Pharmacokinetics and biodistribution of nickel oxide for liver cancer cure

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Use of Nickel Oxide (NiO) nanoparticles as photosensitizers has undergone a strong development in the last few years due to their ability and significant bioavailability. NiO NPs are emerging milestones in photodynamic therapy (PDT) ongoing research with diverse marvelous biomedical applications e.g. cancer diagnostic as well as treatment, in many antibacterial and microbial therapy purposes and plays a dynamic role in such applications because of their unique characteristics like size dependent tunable emission spectrum of broad wavelength of light and high quantum yield. At this time, nanodependent PDT technique involving nanoparticles and NRs is effective, sophisticated and up to the mark due to high drug accumulation in the cancerous tissue and normal safety and biosafety.

The main object of current research is improving the efficiency of cancer treatment up to certain limit. In ongoing research experiment NiO NPs were grown by applying co-precipitation method. Manifold techniques were employed for justification of crystalline and morphological analysis. In first step author focused the toxic nature of suggested particles. Secondly, biotoxicity of NiO NPs were tested in hepatocellular model by applying NRA and microscopy. More sophisticated, efficient, effective and rapidly developing technique for cancer treatment is thirst of this modern age. Because many peoples due to this are under attack due to this severe disease. In addition, hepatocellular carcinoma (HepG2) is fifth the most common harmful cancer found in liver cells. Finally actual cell killing effect via necrosis/apoptosis was the big challenge for author.

After careful study of toxicity of NiO NPs in HepG2 cellular model the author will be confident to interpret protocol for real treatment of liver cancer patients.

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1. Introduction

Nickel oxide nanoparticles are excellent candidate due to its novel characteristics in industrial and biomedical applications due to due to significant ratio of penetration, oxidation and high toxicity profile in cellular system which plays a vital role for cell apoptosis mechanism [1, 2]. The specific properties of metallic oxide nanoparticles are directly correlated with size, chemical composition, shape, surface charge have been extensively used in engineering science and technology in multidisciplinary applications. Due to some precious metallic oxide nanoparticles nanotechnology got more attention in last two decades. In the light of all above consequences nanobiotechnology give a gateway for the development of many new materials and its interdisciplinary application in field of biomedical engineering. In current study the work has been focused on the cytotoxicity assessment of NiO nanoparticle towards hepatocellular model [3].

Collectively metal oxide nanoparticles like Nickel oxide, Zinc oxide and Titanium dioxide show significant cytotoxic trends for biological model [4-7]. In addition, NiO contributes a lot in a variety of Nano technological applications. Metal oxide nanoparticles proved to be excellent having diverse applications towards cancer diagnostics and treatments [8]. It is also quoted in some published data that the cytotoxic and phototoxic effects show various toxic behaviors towards various cellular models. Different synthesis technique was employed for growth of various nanomaterials. Sometimes toxicity of nanomaterial depends upon the size, shape and morphology of grown nanoparticles and dose dependent [9, 10]. Owing to fabulous applications in basic and applied research field development, NiO nanoparticles have attained a great devotion for international research scholars especially in the field of science and technology. In current scenario, NiO is the most efficient and anticancer and antimicrobial material might be successfully tumoricidal material, might be convenient for drug replacement and efficient involvement in pharmaceutical drug preparation [11]. Besides all these usage, metal oxides nanoparticles having many diverse applications like as sun screen filter/blocker, cosmetic products, catalysis, water filtration and antireflective

coating for glasses. Metal oxide nanoparticles have an amusing history with applications especially in food, chemical and biological studies. Due to thermodynamically stability, many oxides like TiO_2, ZnO and SiO_2 have been approved by Food and Drug Administration. In short about toxicity of NiO, it is still not clear cell killing occurred due to chemical reaction via apoptosis or due to size shape and morphological parameters of NiO NPs. We expect that development of this precious material will be the big breakthrough in field of science and technology.

Hepatocellular carcinoma (HepG2) is globally fifth the most common cancer associated with gene mutation and viral infection. For treatment of such premalignant and malignant disease efficient, direct effective and rapid healing developed technique is required. It is therefore, prior to this experimental work some parameters calibration is required.

2. Experimental

2.1 Materials and methods

Nickel Oxide (NiO) nanoparticles are fabricated by chemical solution methods. Initially, 4 grams of Nickel sulphate hexa hydrate ($NiSO_4.6H_2O$) was mixed in 200ml deionized water, on the other hand 2.3 grams Sodium hydroxide (NaOH) was added in 200ml deionized water. Mixtures of the both of the solutions were separated at room temperature under magnetic stirring. Sodium Hydroxide (NaOH) solution was blended drop wise into Nickel sulphate hexa hydrate ($NiSO_4.6H_2O$) at 50 °C. A light green precipitate with pH 11.3 was obtained after 40 minutes of continuous stirring. The solution was rinsed with acetone and deionized water for many times until the pH of the solution was reached to 7.30; acquired precipitates were dispersed into a beaker and kept into oven at 105 °C for 24 hours. The obtained light green product was $Ni(OH)_2$ nanoparticles, which was then calcinated at 500 ^oC for 2 hours. At that temperature water was removed and black nickel oxide NiO nanoparticles were collected.

2.2 Material characterization

X-ray diffraction (XRD) investigation of NiO NPs was determined using PANalytical X'Pert-PRO equipment. The working voltage and operative current was kept as 40 kV and 30 mA individually. The scanning range was from 20^o-80^o (2θ) while step size ($\approx 0.025^o$). XRD patterns of nano-NiO are shown in Fig. 1. In figure, XRD patterns exhibited strong diffraction peaks at 37.4°, 43.5°, 62° and 75.2° demonstrating NiO nano-crystalline structures. All peaks are in good agreement with the previous reported data [12].

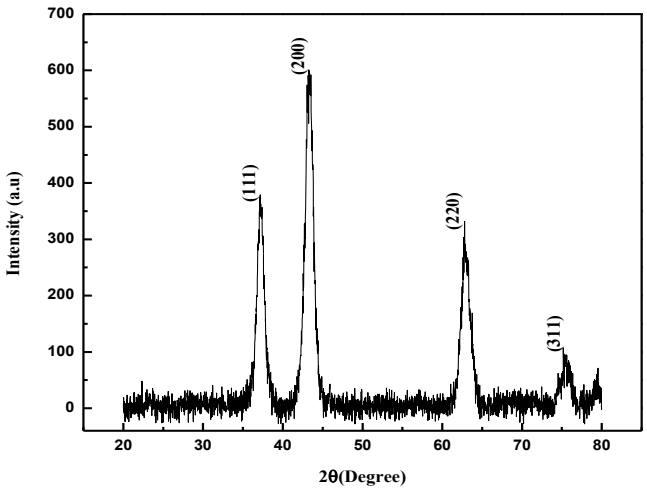

Fig. 1. XRD patterns of NiO nanoparticles.

Average crystallite size of the nanoparticles has been calculated by Scherer's formula. Crystalline size of the grown nanoparticles was determined as (≈97.12 ± 23.30).

Fig. 2 shows that the SEM consists of small nanoparticles of size 95-120 nm which are agglomerate and form a large grain of average size 8-10 µm. The particle size also confirms the results obtained by XRD pattern.

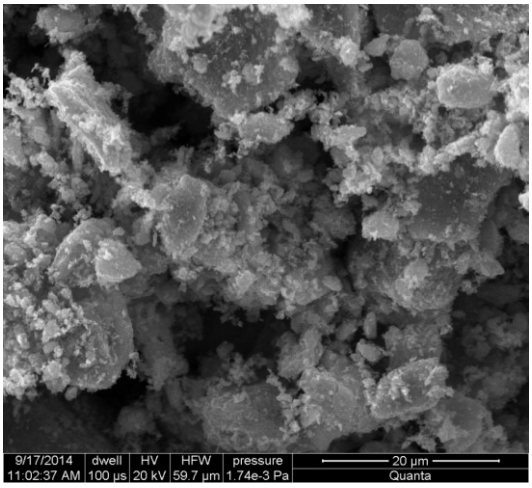

Fig. 2. SEM image of NiO nanoparticles

2.3 Cell culturing

In cell culturing process, HepG2 cell line was cultured in tissue-culture plastic flasks (NuncWiesbaden Germany) in MEM (Minimum Essential Medium) with Hanks salts, also supplemented with 10% fetal bovine serum (FBS), 2 ML glutamine and with some nonessential amino acids. Moreover for suitable connection with the substratum, the cells were incubated for 24 hours at 37C temperature. The cells were also sub cultured for two or three times in a week. After that, the cells were harvested via trypsin 0.25% once reached to the confluence of 75-85 % [13-15].

2.4 Cell labeling

HepG2 cells were seeded in 96 well plates having concentration of 1×10^5 cells/well and were incubated with different concentrations of NiO NPs ranging 0-80µg/ml at 37 °C for 24 hours having 10% FBS and 5% CO_2. In recent study, 96 well plates were arranged in 10 columns while each consists of 4 wells. First 8 columns were labeled with varying concentrations of NiO nanoparticles dispersion solution ranging 0-80µg/ml and the last two columns were kept as standard. The said cell line was treated with NiO nanoparticles with and without the presence of laser light (≈ 630nm of red light) and their cellular viability was determined after 24 hours of incubation period. Same experimental steps were performed as in [16, 17].

2.5 Reactive Oxygen Species (ROS) detection

Working solutions of different concentrations (0-80 µg/ml of NiO NPs) were added in wells and incubated for 48 hours. Three columns were used as control without serum. The cells were incubated in humidified air with 5% CO_2 at 37°C. Intracellular reactive oxygen species (ROS) production was detected after 48 hours of incubation, using the 2', 7'-Dichlorofluorescein diacetate via damaging of cell membrane, mitochondria, nucleus or lysosomal injury.

2.6 Cell viability measurement

Standard protocol of neutral red assay (NRA) analysis were performed after exposing the HepG2 cellular model with optimal concentration of NiO NPs[2, 3]. Cell viability loss reaches to about 60% in the presence of 80 microgram/ml of NiO NPs concentration under laser irradiation of 80 J/cm^2 (consider as suitable value). It is investigated mutual effect of light as well as drug approaches to significant loss in cell viability.

3. Results and discussion

Ongoing research work was carried out for the real understanding of either toxic or nontoxic nature of the NiO NPs towards hepatocellular model. After successful fabrication of desired shape of NiO NPs were exposed to HepG2 cellular model. In this modern age the replacement of photosensitizer is the basic challenge due to significant toxicity towards normal cell model [17]. After perfect investigation about NiO NPs towards HepG2 cell mode, some calibration parameters like optimal dose of solution concentration and light dose, suitable time of incubation is debatable. Data reported by Fakhar et al. [18] that some varieties of nanomaterials are very useful for drug transportation. Many of them might be toxic can be used for treatment of cancerous model [19]. Current research study focused on NiO NPs nature can be helpful for basic and real understanding of NiO NPs characteristics. In order to explore the said finding HepG2 cell model were selected as investigational biological model. Many communities in Asia are under the attack of hepatocellular disease either directly or indirectly. Cure is the basic need of this modern age [20]. However, the significant NiO NPs uptake/bioavailability is basic and important factor in therapy purpose.

3.1 Optimal density (concentration of NiO NPs)

In current experimental piece of work, author was committed to trace the maximum absorbance vs. incubation time and significant absorbance vs. NiO NPs concentration. In fig. 3, graph depicted the scientific measurement/absorbance nickel oxide nanoparticles in hepatocellular model after 24 hours of incubation time. It is obvious that cell uptake/absorbance is totally concentration dependent. Cell were exposed to (10-80 microgram/ml) of NiO NPs dispersion.

Again figure 3 describes the optical density vs multiple concentrations (10-80µg/ml) of NiO nanoparticles after 24 hours of incubation time and absorbance reached to 60%. It again shows non-significant uptake of nanoparticles after 24 hours. However, it is revealed that the absorbance/optimal density of the NiO NPs in HepG2 cells increase with increase in concentration as well as incubation time. Results are agreed with the previous published data by many researchers [2, 10-12].

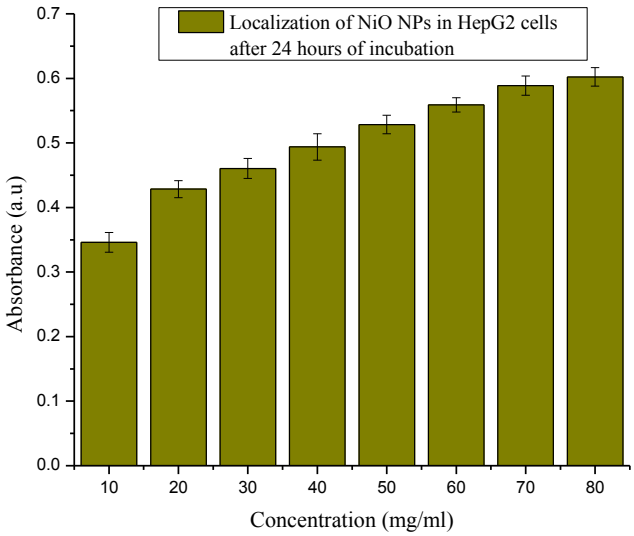

Fig. 3. Absorbance vs concentration of NiO NPs after 24 hours of time of Incubation.

Fig. 4 illustrates the absorption spectrum of NiO in the given biological model after 48 hours of incubation. HepG2 cell line was exposed to NiO nanoparticles for 48 hours of incubation. For the first 24 hours of time of span, non-significant results were obtained and particles showed low absorbance. After 48 hours of time, meaningful results were confirmed by the analysis of our present data and nanoparticles showed significant absorbance. Additionally, particles uptake approaches to 70% after 48 hours of incubation.

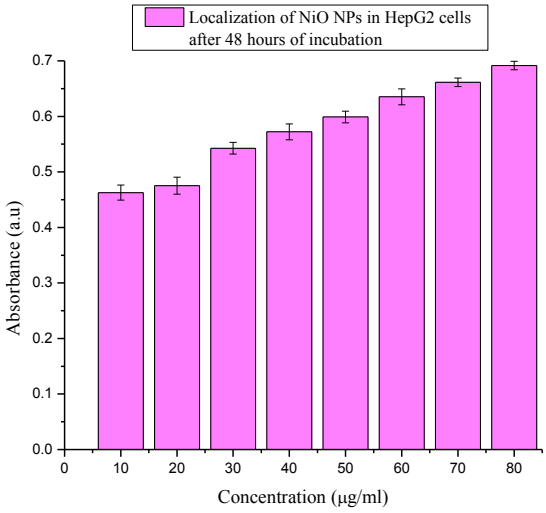

Fig. 4. Absorbance vs concentration of NiO NPs after 48 hours of time of Incubation

3.2 Cellular viability

Cell viability of NiO exposed cell was assessed by applying the standard protocol of neutral red assay (NRA) analysis [2, 3]. In first step cell were seeded in 96 well plates and exposed with different concentration of NiO NPs dispersion in the presence and absence of light exposure. After 48 hours of cell incubation with NiO NPs optimal concentration 50 microliter of neutral red assay (Ratio 50mg/ml) were incorporated in treated cultured plate and incubate it for 3 hours [4]. The medium was removed and cells were washed with 40% formaldehyde and 10% $cacl_2$ (v/v, 4:1). In the next step fusion of 45% ethanol and 15% acetic acid (1:1) was assimilated to extract NR. In further step NR mixed plate was shaken for 50 sec. and kept free for 15 minutes. Absorbance of incorporated dye was gently examined at 510 nm. Quantification of solubilized dye was statistically analyzed with the living cell numbers.

3.3 Reactive Oxygen Species (ROS) measurement

After justification of morphological analysis, production of intracellular reactive oxygen species (ROS) was examined by applying 2', 7'-Dichlorofluorescein diacetate (Sigma Aldrich). The treated HepG2 cells having various concentrations range (0-80µg/ml) were experimented in this research work. Nickel oxide nanoparticles labeled with HepG2 cell line were washed very softly with PBS and added fresh Dulbecco's minimum essential medium (DMEM) and was examined for reactive oxygen species test. Trailed cells were exposing to 100 µM of 5 µM 2', 7'-Dichlorofluorescein diacetate and kept free for 35 minutes in the dark at 37 °C. Then labeled cells were irradiated with UV light with a dose of 20 J/cm^2 for 4 minutes and ROS liberation in all complexes and non-conjugated HepG2 cells were examined with microplate reader (POLAR star Galaxy having multirange function). The excitation of microscope coupling filter allows 450nm wavelength range of excited beam. For fluorescence detection a filter of 520 nm was used during measurements. The results are shown in fig. 5 and out data resembles with previous published data by many researchers. Multiple research groups dealing with PDT revealed in their experiments that ROS played significant role in cell killing mechanism. These species may damage cell membrane, mitochondria or nucleus as well [21-25].

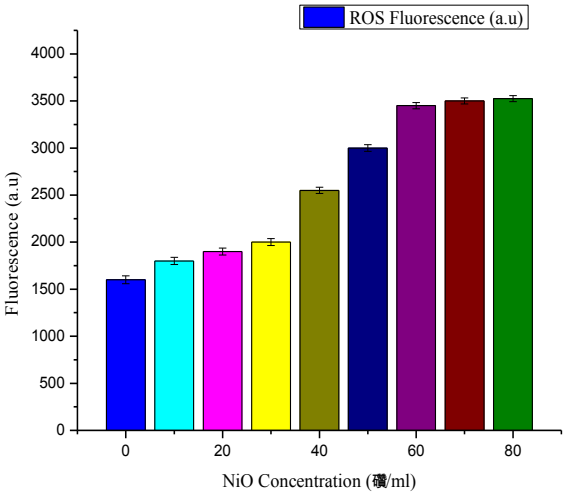

Fig. 5. Reactive oxygen species (ROS) accumulation in HepG2 cell line treated with NiO NPs

3.4 Light exposure

The results demonstrate the phototoxic effects of different NiO NPs (concentration ranging 0-80µg/ml) with optimum concentration of 80µg/ml in HepG2 cells with optimum laser dose of 80 J/cm^2 as depicted in fig. 6. It was found that nickel oxide nanoparticles (NiO NPs) are more effective under exposure of laser light and cellular loss reaches to 60%. Moreover for 0-70 µg/ml of NiO exposed HepG2 cells, the loss in cell viability was insignificant but at 80 µg/ml of NiO a maximum cell death was observed. However for higher concentration of NiO nanoparticles a decreasing trend in the loss in cellular viability was recorded.

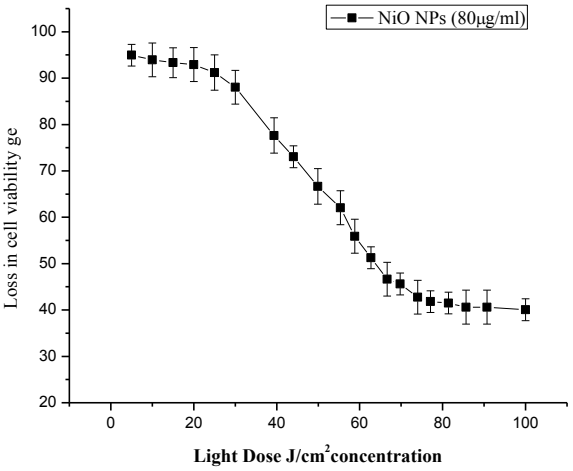

Fig. 6. Loss in cellular viability (% age) in HepG2 cell line treated with NiO NPs (80 microgram/ml) under irradiation of 80 J/cm^2

4. Summary

Nickel Oxide nanoparticles are promising because of high quantum yield, morphology dependent tunable emission of suitable light wavelength. Nano-dependent photodynamic therapy comprising nanoparticles (NPs) is simple, biologically safe, biocompatible in absence of UV light, enhances endogenous fluorescence, noninvasive, fast with their significant permeability in malignant cells. But nickel oxide nanoparticles (NiO NPs) due to high surface to volume ratio show maximum toxicity can be used as an effective drug carrier system which might be fruitful for cell killing mechanism e.g. necrosis or apoptosis. Current experimental study was out to determine the actual finding of cell killing mechanism. Either NiO NPs having capability of cell killing mechanism or can enhance cell proliferation effect. In first step, nickel oxide nanoparticles were grown by applying chemical solution method. Secondly, their characterization steps were performed e.g. XRD and scanning electron microscopy (SEM). In third step, time and concentration based pharmacokinetics of NiO NPs were tested in HepG2 Cellular model.

Furthermore, the suggested cell line was exposed to the NiO NPs under the exposure of threshold light dose of (80 J/cm^2), which leads to the production of reactive oxygen species (ROS). These reactive species liberated photodynamic effect causing DNA and mitochondrial damage resulting in cell apoptosis. Hence, the cell viability was reached to 60% once the material was excited with light. Therefore, it is worth saying that NiO NPs can produce maximum quantity of singlet oxygen into the HepG2 cell line in the presence of suitable dose of laser light which is the novelty of the present research work. Due to this toxic nature of NiO NPs, the author is confident to recommend suggested protocol of cancer treatment by applying photodynamic effects of NiO NPs.

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