Characterization, anticancer properties of Chaetomorpha antennina capped biogenic Zinc oxide, Copper oxide and Nickel oxide nanoparticles

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***Abstract*:** The Zinc oxide (ZnO), Copper oxide (CuO) and Nickel Oxide (NiO) nanoparticles was (NPs) prepared by biosynthesis method using C*haetomorpha antennina* (*C. antennina*) seaweed powder extract. In the XRD patterns, revealed that synthesized ZnO, CuO and NiO NPs exhibits wurtzite hexagonal, monoclinic and cubic structure, respectively. Themorphology and chemical composition were identified by FESEM and EDAX spectra. Anticancer studied were done ZnO, CuO and NiO NPs tested against A549 human lung cancer cell line.

Keywords: Biosynthesis; Seaweed; nanoparticles; C*haetomorpha antennina*; human lung cancer.

I. Introduction

Metal oxide nanoparticles (NPs) have potential applications in the industrial products. The toxic effect of metal oxide NPs is resulted through membrane injury, inflammatory response, DNA damage and apoptosis formation. The toxicity of metal oxide nanoparticles has been ascribed to the release of ions and another way of toxicity of metal oxide nanoparticles is due to their particulate nature which may give rise to Reactive Oxygen Species (ROS). The ROS generation is linked to DNA damage and cellular apoptosis. This toxicity can be reduced by alternative synthesis method, which leads to a stabilization of the material's crystal structure and to slow down the ion release in the material matrix. Early identification of potentially hazardous nanomaterial properties can also able to redesign these materials to improve its quality and safety while still maintaining key nanoscale properties. Such an approach has been implemented by Green method. Chemically synthesized nanoparticles are highly toxic and lead to non-ecofriendly byproducts [1]. Biological approaches using microorganisms, sea weed and plants or plant extracts for metal oxide nanoparticles has been suggested as valuable alternatives to chemical methods. Various biological source are used for green synthesis like bacteria, fungi and yeast [2]. The green synthesis method employing marine seaweed extract has received

more attention as being simple, eco-friendly and less time consuming compared to usual chemical and physical methods.

A novel approaches of ZnO, CuO, and NiO biosynthesized nanoparticles as a natural source, nontoxic barrier of human disease mechanism. One of the major causes is cancer, a world second leading disease with high mortality rate. The treatment of cancer causes many side effects, based on that increasing demand on anticancer therapy. So, conventional methods required for combination research technology and targeted drug delivery. The growing need to develop eco-friendly nanomaterials using without any toxic and uniformly nanosized drug particles for new formulation in pharmaceutical products of various human diseases. Nanotechnology is relatively new and promising scope in the medicine for disease diagnosis, drug delivery at specific target site, molecular imaging etc. Synthesize of nanomaterial is a challenge for the young researcher to modify or rectify the major complications. There is an increased knowledge on marine products, a new finding for drug discovery and development [3-8]. In early literature, the *C.antennnina* extract was used for biomedical applications such as antibacterial, antioxidant, radical scavenging property, antimalarial,antifungal, anti-HIV, anti-inflammatory, anti-hypertensive, antidote, and anti-tumor properties [9].

In the present work, biogenic ZnO, CuO and NiO NPs are prepared by biosynthesized method using *C. antennina* seaweed extract. The biosynthesized nanoparticles and further exploring anticancer activity of *in vitro* cell line. Results of Antitumor potential ZnO, CuO and NiO NPs were established with a cell viability test and morphological characterization.

II. Materials and Method

2.1 Biosynthesis of ZnO, CuO and NiO NPs

The fresh seaweed can be collected from mandapam Rameshwaram. Then, 10 g of *C. antennina* fine seaweed powder were added to 100 mL of double distilled water and boiled at 50-60 °C for 10 min. The obtained extraction was filtered using Whatmann No. 1 filter paper.

The following high purity chemicals such as, zinc nitrate, copper nitrate, and nickel nitrate were used as precursors without further purification.

However, 0.1M of Zn $(NO_3)_2.6H_2O$, Cu $(NO_3)_2.6H_2O$ and Ni $(NO_3)_2.6H_2O$ salts were dissolved in 80 ml double distilled water. 20 ml of *C. antennina* seaweed extract added to 80 mL of aqueous Zinc, copper and nickel nitrate salts solution. This solution was stirred constantly at 80 °C temperature for 6 h.The white and block precipitates were formed on continuous stirring. Finally, the precipitate was dried at 120 ˚C. Thus, ZnO, CuO and NiO samples were obtained. Further the precipitates were annealed at 700 °C for 5h. Thus, ZnO, CuO and NiO NPs were obtained.

2.2 Cell culture

A549 (lung carcinoma cell) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC.

2.3 MTT Assay

The ZnO, CuO, NiO samples were tested for *in vitro* cytotoxicity, using A549cellsby 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured A549cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 h at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the ZnO, CuO, NiO samples in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1_X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percent

ZnO, CuO, NiO samples cell viability and IC_{50} value was calculated using GraphPad Prism 6.0 software (USA).The optical density (OD) value was used to calculate the percentage of viability using the following formula.

$$= \frac{mean\ OD\ of\ untreated\ cells\ (control)\ -mean\ OD\ of\ treated\ cells}{mean\ OD\ of untreated\ cells\ (control)} \text{ x } 100$$

All the *in vitro* experiments were done in triplicate, and the experiments were repeated at least thrice. The statistical software SPSS version 17.0 was used for the analysis. *P* value <0.01 was considered significant.

2.4 Characterization Techniques

The XRD patterns of the ZnO, CuO and NiO samples were collected using a X'PERT PRO PAN alytical X-ray diffractometer with CuKα (40 kV, 30 mA) radiation source. The ZnO NPs samples were gently crushed before being smeared on a clean glass slide. The powder diffraction patterns were collected over 2θ in the range between 10˚-80˚ with a scan speed and sampling width of 2 min^{-1} and 0.05˚ respectively. FESEM was performed on the ZnO, CuO and NiO using a (Carl Zeiss Ultra 55 FESEM) with EDX (model: Inca) microscope operating at 30 kV. The microscope was equipped with a charge-coupled device (CCD) camera. The samples were prepared by using 1mg of ZnO, CuO and NiO samples coated with 1.2 nm gold particle separations on a carbon tape using the low vacuum.

III. Result and Discussion

3.1 X-ray diffraction studies

X-ray diffraction pattern of phytochemical capped biogenic ZnO, CuO and NiO NPs synthesized through *C. antennina* seaweed extract as shown in Fig. 1. The synthesized ZnO, CuO and NiO NPs exhibited wurtzite hexagonal, monoclinic and cubic structure, which are confirmed by (JCPDS card no: 36-1451 (ZnO), 45-0937 (CuO), 44-1159 (NiO)). Average crystallite size NPs can be calculated using Debey-scherrar's formula:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where the constant K is the shape factor = 0.94, λ is the wavelength of X-rays (1.5418 for Cu Kα), θ is Bragg's angle, β is the full width at the half-maximum (FWHM). The average crystallite size 33, 42 and 37 nm for ZnO, CuO and NiO NPs.

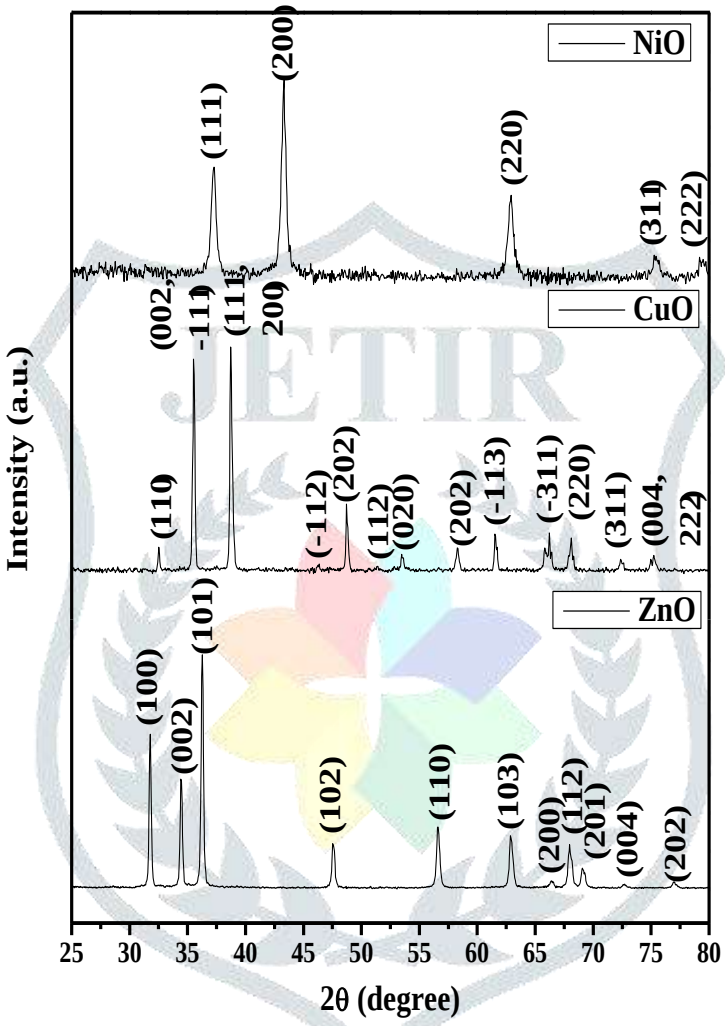

Figure 1 X-ray diffraction patterns of ZnO, CuO and NiO NPs synthesized using *C. antennina* seaweed extract.

3.2 Morphological and chemical composition analysis

Figure 2(a-f) low and high magnification image shows the biosynthesized ZnO, CuO and NiO NPs using *C. antennina* seaweed extract. The synthesized ZnO, CuO and NiO NPs are formed spherical strucure, stone like structure, and spherical structure with porous formation. The chemical composition of ZnO, CuO

and NiO NPs are identified by EDAX spectra as shown in Fig. 3(a-c),this result confirmed that Zn, Cu, Ni and O elements are present in the synthesized nanoparticles samples and metal Zn, Cu, Ni and O elemental peaks are observed at normal energy level.

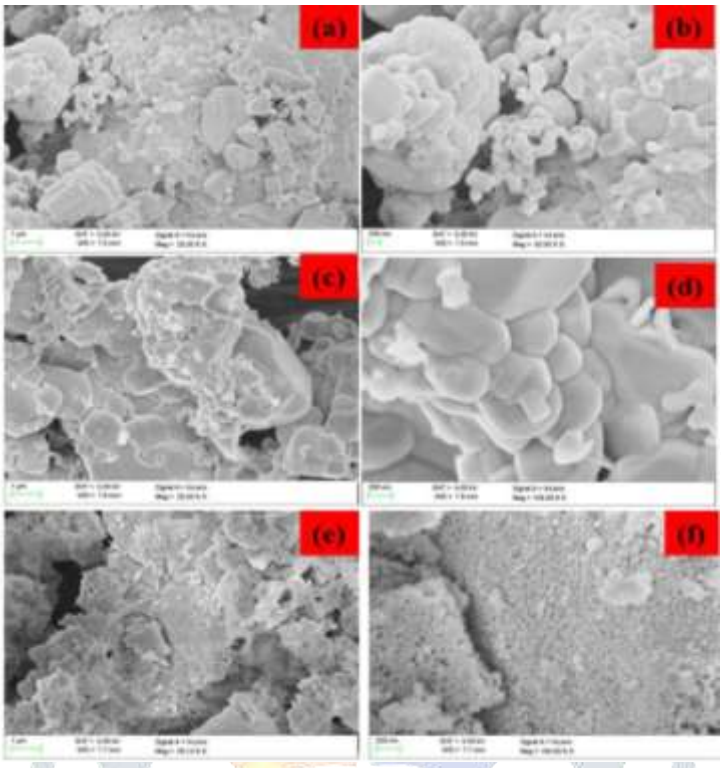

Figure 2(a-f) FESEM image of low and high magnification image of ZnO, CuO and NiO NPs synthesized using *C. antennina* sea weed extract.

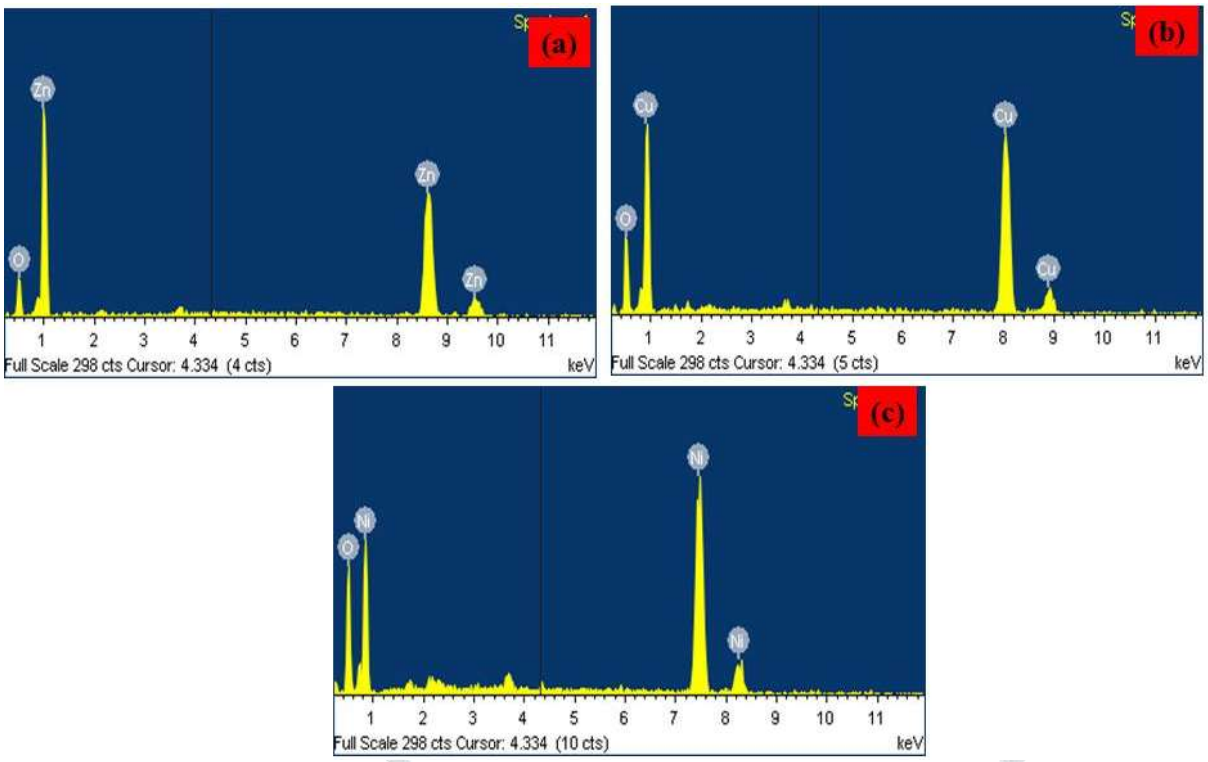

Figure 3(a-c) EDAX spectra of ZnO, CuO and NiO NPs synthesized using *C. antennina* sea weed extract.

3.3 Cytotoxicity analysis

Marine seaweeds are rich in biodynamic compounds for the development of new anti cancer drugs. Secondary metabolite is playing a vital role in biosynthesis based on that design the well-known their cytotoxic properties for nanoparticle during synthesis.The present study was conducted to screen the seaweed *C. antennina* for the synthesis of ZnO, CuO, and NiO NPs and its cytotoxic potential against cancer cell lines (lung carcinoma cell).

The nanoparticles can lead to the spontaneous ROS generation at their surface owing to their chemical and surface characteristics. They can also lead to the generation of free radical (superoxide anion ($^{\cdot}O_2^-$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$)). ROS are generated intrinsically or extrinsically within the cell, after their interaction with cellular components, e.g., mitochondrial damage. The molecular oxygen generates $^{\cdot}O_2$, which is the primary ROS via one electron reduction catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Further reduction of oxygen may either lead

to H_2O_2 or $OH^{\cdot}$via dismutation and metal-catalyzed Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$), respectively [10,11]. A variety of NPs including metal oxide particles induce ROS as one of the principal mechanisms of cytotoxicity [12]. NPs have been reported to influence intracellular calcium concentration, activate transcription factors, and modulate cytokine production via generation of free radicals [13, 14].

The MTT assay was used to evaluate the inhibitory effects of the ZnO, CuO, and NiO NPs tested against A549 lung cancer cells. A549 cells were incubated with different (10-100 µg/ml) concentrations of ZnO, CuO, and NiO NPs. The results were demonstrated that the CuO sample increase cytotoxicity than ZnO and NiO NPs forA549 lung cancer cells in a dose-dependent manner (Fig. 4). In MTT assay, the statistical analysis revealed that CuO induced apoptosis in treating A549 cells compared to ZnO and NiO NPs indicating apoptotic cell death induced toxicity. The IC_{50} value of 54.91 µg/ml (evaluated after 24h) of CuO against A549 cells was ($p \leq 0.05$ P value <0.01). Activity was decreased on other two type of nanoparticles (ZnO and NiO). Cell morphological changes were observed for light microscope with different concentration 10, 50 and 100 µg/ml (Fig 5-7). The results revealed that the occurrence of morphologically altered cells observed nanoparticles treated group as compared to control group.

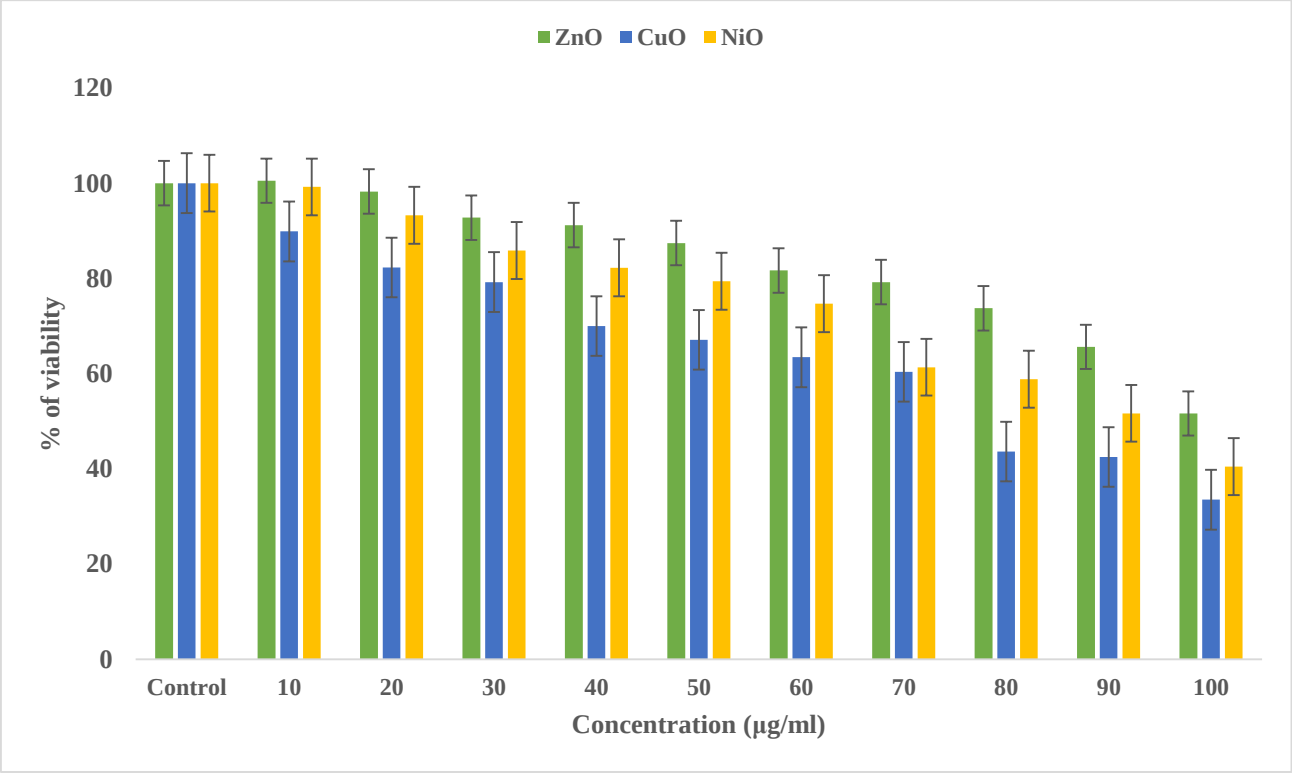

Figure 4 The effect of ZnO, CuO and NiO NPs on the cytotoxicity property in human lung cancer cell line (MCF-7).

The mechanism of cellular toxicity, as an elevated ROS production that exceeds the capacity of the cellular antioxidant defense system to manage causes cells to enter the state of oxidative stress which results in damage of cellular components such as lipids, proteins and DNA [15, 16]. The oxidation of fatty acids then leads to the generation of lipid peroxides that initiates a chain reaction leading to disruption of plasma and organelle membranes and subsequent cell death by induction of apoptosis. The ROS act as the critical signalling mechanism in the induction of apoptosis/cell death by many different stimuli [17, 18]. The possible mechanisms underlying the cytotoxic activities of CuO NPs as shown in **Fig. 8**.

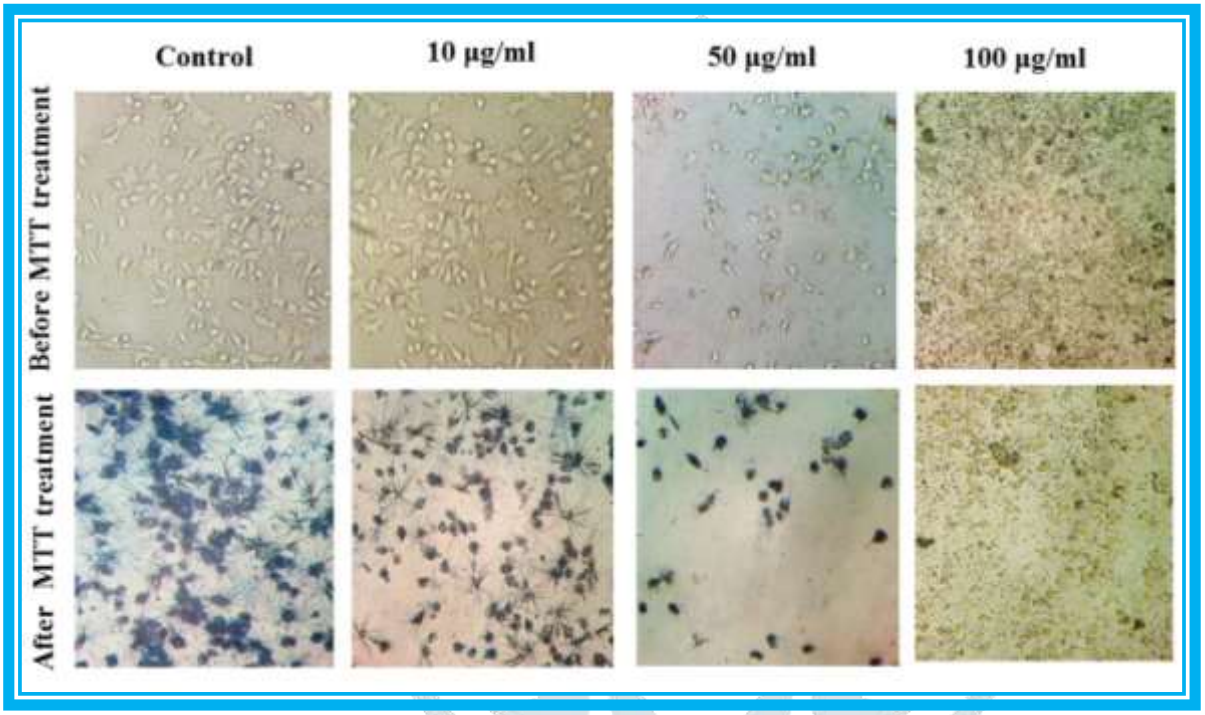

Figure 5 Lung cancer cells treated with ZnO NPs at the respective various concentration 10, 50 and 100 µg/ml.

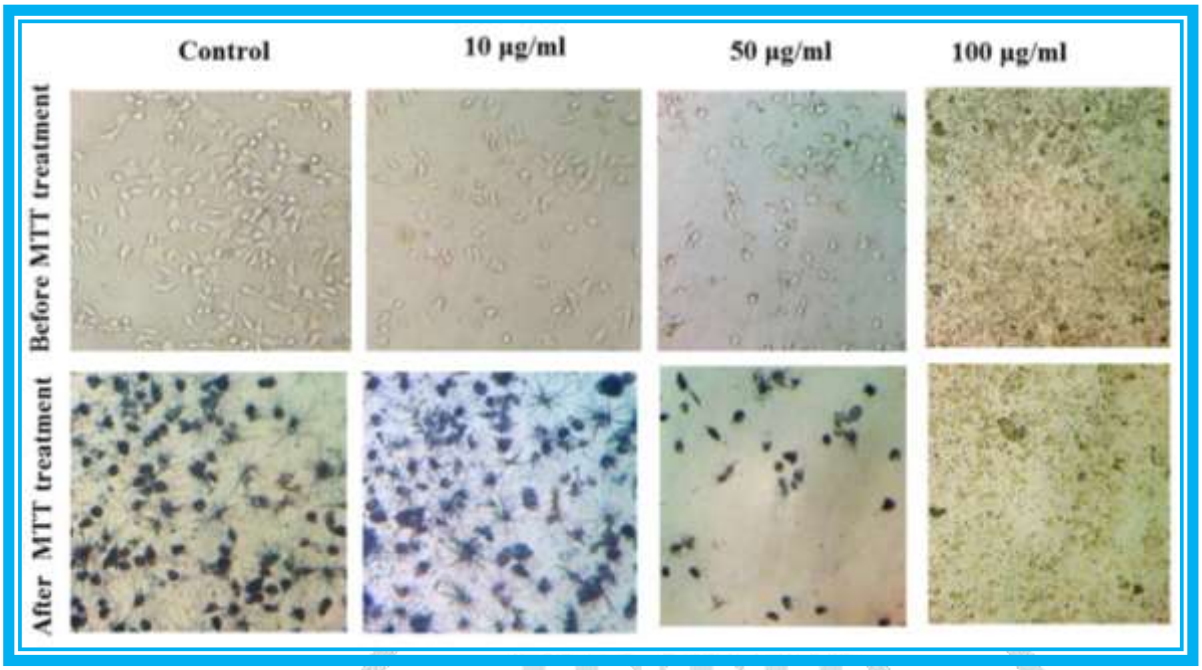

Figure 6 Lung cancer cells treated with CuO NPs at the respective various concentration 10, 50 and 100 µg/ml.

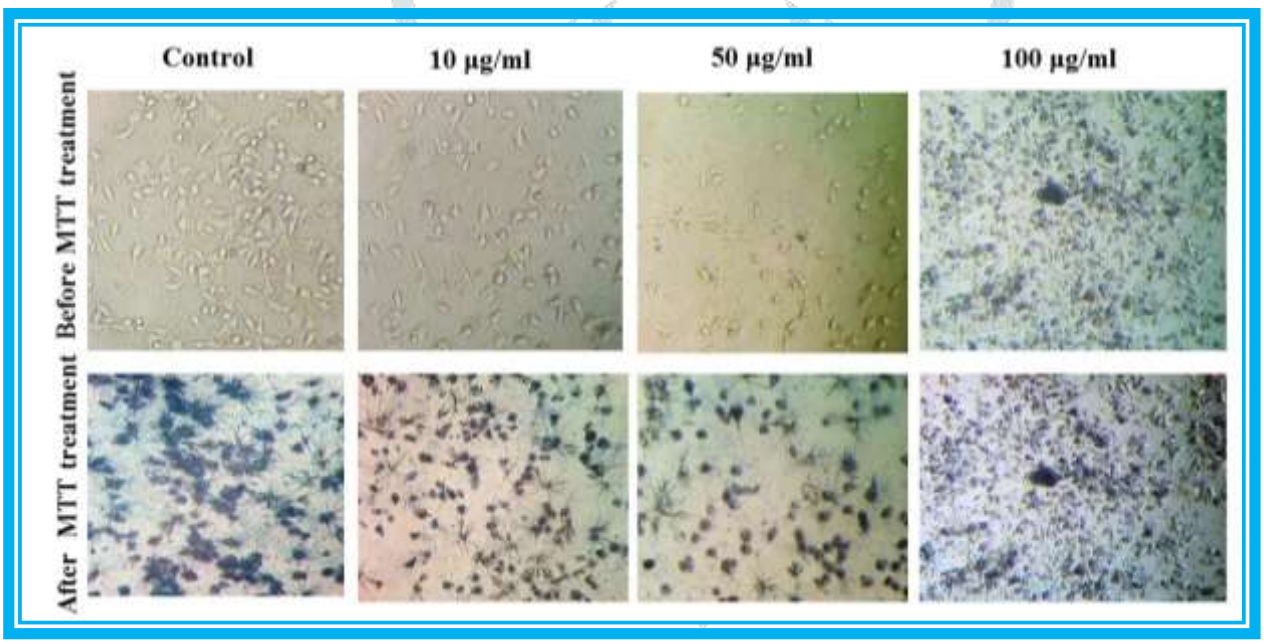

Figure 7 Lung cancer cells treated with NiO NPs at the respective various concentration 10, 50 and 100 µg/ml.

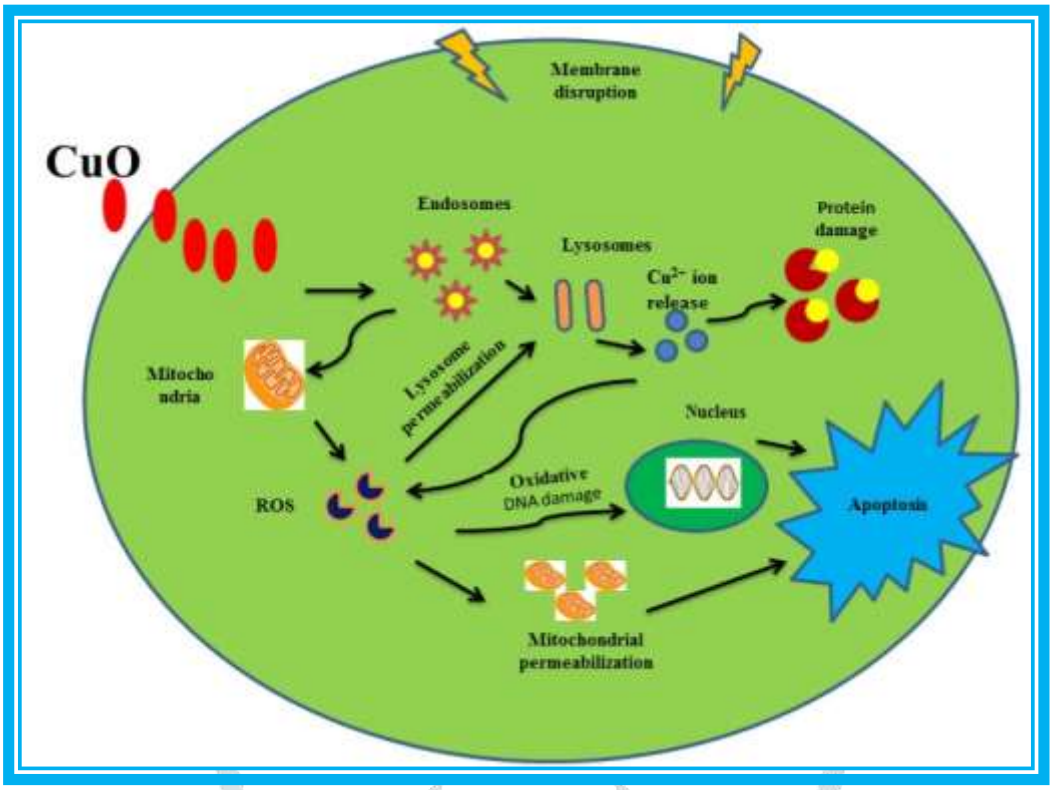

Figure 8 Possible mechanisms underlying the cytotoxic activities leading to cell death as caused by CuO NPs

IV. Conclusions

In the summary, the biogenic ZnO, CuO and NiO NPs were prepared by biosynthesis method using *C. Antennina* seaweed extract**.** The biosynthesized ZnO, CuO and NiO NPs exhibited wurtzite hexagonal, monoclinic and cubic structure and average crystallite size 33, 42 and 37 nm for ZnO, CuO and NiO NPs. FESEM image clearly showed that synthesized ZnO, CuO and NiO NPs were formed spherical strucure, stone like structure, and spherical structure with porous formation. The chemical composition were identified by EDAX spectra. The biosynthesized ZnO, CuO, and NiO NPs and its cytotoxic tested against cancer cell lines (lung carcinoma cell) and CuO NPs sample increased cytotoxicity than ZnO and NiO NPs for A549 lung cancer cells.

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