper-based nanomaterial with improved activity against cell proliferation. The *in vitro* cytotoxic potential of increasing concentrations of the corresponding copper NPs (1-500 $\mu g \cdot ml^{-1}$) on the growth and morphological characteristics of the human HepG2 cancer cell line, estimated via the MTT assay, indicated cellular toxicity values of up to 54.5% [113].

Prosopis cineraria are a well-known therapeutic tree species with excellent analgesic, antihyperglycemic, antipyretic, antihypercholesterolemic, antioxidant, and antitumor properties. Examination of the anticancer potential of *Prosopis cineraria* leaf extracts indicated growth inhibition of human HeLa and MCF-7 cancer cells [114,115].

Table 1: Determined characteristics and attributes of each morphological and physico-chemical characterization technique [**67**].

Morphological and physico-chemical characterization techniques	Determined characteristics and attributes
Ultraviolet-visible spectroscopy (UV-vis)	Concentration and shape of NPs
Fourier transform infrared spectroscopy (FTIR)	Nature of bonds and functional groups
X-ray diffraction (XRD)	Size and crystallinity of NPs
Scanning electron microscopy (SEM)	Shape, size and structure of nano-formulations
Field emission scanning electron microscopy (FESEM)	Structural and morphological characteristics
Transmission electron microscopy (TEM)	Shape, size and structure of nano-formulations
Particle size analysis (PSA)	Size distribution of solid or liquid particulate materials
Malvern Zetasizer (MZS)	Size, zeta potential, and protein mobility
Energy-dispersive X-ray spectroscopy (EDX/EDS)	Composition of NPs
Nanoparticle tracking analysis (NTA)	Particle size, concentration, and fluorescent properties
Small-angle X-ray scattering (SAXS)	Shape and size conformation
X-ray reflectometry (XRR)	Thickness, density, and roughness
X-ray fluorescence spectroscopy (XRF)	Chemical composition and concentration
X-ray photoelectron spectroscopy (XPS)	Elemental composition
Brunauer-Emmett-Teller analysis (BET)	Specific surface area
Selected area electron diffraction (SAED)	Shape, size and structure of nano-formulations
Atomic force microscopy (AFM)	Particle size and surface characterization
Atomic absorption spectroscopy (AAS)	Amount of metal present in metallic nano-formulations
Inductively coupled plasma mass spectrometry (ICP-MS)	Amount of metal present in metallic nano-formulations

Figure 1: Sargassum polycystum brown seaweed.

Studies on the microwave-assisted synthesis of "eco-engineered" copper NPs using *P. cineraria* leaf extracts and their cytotoxic and antiproliferative effects against human MCF-7 cancer cell line, estimated by the MTT assay, indicated that stabilized copper NPs improved the cytotoxic activity strongly associated with the DNA-fragmentation procedure, significantly more effectively compared to that of stabilized silver NPs and plant leaf extracts [116].

Quisqualis indica Linn is an evergreen plant species with therapeutic phytoconstituents exhibiting anti-inflammatory, antipyretic, immuno-modulatory, and antibacterial properties [117]. Efforts on the development of a green synthetic procedure of copper NPs utilizing the *Quisqualis indica Linn* floral extract resulted in the production of mono-disperse nano-formulations with a dose-dependent (40-120 $\mu g \cdot ml^{-1}$) cytotoxic activity, as evidenced through extracellular Lactate Dehydrogenase (LDH) release, reactive oxygen species (ROS) generation, and intracellular reduced glutathione (GSH) content depletion in B16F10 melanoma cells and with an IC_{50} value lower compared to that of the floral extract [118].

In recent studies, the eco-friendly synthetic approach of highly crystalline and small-sized copper NPs using Broccoli Green Extract (BGE) resulted, initially, in rapid reduction of $CuSO_4$ precursor and, subsequently, in the production of relatively small and spherical shaped copper nano-formulations. Cytotoxicity studies of the corresponding surface modified copper NPs via cellular density measurements presented no effect on prostate cancer (PC-3) cells in concentrations ranging between 0.5-1.5 μM and after 2 h of exposure time. The collective anti-proliferative outcome is comparable to reported results on the observed 44-times more intense inhibitory effect of silver NPs against HepG2 cells compared to normal cells [119,120].

A relatively rapid and simple synthetic procedure of peptide capped copper NPs in the latex of the therapeutic *Euphorbia nivulia* plant species [121] has also been reported [122]. Further studies on the cytotoxic potential of the resulting biocompatible latex-capped nano-formulations in human A549 lung carcinoma cells indicated the dose-dependent anti-proliferative activity of the corresponding nano-formulations in concentrations ranging between 1-100 $\mu g \cdot ml^{-1}$. These observations confirm the ability of copper NPs to induce structural damages of the cellular milieu, mitochondrial dysfunction, and high indices of oxidative stress [104].

Research reports on the "green" and cost-effective production of copper NPs utilizing green tea aqueous extract as reducing, stabilizing and capping agent indicated the synthesis of nano-sized copper formulations (≈ 20 nm) with a concentration-dependent (2-1024 $\mu g \cdot ml^{-1}$) cytotoxic activity against animal cells as evidenced via the MTT assay [123].

2. Anticancer activity of stabilized copper NPs

2.1. Anticancer activity of chemically stabilized copper NPs: In an effort to produce bioactive metallic NPs of variable sizes and shapes, with the desired stability and resistance against atmospheric parameters, researchers developed several synthetic approaches such as the Brust-Schiffrin technique [124], as a two-step phase transfer synthetic procedure with a mediated phase transfer agent (PTA). The unstable nature of the as-produced NPs can be controlled through the utilization of a capping stabilizing ligand including the biologically effective Schiff base ligands [125]. Increasing concentrations (0-250 $\mu g \cdot ml^{-1}$) of newly synthesized Schiff base stabilized copper NPs with pyrimidine derivatives of 2-(4,6-dimethoxypyrimidin-2-ylimino)methyl)-6-methoxyphenol (**DPMM**) showed enhanced CT-DNA binding affinity through hydrophobic interactions and increased anti-proliferative activity against MCF-7 cancer cells as evidenced via the MTT assay after 24 h of post-exposure [126].

Benzimidazole (BMZ) is considered an effective antibacterial agent and its derivatives have been proved to possess anticancer properties [127,128]. Recent studies on nanoparticulate copper based benzimidazole complexes (≈ 100 nm), produced via the re-precipitation method, demonstrated the improved therapeutic potential of the corresponding nano-

formulations against malignant cells [129], such as NIH/3T3 and A549 cell lines. The novel nanomaterials operated as adequate photothermal transducers, during the application of the Photothermal Therapeutic method (PTT), presenting enhanced binding ability to the target cells, energy absorbance, cytotoxic efficacy, and limited interaction with the healthy cells [130].

2.2. Anticancer activity of physically stabilized copper NPs

Efforts on the production of nano-formulations with enhanced bactericidal activity resulted in the development of gelatin stabilized copper NPs by reduction of copper chloride ($CuCl_2$) [131]. The corresponding NPs presented exceptional stability and sizes of around 50-60 nm and were further attested against three cancer cell lines such as: human skin melanoma A-375, human lung cancer A-549, and rat glioblastoma C6-G. The estimated IC_{50} values of the novel copper NPs were 1.71 $\mu g \cdot ml^{-1}$ for the first cancer cell line, 1.81 $\mu g \cdot ml^{-1}$ for the second, and 1.88 $\mu g \cdot ml^{-1}$ for the third, respectively. These results implied a similar cytotoxic profile of the produced copper NPS to all the attested cancer cell lines, indicated the effectiveness of the corresponding nanomaterials compared to other alternatively synthesized types of copper NPs [132,133], and stressed the ability of these copper nano-formulations to selectively target only the cancer cells leaving the peripheral healthy cells unaffected [134].

Naturally occurring active biomolecules have gained great attention due to their utilization in the synthesis of metallic NPs [135]. Among various functional and therapeutic food ingredients, polyphenols, and more specifically curcumin, have been proved effective anticancer agents [136]. Efforts on the development of adequate curcumin diverse nanocarriers, in order to improve the limited aqueous solubility and bioavailability of the corresponding polyphenol, led to the synthesis of curcumin-capped copper NPs. The novel copper nano-formulations were synthesized according to the Creighton method [137], and were further evaluated for their anticancer potential via the MTT and cell migration assays against human breast cancer cells (MDA-MB 231), treated with concentrations ranging between 5-25 μM. Furthermore, their antiangiogenic potential was further attested using Human Angiogenesis ELISA Strip I Kit for profiling eight cytokines and *in vivo* chorioallantoic membrane model (CAM assay). The overall results pointed out that the anticancer and antiangiogenic activity of the corresponding copper NPs did not exceed that of native curcumin [138].

Recently, studies on the synthesis of colloidal copper nanomaterials led to the production of glycerin stabilized copper NPs generated via the reduction of $CuSO_4$ by utilizing oxalic acid. The emerged nano-formulations were further capped with Poly vinyl alcohol (PVA). The anticancer activity of the synthesized colloidal suspension of copper NPs was determined against MCF-7 cancer cells (breast carcinomas) via the MTT assay. The results at hand showed that 50% cell inhibition was observed after the addition of 250 $\mu g \cdot ml^{-1}$ of colloidal copper NPs [139].

Anticancer Nanocomposites of Copper NPs

Polymer/metal nanocomposites represent a category of hybrid materials deriving from the synthesis of an organic polymer matrix bearing dispersed metallic NPs. They are widely applied in drug delivery, molecular imaging, cell labeling, diagnosis/treatment, cancer therapy, material chemistry and bio-sensing [140-147].

Chitin is a non-toxic, renewable, biodegradable, non-immunogenic, and biocompatible natural polymer that can be easily modified into membranes, hydrogels, beads, nanofibers, spongesm scaffolds, and micro/nanoparticles [148-154].

Recently, chitin nanocomposites with several metallic NPs have attracted extensive scientific interest for their electronic, optical, catalytic, and mechanical properties. In an experimental study on the synthesis of chitin-copper nanocomposites, the novel nano-formulations were further attested for their anticancer activity against MCF-7 cancer cells and normal HEK-293T cells. The corresponding nanomaterials exhibited enhanced anticancer effect, induction of apoptosis, and significant

cytotoxicity only to the cancer cells, implying extended selectivity and targeted anticancer potential [155].

Chitosan-copper nanocomposites are a specific type of nanomaterials widely used in the fields of biological application and environmental remediation [156-158]. Studies on newly synthesized spherical chitosan-copper nano-formulations proved the ability of the novel nanomaterials to induce blebbing and shrinkage of A549 cancer cells, reduce cell density and the cell-to-cell contact in comparison to the corresponding behavior of untreated cells. The observed cytotoxic effect increased from 0.2% to 10.5%, with increasing nanocomposite concentrations from 10 $\mu g \cdot mL^{-1}$ to 100 $\mu g \cdot mL^{-1}$, and with the highest IC_{50} value estimated around 434.6 $\mu g \cdot mL^{-1}$ [159].

In general, the basic characteristic of a potent chemotherapeutic drug is its ability to destroy selectively only the targeted cancer cells without altering the cellular integrity of the peripheral healthy tissues. Albumin NPs, as nano-carriers for targeted chemotherapeutic drug delivery applications, have attracted the scientific attention due to their main ability to enhance the endocytic drug uptake by cancer cells [160]. This attribute relies heavily on: a) the strong permeability and retention effect (EPR) of albumin NPs mediated by passive albumin uptake in cancer cells, and b) the enhanced active drug absorption by cancer cells, initiated by albumin NPs via the albumin receptor. As a result, novel drug delivery nano-formulations have been designed based on albumin such as prodrugs, drug-albumin conjugates, albumin-binding drug derivatives, and albumin NPs [161]. Another important advantage of albumin NPs is their ability to eliminate ethanol, cremophor and emulsifiers due to the enhanced drug solubility [162]. Serum albumin is considered a very functional blood protein due to its ability to maintain osmotic pressure and blood pH, transport various types of exogenous and endogenous molecules [163], bear several binding sites, and possess high half-life in blood circulation, great stability and solubility [164,165].

Recent efforts on the synthesis of a potent chemotherapeutic nano-formulation based on albumin led to the production of albumin nano-carriers of copper NPs. The novel nanomaterials were further attested for their anticancer potential against invasive MDA-MB 231 cancer cells and normal MCF-10A counterparts. The overall findings suggested that the synthesized spherical nanomaterials (≈ 100 nm) induced cell death via a dose-dependent manner through apoptosis. Their cytotoxic activity was estimated 5.7 times more acute compared to that on healthy cells as evidenced through the observed cyto-morphological changes, cell permeability, and the created DNA ladder pattern [166].

Comparison of the Anticancer Activity between Copper NPs and other Types of Metallic NPs

Various types of metallic NPs have been extensively studied for their anticancer properties such as gold [167], cobalt [168], silver [169], cerium [170], and others. However, copper NPs have become increasingly applicable due to their cost effectiveness, enhanced cytotoxic potency against cancer cells at low doses and longer stability period compared to Au and Ag NPs [171-176].

As an alternative to silver or gold NPs, CuS NPs and generally copper based chalcogenide semiconductor NPs represent a novel group of photothermal conducting agents that can be easily and cost-effectively synthesized and possess a remarkable translational potential. Moreover, in contrast to gold NPs, they possess a tunable absorption, based on the particle size, which can be formulated below 20 nm ensuring near-infrared absorption, improved clearance and pharmacokinetic properties, high tumor accumulation, and efficient elimination from the body when their size is below 6 nm [177].

Compared to silver and gold, the cytotoxic activity of copper has been less studied [178]. However, recent investigations on the toxicity of several types of metallic NPs (Au, Ag, Cu and Co NPs) through the laser ablation method that generates uncapped NPs in solution [179], indicated that all types of NPs possess moderate cytotoxic behavior to PC3 and HeLa human cell lines

with copper NPs presenting the lowest cytotoxicity [180].

Recent studies on the synthetic procedure of water soluble starch stabilized copper NPs produced via the utilization of ascorbic acid as the reducing agent resulted in the generation of 10 nm copper NPs with relatively low cytotoxic levels and enhanced bactericidal effect [181].

Evaluation studies of the cytotoxic activity of **DPMM**-stabilized copper and nickel NPs revealed that the newly synthesized **DPMM-Cu NPs** are more biologically active compared to the corresponding nickel NPs due to their lower 50% cell viability, better binding affinity with CT-DNA, increased interactions with DNA and enhanced cytotoxicity against MCF-7 cancer cells [126].

Comparative studies through MTT assay on the cytotoxic and antiproliferative activity of *Prosopis cineraria* biofabricated copper and silver NPs against MCF-7 cancer cells proved that the bioengineered copper NPs presented better cytotoxic effect (lower IC_{50}) compared to that of silver NPs [116].

Comparative Analysis of the Anticancer Activity of Copper-Derived NPs

There are several types of copper-derived NPs with proved anticancer activity such as CuI, CuO, $CuCO_3$, $Cu(PO_4)_2$, Cu_2O, CuS [171-176].Recent reports on the anticancer potential of CuI and $Cu(PO_4)_2$ NPs, with sizes ranging from 35 nm to 67 nm, respectively, showed that low doses of CuI NPs were more effective in comparison to $Cu(PO_4)_2$ NPs with 2.5 $\mu g \cdot ml^{-1}$ LD50 value for CuI and 10 $\mu g \cdot ml^{-1}$ for the corresponding $Cu(PO_4)_2$ NPs. Both types of NPs cause apoptotic mediated cell death by inducing ROS-mediated DNA damage [171].

Newly synthesized crystalline and amorphous CuS NPs of similar sizes(≈50(-60nm) presented enhanced cytotoxicity on tumor cells. The LD50 values on HL-60 cell lines were 29 $\mu g \cdot ml^{-1}$ for CuS nanocrystals and 18.5 $\mu g \cdot ml^{-1}$ for amorphous CuS NPs. Moreover, both types of NPs mediated targeted apoptosis in tumor cells unaffecting peripheral healthy cells [176].

Research studies on the anticancer potential of Cu_2O NPs indicated the specific and selective anticancer activity of the corresponding type of copper NPs only against cancer cells, even at low concentrations, without affecting the normal cells [175]. Novel synthetic approaches resulted in the production of $CuCO_3$ NPs of 20 nm average size that can induce ROS-mediated DNA and mitochondria damage, resulting in apoptosis in tumor cells. The corresponding $CuCO_3$ NPs were modified with folic acid in order to obtain targeted action against cancer cells. Folic acid is used as a precursor for DNA synthesis and its high effectiveness depends on its demand for fast reproducibility. Furthermore, the corresponding folate receptor is over-expressed in the majority of cancer cells leading to the internalization of folic acid. Consequently, upon treatment of mice cancer models with folic acid modified $CuCO_3$ NPs, toxicity decreased to the lowest level and survivability increased significantly in comparison to the control group. Additionally, a reduction of the tumor volume was also observed [173].

Newly synthesized CuO NPs of spherical shape from "green" synthetic approaches and chemical reduction processes, resulted in the production of NPs with sizes ranging from 5-100 nm depending on the employed plant species and the type of the applied chemical reduction process. The corresponding group of CuO NPs showed enhanced effectiveness against cervical, HeLa and MCF-7 breast cancer cells. The proposed anticancer mechanism includes autophagy which is provoked by the intracellular stress induced by the ROS mediated DNA damage leading to apoptosis. Furthermore, conjugation of folic acid to the corresponding CuO NPs utilizing APTS enhanced their targeted activity and selectivity towards cancer cells when attested *in vivo* [173-176,182].

Several novel types of copper-containing NIR-absorbing nano-formulations have been synthesized and further attested for PTT such as copper selenide ($Cu_{2-x}Se$) nanocrystals, monodispersed CuTe nanorods, nanoplates, and nanocubes, copper bismuth sulfide (Cu_3BiS_3) nano-structures. All of the above-mentioned copper-derived nano-molecules present enhanced photothermal heating efficiency and significant anticancer potential [177].

Developing Approaches of Targeted Anticancer Copper and Copper-Derived Nano-Formulations

The development of lipid and polymer nanocarriers has eased the delivery of several therapeutic molecules to diseased tissues and cells. Copper NPs, copper-derived NPs or copper complexes with organic substrates can be easily encapsulated into bioavailable, biodegradable, and non-toxic nano-vehicles for targeted administration to specific cellular targets.

Recent scientific results on the encapsulation of anticancer copper complexes indicated the development of a hydrophobically stearic acid modified chitosan nano-formulation bearing hydrophobic copper complexes [183,184]. The resulting nanomaterials targeted effectively the folate and HER 2 receptors of tumor cells, resulting in tumor regression when attested *in vivo* [183].

Recently, a novel liposomal nanocarrier bearing the antialcoholic drug disulfiram (**lipo-DS**) combined with copper gluconate, was tested *in vivo* against CSCs (tumor initiating cells) in order to avoid pan-chemoresistance. The corresponding nano-formulations targeted the NFκB pathway, presenting an enhanced anti-CSC efficacy towards hypoxia-induced CSCs [185].

Furthermore, newly synthesized soft copper oleate NPs were proved to possess effective photoacoustic (PA) contrast, equivalent to gold-based nano-formulations but more cost-effective and with higher availability of the starting materials. The corresponding type of copper NPs presented a strong PA contrast, similar to that of gold nanobeacons and relative to blood. Additional *in vivo* studies in the Matrigel angiogenesis model showed that **$\alpha_v\beta_3$-CuNPs** significantly increased the neovessel sprout formation levels and operated as efficient anti-angiogenic fumagillin-prodrug nanocarriers providing the first applied targeted approach for the development of a drug delivery treatment combined with a PA contrast agent [186].

Research studies on the development of double-strand DNA-hosted copper nanoclusters by utilizing random dsDNA (double-stranded DNA) or poly(thymine) ssDNA (single-stranded DNA) as the template, indicated that the fluorescent properties of the corresponding nanomaterials could be applied in the identification processes of SNPs (Single nucleotide polymorphisms), providing a tool for the fluorimetric diagnosis of DNA mismatches [187].

Experimental studies on the development of targeted drug delivery and bioimaging molecules resulted in the formation of transferrin (Tf) templated copper nanoclusters (**Tf-CuNCs**) with enhanced luminescence. The corresponding nano-molecules were further formulated into spherical transferrin copper nanocluster-doxorubicin (Dox) NPs (**Tf-CuNC-Dox-NPs**) of spherical shape, based on the electrostatic interactions with doxorubicin. The newly synthesized nanomaterials were further assesed *in vivo* on TfR (Transferrin Receptor) positive DLA (Daltons Lymphoma Ascites) bearing mice revealing enhanced inhibition of tumor growth and prolongation of the survival of the animals [188].

Additionally, integration of ^{64}Cu radionuclide into CuS NPs without the introduction of radiometal chelators, led to the production of the radioactive [^{64}Cu]CuS NPs, adequate for quantitative tissue analysis and PET (positron emission tomography) imaging. Quantitative tissue analysis plays a significant role in the design of targeted approaches for NP-based therapy [177].

In Vivo Effects of Copper and Copper-Derived NPs

Studies on the systematic comparison of renal clearance rates of luminescent degradable glutathione-coated copper NPs (**GS-CuNPs**) and their dissociated products, Cu(II)-glutathione disulfide (GSSG) complexes (Cu(II)-GSSG), *in vivo*, indicated that GS-CuNPs can be accumulated in the liver less than their dissociation counterparts and eliminated through the urinary system significantly faster. This behavior can be attributed to the increased resistance of GS-CuNPs against serum protein adsorption compared to that of Cu(II)-GSSG. Additionally, the biodistribution and renal clearance of GSCuNPs depend on the type of the dissociation process (zero-order chemical kinetics) and the primary injection doses [189].

Physicochemical studies on copper NPs indicate their tendency to form aggregates of low solubility in vehicle

or simulated intestinal environment. It has also been proved that copper NPs present a satisfactory dissolution level in acidic environment but lower than that of copper ions. These observations indicate the existing interdependence of the biological system and the physicochemical characteristics of copper NPs that further affect their *in vivo* toxic behavior. Copper NPs, via kinetic studies, have been proved to present delayed and lower concentrations of accumulated or absorbed copper levels in tested organs and blood compared to copper ions. Additionally, the *in vivo* toxic behavior of copper NPs is more profound at high doses compared to that of copper ions, eliciting similar response with distinct sex-related differentiations. Furthermore, copper NPs can induce morphological and functional changes in the spleen, kidneys, and liver due to *in vivo* systemic toxic effects associated with their biodistribution rate and solubility [190]. Further *in vivo* studies have proved that the decreased particle size of copper NPs along with their morphological characteristics and the species-specific vulnerability of cells can affect their toxicity [191].

Conclusion

Several diversified strategies utilize metallic NPs against cancer disease. It is proved that NPs can present either direct antitumor effects or indirect hyper thermic anticancer activity, *in vitro* and *in vivo*. Moreover, NPs can operate as a conventional anticancer drug, reducing both the side effects and the required dose. Despite the fact that research on the anticancer potential of metallic NPs has been increased, there are still limitations due to the cell heterogeneity utilized for each tumor environment which hinders the comparative studies. Another limitation regards the formation of protein corona after the interactions of NPs with the blood and the plasma proteins, affecting the *in vivo* clearance and distribution. New research efforts will enable the development of novel types of metallic NPs, such as copper NPs, with improved and selective anticancer activity, enhanced biocompatibility and bio-distribution, and low toxicity for normal tissues.

In this review, we have thoroughly discussed the anticancer therapeutic applications of copper and copper-derived NPs *in vitro* and *in vivo* including drug delivery, cancer imaging, image-guided therapy, PTT, and developing approaches on selective targeting and mitigation of potential toxicities. In comparison to other inorganic nanomaterials, such as carbon nanotubes, gold nanoshells or nanorods, and other types of metallic NPs, copper NPs have not been extensively studied, especially *in vivo*. Their multifunctional features and characteristics render copper NPs ideal nanomaterials for theranostic applications as contrast agents or nuclear tracers for various diagnostic and imaging techniques, photothermal cancer cell destruction, and controlled drug release applications. Despite many experimental reports *in vitro*, more effort should be focalized on the *in vivo* efficiency, disposition, and properties of copper NPs, including their potential cytotoxicity, pharmacokinetics, and pharmacodynamics in order to evaluate their limitations and advantages along with their controllable long-term cytoxicity, favorable *in vivo* pharmacology, and photothermal conversion capacity.

References

1. Schaming D, Remita H. (2015). Nanotechnology: from the ancient time to nowadays. Found Chem. 17: 187–205.

2. Gopinath M, Subbaiya R, Selvam MM, Suresh D. (2014). Synthesis of copper nanoparticles from nerium oleander leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol App Sci. 3: 814-818.

3. Bicer M, Sisman I. (2010). Controlled synthesis of copper nano/microstructures using ascorbic acid in aqueous CTAB solution. Powder Technol. 198: 279–284.

4. Brust M, Kiely CJ. (2002). Some recent advances in nanostructure preparation from gold and silver particles, a short topical review. Colloids Surf. A. 202: 175-186.

5. He Y. (2007). A novel solid-stabilized emulsion approach to CuO nanostructured microspheres. Mater. Res. Bull. 42: 190-195.

6. Hussain JI, Kumar S, Hashmi AA, Khan Z. (2011). Silver nanoparticles: Preparation, characterization, and kinetics. Adv Mater Lett. 2: 188-194.

7. Khan I, Saeed K, Khan I. (2017). Nanoparticles: Properties, applications and toxicities. Arab J Chem. (In press, Corrected proof).

8. Kumar KM, Sinha M, Mandal BK, Ghosh AR, Kumar KS, et al. (2012). Green synthesis of silver nanoparticles using Terminalia chebula extract at room temperature and their antimicrobial studies. Spectrochim Acta A. 91: 228–233.

9. Park CS, Lee C, Kwon OS. (2016). Conducting polymer based nanobiosensors. Polymers. 8: 249.

10. Jain S, Hirst DG, O'Sullivan JM. (2012). Gold nanoparticles as novel agents for cancer therapy. Br J Radiol. 85: 101–113.

11. Jannathul FM, Lalitha P. (2015). Apoptotic efficacy of biogenic silver nanoparticles on human breast cancer MCF-7 cell lines. Prog Biomater. 4: 113–121.

12. Sanpui P, Chattopadhyay A, Ghosh SS. (2011). Induction of apoptosis in cancer cells at low silver nanoparticle concentrations using chitosan nanocarrier. ACS Appl Mater Interfaces. 3: 218–228.

13. Jose GP, Santra S, Mandal SK, Sengupta TK. (2011). Singlet oxygen mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells. J Nanobiotechnol. 9: 9.

14. Castro L, Blázquez ML, Muñoz JÁ, González F, Ballester A. (2014). Mechanism and applications of metal nanoparticles prepared by bio-mediated process. Rev. Adv. Sci. Eng. 3: 199-216.

15. Dahoumane SA, Jeffryes C, Mechouet M, Agathos SN. (2017). Biosynthesis of inorganic nanoparticles: A fresh look at the control of shape, size and composition. Bioengineering (Basel). 4: 14.

16. Theivasanthi T, Alagar M. (2011). Nano sized copper particles by electrolytic synthesis and characterizations. Int. J. Phys. Sci. 6: 3662-3671.

17. Wang L, Hu C, Shao L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int. J. Nanomedicine. 12: 1227–1249.

18. Giannousi K, Pantazaki A, Dendrinou-Samara C. (2017). Copper based nanoparticles as Antimicrobials. Multi Volume SET (I-V) Therapeutic Nanostructures in Medicine. Grumezescu AM. (editors). Chapter No 23. (published by Elsevier). Subject: Nanostructures for Antimicrobial Therapy. 515-529.

19. Giannousi K, Lafazanis K, Arvanitidis J, Pantazaki A, Dendrinou-Samara C. (2014). Hydrothermal synthesis of copper based nanoparticles: Antimicrobial screening and interaction with DNA. J. Inorg. Biochem. 133: 24-32.

20. Giannousi K, Sarafidis G, Mourdikoudis S, Pantazaki A, Dendrinou-Samara C. (2014). Selective Synthesis of Cu2O and Cu/Cu2O NPs: Antifungal Activity to Yeast Saccharomyces cerevisiae and DNA Interaction. Inorg. Chem. 53: 9657-9666.

21. Giannousi K, Hatzivassiliou E, Mourdikoudis S, Vourlias G, Pantazaki A, et al. (2016). Synthesis and biological evaluation of PEGylated CuO nanoparticles. J. Inorg. Biochem. 164: 82-90.

22. Antonoglou O, Giannousi K, Pantazaki A, Arvanitidis J, Mourdikoudis S, et al. (2017). Beneficial role of CuFe NPs as antimicrobials. J Inorg Biochem. 177: 159-170.

23. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, et al. (2014). "Green" nanotechnologies: Synthesis of metal nanoparticles using plants. Acta Naturae. 6: 35–44.

24. Satlavati-Niasari M, Davar F, Mir N. (2008). Synthesis and characterization of metallic copper nanoparticles via thermal decomposition. Polyhedron. 27: 3514 – 3518.

25. Patil MP, Ngabire D, Thi HHP, Kim MD, Kim GD. (2017). Eco-friendly synthesis of gold nanoparticles and evaluation of their cytotoxic activity on cancer cells. J Clust Sci. 28: 119-132.

26. Zharov VP, Kim JW, Curiel DT, Everts M. (2005). Self-assembling nanoclusters in living systems: application for integrated photothermal nanodiagnostics and nanotherapy. Nanomedicine. 1: 326-345.

27. Fang J, Yu L, Gao P, Cai Y, Wei Y. (2010). Detection of protein-DNA interaction and regulation using gold nanoparticles. Anal. Biochem. 399: 262-267.

28. Thanh NTK, Green LAW. (2010). Functionalisation of nanoparticles for biomedical applications. Nano Today. 5: 213-230.

29. Wang P, Wang X, Wang L, Hou X, Liu W, et al. (2015). Interaction of gold nanoparticles with proteins and cells. Sci Technol Adv Mater. 16: Article id. 034610.

30. An FF, Zhang XH. (2017). Strategies for preparing albumin-based nanoparticles for multifunctional bioimaging and drug delivery. Theranostics. 7: 3667–3689.

31. Pissuwan D, Niidome T, Cortie MB. (2011). The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J. Control. Release. 149: 65-71.

32. Jahangirian H, Lemraski EG, Webster TJ, Moghaddam-Rafiee R, Abdollahi Y. (2017). A review of drug delivery systems based on nanotechnology and green chemistry: green nanomedicine. Int J Nanomedicine. 12: 2957–2978.

33. Liu Y, Miyoshi H, Nakamura M. (2007). Nanomedicine for drug delivery and imaging: A promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. Int J Cancer. 120: 2527-2537.

34. Sharma N, Bhatt G, Kothiyal P. (2015). Gold nanoparticles synthesis, properties, and forthcoming applications – A review. Indian J Pharm Biol Res. 3: 13-27.

35. Umer A, Naveed S, Ramzan N, Rafique MS, Imran M. (2014). A green method for the synthesis of copper nanoparticles using L-ascorbic acid. Matéria (Rio J). 19: 197-203.

36. Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GEJ. (2015). Green synthesis of metallic nanoparticles via biological entities. Materials. 8: 7278-7308.

37. Akter M, Sikder T, Rahman M, Ullah AKMA, Hossain KFB, et al. (2018). A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. J Adv Res. 9: 1-16.

38. Vinod KV, Nithya S, Aswin S, Sai SN, Tennis AJ, et al. (2016). Natural amino acid based phenolic derivatives for synthesizing silver nanoparticles with tunable morphology and antibacterial studies. Bull Korean Chem. Soc. 34: 2702-2706.

39. Sangeetha S, Kalaignan GP, Anthuvan JT. (2015). Pulse electrodeposition of self-lubricating Ni-W/PTFE nanocomposite coatings on mild steel surface. Appl Surf Sci. 359: 412-419.

40. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, et al. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix, a novel biological approach to nanoparticle synthesis. Nano Lett. 1: 515-519.

41. Kuppusamy P, Yusoff MM, Maniam GP, Govindan N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharm J. 24: 473-484.

42. Benelli G, Pavela R, Canale A, Mehlhorn H. (2016). Tick repellents and acaricides of botanical origin, a green roadmap to control tick-borne diseases? Parasitol. Res. 115: 2545-2560.

43. Jabir NR, Tabrez S, Ashraf GM, Shakil S, Damanhouri GA, et al. (2012). Nanotechnology-based approaches in anticancer research. Int J Nanomedicine. 7: 4391–4408.

44. Wang Z, Li N, Zhao J, White J C, Qu P, et al. (2012). CuO nanoparticle interaction with human epithelial cells: cellular uptake, location, export, and genotoxicity. Chem Res Toxicol. 25: 1512−1521.

45. Siddiqui MA, Alhadlaq HA, Ahmad J, Al-Khedhairy AA, Musarrat J, e al. (2013). Copper oxide nanoparticles induced mitochondria mediated npoptosis in human hepatocarcinoma cells. PLoS ONE. 8: e69534.

46. Sun T, Yan Y, Zhao Y, Guo F, Jiang C. (2012). Copper oxide nanoparticles induce autophagic cell death in A549 cells. PLoS ONE. 7: e43442.

47. Wang Y, Yang F, Zhang HX, Zi XY, Pan XH, et al. (2013). Cuprous oxide nanoparticles inhibit the

growth and metastasis of melanoma by targeting mitochondria. Cell Death Dis. 4: e783.

48. Laha D, Pramanik A, Maity J, Mukherjee A, Pramanik P, et al. (2014). Interplay between autophagy and apoptosis mediated by copper oxide nanoparticles in human breast cancer cells MCF7. Biochim Biophys Acta. 1840: 1–9.

49. Chena Z, Menga H, Xing G, Chena C, Zhaoa Y, et al. (2006). Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett. 163: 109–120.

50. Ahmad MZ, Akhter S, Jain GK, Rahman M, Pathan SA, et al. (2010). Metallic nanoparticles: technology overview & drug delivery applications in oncology. Expert Opin Drug Deliv. 7: 927-942.

51. Jia F, Zhang L, Shang X, Yang Y. (2008). Non-aqueous sol–gel approach towards the controllable synthesis of nickel nanospheres, nanowires, and nanoflowers. Adv Mater. 20: 1050–1054.

52. Imran DM, Rani A. (2016). Recent advances in the synthesis and stabilization of nickel and nickel oxide nanoparticles: A green adeptness. Int J Ana Chem. 2016: 3512145.

53. Tanori J, Pileni MP. (1997). Control of the shape of copper metallic particles by using a colloidal system as template. Langmuir. 13: 639-646.

54. Bajaj G, Chaudhary A, Naaz H, Kumar B, Soni R. (2007). Laser ablation synthesis of Zn/ZnO core-shell nanoparticles. IEEE Xplore. 2007: 940–942.

55. Treguer M, de Cointet C, Remita H, Khatouri J, Mostafavi M, et al. (1998). Dose rate effects on radiolytic synthesis of gold−silver bimetallic clusters in solution. J Phy Chem B. 102: 4310–4321.

56. Lisiecki I, Pileni MP. (1993). Synthesis of copper metallic clusters using reverse micelles as microreactors. J Am Chem Soc. 115: 3887–3896.

57. S-H W, Chen D-H. (2004). Synthesis of high-concentration Cu nanoparticles in aqueous CTAB solutions. J Colloid Interf Sci. 273: 165–169.

58. Zhang G, Wang D. (2008). Fabrication of heterogeneous binary arrays of nanoparticles via colloidal lithography. J Am Chem Soc. 130: 5616–5617.

59. Chen W, Cai W, Zhang L, Wang G, Zhang L. (2001). Sonochemical processes and formation of gold nanoparticles within pores of mesoporous silica. J Colloid Interf Sci. 238: 291–295.

60. Eustis S, Hsu H-Y, El-Sayed MA. (2005). Gold nanoparticle formation from photochemical reduction of Au3+ by continuous excitation in colloidal solutions. A proposed molecular mechanism. J Phy Chem B. 109: 4811–4815.

61. Khaydarov RA, Khaydarov RR, Gapurova O, Estrin Y, Scheper T. (2009). Electrochemical method for the synthesis of silver nanoparticles. J Nanopart Res. 11: 1193–1200.

62. Shanmugavadivu M, Kuppusamy S, Ranjithkumar R. (2014). Synthesis of pomegranate peel extract mediated silver nanoparticles and its antibacterial activity. Drug Deliv. 2: 174–182.

63. Frattini A, Pellegri N, Nicastro D, De Sanctis O. (2005). Effect of amine groups in the synthesis of Ag nanoparticles using aminosilanes. Mater Chem Phys. 94: 148–152.

64. Chen D, Xu R. (1998). Hydrothermal synthesis and characterization of nanocrystalline γ-Fe2O3 particles. J. Solid State Chem. 137: 185-190.

65. Chen D-H, S-H W. (2000). Synthesis of nickel nanoparticles in water-in-oil microemulsions. Chem. Mater. 12: 1354-1360.

66. Basak S, Chen D-R, Biswas P. (2007). Electrospray of ionic precursor solutions to synthesize iron oxide nanoparticles: modified scaling law. Chem. Eng. Sci. 62: 1263-1268.

67. Din MI, Arshad F, Hussain Z, Mukhtar M. (2017). Green adeptness in the synthesis and stabilization of copper nanoparticles: Catalytic, antibacterial, cytotoxicity, and antioxidant activities. Nanoscale Res. Lett. 12: 638.

68. Philip D. (2010). Green synthesis of gold and silver nanoparticles using Hibiscus rosa sinensis. Phys. E. 42: 1417-1424.

69. Kumar P, Singh P, Kumari K, Mozumdar S, Chandra R. (2011). A green approach for the synthesis

of gold nanotriangles using aqueous leaf extract of Callistemon viminalis. Mater. Lett. 65: 595-597.

70. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R. et al. (2003). Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, Rhodococcus species. Nanotechnol. 14: 824-828.

71. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M. (2005). Extra-/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, Trichothecium sp. J. Biomed. Nanotechnol. 1: 47-53.

72. Bhainsa KC, D'Souza S. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloid. Surface B. 47: 160-164.

73. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M. (2003). Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, Thermomonospora sp. Langmuir. 19: 3550-3553.

74. Sastry M, Ahmad A, Khan MI, Kumar R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. Current Sci. 85: 162-170.

75. Dameron C, Reese R, Mehra R, Kortan A, Carroll P, et al. (1989). Biosynthesis of cadmium sulphide quantum semiconductor crystallites. Nature. 338: 596-597.

76. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, et al. (2002). Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnol. 14: 95-100.

77. Mohanpuria P, Rana NK, Yadav SK. (2008). Biosynthesis of nanoparticles: technological concepts and future applications. Nano Res. 10: 507-517.

78. Roh Y, Lauf R, McMillan A, Zhang C, Rawn C, Bai J, Phelps T. (2001). Microbial synthesis and the characterization of metal-substituted magnetites. Solid State Commun. 118: 529-534.

79. Lengke M, Southam G. (2006). Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold (I)-thiosulfate complex. Geochim. Cosmochim. Ac. 70: 3646-3661.

80. Nair B, Pradeep T. (2002). Coalescence of nano-clusters and formation of submicron crystallites assisted by Lactobacillus strains. Cryst. Grow. Design. 2: 293-298.

81. Klaus-Joerger T, Joerger R, Olsson E, Granqvist C-G. (2001). Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trend. Biotechnol. 19: 15-20.

82. Husseiny M, El-Aziz MA, Badr Y, Mahmoud M. (2007). Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa. Spectrochim. Acta Part A. 67: 1003-1006.

83. Lee S-W, Mao C, Flynn CE, Belcher A. (2002). Ordering of quantum dots using genetically engineered viruses. Science. 296: 892-895.

84. Merzlyak A, Lee S-W. (2006). Phage as templates for hybrid materials and mediators for nanomaterial synthesis. Curr. Opinion Chem. Bio. 10: 246-252.

85. Song JZ, Kim BS. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng. 32: 79-84.

86. Singaravelu G, Arockiamary JS, Kumar VG, Govindaraju K. (2007). A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wightii Greville. Colloids Surf. B Biointerfaces. 57: 97-101.

87. Ghodake G, Lee DS. (2011). Biological synthesis of gold nanoparticles using the aqueous extract of the brown algae Laminaria japonica. J. Nanoelectron Optoe. 6: 268-271.

88. Vijayaraghavan K, Mahadevan A, Sathishkumar M, Pavagadhi S, Balasubramanian R. (2011). Biosynthesis of Au(0) from Au(III) via biosorption and bioreduction using brown marine alga Turbinaria conoides. Chem. Eng. J. 167: 223-227.

89. Rajathi FAA, Parthiban C, Ganesh KV, Anantharaman P. (2012). Biosynthesis of antibacterial gold nanoparticles using brown alga, Stoechospermum marginatum (kützing). Spectrochim. Acta Part A. 99: 166-173.

90. Selvin J, Huxley AJ, Lipton AP. (2004). Immunomodulatory potential of marine secondary metabolites against bacterial diseases of shrimp. Aquaculture. 230: 241-248.

91. Selvin J, Lipton AP. (2002). Development of a rapid 'mollusc foot adherence bioassay' for detecting potent antifouling bioactive compounds. Curr. Sci. 83: 735-737.

92. Lipton AP, Jose J. (2006). Anticoagulant and immune enhancing activities of marine macroalgae explored. Spectrum-ICAR News. 12: 8.

93. ISO. (1986). Water quality — Determination of cobalt, nickel, copper, zinc, cadmium and lead — Flame atomic absorption spectrometric methods. Geneva, International Organization for Standardization (ISO 8288–1986 (E)).

94. ISO. (1996). Water quality — Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy. Geneva, International Organization for Standardization (ISO 11885:1996 (E)).

95. ASTM. (1992). Annual book of ASTM standards 11.01. Philadelphia, PA, American Society for Testing and Materials.

96. ASTM. (1994). Annual book of ASTM standards 11.02. Philadelphia, PA, American Society for Testing and Materials.

97. US EPA. (1994). Methods for determination of metals in environmental samples. Supplement I. Washington, DC, US Environmental Protection Agency, Office of Research and Development (EPA600/R-94-111).

98. Marcõ PLM, Jiménez E, Hernndez CEA, Rojas A, Greaves ED. (2001). Determination of Zn/Cu ratio and oligoelements in serum samples by total reflection X-ray fluorescence spectrometry for cancer diagnosis. Spectrochim. Acta B. 56: 2195-2201.

99. Zowczak M, Iskra M, Paszkowski J, Mańczak M, Torliński L, et al. (2001). Oxidase activity of ceruloplasmin and concentrations of copper and zinc in serum of cancer patients. J. Trace Elem. Med. Biol. 15: 193-196.

100. Majumder S, Chatterjee S, Pal S, Biswas J, Efferth T, Choudhuri SK. (2009). The role of copper in drug-resistant murine and human tumors. Biometals. 22: 377-384.

101. Bhattacharya PT, Misra SR, Hussain M. (2016). Nutritional aspects of essential trace elements in oral health and disease: An extensive review. Scientifica (Cairo). 2016: 5464373.

102. IOM. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. A report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Food and Nutrition Board, Institute of Medicine. Washington, DC, National Academy Press.

103. WHO. (1996). Trace elements in human nutrition and health. Geneva, World Health Organization.

104. Institute of Medicine (US) Panel on Micronutrients. (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.

105. Plantone D, Primiano G, Renna R, Restuccia D, Iorio R, et al. (2015). Copper deficiency myelopathy: A report of two cases. J. Spinal Cord. Med. 38: 559–562.

106. Shackel NA, Day RO, Kellett B, Brooks PM. (1997). Copper-salicylate gel for pain relief in osteoarthritis: a randomised controlled trial. Med. J. Aust. 167:134-136.

107. Strain JJ. (1988). A reassessment of diet and osteoporosis--possible role for copper. Med. Hypotheses. 27: 333-338.

108. Willis MS, Monaghan SA, Miller ML, McKenna RW, Perkins WD, et al. (2005). Zinc-induced copper deficiency: a report of three cases initially recognized on bone marrow examination. Am. J. Clin. Pathol. 123: 125-131.

109. Chhetri SK, Mills RJ, Shaunak S, Emsley HCA. (2014). Copper deficiency. BMJ. 348: g3691.

110. Ramaswamy SVP, Narendhran S, Sivaraj R. (2015). Potentiating effect of ecofriendly synthesis of copper oxide nanoparticles using brown alga:

antimicrobial and anticancer activities. Bull. Mater. Sci. 39: 361-364.

111. Gopinath M, Subbaiya R, Masilamani Selvam M, Suresh D. (2014). Synthesis of copper nanoparticles from Nerium oleander leaf aqueous extract and its antibacterial activity. Int. J. Curr. Microbiol. App. Sci. 3: 814-818.

112. Lee H, Song JY, Kim BS. (2013). Biological synthesis of copper nanoparticles using Magnolia kobus leaf extract and their antibac¬terial activity. J. Chem. Technol. Biotechnol. 88: 1971-1977.

113. Chung IM, Rahuman AA, Marimuthu S, Kirthi AV, Anbarasan K, et al. (2017). Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidant and cytotoxic activities. Exp. Ther. Med. 14: 18-24.

114. Sumathi S, Dharani B, Sivaprabha J, Raj KS, Padma PR. (2013). Cell death induced by methanolic extract of Prosopis cineraria leaves in MCF-7 breast cancer cell line. Inter. J. Pharma. Sci. Inven. 2: 21-26.

115. Roberson M, Rangari V, Jeelani S, Samuel T, Yates C. (2014). Synthesis and characterization silver, zinc oxide and hybrid silver/zinc oxide nanoparticles for antimicrobial applications. Nano LIFE. 4: 1440003.

116. Jinu U, Gomathi M, Saiqa I, Geetha N, Benelli G, et al. (2017). Green engineered biomolecule-capped silver and copper nanohybrids using Prosopis cineraria leaf extract: Enhanced antibacterial activity against microbial pathogens of public health relevance and cytotoxicity on human breast cancer cells (MCF-7). Microb Pathog. 105: 86-95.

117. Sahu J, Patel PK, Dubey B. (2012). Quisqualis indica linn: a review of its medicinal properties. Int. J. Pharm. Phytopharmacol. Res. 1: 313–321.

118. Mukhopadhyay R, Kazi J, Debnath M C. (2018). Synthesis and characterization of copper nanoparticles stabilized with Quisqualis indica extract: Evaluation of its cytotoxicity and apoptosis in B16F10 melanoma cells. Biomed. Pharmacother. 97: 1373-1385.

119. Faedmaleki F, Shirazi HF, Salarian AA, Ahmadi AH, Rastegar H. (2014). Toxicity effect of silver banoparticles on mice liver primary cell culture and HepG2 cell line. Iran. J. Pharm. Res. 13: 235-242.

120. Prasad PR, Kanchi S, Naidoo EB. (2016). *In-vitro* evaluation of copper nanoparticles cytotoxicity on prostate cancer cell lines and their antioxidant, sensing and catalytic activity: One-pot green approach. J. Photochem. Photobiol. B. 161: 375-382.

121. Daniel M. (2009). Medicinal Plants Chemistry and Properties, 1st Ed., Science, United States.

122. Valodkar M, Jadeja RN, Thounaojam MC, Devkar RV, Thakore S. (2011). Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Mater. Chem. Phys. 128: 83-89.

123. Keihan Ah, Veisi H, Veasi H. (2016). Green synthesis and characterization of spherical copper nanoparticles as organometallic antibacterial agent. Appl. Organometal. Chem. 31: e3642.

124. Brust M, Walker M, Bethell D, Schiffrin DJ, Whyman R. (1994). Synthesis of thiolderivatised gold nanoparticels in a two-phase Liquid-Liquid system. J. Chem. Soc., Chem. Comm. 0: 801-802.

125. Ejidike LP, Ajibade PA. (2015). Transition metalm compelxes of symmertrical and asymmetrical schiff bases as antibacterial, antifungal, antioxidant and anticancer agents: Progress and Prospects. Rev. Inorg. Chem. 35: 191-224.

126. Jose PA, Raja JD, Sankarganesh M, Rajesh J. (2017). Evaluation of antioxidant, DNA targeting, antimicrobial and cytotoxic studies of imine capped copper and nickel nanoparticles. J. Photochem. Photobiol. B. 178: 143-151.

127. El-Masry AH, Fahmy H, Ali Abdelwahed S. (2000). Synthesis and antimicrobial activity of some new benzimidazole derivatives. Molecules. 5: 1429-1438.

128. Penning TD, Zhu GD, Gandhi VB, Gong J, Liu X, et al. (2009). Discovery of the Poly(ADP-ribose) polymerase (PARP) inhibitor 2-[(R)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide (ABT-888) for the treatment of cancer. J. Med. Chem. 52: 514-523.

129. El-Sayed IH, Huang X, El-Sayed MA. (2006). Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. Cancer Lett. 239: 129-135.

130. Praveen PA, Babu RR, Balaji P, Murugadas A, Akbarsha MA. (2018). Laser assisted anticancer activity of benzimidazole based metal organic nanoparticles. J. Photochem. Photobiol. B. 180: 218-224.

131. Chatterjee AK, Sarkar RK, Chattopadhyay AP, Aich P, Chakraborty R, et al. (2012). A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against E. coli. Nanotechnology. 23: 085103.

132. Studera AM, Limbach LK, Duc LV, Krumeich F, Athanassiou EK, et al. (2010). Nanoparticle cytotoxicity depends on intracellular solubility: Comparison of stabilized copper metal and degradable copper oxide nanoparticles. Toxicol. Lett. 197: 169–174.

133. Midander K, Cronholm P, Karlsson HL, Elihn K, Möller L, et al. (2009). Surface characteristics, copper Release, and toxicity of nano- and micrometer-sized copper and copper (II) oxide particles: A cross-disciplinary study. Small. 5: 389–399.

134. Chakraborty R, Basu T. (2017). Metallic copper nanoparticle induces apoptosis in human skin melanoma, A-375 cell line. Nanotechnology. 28: 105101.

135. Sreelakshmi CH, Goel N, Datta KK, Addlagatta A, Ummanni R, et al. (2013). Green synthesis of curcumin capped gold nanoparticles and evaluation of their cytotoxicity. Nanosci. Nanotechnol. Lett. 5: 1258–1265.

136. Bachmeier B, Nerlich AG, Iancu CM, Cilli M, Schleicher E, et al. (2007). The chemopreventive polyphenol curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. Cell. Physiol. Biochem. 19: 137–152.

137. Evanoff DD, Chumanov G. (2005). Synthesis and optical properties of silver nanoparticles and arrays. Chemphyschem. 6: 1221–1231.

138. Kamble S, Utage B, Mogle P, Kamble R, Hese S, et al. (2016). Evaluation of curcumin capped copper nanoparticles as possible inhibitors of human breast cancer cells and angiogenesis: a comparative study with native curcumin. AAPS PharmSciTech. 17: 1030-1041.

139. Saranya R, Ali MM. (2017). Synthesis of colloidal copper nanoparticles and its cytotoxicity effect on MCF-7 breast cancer cell lines. J. Chem. Pharm. Sci. 1: 1-5.

140. Giannelis EP. (1996). Polymer layered silicate nanocomposites, Adv. Mater. 8: 29-35.

141. Rangari VK, Mohammad GM, Jeelani S, Hundley A, Vig K, et al. (2010). Synthesis of Ag/CNT hybrid nanoparticles and fabrication of their nylon-6 polymer nanocomposite fibers for antimicrobial applications. Nanotechnology. 21: 95102-95112.

142. Bhattacharya D, Gupta RK. (2005). Nanotechnology and potential of microorganisms. Crit. Rev. Biotechnol. 25: 199-204.

143. Gittins DI, Bethell D, Schiffrin DJ, Nichols RJ. (2000). A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups. Nature. 408: 67-69.

144. Meng H, Liong M, Xia T, Li Z, Ji Z, et al. (2010). Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. ACS nano. 4: 4539-4550.

145. Marcato PD, Durán N. (2008). New aspects of nanopharmaceutical delivery systems. J. Nanosci. Nanotech. 8: 2216-2229.

146. Singh R, Nalwa HS. (2011). Medical applications of nanoparticles in biological imaging, cell labeling, antimicrobial agents, and anticancer nanodrugs. J. Biomed. Nanotech. 7: 489-503.

147. Wise K, Brasuel M. (2011). The current state of engineered nanomaterials in consumer goods and waste streams: the need to develop nanoproperty-quantifiable sensors for monitoring engineered nanomaterials. Nanotech. Sci. App. 4: 73-86.

148. Tamura H, Furuike T, Nair SV, Jayakumar R. (2011). Biomedical applications of chitin hydrogel membranes and scaffolds. Carbohydr. Polym. 84: 820-824.

149. Madhumathi K, Binulal NS, Nagahama H, Tamura H, Shalumon KT, et al. (2009). Preparation and characterization of novel β-chitin–hydroxyapatite composite membranes for tissue engineering applications. Int. J. Biol. Macromol. 44: 1-5.

150. Shalumon KT, Anulekha KH, Girish CM, Prasanth R, Nair SV, et al. (2010). Single step electrospinning of

chitosan/poly (caprolactone) nanofibers using formic acid/acetone solvent mixture. Carbohydr. Polym. 80: 414-420.

151. Jayakumar R, Reis RL, Mano JF. (2006). Phosphorous containing chitosan beads for controlled oral drug delivery. J. Bioact. Compat. Polym. 21: 327-340.

152. Dev A, Mohan JC, Sreeja V, Tamura H, Patzke GR, et al. (2010). Novel carboxymethyl chitin nanoparticles for cancer drug delivery applications. Carbohydr. Polym. 79: 1073-1079.

153. Peter M, Binulal NS, Soumya S, Nair SV, Furuike T, et al. (2010). Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications. Carbohydr. Polym. 79: 284-289.

154. Portero A, Teijeiro-Osorio D, Alonso MJ, Remuñán-López C. (2007). Development of chitosan sponges for buccal administration of insulin. Carbohydr. Polym. 68: 617-625.

155. Solairaj D, Rameshthangam P, Arunachalam G. (2017). Anticancer activity of silver and copper embedded chitin nanocomposites against human breast cancer (MCF-7) cells. Int. J. Biol. Macromol. 105: 608-619.

156. Usman MS, Ibrahim NA, Shameli K, Zainuddin N, Yunus WMZW. (2012). Copper nanoparticles mediated by chitosan: Synthesis and characterization via chemical methods. Molecules. 17: 14928-14936.

157. Wu SJ, Liou TH, Mi FL. (2009). Synthesis of zero-valent copper-chitosan nanocomposites and their application for treatment of hexavalent chromium, Bioresour. Technol. 100: 4348–4353.

158. Tokarek K, Hueso JL, Kustrowski P, Stochel G, Kyziol A. (2013). Green synthesis of chitosan-stabilized copper nanoparticles. Eur. J. Inorg. Chem. 28: 4940–4947.

159. Nithya A, Mohan SC, Jeganathan K, Jothivenkatachalam K. (2017). A potential photocatalytic, antimicrobial and anticancer activity of chitosan-copper nanocomposite. Int. J. Biol. Macromol. 104: 1774-1782.

160. Wartlick H, SpaÈnkuch-Schmitt B, Strebhardt K, Kreuter J, Langer K. (2004). Tumour cell delivery of antisense oligonuclceotides by human serum albumin nanoparticles. J. Control. Release. 96: 483-495.

161. Mirtsching B, Cosgriff T, Harker G, Keaton M, Chidiac T, et al. (2011). A phase II study of weekly nanoparticle albumin-bound paclitaxel with or without trastuzumab in metastatic breast cancer. Clin. Breast Cancer. 11: 121-128.

162. Kim TH, Jiang HH, Youn YS, Park CW, Tak KK, et al. (2011). Preparation and characterization of watersoluble albumin-bound curcumin nanoparticles with improved antitumor activity. Int. J. Pharm. 403: 285-291.

163. Wang Z-m, Ho JX, Ruble JR, Rose J, RuÈker F, et al, (2013). Structural studies of several clinically important oncology drugs in complex with human serum albumin. Biochim. Biophys. Acta. 1830: 5356-5374.

164. Poór M, Li Y, Matisz G, Kiss L, Kunsági-Máté S, et al. (2014). Quantitation of species differences in albumin-ligand interactions for bovine, human and rat serum albumins using fluorescence spectroscopy: A test case with some Sudlow's site I ligands. J. Lumin. 145: 767-773.

165. Yanase K, Arai R, Sato T. (2014). Intermolecular interactions and molecular dynamics in bovine serum albumin solutions studied by small angle X-ray scattering and dielectric relaxation spectroscopy. J. Mol. Liq. 200: 59-66.

166. Azizi M, Ghourchian H, Yazdian F, Dashtestani F, AlizadehZeinabad H. (2017). Cytotoxic effect of albumin coated copper nanoparticle on human breast cancer cells of MDA-MB 231. PLoS One. 12: e0188639.

167. Geetha R, Ashokkumar T, Tamilselvan S, Govindaraju K, Sadiq M, et al. (2013). Green synthesis of gold nanoparticles and their anticancer activity. Cancer Nanotechnol. 4: 91-98.

168. Khan S, Ansari AA, Khan AA, Ahmad R, Al-Obaid O, et al. (2015). In vitro evaluation of anticancer and antibacterial activities of cobalt oxide nanoparticles. J. Biol. Inorg. Chem. 20: 1319-1326.

169. Sreekanth TVM, Pandurangan M, Kim DH, Lee YR. (2016). Green Synthesis: In-vitro Anticancer Activity

of Silver Nanoparticles on Human Cervical Cancer Cells. J. Clust. Sci. 27: 671- 681.

170. Pesic M, Podolski R-A, Stojkovic S, Matovic B, Zmejkoski D, et al. (2015). Anti-cancer effects of cerium oxide nanoparticles and its intracellular redox activity. Chem. Biol. Interact. 5: 85-93.

171. Laha D, Pramanik A, Maity J, Mukherjee A, Pramanik P, et al. (2014). Interplay between autophagy and apoptosis mediated by copper oxide nanoparticles in human breast cancer cells MCF7. Biochim. Biophys. Acta 1840: 1-9.

172. Dipranjan L, Debalina B, Arindam P, Chitta Ranjan S, Panchanan P, et al. (2012). Evaluation of copper iodide and copper phosphate nanoparticles for their potential cytotoxic effect. Toxicol. Res. 1: 131-136.

173. Pramanik A, Laha D, Chattopadhyay S, Dash SK, Roy S, et al. (2015). Targeted delivery of "copper carbonate" nanoparticles to cancer cells in-vivo. Toxicol. Res. 4: 1604-1612.

174. Nagajyothia PC, Muthuramanb P, Sreekanthc TVM, Kim DH, Shima J. (2017). Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human cervical carcinoma cells. Arab. J. Chem. 10: 215-225.

175. Wang Y, Zi XY, Su J, Zhang HX, Zhang XR, et al. (2012). Cuprous oxide nanoparticles selectively induce apoptosis of tumor cells. Int. J. Nanomedicine 7: 2641-2652.

176. Guo Y, Zhang J, Yang L, Wang H, Wang F, et al. (2010). Syntheses of amorphous and crystalline cupric sulfide nanoparticles and study on the specific activities on different cells. Chem. Commun. (Camb) 46: 3493-3495.

177. Zhou M, Tian M, Li C. (2016). Copper-based nanomaterials for cancer imaging and therapy. Bioconjugate Chem. 27: 1188–1199.

178. Valodkar V, Jadeja RN, Thounaojam MC, Devkar RV, Thakore S. (2011). Biocompatible synthesis of peptide capped copper nanoparticles and their biological effect on tumor cells. Mater. Chem. Phys. 128: 83-89.

179. Ullmann M, Friedlander SK, Schmidt-Ott A. (2002). Nanoparticle formation by laser ablation. J. Nanopart. Res. 4: 499-509.

180. Tauran Y, Brioude A, Coleman AW, Rhimi M, Kim B. (2013). Molecular recognition by gold, silver and copper nanoparticles. World J. Biol. Chem. 4: 35-63.

181. Valodkar M, Rathore PS, Jadeja RN, Thounaojam M, Devkar RV, et al. (2012). Cytotoxicity evaluation and antimicrobial studies of starch capped water soluble copper nanoparticles. J. Hazard. Mater. 201-202: 244-249.

182. Dipranjan L, Arindam P, Sourav C, Sandip KD, Somenath R, et al. (2015). Folic acid modified copper oxide nanoparticles for targeted delivery in in-vitro and in-vivo systems. RSC Adv 5: 68169-68178.

183. Hadinoto K, Sundaresan A, Cheow WS. (2013). Lipid–polymer hybrid nanoparticles as a new generation therapeutic delivery platform: A review. Eur. J. Pharm. Biopharm. 85: 427-443.

184. Pramanik A, Laha D, Pramanik P, Karmakar P. (2014). A novel drug "copper acetylacetonate" loaded in folic acid-tagged chitosan nanoparticle for efficient cancer cell targeting. J. Drug Target. 22: 23-33.

185. Liu P, Wang Z, Brown S, Kannappan V, Tawari PE, et al. (2014). Liposome encapsulated Disulfiram inhibits NFκB pathway and targets breast cancer stem cells in vitro and in vivo. Oncotarget. 5: 7471–7485.

186. Zhang R, Pan D, Cai X, Yang X, Senpan A, et al. (2015). αvβ3-targeted copper nanoparticles incorporating an Sn 2 lipase-labile fumagillin prodrug for photoacoustic neovascular imaging and treatment. Theranostics. 5: 124-133.

187. Tao Y, Li M, Ren J, Qu X. (2015). Metal nanoclusters: novel probes for diagnostic and therapeutic applications. Chem. Soc. Rev. 44: 8636-8663.

188. Goswami U, Dutta A, Raza A, Kandimalla R, Kalita S, et al. (2018). Transferrin-copper nanocluster-doxorubicin nanoparticles as targeted theranostic cancer nanodrug. ACS Appl. Mater. Interfaces. 10: 3282-3294.

189. Zheng J, Yang S, Sun S, Zhou C, Hao G, et al. (2015). Renal clearance and degradation of

glutathione-coated copper nanoparticles. Bioconjugate Chem. 26: 511–519.

190. Lee IC, Ko JW, Park SH, Lim JO, Shin IS, et al. (2016). Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats. Int. J. Nanomedicine. 11: 2883-2900.

191. Song L, Connolly M, Fernández-Cruz ML, Vijver MG, Fernández M, et al. (2014). Species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. Nanotoxicology. 8: 383-393.