RESEARCH ARTICLE

CuO-NiO Nano composites: Synthesis, Characterization, and Cytotoxicity evaluation

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ABSTRACT

Objective(s): In this work, CuO- NiO nano-composites were synthesized via free-surfactant co-precipitation method and then their physiochemical properties, as well as cytotoxicity and antifungal effects, were studied.

Methods: The structural and optical properties of CuO-NiO nanostructures were analyzed by X-ray diffraction (XRD), scanning electron microscopy (SEM), Atomic force microscope (AFM), UV–Vis absorption, and vibrating sample magnetometer (VSM) techniques. MTT assay was used to evaluate the cytotoxicity of nanostructures.

Results: TThe cubical structure of CuO- NiO nano-composites was confirmed by the XRD technique. The optical study of the samples by UV-Vis indicted a blue shift in absorption wavelength with decreasing particle size due to quantum size effect. The super magnetic behavior of CuO-NiO nano composites after calcination was confirmed by magnetic characterization instrument. Finally, the results of cytotoxicity evaluation of CuO-NiO nano-composites at the lower concentrations on Breast cancer MDA cell lines demonstrate no significant toxicity. Minimum inhibitory concentration range and Minimum fungicidal concentration of nanoparticle were determined 0.97-15.62, 7.81µg/ml and for fluconazole were 1.75-25 µg/ml and 12.58 µg/ml, respectively.

Conclusions: The study result of antimicrobial of CuO-NiO nano composites indicated an MIC90 antifungal activity with a concentration of 3.90µg/ml against vaginal isolates of C. albicans. The results of cytotoxicity study of nano-composites at concentration of 50µg/ml and 10µg/ml on the cell line of Breast cancer MDA was equivalent to %60 and %80, respectively.

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INTRODUCTION

The crystalline nanometer-sized particles have attracted a wide attention due to not only their major applications in the different scientific fields but also their biological applications to improve the environment and human health [1]. Nickel Oxide (NiO) and Copper oxide (CuO) with a optical band gap of 3.6-4eV (Eg=3.6-4 eV) and 1.2eV (Eg= 1.2eV), respectively, act as a P-type semiconductor with application in wide range of technologies such as sensors, optical devices, electrochemical super capacitor, as a transparent P-type semiconducting

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layer and as an antiferromagnetic film and so on due to is the optical and magnetical unique properties [1-5].

The conducted researches state that the technological application of semiconductor materials can be enhanced via mixing and decreasing their dimensions in nanometer-sized scale [1-5]. Moreover, it must be pointed out that applied methods to synthesis nano-particles have a marked effect on the particle size. To this end, many methods have been developed to prepare nickel oxide nonmaterial, including low-pressure spray pyrolysis, surfactant-mediated method, simple liquid phase process, electro spinning, etc [10–17].

On the other hand, reported studies in the field of study of CuO-NiO nano composites have only been focused on their optical and magnetically properties [8-9], but not their biological study including the cytotoxicity and antifungal effects, to our knowledge.

It is important to mention that metal oxides nanostructures such as CuO–NiO nanocomposites. Due to their unique magnetical, optical, and molecular properties as a consequence of mixing the metal oxides of Nickel Oxide (NiO) and Copper oxide (CuO) [1-5] can be used in different biomedical fields such as nano-biosensor for clinical and non-clinical diagnostic applications [6-7], drug delivery, immunoassays, etc.

In a study conducted by Mahmoodi et al, CuO–NiO nanocomposites were suggested as an inorganic adsorbent to remove dye from water [36]. In addition, Devadathan and Raveendran studied the antifungal properties of metal oxide nanocomposites of nickel-zinc, and they reported an antifungal property of the nanocomposites for the fungi Penicillium Chrysogenum [37].

In another study, fabricated-bioactive glasses based on nanocomposites containing different CuO contents were introduced to inhibit infections resulting from implants [38]. With this in mind, in the current study, the CuO- NiO nanoscale-sized composites were synthesized via co-precipitation method in the absence of capping agent to avoid their toxic effect on biological systems, and changing copper percent in the solution, and then samples were characterized by the scanning electron microscopy (SEM), and X-ray diffraction (XRD) vibrating sample magnetometer (VSM),Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), and atomic force microscopy (AFM) techniques, and then the cytotoxicity and antifungal effects of CuO- NiO nano composites on MDA cell line of Breast cancer and against vaginal isolates of Candida albicans were also studied as part of the study. In addition, it is of interest to note that C. albicans is an important human fungal pathogen. Although this opportunistic pathogen is a natural component of the human flora, it can cause life-threatening infections in immune-suppressed patients, Due to the multiple pathogenic factors. On the other hand Due to the increasing drug resistance of fungal infections caused by Candida albicans discovery of new antifungal drugs are necessary and essential.

MATERIALS AND METHODS

Reagents

$NiCl_2.6H_2O$, $CuCl_2.2H_2O$ and NaOH (99.9 %, Merck Co.) were analytical grade , Breast cancer MDA cells (Pasteur Institute (Iran), Fluconazole (Amin pharmaceutical company, Iran), CHROMagar Candida medium (Paris, France), and Corn meal agar (Difco, USA) used as received without further purification and deionized water as solvent were used.

Synthesis of CuO-NiO Nano composites

To prepare CuO-NiO Nano composites via co-precipitation method without the surfactant, initially, a weight amount of 5.9412g of $NiCl_2.6H_2O$ and 3.3409g of $CuCl_2.6H_2O$ were separately dissolved in 50 mL of deionized water under stirring (A sample containing high Cu content), a weight amount of 5.9412g of $NiCl_2.6H_2O$ and 4.2331g of $CuCl_2.6H_2O$ were separately dissolved in 50 mL of deionized water under stirring (B sample containing low Cu content) at room temperature [4,8-9]. Then, intermixed solution containing a precursor of Nickel and Copper was magnetically stirred for 50min at 50oC temperature. Afterward, the 10cc NaOH (1M) was added drop by drop to the solution with constant Stirring until the pH becomes 8.

In the final step, the obtained green gel washed with deionized water to remove formed byproducts during the reaction process and dried at 60°C temperature for 14 hr. Then, dried samples were annealed at 500°C temperature for 2h to obtain CuO-NiO Nano composites. The color of samples

changed from green to black due to calcination process.

Characterization

X-ray diffraction pattern of nanoscale-sized structures was carried out by a diffract meter (Rigaku-Miniflex model equipped with a source of X-ray was Cukα and a wavelength of 1.5406 Å).
Magnetization measurement of samples was performed by a vibrating sample magnetometer (VSM) tool (MDKFT, Kashan, Iran). The scanning electron microscopy (Philips XL10 model voltage 17kV) was used to study the morphology of particles. The elemental analysis of nano-composites was done by The EDAX tool (Philips XL10 model and the working voltage was 17 kV). Zetasizer Nano (Malvern Instruments, Germany) equipped with a He–Ne laser source (633nm) with vertically polarized light was used to calculate the hydrodynamic diameter of nanoparticles. The UV/Vis absorption spectrum of nanostructures was carried out by using a UV-1650 PC spectrometer (Shimadzu) in the wavelength of 200 to 700 nm.

Sample preparation

In this experimental study, we used of 41 clinical vaginal isolates of Candida albicans that were isolated from patients with vulvovaginal candidacies. All isolates were identified by germ tube production test, culture on CHROMagar Candida medium (Paris, France), and Cornmeal agar with 1% of tween 80 (Difco, USA).
The determination of the minimum inhibitory concentration (MIC(was performed in 96-well plates using serial dilutions of a stock of sonicating (The Model 3000MP Ultrasonic Homogenizer) nano-composites according to the Clinical and Laboratory Standards Institute (NCCLS) M37-A3 document [30]. Fluconazole (Amin pharmaceutical company, Iran) was obtained for the preparation of the broth micro-dilution based on CLSI recommendations.

Cell viability assay

MDA cells were exposed to CuO-NiO nano-composites (10–50 μg/ml) for 24 h to evaluate their cytotoxicity by MTT assays. MDA cells of Breast cancer were cultured in the usual fashion as monolayers in RPMI1640 media enriched with 10% fetal bovine serum. The MDA cells (1×104 cells/well) were moved onto 96-well plates and at 24h post-seeding, different concentrations (i.e. ranging from 10 to 50 μg/ml) of nanometer-sized particles were added to each well. The treated cells were then incubated for 24h. After the defined time, cells were treated with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium)bromide (MTT) [27-28]. The plates were then incubated for 3.5h at 37°C in a CO_2 incubator. Eventually, after removing the medium absorbance of the formed formazan crystals was read by adding 200 μl DMSO. Then the absorbance was evaluated at a wavelength of 570 nm using a spectrophotometer (Elisa reader, Biochrom Anthos 2020 microplate reader) and cell viability was studied according to the standard protocol [29].

In vitro antifungal assay

The determination of the minimum inhibitory concentration (MIC) was performed in 96-well plates (The Model 3000MP Ultrasonic Homogenizer) nano-composites according to the Clinical and Laboratory Standards Institute (NCCLS) M37-A3 document [30]. In this study, we used of 41 clinical vaginal isolates of Candida albicans that were isolated from patients with vulvovaginal candidacies. All isolates were identified by germ tube production test, culture on CHROMagar Candida medium (Paris, France), and Corn meal agar with 1% of tween 80 (Difco, USA). Fluconazole (Amin pharmaceutical company, Iran) was obtained for preparation of the broth micro-dilution based on CLSI recommendations.

RESULTS AND DISCUSSION

As stated in the Introduction, this research is aimed to understand physiochemical properties as well as cytotoxicity and antifungal effects of CuO-NiO nano-composites. In order to do this, the synthesized nano composites were characterized by different techniques including the scanning electron microscopy (SEM), X-ray diffraction (XRD) vibrating sample magnetometer (VSM), Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS), and atomic force microscopy (AFM), and in final step, the cytotoxicity and antifungal effects of CuO- NiO nano composites on MDA cell line of Breast cancer and against vaginal isolates of Candida albicans were also studied as part of the study. Considering the above objectives, Fig. 1, shows

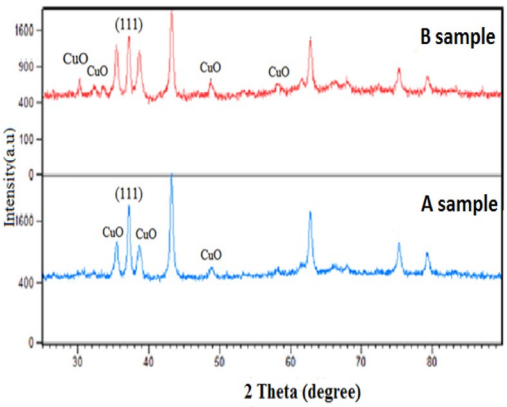

Fig. 1. XRD patterns of CuO-NiO Nanocomposites after calcinations

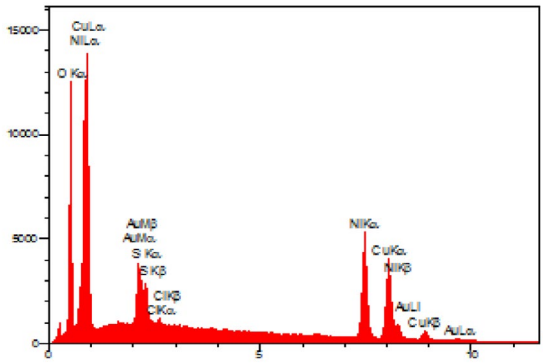

Fig. 2. EDX spectra of B sample (nano-composite containing high Cu content) after calcinations.

X-ray diffraction (XRD) pattern of the CuO-NiO Nano composites prepared via Co-Precipitation route at room temperature.

As seen in Fig.1 , the Bragg peaks were obtained at 2Theta values of 37.1775, 43.1897, 62.7810, 75.2675, 79.2376 degrees correspond to planes of (111), (200), (220), (311) and (222) related to cubic structure of NiO and other 2Theta values of 35.5651, 38.6481, 48.8422 related to monoclinic structure of CuO for A sample (nano-composite containing low Cu content), that these results were matched with the observed values in JCPDS files (No.01-078-0648 and No. 00-049-1830, respectively).

As seen in Fig.1, the number of planes related to CuO increases with increase in Cu content in the nano-composite (B sample containing high Cu content). In addition, it observed that the peak intensity related to Ni decreases and the peak intensity of Cu increase as Cu content increases in the system.

It is clear from Fig. 1 that no other characteristic peak due to any impurities and sharp peaks are present in the pattern, indicating that the prepared samples are of high purity and crystallinity.

The average crystallite size of 39, 34 nm were obtained for Nano composites A and B, respectively, using the Scherer's equation [18-20] by (111) plane reflection from the XRD pattern. The EDX spectrum of nano-composite (A) is shown in Fig.2.

Results in Fig. 2 confirm the presence of Ni, O and Cu as the only elementary species in the sample without any other impurity species. The magnetic properties of the synthesized nano-composites were studied with the VSM tool at room temperature. Fig. 3 shows the magnetization curve for prepared nano-structures. The values of the magnetic field [H] and magnetization [M] of the sample were listed in Table 1.

Super paramagnetic properties of the nano composites at magnetic field of -8000 to 8000Oe were confirmed by hysteresis loop of samples shown in the Fig. 3, which means Coercively force (Hc) and Magnetic Remanence (Mr) of nanometer-sized composites are zero or very small. On the other hand, the results in the Fig.3 highlight that the magnetic properties of nano composites are correspond with that of in magnetic materials with sizes greater than 10 nm [22-25]. The hysteresis loop of nanometer-sized composites was attributed to domain structure due to pinning effect of magnetic domain walls at grain boundaries, the magnetic anisotropy of the crystalline lattice as well as impurities within the nano-meter sized structures [22-25, 32]. As can be seen in Fig.3, the saturation magnetization of the magnetic samples

Table 1. The data related to the Optical band gap, Magnetization (M), magnetic field (H), and the average crystallite size of CuO-NiO nano-composites.

sample	Band-gap (ev) after calcination	M (emu/g) before calcination	M (emu/g) after calcination	The average crystallite size (D) of Nano composites	H(Oe) after calcination
A	3.85	0.19536	0.10645	39	150
B	3.99	0.18693	0.07711	34	0

Table 2. In vitro susceptibility testing of 21 clinical vaginal isolates of C. albicans (µg/ml).

Antifungal	MFC	MIC50	MIC90	MIC range
Nanoparticles	7.81	1.95	3.90	0.97-15.62
Fluconazole	12.5	3.12	6.25	1.75-25

is depends on the Cu content in nano composite, as well as less than that of the Nio bulk materials (55 emu/g) [22-26].

It is clear from data in Table 2 that the magnetization (M) and coercively force (Hc) of CuO-NiO nano materials decreased with the reduction in the size of nano-composite. This magnetic behavior of nano materials can be assigned the competition between the F-centre exchange coupling (FCE) coupling and super-exchange coupling in the nano composite with a change in their size due to variation in the structure of the domain, size and surface effect in nanoscale-sized structures [24, 32-33]. The SEM image of synthesized nanometer-sized particles is shown in Fig.4. It can be seen in Fig.4 that the synthesized nano-composites are to form of shape-like aggregated nano-clusters due to van der Waals force between particles [22-25]. The observed average size of sample (A) from SEM images was about 30nm. Hydrodynamic size of B sample in cell culture medium is shown in Fig.5.

The UV–Vis spectra of CuO-NiO nano composites after calcinations are shown in Fig.7 that was measured using a USB-2000 UV–Vis spectrophotometer.

Fig.7 shows that the absorption edge of nano-composites there is in the range of 200-350 nm. The blue shift of absorption edge for nanometer-sized samples was attributed to decrease in size of particles due to increase in Cu content in the composite based on the quantum size effect of nano structures.

The obtained direct optical band gap values for samples are shown in Fig.8. It is necessary to mention that the optical direct band gap values of the samples were determined by Tauc's relation [21-22]:

$$\alpha h\upsilon = \alpha_0 (h\upsilon - E_g)^{1/2} \quad (1)$$

Where hν, αo and Eg are photon energy, a constant and optical band gap of the nanoparticles, respectively. Absorption coefficient (α) of the samples can be calculated from the absorption spectra at different wavelengths. The values of Eg were determined by extrapolations of the linear regions of the plot of versus .

As seen in Fig. 8, value of optical band gap increases with increase Cu content in the system.

Cytotoxic evaluation

The result of the cytotoxic study of nano-composites on MDA cells of Breast cancer is shown in Fig.9. From Fig.9 it can be seen that nano-composites demonstrate no significant toxicity at the lower concentrations. However, mean cell viability at a concentration of 50µg/ml and 10µg/ml was equivalent to %60 and %80, respectively.

In the current research, three wells of 96-well culture plates were the mixture of nanoparticles and MTT reagent without cells, which were used as blanks and for evaluating of different dilution of

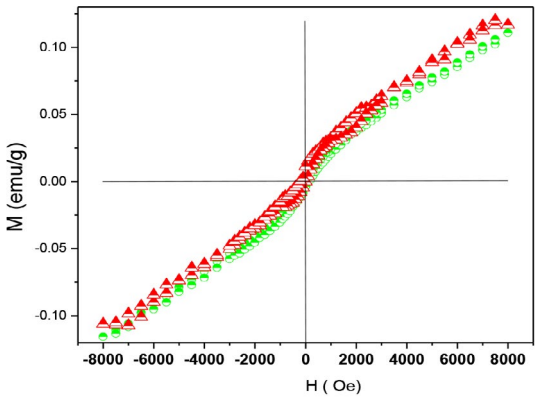

Fig. 3. M-H curves of CuO- NiO Nano composite (up triangle): A sample and (circle): B sample after calcination.

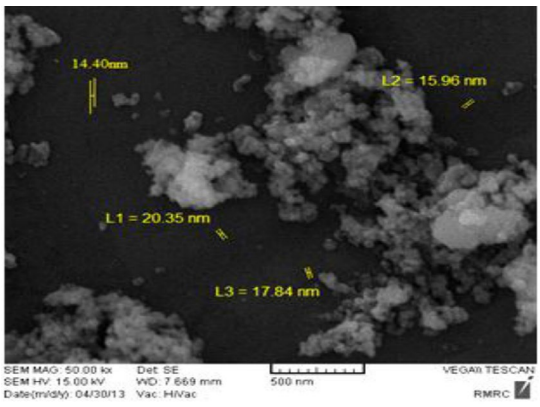

Fig. 4. SEM image of a sample (nano composite containing low Cu content)after calcinations.

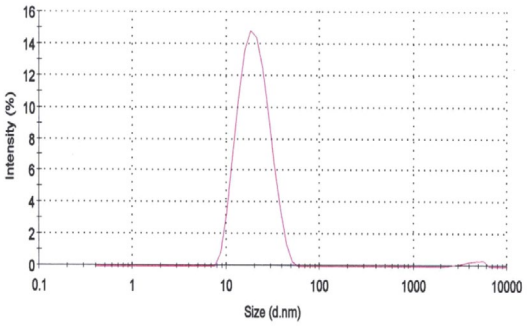

Fig. 5. Hydrodynamic size of B sample (nano-composite containing high Cu content) in cell culture medium

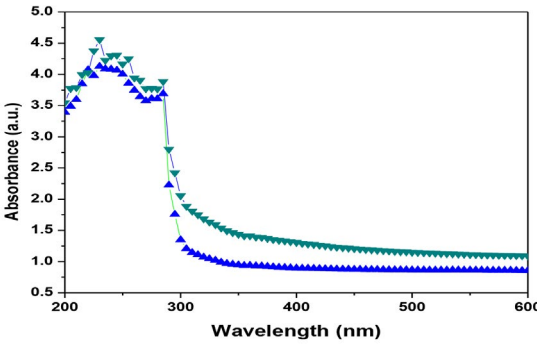

Fig. 7. UV– Vis spectra of CuO-NiO nano-composites after calcinations.

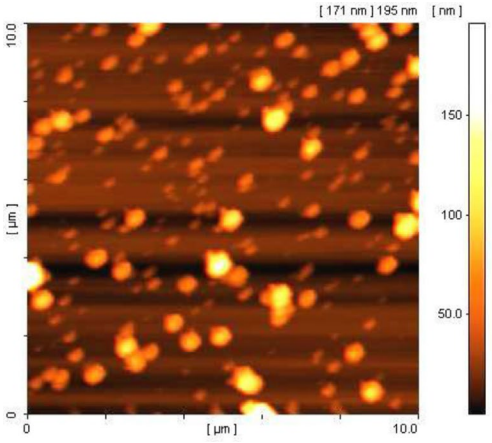

Fig.6. AFM image of sample with B sample (nano-composite containing high Cu content) before calcinations.

nanoparticle was added cell suspension from the petri dish into each well of the required 96-well culture plates. In comparison, Moses et al (2015) based on their study about Cytotoxicity effects of nanoparticles (AuNPs) on MCF-7 and MDA-MB-231 Breast Cancer Cells reported that MDA-MB-231 cells greater sensitivity to the mixture than the MCF-7 cells, particularly using the extra strength mixture (IC50 = 0.013% vs. 0.04%) [33].
Similarly, Suganya et al (2016) assessed the cytotoxic effect of synthesized AuNPs on MDA-MB-231 cells using MTT assay and They not observed 50% inhibition in cell proliferation in the cell lines for all the dosages after 24 h incubation [39]. In another paper, Abbasali pourkabir et al found that cytotoxicity effects of solid lipid nanoparticle (SLN) on MDA-MB231cell lines were low [40]. In another study, the cytotoxicity assay by MTT method for Nanobiocide has been performed [41].
So, although there were several studies about non-cytotoxicity effects of other nanoparticles including AuNPs on MDA-MB cells; however, there is no published report about the CuO- NiO nano-composites effects on MDA-MB cells.

In vitro Minimum inhibitory concentration (MIC)

Table 2 includes the study result of MIC of Fluconazole and nano-composites (B sample) against vaginal isolates of C. albicans.
The data in Table 2 clearly demonstrates that the nano-composites have potent antifungal activity against vaginal isolates of C. albicans in comparison with Fluconazole. It is important to mention that Ghahremanloo et al conducted a study based on the antifungal activity of the silver nanoparticles with different concentration against Candida albicans. They reported that the highest mean difference for standard Candida albicans for 2.5% concentration of nanoparticles after 24 hours of exposure time was 501.0±23.1, whereas for concentrations of 5% and 10% of nano-meter sized particles after 6 hours of exposure time was

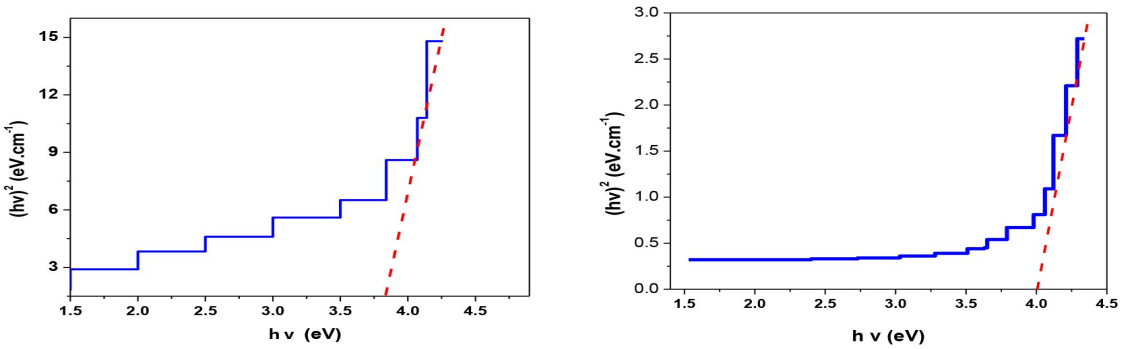

Fig .8. The (αhν)2 as a function of hν spectra of (right): A sample and (left): A sample

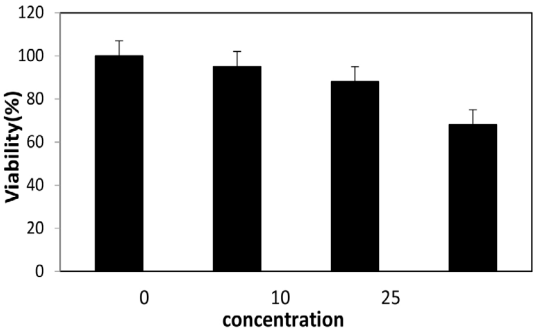

Fig.9. Cytotoxic effects of different concentrations of B sample on MDA cells of Breast cancer.

953±87, 1000±24.9, respectively [34]. In another study by Alipoor et al, Minimum inhibitory and fungicidal concentrations of gold nanoparticle were determined 6.25, 12.5 ppm and for Fluconazole were 50.25±19.48 and 100.50±38.96, respectively, and they reported that antifungal activity of gold nanoparticle was dependent on concentration [35].

CONCLUSIONS

The CuO-NiO nano-composites synthesized by precipitation method in the absence of capping agent. The absorption peak of nanoparticles appears at around 200-350 nm. The reduction in size and increase in the band gap of nanoparticle was observed with increase in Cu content in nano-composite. The XRD pattern analysis of CuO-NiO nano-composites showed that the synthesized samples are in a cubical phase. The average size of samples was found about 20–40nm as calculated by XRD and SEM techniques. Spherical-like morphology of nano composites was confirmed by the AFM and SEM images. Magnetization characterization of CuO-NiO nano-composite by using VSM technique indicated a decrease in their magnetic property with the increase in Cu content in the system.

Antifungal study of CuO-NiO nano-composites on against vaginal isolates of C.lbicans indicated an MIC90 antifungal activity with 3.90µg/ml. Results of cytotoxicity study of nano- composites at the lower concentrations on Breast cancer MDA cell lines demonstrate no significant toxicity.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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