Biogenic Metal and Metal Oxides Nanoparticles as Anticancer Agent: A Review

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Abstract. Herein this review we have summarized a number of cytotoxic studies which have been done using various biosynthesized metal nanoparticles (MNPs) and metal oxide nanoparticles (MONPs) on different cancer cell lines. Plants can serve as a good candidate to provide natural surfactants for the green approach in the preparation of nanoparticles. Numerous biomolecules are present in the plants. Also, numerous plant extract-based MNPs and MONPs have been synthesized and used in several fields of applications particularly in biomedicine. This property can be attributed because of their low cost, biocompatibility and favourable to the environment. In the past few years, the utilisation of these biogenic nanoparticles has increased tremendously particularly in cancer therapy. These biogenic nanoparticles considered as an excellent tool for cancer diagnosis and drug delivery at the tumour site preferentially. By utilising the unique properties of nanoparticles and antioxidant and antitumor nature of plants, these biosynthesised nanoparticles selectively destroy the tumour cells and do not harm the normal healthy cells. In this review, we have compiled the most significant results obtained by the biosynthesized MNPs and MONPs like silver (Ag), gold (Au), Fe₂O₃/Fe₃O₄, ZnO, and CuO respectively.

Keywords: Biogenic synthesis, Apoptosis, Reactive Oxygen Species, Cytotoxicity, Antitumor

1. Introduction

1.1. Importance of metal /metal oxide nanoparticles

Nanoparticles are the particles whose size lie between 1nm to 100 nm. Owing to very small size, their chemical and physical properties are size dependent. This feature is different from properties associated with bulk material [1]. The utilization of MNPs and MONPs in various areas is increasing day by day. Nanoparticles have immense possibilities to be used in diverse area of applications like electronics, biotechnology, biomedical and environmental sciences. The use of these nanoparticles has increased tremendously in several everyday care products like soaps, cosmetics, shampoos, detergent, medical and pharmaceutical products [2,3]. Nature has the ingenious beauty of developing most effi-
cient small sized functional materials. These materials can be synthesized by utilising either green approach or the conventional physical and chemical approaches. However, the conventional methods of synthesis are suffered from various disadvantages like high energy requirements, use of toxic chemicals, time consuming and expensive respectively. However, the biosynthesis is the promising way of synthesizing variety of nanoparticles with unique size and shape. Green synthesis avails non-toxic, sustainable and economical way to produce nanoparticles. The biosynthesis of nanoparticles is a bottom-up technique where biological entities like as the extracts of various parts of plants (leaf, flower, fruit peel, bark, root and stem), microorganisms such as algae, fungus and bacteria are taken in use for the preparation of nanoparticles. Herein, Figure 1 displays the use of different sources of the green approach for preparation of nanoparticles.

![Green Synthesis Diagram]

**Figure 1.** Schematic representation showing utilization of various sources for green synthesis

**1.2. Importance of plants for nanoparticle synthesis**

Very significant phytochemicals such as flavonoids, derivatives of phenols, polyphenolic acids, monoterpenes, quercetin and many more have been investigated in the extracts of various parts of different plants. These phytochemicals serve the role as capping and reducing agents in the synthesis of variety of MNPs and MONPs which are non-toxic. Since the last few years, the extensive study has been performed on utilisation of extract of different plants parts for the preparation of nanoparticles and their anticancer studies. Metal oxide nanoparticles are prepared from metal precursors. These nanoparticles can be formed by several ways like electrochemical, sol-gel, co-precipitation, hydrothermal, solvothermal, microemulsion technique, laser ablation technique, thermal decomposition, high-energy ball milling technique etc. The biogenic synthesis of nanoparticles with fungi, bacteria and plant extracts provides several benefits over the abovementioned methods. Similarly, if we compare the biosynthesis with fungi, bacteria and extract of plants, then synthesis with extract of plants is easier in terms of decreased number of synthesis steps like elaborate culturing procedures of bacteria and other things which are required for maintenance. In green approach for preparation of nanoparticles with extract of plants, unique size and shape of nanoparticles can be obtained for cancer targeted properties.
Because the extract of various plants has specific biomolecules which have their own biological significance namely anticancer, antimicrobial, antioxidant, drug delivery and antiflammery respectively. The plant extracts have specific biomolecules such as polyphenols, polysaccharides, terpenoids, flavonoids, amide, aldehydes, carboxylic acids and heterocyclic compounds respectively [4-8]. Very recently, Nutan et al also reported in vitro investigation of ZnO nanoparticles prepared by green approach on (A549) human lung cancer cell lines [92]. Gupta et al have reported the synthesis of MNPs and MONPs (inorganic nanoparticles) and their implementation in cancer therapy and as antibacterial agent. Gupta et al have prepared gold nanoparticle with green tea extract and utilized in catalytic reduction of methylene blue [93]. Singh et al have synthesized metal nanoparticles namely platinum, silver and gold using green approach [94]. Herein, these nanoparticles have been used for degradation of methyl orange. Herein, they have studied an electron relay effect. Byrne et al have synthesized biogenic magnetite nanoparticle (Fe₃O₄) using extract of Geobacter sulfurreducens at industrial level [95]. Herein, Geobacter sulfurreducens is a gram-negative metal and sulphur-reducing roteobacterium. **Figure 2** shows the general procedure which is followed for the preparation of plant extracts based MNPs and MONPs. Herein, first step demonstrates the preparation of fresh extract with various parts of plants and second step demonstrates preparation of aqueous metal salts precursor solution after that by adding definite amount of extract in the metal salt precursor solution, specific morphology of MNPs and MONPs are obtained.

**Figure 2.** Showing the common method for green synthesis of MNPs and MONPs using plant parts.

Literature survey reveals that numerous MNPs and MONPs have been synthesized using extract of different plants parts such as stem, bark, seed coat, fruit etc. **Table 1** summarizes the various biosynthesized MNPs and MONPs having different shape and size. These biosynthesized nanoparticles have also been studied for their application as anticancer agent on different cancer cell lines.
Table 1. Showing various metal/metal oxide nanoparticles synthesized by utilizing green methods and their study on various cancer cell lines.

| Sr. no. | Source                      | Plant part utilised | Metal/metal oxide prepared | Size/Shape               | Cancer cell line used       | Ref. |
|---------|-----------------------------|---------------------|-----------------------------|--------------------------|-----------------------------|------|
| 1       | Costus pictus D. Don         | leaf                | Zinc oxide                  | 20-80 nm/rod shaped      | DLA                         | 75   |
| 2       | Tabernaemontana divaricata  | leaf                | Zinc oxide                  | ~ 36 nm/ spherical       | MCF-7                       | 9    |
| 3       | Capparis zeylanica          | leaf                | Zinc oxide                  | 28-30 nm                 | A549                        | 10   |
| 4       | Deverra tortusa             | Aerial parts        | Zinc oxide                  | 9.26-31.18 nm/ spherical | Caco-2, A549                | 84   |
| 5       | Albizia lebbeck             | Stem bark           | Zinc oxide                  | 66.25 nm/ spherical       | MDA-MB 231, MCF-7           | 65   |
| 6       | Borassus flabelifer         | Seed coat leaf      | Iron oxide                  | 35 nm/ hexagonal          | NIH3T3                      | 57   |
| 7       | Rosemary extract            | leaf                | Iron oxide                  | 20-80 nm/ spherical       | 4T1, C26                    | 58   |
| 8       | Juglan regia                | Green husk leaf     | Iron oxide                  | ~5.77 nm/ cubical         | NIH-3T3/ HT-29, Hep G2      | 11   |
| 9       | Rhamnella giglitica         | leaf                | Iron oxide                  | ~21 nm/ spherical         |                             | 12   |
| 10      | Punica Granatum             | Fruit peel          | Iron oxide                  | 11 nm/ spherical, cubical | HCT-116, MCF-7, HeLa, A549, HONE1 | 13   |
| 11      | Acalypha indica             | leaf                | Copper oxide                | 26-30 nm/ spherical       |                             | 88   |
| 12      | Ficus religiosa             | leaf                | Copper oxide                | 577 nm/ spherical         | A549                        | 89   |
| 13      | Ormocarpum cochinense       | leaf                | Copper oxide                | 1-2 μm/agglomerated       | HCT-116                     | 85   |
| 14      | Black bean                  | Seed extract        | Copper oxide                | ~ 26 nm/ spherical        | HeLa cells                  | 90   |
| 15      | Syzygium alternifolium      | Bark                | Copper oxide                | ~ 17 nm/ spherical        | MDA-MB 231                  | 14   |
| 16      | Cotton plant                | Saffron stigma      | Gold                        | 4-10 nm/ spherical        | MCF-7                       | 54   |
Table 1: Properties of different natural materials

| No | Plant Name           | Part of the Plant | Material | Size/Shape         | Tumor Lines | IC50 \(\mu \text{M}\) |
|----|----------------------|-------------------|----------|---------------------|-------------|------------------------|
| 17 | Carica papaya and Catharanthus | leaf | Gold | 15-28 nm/ spherical | MCF-7, Hep G2 | 53 |
| 18 | Curcuma wenyujin | Rhizome | Gold | 200 nm/ spherical | A498, Sw-156 | 52 |
| 19 | Rabdosia rubescens | leaves | Gold | 130 nm/ spherical | A549 | 47 |
| 20 | Scutellaria barbata | Whole plant | Gold | 0.4-1 \(\mu\)m/ spherical | PANC-1 | 46 |
| 21 | Dolichos lablab | leaf | Silver | 9 nm/ spherical | Hep G2 | 15 |
| 22 | Plumeria alba | flower | Silver | 36.19 nm/ spherical | COLO 205 | 16 |
| 23 | Syzygium aromaticum | Fruit | Silver | 5-20 nm/ spherical | MCF-7, A549 | 17 |
| 24 | Punica granatum | peel | silver | 15.6 nm/ spherical | MCF-7, BT-20 | 32 |
| 25 | Neptia deflersiana | Aerial parts of the plant | silver | 33 nm/ spherical | HeLa | 33 |

2. Conditions required by MNPs and MONPs for anticancer application

2.1. Size

The size of MNPs and MONPs is a foremost criterion to be considered for biomedical application [21-23]. Since, the size of nanoparticles controls their movement inside the blood stream and tumour vasculature. In cancer treatment, the nanoparticles follow either active or passive pathway to treat tumour cells. The utilisation of nanoparticle has been observed as an important tool for the discovery of new age cancer drug [18]. For the application of nanoparticles in medicine and in vivo studies, the biocompatibility, biodegradability, long retention time and non-toxic properties are required to minimize the aftereffects [19]. The anticancer activity of various metal and metal oxide nanoparticles might be either due to intrinsic effect or by external stimuli such as hyperthermia. The intrinsic antitumor effect such as antioxidant behaviour of nanoparticles is due to specific physicochemical properties of nanoparticles. When external applied magnetic field or infrared radiations are applied, the external stimuli work by producing reactive oxygen species (ROS) which kills the tumour cells [20]. Enhanced permeability and retention (EPR) effect is applied in passive drug delivery system. The leaky vascular nature of cancerous cells permits metal and metal oxide nanoparticles to enter easily and kill the tumour cells [24]. In active pathway the nanoparticles are functionalized with different biomolecules or ligand to directly target at the cancerous cell site.
2.2. Shape

The shape of nanoparticles is another important criterion. Nanoparticles with spherical shape is most preferred for drug delivery application [25], because these have higher surface volume ratio on which sufficient drug amount can be loaded for better efficacy. Finally, by conjugating different targeting ligand the efficacy of the delivery system can be further increased. Li et al has observed the increased cytotoxicity into different tumour cells by N-(2-hydroxylpropyl) methacryl amide copolymer loaded with doxorubicin through the folate receptor [26]. Herein, Figure 3 illustrates the graphical representation of biosynthesized MNPs and MONPs in the treatment of cancer as anticancer agent. Where three important anticancer applications of various biosynthesized MNPs and MONPs have been demonstrated. Those are cytotoxic study in mouse model, DNA damage of cancerous cell (apoptosis) and targeted drug delivery in the human beings due to biocompatibility.

![Figure 3. Graphical Illustration of utilisation of biosynthesized MNPs and MONPs as anticancer agents in cancer treatment.](image)

3. Cancer

Cancer, considered as a deadly disease where an uncontrolled growth of abnormal cells occurs in any organ or tissue of the body. The most common types of cancer are carcinoma, melanoma (mainly skin cancer), sarcoma, lymphoma (cancer of lymphocytes) and leukemia (cancer of bone marrow where blood cells generate) respectively. The sarcoma is a cancer of connective tissues like as blood vessels muscles, cartilage and bones. We know that the carcinoma is the most common type of cancer. As we also know that the carcinoma starts in the cells or the tissue that covers the surface of internal organs, system and glands respectively. Some carcinoma are like as pancreatic cancer (in pancreas),
prostatic cancer (in prostate), colorectal cancer (in colon) and so on. GLOBOCAN 2020 survey estimates that almost 10 million cancer death and 19.3 million new cancer cases occurred worldwide [27]. The common approaches utilised for the diagnosis and treatment of cancer involve chemotherapy, surgery, radiotherapy and use of alkylating agents. But the major problem of these approaches are the side effects which occurred due to problems in identifying the normal and cancerous cells which leads to systemic toxicity [28]. Hence there is an utmost requirement to discover alternate methods and drug development in the field of cancer treatment.

4. General mechanism of cytotoxic activity

The exact mechanism is not yet clear about the cytotoxic effect of various MNPs and MONPs towards various cancer cell lines. The vital mechanism of cell death has been considered as apoptosis till yet [29-31]. The MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay is commonly used to measure the cytotoxic effect of various MNPs and MONPs in tumour cells. The MTT assay is generally used to access the metabolic activities of cells. This assay involves the conversion of soluble MTT yellow salt to formazan crystals which are insoluble in water by the living cells. **Figure 4** demonstrates the various steps involved in the cytotoxicity caused by biosynthesized MNPs and MONPs.

**Figure 4.** Schematic representation showing general mechanism of cytotoxicity
5. Silver Nanoparticles

During the past few years, many researchers have proved and explained the cytotoxic effects of silver nanoparticles which are synthesized using green methods on various cancer cell lines. The researchers have used the water mediated leaf extract of pomegranate (Punica granatum) for synthesizing the silver nanoparticles [32]. The size obtained in the range from 41.69 nm to 69.1 nm of the as formed silver nanoparticles. The cell viability of human cervical cancer cells has been tested by employing MTT assay. Herein, the cell viability decreases dose dependently with increase in concentration of silver nanoparticles. Similarly, the lactate dehydrogenase (LDH) assay has been performed to know the cytotoxic effect of synthesised silver nanoparticles. All the normal mammalian cells contain lactate dehydrogenase (LDH) but only damaged and necrotic cells release LDH. Therefore, by using the obtained nanoparticles on the above mentioned cancer cell lines the cytotoxic effect has been explored by knowing the released amount of LDH. The experimental results show that the released amount of LDH increases as the concentration of silver nanoparticles increased from 50 - 250 μg/mL. The DNA fragmentation assay of human cervical cancer cells also demonstrates that ladder pattern has been observed for the cleaved DNA. Which indicates that apoptosis has been induced by silver nanoparticles. No fragmentation of DNA has been observed in the untreated cells. These findings show that biosynthesised silver nanoparticles serve the role as therapeutic agent for cancer treatment. Similarly, Nepeta Deflersiana aqueous extract has been taken for synthesizing the silver nanoparticles [33]. The cell viability of HeLa cells was assessed by both MTT and neutral red uptake (NRU) assay. Herein, the cell viability of human cervical cancer cells affects very significantly on variation of concentration of silver nanoparticles. Where, very low value of IC₅₀ (5 μg/mL) has been reported. These biosynthesised Nepeta Deflersiana mediated silver nanoparticles (ND-Ag NPs) show cytotoxicity on increasing the lipid peroxidation level and ROS level and simultaneously decreasing the glutathione (GSH) level. Herein, the flow cytometry analysis has been done through the arrest of SubG1 cell cycle. These results show that cell death occurs due to the induced apoptosis and necrosis in the human cervical cancer cells. Khatami et al have researched on MCF-7 cancer cell lines, herein they have investigated the inhibitory effect of phytomediated silver nanoparticles with extract of waste grass [34]. Furthermore, Western blot assay has been performed to extract the Cyclin D1 protein and to study the effect of the synthesised silver nanoparticles on the cell division and growth of MCF-7 cell line. It has been found that the expression of Cyclin D1 protein has reduced to 64.42% at 20 μg/mL of concentration of silver nanoparticles.

Khorrami et al have prepared silver nanoparticles with black peel pomegranate which have been found highly selective and show enhanced cytotoxicity against two tumour cell lines namely MCF-7 and BT-20 [35]. The maximum death of MCF-7 cell has been found to be 89.6% after 48 h incubation. Similarly, 81% cell death occurs in BT-20 cancer cell lines. Herein, same cytotoxicity study has been performed against non-tumour L-929 cell lines which indicates that these silver nanoparticle as well as only the extract of black peel pomegranate do not exhibit any cytotoxic effect.

Sankar et al have synthesised silver nanoparticles using Origanum vulgare aqueous extract [36]. In vitro cytotoxic study has been done A549 cell lines by performing MTT assay. The experimental findings show the 50% cell death occurs in A549 cell line at 100 μg/mL concentration. The researchers have reported the presence of bioactive compounds like thymol, linalool, quercetin, apigenin, sabinene, carvacrol, terpinolene, terpinene in Origanum vulgare helps in increasing the cytotoxic activity of silver nanoparticles by acting as capping agent. Kathiravan et al have investigated the potent anticancer activity of phytomediated silver nanoparticles against MCF-7 cell line [37]. The aqueous leaf extract of Melia dubia has been taken for the preparation of silver nanoparticles. Herein, the cell death of MCF-7 cell line enhanced with increasing the concentration of biosynthesised silver nanoparticles. The IC₅₀ value (31.2 μg/mL) has been determined with crude leaf extract of Melia dubia.
Subramanian et al also have formed silver nanoparticles with _Sargassum polycystum_ and used them in vitro cytotoxic study of human colon cancer (HT-29) [38]. The authors have found that the cell viability decreases as the concentration of silver nanoparticles increases from 5 to 50 μg/mL. Herein, the IC₅₀ value has been reported 20 μg/mL. The lower value of IC₅₀ indicates the higher cytotoxic effect of the biosynthesised silver nanoparticles on cancer cell lines. Herein, a study on nuclear condensation has been done using the propidium iodide method. A large number of propidium iodide-stained cells have been observed when the HT-29 cells are treated with silver nanoparticles at 20 μg/mL after 24 h.

Lakshmanan et al have similarly synthesized silver nanoparticles by taking the fruit extract of _Cleome viscosa_ [39]. Herein, the biosynthesized silver nanoparticles have been assessed for checking the in vitro anticancer activity via MTT assay against human lung (A549) and ovarian (PA1) cancer cell lines. Where, DAPI (4,6-diamidino-2-phenylindole) nuclear staining method has been used for finding the morphological changes happened in lung (A549) and ovarian (PA1) cancer cell lines. The IC₅₀ values have been observed to be 28 μg/mL and 30 μg/mL respectively for lung and ovarian cancer cell lines. In another study, Afurayadi et al have reported the biosynthesis of silver nanoparticles by utilising the sesame oil cake [40]. Herein, the antitumor activity has been investigated at two distinct concentrations (2.5 μg/mL and 7.5 μg/mL) of silver nanoparticles on MCF-7 cell lines.

Similarly, the aqueous extract has prepared by taking aerial parts of _Lippia nodiflora_ [41]. Silver nanoparticles have been synthesised using this water mediated extract which act as a reducing agent. The MCF-7 cancer cell line has been utilised for determining the cytotoxic activity of biosynthesised silver nanoparticles. The observed cytotoxicity is time and dose dependently, where the value of IC₅₀ has been obtained 40 μg/mL for incubation time of 24 h. The morphological changes have been seen in the MCF-7 cancer cell lines using phase contrast microscopy. The Retraction, detachment and suspended cells are seen, when the MCF-7 cells are treated with silver nanoparticles. Whereas untreated cells show flattened and smooth morphology.

From the literature survey, it is clear that _Alternanthera sessils_ mediated synthesis of silver nanoparticles show better cytotoxic effect as compared to standard drug cisplatin on breast cancer cells [42]. Herein, the cytotoxic effect has been studied on (A431) cell line using various shaped silver nanoparticles such as cuboidal and spherical respectively. Nayak et al have reported IC₅₀ values from 78.58 to 83.57 μg/mL for epidermoid (A431) carcinoma [43]. Asharani et al have reported an anti-Proliferative activity of as prepared silver nanoparticles [44]. The specific participation of silver nanoparticles in the interference of mitochondrial respiratory chain which leads to generation of ROS and disturbance in the synthesis of ATP cycle that is cause of nuclear damage. Abovementioned these activities of silver nanoparticles show that it is a promising anticancer agent.

6. Gold Nanoparticles

_Albutilon indicum_ leaf extract mediated gold nanoparticles (AIGNPs) have been synthesized by Mata et al [45]. Herein, the cytotoxic effect of these biosynthesized nanoparticles has been studied on HT-29 colon cancer cells. The cytotoxicity has been observed in term of the damage in cell membrane as well as nuclear morphological changes. This observed cytotoxicity has been explained on the basis of increase in ROS and concurrent decrease in cellular antioxidants like glutathione (GSH), catalase and superoxide dismutase (SOD). Further, there has been seen loss in mitochondrial membrane potential, G1/S phase cell cycle arrest and DNA damage due to reduction of antioxidants. Herein, the results also show that increase in the expression level of particular protease markers like Caspase-3, Caspase-8, Caspase-9, poly (ADP-ribose) polymerase (PARP) and Lamin A/C supports the induction of apoptosis by intrinsic and extrinsic pathways in the cells treated with AIGNPs. With increasing the concentration of AIGNPs, cause simultaneous increase in cytotoxic effect and IC₅₀ values have been obtained 210 μg/mL at 24 h and 180 μg/mL at 48 h incubation respectively. The experimental data also reveal the
selective cytotoxicity of AIGNPs against HT-29 cells. Herein, in vitro study has been done on normal HaCaT cells. This study demonstrates that these gold nanoparticles do not show cytotoxic effect on these normal cell lines.

The gold nanoparticles have been synthesised using the aqueous extract of Scutellaria barbata [46]. The cytotoxic study has been done using MTT assay against pancreatic cancer cells (PANC-1) at two concentration (25 μg/mL and 50 μg/mL) of gold nanoparticles. The oxidoreductase enzyme presents in the mitochondria of viable cells react with MTT dye and gets converted into formazan crystals. Herein, the cell viability decreases with time in dose dependent manner. Herein, the anticancer activity of Scutellaria barbata mediated gold nanoparticles have been reported due to generation of ROS in the cancer cell line. There are also some factors such as upregulation of Caspase 3, Caspase 9 and Bax and down regulation of Bid and Bcl-2 which occur at 50 μg/mL concentration of gold nanoparticles.

Similarly, Zhang et al have also revealed the apoptotic study of gold nanoparticles which are biosynthesised using Rabdosia rubescens [47]. Herein, the ROS production, protein expression of apoptotic signalling pathways has been confirmed on the basis of estimation of caspase level which show the marked anticancer activity of biosynthesized gold nanoparticles.

Zheng et al have synthesized gold nanoparticles using extract of Magnolia officinalis. These nanoparticles also demonstrate anticancer activity on lung cancer cell line (A549). Where, the apoptosis has been reported through intrinsic pathway [48]. Herein, DAPI (4’,6-diamidino-2-phenylindole) and terminal deoxynucleotidyl transferase (TdT) dUTP nick end labelling (TUNEL) staining have been used for assessing the apoptosis in A549 cancer cell lines. Herein, the gold nanoparticles cause fragmentation of DNA and western blotting results shows the decrease in expression level of Bcl-2, Bid and increase in expression level of Bax, Caspase-3 and Beclin-1 in A549 cancer cells.

The preparation of gold nanoparticles by a green route method using Cordyceps militaris extract have reported [49]. The formed gold nanoparticles have been assessed for their antiproliferative study against liver cancer cells (HepG2). The IC$_{50}$ value has been found to be 12.5 μg/mL. Herein, the experimental data show that induction of apoptosis in liver cancer cells occur via the pathway that is caspase dependent. The Caspase are the family of cysteine protease enzymes demonstrates a significant part in apoptosis process. Herein, various parameters have been reported like as up regulation of apoptosis pathway genes (Bax, Bid, Caspase 3 and Caspase 9) expression and downregulation of anti apoptotic genes (Bcl-2) expression of HepG2 cells.

Mishra et al have synthesized gold nanoparticles using the extract of Hibiscus sabdariffa and their stability has been observed in different concentration of glucose [50]. The literature survey reveals that for production of energy, most cancer cells make use of glucose. Moreover, it was observed that gold nanoparticles are stable in glucose solution (concentration 1-30mM) for seven days. Under hyperglycaemia condition the cytotoxic study has been performed with the synthesized gold nanoparticles against U87 Glioblastoma cells. The experimental data reveal that 85% and 80% cell death occur under normal and hyperglycaemia conditions respectively. The cytotoxic effect also depends on the amount of gold nanoparticles used in study of U87 Glioblastoma cells.

The utilisation of gold nanoparticles in biomedical applications, mainly in molecular imaging specifically depends on the evaluation of particular biomarkers and the adherence of gold nanoparticles to particular ligands on the tissue of tumour cells [51]. In vitro apoptotic effect of biogenic gold nanoparticles synthesized using wenyujin extract has been evaluated by MTT assay against human renal cell carcinoma A498 cells [52]. The study shows that induction of apoptosis in the A498 was due to enhancement of caspase3, caspase 9, Bax, Bid and decrease in the expression of antiapoptotic protein Bcl-2 in a dose dependent manner which has been analyzed by qPCR and immunoblotting assay. Herein, the Rhodamine 123 staining results show that there is an increase in mitochondrial membrane potential that leads to induction of apoptosis. This observation has been further confirmed by flow cytometry assay.

Similarly, Kumar et al have prepared the gold nanoparticles using the leaf extracts of two plants namely Carica papaya (CP), Catharanthus roseus (CR) and mixture of these two extracts (CPCRM) [53]. The gold nanoparticles have been tested for their antibacterial and antitumor activity. MTT assay
has been used for testing the cytotoxic effect against two cell lines, MCF-7 and HepG2 cell. The as formed gold nanoparticles have been found biocompatible against 3T3 fibroblast cell line at a concentration of 250 μg/mL of gold nanoparticles. Herein, gold nanoparticles have been synthesized using combined (mixture) extract (CPCRM) showed the enhanced cytotoxic effect. Where, a comparative study has been performed on the gold nanoparticles prepared using individual extract and combined (mixture) extract to two plants respectively. The experimental data reveal that the live cells percentage decreases as the concentration of the gold nanoparticles increases. Crocin, an important component of saffron stigma has been taken for the phytosynthesis of gold nanoparticles and investigated for their in-vitro anticancer effect on MCF-7 cells using MTT and LDH assay [54]. It has been found that crocin mediated gold nanoparticles prevent the growth of MCF-7 cell lines in both manner i.e., dose and time dependent. The cytotoxic effect of crocin-gold nanoparticles was less on MCF-7 cell lines than with only crocin.

7. Iron oxide Nanoparticles

The iron oxide nanoparticles have also been applied in magnetic resonance targeted drug delivery, theranostics, imaging (MRI) and CT-scan for detection of several types of cancer. Kudr and Val-labani et al have reported various medical applications for cancer treatment and diagnosis like targeted drug delivery, thermoablation, magnetic fluid hyperthermia, contrast agent in MRI etc [55,56]. An environmentally benign green synthesis pathway has been suggested as an efficient method for the production of iron oxide nanoparticles because of their potential cytocompatibility for biological system. The iron oxide nanoparticles have been prepared with taking the seed coat extract of B. flabelifer which are highly biocompatible [57]. The cytocompatibility of these magnetic iron oxide nanoparticles has been studied in NIH-3T3 fibroblast cell lines using MTT assay. Different concentrations of iron oxide nanoparticles (50-500 μg/mL) have been taken for studying the cytotoxicity at incubation time of 24 h and 48 h respectively. Herein, the cell viability has been reported more than 80% which indicates their high biocompatibility towards the fibroblast cells. The hemolytic study has also been performed for finding the biocompatibility by taking different concentration of iron oxide nanoparticles 25, 50, 75 and 100 μg/mL respectively. These iron oxide nanoparticles do not show the hemolytic activity at abovementioned different concentrations.

Farschi et al have prepared iron (Fe) nanoparticles with Rosemary aqueous extract [58]. In vitro, also cytotoxic activity of biosynthesised Fe nanoparticles has been done on two different cancer cell lines i.e., breast cancer 4T1 cell line and colon carcinoma C26 cell line using MTT assay. The cytotoxicity of biosynthesized Rosemary-Fe nanoparticles and rosemary extract has been determined at concentration of 6.25-200 μg/mL against 4T1 cell line at 48 h incubation time. The cell viability was investigated 80% and 45% with Rosemary-Fe nanoparticles and only rosemary extract respectively. These values of cell viabilities have been reported that at the same concentration of Rosemary-iron nanoparticles and only extract. Similarly, Rosemary-iron nanoparticles shows 20-90% cytotoxicity in the colorectal cancer C26 cell line at concentration of 3.125- 200 μg/mL. The IC_{50} values have been obtained 20.98 μg/mL for Rosemary-Fe nanoparticles and 47.87 μg/mL for rosemary aqueous extract respectively. Which indicates the more cytotoxicity of biosynthesized Rosemary-iron nanoparticles against C26 colon cancer cell line as compared to 4T1 cell line.

The functionalized iron oxide nanoparticles with conventional drugs have also used in cancer treatment and diagnosis. The biosynthesized iron oxide nanoparticles could be conjugated with enzymes, drugs or proteins and can be targeted to a particular organ, tissue or tumour site with the aid of external applied magnetic field or for hyperthermia application could be heated up in alternating magnetic field [59]. The core shell systems of iron oxide nanoparticles show marked activity for cancer treatment and diagnosis when they are functionalized with natural compounds [60]. Iron oxide nanoparticles have been biosynthesized by co-precipitation method using glucose. The average size of iron ox-
ide nanoparticles formed has been reported 20 nm. These nanoparticles have also utilised in targeted hyperthermia process for cancer cells [61].

Similarly, in the hyperthermia treatment of breast cancer, iron oxide nanoparticles have been conjugated with dextran-spermene. Avazzadeh et al have found that 63% of cancer cells are died within 20 minutes when these cells are treated with iron oxide nanoparticles [62]. The stability of iron oxide nanoparticles can be improved by coating with metal oxides like ZnO, SnO, TiO₂, ZrO₂ and WO₃ and it also helps in increasing the generation of heat by iron oxide nanoparticles in hyperthermia treatment. Kandasamy et al have reported in vitro study on liver cancer cell lines (HepG2) at temperature (42 °C) [96]. They have reported the heating profiles of the synthesized surface functionalized superparamagnetic iron oxides (SPIOs). Herein, these SPIOs have been used as nanomedicine for treating liver cancer. Herein, these SPIOs have been used in the form of alternating magnetic fields (AFFs). Herein, the calorimetric magnetic fluid hyperthermia (C-MFH)-based theromotherapy has been demonstrated using a magnetic hyperthermia instrument (magneTherm-nanoTherics). The researchers have reported that by using superparamagnetic Fe₃O₄ nanoparticles heat dissipation occurred due to spin resonance and rise in temperature was seen [97].

The characteristic small size and advantage of having more surface area of the iron oxide nanoparticles permits their use as in targeted drug delivery application. The researchers synthesized iron oxide nanoparticles by coprecipitation method. These iron oxide nanoparticles were conjugated with bovine serum albumin and curcumin exhibiting more than 90% cytocompatibility in HFF-2 cells. Moreover, the IC₅₀ value of conjugated iron oxide nanoparticles have been found to be 915 μM and 275μM at 72 h and 96 h incubation respectively. Whereas the IC₅₀ value of curcumin only has been found 730 μM and 300 μM at 72 h and 96 h incubation. An enhanced cytotoxic activity has been investigated by conjugating these nanoparticles with bovine serum albumin and curcumin towards MCF 7 cell lines [63]. Pirayesh et al have coated SPIO nanoparticle with mesoporous hydroxyapatite nanocomposites (mHANs) for the treatment of breast cancer cells [64]. These nanocomposites have been loaded with doxorubicin and 2-Deoxy-D-glucose by diffusion process. T47D and SKBR3 human cancer cell lines have been used for studied the effect of these synthesised binary nanocomposites. The researchers have investigated that combined chemoradiotherapy is best method to treat the cancerous cells. Where, the cytotoxicity effect can be increased towards the tumour cells and side effect can be minimized on healthy cells due to single mode of cancer treatment. The authors have explained that increased cytotoxicity may be due to more localization of chemodrug at the tumour site. Since the loading efficiency of iron oxide nanocomposites has been reported experimentally 93% and 45% for doxorubicin and 2-Deoxy-D-glucose respectively.

8. Zinc Oxide Nanoparticles

Umar et al, in their research work reported the significant cytotoxic activity of the Zinc oxide on two breast cancer cell lines namely MDA-MB 231 and MCF-7 in a concentration dependent manner [65]. Herein, these nanoparticles have been biosynthesized using Albizia lebbek stem bark extract. They have also observed the formation of membrane blebs in the cells of as taken breast cancer cell lines. When these cells have been treated with zinc oxide nanoparticles in a concentration dependent manner at 48 h incubation. Herein, the formation of plasma membranes blebs indicates the induced apoptosis in the cells. These plasma membrane blebs have not seen in the control cells. Prashant et al have demonstrated cytotoxic effect of zinc oxide nanopowder against MCF-T Bca cell lines in a concentration dependent manner [66]. These zinc oxide nano powders have been prepared using Punica granatum and Tamarindus indica L extracts.
Hanley et al have investigated that the increase in Zn\(^{2+}\) ions and other molecules which are released from the zinc oxide in the intracellular level causes the increase in generation of ROS and apoptosis [67]. For improved targeted therapy, in-vitro and in-vivo tumour model have been developed. Where quercetin loaded PBA functionalised zinc oxide nanoparticles (PBA-ZnO-Q) have been utilised for monitoring the anti-tumour efficacy. Herein, for in-vitro study human breast cancer MCF-7 cells were taken. In vivo study has been performed on Swiss albino mice having ehrlich ascites carcinoma (EAC) solid. These synthesised quercetin loaded PBA functionalised nanohybrids show a strong green and blue fluorescence under a fluorescent microscope. Hence due to fluorescent nature of these nanohybrids, they can also be used as bioimaging agent. The cancer cells are characterised by the expression of sialic acid on its cell membrane [68]. Also, it has been reported that presence of sialic acid on the membrane of cancer cells are more than the normal cells. The PBA-ZnO-Q have showed the cytotoxicity in a dose dependent manner. Herein, the amount of dose has been taken from 3.5 to 35 \(\mu\)g/mL. These nanocarriers have shown a notable cytotoxic effect in breast cancer cells. The particular cytotoxic effect has not been seen in non-tumorigenic (normal) human epithelial cell lines. These nanohybrids induce mitochondrial dysfunction according to the dose amount taken by decreasing the mitochondrial membrane potential (MMP). Saha et al have found that the oxidative stress occurred with increase in the level of intracellular ROS [69]. They have also reported that downregulation of Bcl2 and proteins which are present on the mitochondrial membrane and upregulation of Bax are associated with the oxidative stress. From the literature survey it has been suggested that functionalization of nanoparticles with PBA increases their stability and more absorption in the tissues [70]. It has been found that these nanohybrids reduces toxicity associated with tumour in liver, kidney and spleen respectively. Due to tumour targeting ability of PBA-ZnO-Q nanoparticles, the accumulation of quercetin has been found to be very less in kidney and liver. The biocompatible nature of zinc oxide nanoparticles makes the significant loading of quercetin at the tumour site. The prominent increase of anticancer effect of quercetin based zinc oxide nanoparticles has been observed due to increase in their bioavailability. These naturally occurring anticancer bioactive compounds show distinctive cytotoxicity in cancer cells. These are seemed to be non-toxic to the other body organs [71, 72]. Sadhukhan et al have developed quercetin loaded PBA functionalised zinc oxide nanoparticles (PBA-ZnO-Q) in monitoring the anti-tumor efficacy [72]. Using this type of nanohybrids, the antitumor efficacy of quercetin at the tumor site has increased due to high bioavailability of zinc oxide nanoparticles. The authors have demonstrated that the PBA-ZnO-Q nanohybrids do not show the systemic toxicity in the mice having tumour. They also concluded that zinc oxide nanoparticles can behave like a remarkable system for delivering and loading of anticancer drugs which are hydrophobic in nature.

There is a need of a lot of research in therapeutic technique to improve cancer therapy in order to minimise the tumour associated side effects. Kandasamy et al synthesized Trichoderma strains mediated zinc oxide nanoparticles which have been conjugated with \(\beta\)-D glucan from barley (T-\(\beta\)-D-glu-ZnO) nanoparticles [73]. These synthesised nanoformulation have been found non-toxic against NIH3T3 cells. Also, they show cytotoxic effect in (A549) human lung carcinoma cells with IC\(_{50}\) 56.25 \(\mu\)g/mL. Similarly, the authors have found that dose dependent cytotoxicity of zinc oxide nanoparticles in-vitro conditions. These nanoparticles have been synthesized using pure bioflavonoid rutin [74]. The cytotoxicity has been reported against MCF-7 cell lines. Herein, it is seen that when the concentration of synthesised zinc oxide nanoparticles increases from 10-50 \(\mu\)g/mL, viability of (MCF-7) breast cancer cells increase. Similar observations have been investigated by Suresh et al where the researchers synthesized zinc oxide nanoparticles with the extract of leaves of Costus pictus. In-vitro cytotoxicity study has been done on mice having DLA (Dalton’s ascites cells) cell lines [75]. Herein, the investigation show that cell viability and cell growth inhibition increase as the concentration of zinc oxide nanoparticles increases from 10-50 \(\mu\)g/mL. The maximum cell growth inhibition has been observed at 50 \(\mu\)g. Also, it has been revealed that zinc oxide nanoparticles show the cytotoxic effect towards highly proliferating cells because of increased ROS generation [76]. The increase in lipid peroxidation level in the liposomal membrane was due to production of ROS by zinc oxide na-
noparticles [77]. Many reported studies have demonstrated that the zinc oxide nanoparticles are very selective and highly cytotoxic towards various cell lines but do not show toxic effect towards the normal cells. The selective anticancer activity of zinc oxide nanoparticles has been shown against (HepG2) i.e., human hepatocellular carcinoma, (BEAS-2B) i.e., human bronchial epithelial and (A549) i.e., human lung adenocarcinoma as a result of apoptosis. These nanoparticles do not show the cytotoxicity towards the normal cells such as astrocytes and hepatocytes respectively [78].

It has also been suggested that the apoptosis is considered the vital mechanism of cell death due to cytotoxicity caused by zinc oxide nanoparticles [79]. Similar results have been reported about the apoptosis which occurs in HT29 colon cancer cells on exposing with zinc oxide nanoparticles [80]. The increase in early stage apoptotic cell number with increase in concentration of zinc oxide nanoparticles have been seen. The zinc oxide nanoparticles react with the chemical species present inside the cells and produce larger amount of ROS, that is due to output of oxidative stress. Owing to this stress inside the cell, there occurs damage to proteins, nucleic acid and lipids through protein denaturation and lipid peroxidation, followed by DNA damage and necrosis, finally resulting in cell death by apoptosis [81,82]. Literature survey reveals that surface modifications of zinc oxide quantum dots with silver and polyvinylpyrrolidone (PVP) show excellent anticancer activity in (MCF-7) breast cancer cell lines [83]. Similarly, high cytotoxicity effect has been seen in HT29 colon cancer cells using zinc oxide nanoparticles conjugated with hydrophobic peptides. The authors have reported that the expense of cytotoxicity depends on the type of cells and also on the modification of nanoparticles by using various formulations [84]. The researchers have prepared the zinc oxide nanoparticles with the water mediated plant extract of Deverra tortuosa and studied the cytotoxic activity of these nanoparticles against two cancer cell lines i.e., (Caco-2) human colon adenocarcinoma and (A549) human lung adenocarcinoma and compared their activities using MTT assay on human lung fibroblast cell line(W138). It has been found that both zinc oxide nanoparticles and plant extract displayed a marked particular cytotoxic effect on (A549) and (Caco-2) cancer cell lines and remarkable lower cytotoxic effect on the normal W138 cells.

The ZnO nanoparticles synthesized with the leaves of Hyssopus officinalis have been tested in a mouse model to control tumour growth in liver and spleen cancers [98]. These nanoparticles also have been used in vitro study of and then in HUH7 and HepG2 human liver cancer cell lines respectively. Although in vivo application of ZnO NPs has been rare, this study is remarkable in revealing a synergistic mechanism for cytotoxicity wherein apart from increased ROS concentration, the NPs also activate expression of pro-apoptotic genes Bax and p53.

9. Copper Oxide Nanoparticles

Gnanavel et al have also prepared copper oxide nanoparticles with the leaves of Ormocarpum cochinchinense [85]. These biosynthesized copper oxide nanoparticles have been assessed for their anticancer activity against (HCT-116) human colon cancer cell lines by performing the MTT assay. These results show that biosynthesized copper oxide nanoparticles have strong cytotoxic efficiency against HCT-116 with IC50 value observed at 40 μg/mL. The cell viability also decreases to 22% at 100 μg/mL concentration of copper oxide nanoparticles.

Rehana et al have reported the in vitro investigation to show the cytotoxic effect of biosynthesized copper oxide nanoparticles against the cancer cell lines namely (A549, HeLa, Hep-2, MCF-7) and normal human dermal fibroblast cell line (NHDF) respectively [86]. Herein, the cisplatin has been used as a standard. The Hoechst 33258 staining method has been used to see the morphological changes in the cell lines. Herein, the utilised Copper oxide nanoparticles have been phytofabricated with the leaves extract of five different plants namely Azadirachta indica, Hibiscus rosa-sinensis, Moringa oleifera, Murraya Koenigi, Tamarindus indica. The experimental results show that the cell
viability decreases as the concentration of CuO nanoparticles increases. The cytotoxic effect of copper oxide nanoparticles on normal cell line (NHDF) has been reported less than 20%. The copper oxide nanoparticles show enhanced cytotoxic effect against human breast cancer cell line (MDA-MB-231). Herein, these copper oxide nanoparticles have been prepared with the extract of Pterolobium hexapetalum leaves [87]. The authors have synthesized the copper oxide nanoparticles by utilizing aqueous extract of Acalypha indica [88]. The cytotoxic effect has been studied on MCF-7 cancer cell line at various concentrations of copper oxide nanoparticles (from 6.5 to 100 μg/mL). The IC50 value has been obtained 56.16 μg/mL.

Sankar et al have reported Ficus religiosa arbitrated preparation of copper oxide nanoparticles and their cytotoxicity effect towards A549 cell lines [89]. Herein, highly crystalline copper oxide nanoparticles have been synthesized. These synthesized nanoparticles exhibit cytotoxicity in vitro in the concentration range 50-500 μg/mL. Herein, strong cytotoxic effect has been studied against A549 lung cancer cells by apoptosis induction with more generation of ROS and altering the potential of mitochondrial membrane. The phase contrast microscope has been used for visualising the change in cell morphology. The results prove that the cytotoxic effect of biogenic copper oxide nanoparticles has observed dose dependently where IC50 has been determined 200 μg/mL.

In another research paper, Nagayjiyothi et al have described the induction of apoptosis and suppression of proliferation of human cervical carcinoma cells using copper oxide nanoparticles [90]. The water mediated black bean extract has been taken for the preparation of copper oxide nanoparticles. The sulphordamine-B assay has been performed for studying the cytotoxic effect of as prepared copper oxide nanoparticles. These copper oxide nanoparticles have been found very efficient to reduce colonies of cervical carcinoma as depicted by clonogenic assay. In another study, Kalatara et al have concluded that copper oxide nanoparticles are responsible for the regulation of several classes of enzymes called histone deacetylases (HDACs) [91]. Herein, the induction of apoptosis might be due to inhibition of HDACs which control the transcription process. Herein, the copper oxide nanoparticles have been prepared with the leaf extract of Ficus religiosa. It has been observed that copper oxide nanoparticles inhibited the HDAC level in lung cancer cells (A549). When, lung cancer cells are treated with copper oxide nanoparticle then the expression of inflammatory responsible genes (TNF-, COX-2) are downregulated. The results also show that the significant activation of caspase i.e., (mRNA expression of caspase 3, caspase 8 and caspase 9) pathway and downregulation of oncogenes (MMP-2, MMP-9) occurred in the lung cancer cells (A549).

10. Conclusion and future

The unique physicochemical properties of various biogenic MNPs and MONPs makes the enhanced application in cancer research and therapy. Nanoparticles can be regarded as modern structures medicines for the cancer treatment. The application of nanosized metal and metal oxide nanoparticles leads to the better efficacy and minimal toxicity of novel anticancer drugs. Herein, this review specifically focussed on the cytotoxic effect of several metals and metal oxides on different cancer cell lines in vitro and in vivo respectively. Presently the use of biosynthesized nanoparticles in medical field is a new environmentally benign emerging field of research. Various cell lines of cancer have been taken for in vitro study for showing the cytotoxicity of biogenic nanoparticles. For in-vivo study and clinical trials, the nanoparticles should have higher selectivity and efficacy towards cancer cells and less toxicity on normal cells. So, there is need of thorough understanding of cancer biology and metal and metal oxide nanoparticles for clinical purpose. Cancer is an epidemic disease, affecting people worldwide at a large scale. Inorganic nanoparticle has been classified into superparamagnetic (SPIO), nanoshells, nanocages silica nanoparticles, gold nanoparticles and quantum rods, quantum dots and some antibody-conjugated nanoparticles (ACNPs) have been developed as diagnostic biomarkers which can be used as screening tool for anticancer and other biomedical applications [99]. Anselmo et
al have performed the clinical trials on few drugs based on nanoparticles [100]. Various modification in synthesis of nanoparticles and their formulations with cancer drug can be best way to cure various cancers in the future. Hence, in future detailed investigations and efforts are needed for controlled morphology, biocompatibility and pharmacokinetic studies for making MNPs and MONPs as a major tool for treatment of cancer.

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Disclosure

The authors hereby declare no conflict of interest.

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