Apoptotic effect of TiO_2 in HepG2 cellular model

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Liver cancer being the fifth most common lethal cancer in the world requires prompt emergent, convincing, effective treatment, which can be acquired by the PDT. Current study demonstrates the cytotoxic response of TiO_2 NPs in hepatocellular (HepG2) model. However, underlying mechanism of apoptosis in HepG2 cells due to TiO_2 NPs exposure is largely lacking. We explored the possible mechanism of apoptotic effect induced by titanium dioxide nanoparticles in human hepatocellular model. Due to high surface to volume ratio and extremely toxic nature, said nanomaterial can be used as tumoricidal drugs which can be act/proved as milestone for cancer treatment. TiO_2 NPs were prepared via Sol-Gel technique by using Titanium Isopropoxide as a main precursor. Crystal structures of the particles were examined XRD and the surface morphology of synthesized nanoparticles investigated by SEM. In said research work, focus of our study was to explore the actual cytotoxicity and photo-toxicity of TiO_2 NPs individually having broad range concentration when expose to HepG2 cellular model for 48 hours as time of spam. After successful investigation of toxicity of said nanoparticles in said cellular model, the author is confident to demonstrate the actual reason of cell killing mechanism which will be big turnip of scientists, might be helpful especially for real treatment of malignant/pre-malignant patients. Additionally, a comparison of cytotoxicity between ZnO and TiO_2 nanoparticles is also explored in this article.

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1. Introduction

Nanotechnology is manipulating at a scale of billionth of a meter that is termed as nanometer. At nanometer scale, a particle may be defined as a tiny object which acts as an entire entity with respect to its properties [1] and transportation [2]. Additionally, the surface area to volume ratio increases due to which nanomaterials becomes more effective [3]. Nanomaterials have great importance in numerous areas such as information technology, engineering and medicine, etc. Furthermore, nanomaterials are involved in multidisciplinary field of science and technology such like in health and medical field, particularly as drug delivery agents, biosensors etc. In reported data, it has been investigated that nanoparticles (NPs) having 1-100 nm size can be localized into the targeted site of the human body, very frequently [4]. Owing to several properties and numerous technological applications, titanium dioxide (TiO_2) nanoparticles have attained great attention of materials scientists and physicists [5]. It has been used for different applications including sensors, selfcleaning surfaces, antimicrobial precursors, pharmaceutical drugs etc after making morphological and structural modifications in the said material [6-9]. With the integration of nanotechnology and increased use of TiO_2 NPs, researchers are led to cure the effects of these materials.

Recently, titanium dioxide (TiO_2) NPs have been synthesized by different techniques and used for several purposes. TiO_2 has many distinct properties just like catalytic, anticorrosion, high stability. However, many researchers have reported that TiO_2 NPs had diverse hazards to health of the human beings [10-11] as compared to their average size complements. It has been shown that reactive oxygen species (ROS) are produced by TiO_2 NPs which is a key factor to cytotoxicity [12]. Significant quantity of ROS and free radical production is the basic need of particle toxicity. The said oxidative entities are primary mechanisms, which may result in oxidative stress, inflammatory response and destruction of the mitochondria/DNA damage. As mentioned above, small size of the particle possesses large surface area to volume ratio and shows high chemical reactivity as well as interaction with biotic systems. The high chemical reactivity of NPs indicates the greater ROS production and free radicals [13, 14].

In previous research studies, lungs were the main focus part for the potential toxicity of TiO_2 NPs. It has been revealed from animal studies that TiO_2 NPs could induce inflammation and unreasonable changes to lung tissues after they inhaled to lungs [15]. TiO_2 NPs have shown apoptotic effects in numerous cell lines like brain cells [16] osteoblasts [17] as well as induce necrosis in fibroblasts [18]. TiO_2 is an excellent candidate for liberation of cytotoxic and apoptotic effect in human lymphomas [12]. Non-significant evidence regarding cell killing mechanism by labeling TiO_2 was reported, while cytotoxic effect of TiO2 was tested in HEK-293 (human

embryonic kidney cell line) used as an experimental biological model [19].

Hepatocellular carcinoma (HepG2) is fifth the most common harmful cancer found in liver cells. For the treatment of such cancer, more sophisticated, efficient, effective and rapidly developing technique is required, which is possible through photodynamic therapy (PDT) [20]. In 2008, World Health Organization reported (WHO) that liver cancer is still at fifth position in annually death rate as compared with other cancerous infections [21]. The major causes of the said serious disease include alcoholism, liver damage and prolonged viral hepatitis [20, 22]. In current study, we applied TiO_2 and ZnO nanoparticles in HepG2 (Liver carcinoma) cell line. By comparing the cytotoxicity of the said nanoparticles, we demonstrated the cell killing mechanism due to the exposure of TiO_2 nanoparticles in the present experimental biological model.

2. Experimental

2.2 Synthesis of Nanoparticles

Titanium dioxide (TiO_2) nanoparticles were prepared via Sol-Gel method using Titanium Isopropoxide [Ti $(OC_3H_7)_4$] (97% supplied by Sigma Aldrich) as a starting material. Ethanol and distilled water were used as solvents. Oxalic acid was used in order to control the pH of the solution. The flowchart of the complete process is shown as below in fig. 1.

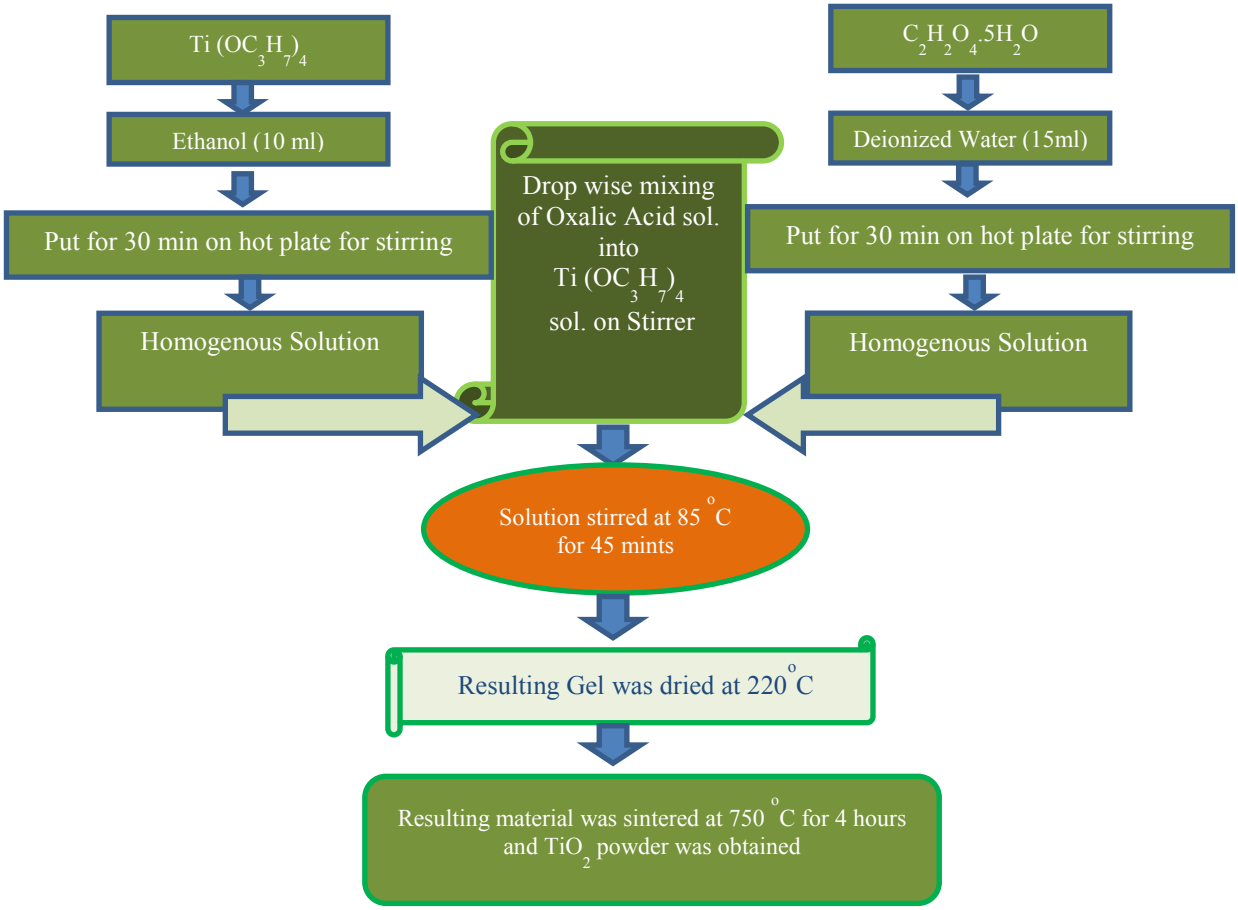

Fig. 1 Schematic diagram of the process of preparation of TiO_2 nanoparticle..

ZnO NPs were prepared by using hydrothermal growth technique. Details of mentioned technique can be viewed from Y. Khan *et al* published data [23].

2.2 Material characterization

XRD result shows that anatase phase is stabilized at all pH values without any impurity. The spectrum indicates that all samples are crystalline, no amorphous phase is observed. The peaks indicate the presence of anatase phase with (101) planes respectively. XRD card verifying the result is 01-075-1537. The average crystallite calculated by Scherer formula is 12.9 nm. XRD pattern of said grown material were shown in fig. 2.

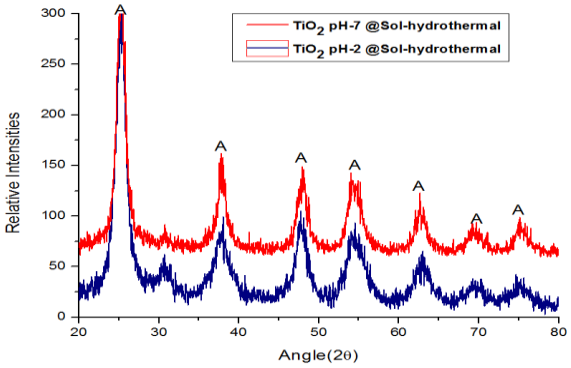

Fig. 2 XRD results for Sol-Gel synthesis of TiO_2.

SEM results of pure TiO_2 synthesized via Sol-Gel technique shows the well dispersed with probably spherical morphology. The SEM image of TiO_2 was shown in fig. 2.

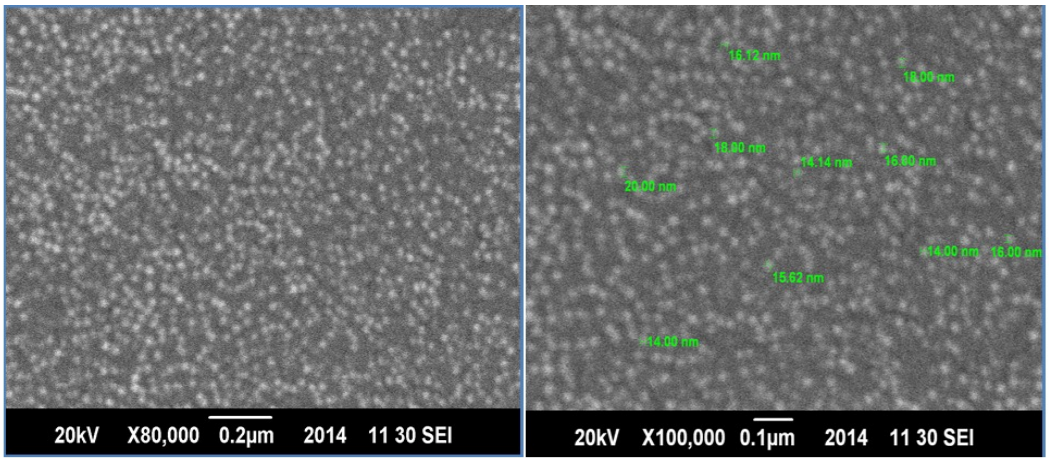

Fig. 3 SEM results of un-doped TiO_2 synthesized via Sol-Gel process.

The sample was dispersed in ethanol. From fig. 3, it is clear that maximum size of said grown nanoparticles is about ≈14 nm to 20 nm which shows much resemblance with the XRD calculated results by Scherer formula.

3.3 Cell culturing and labeling

HepG2 (Hepatocellular carcinoma) cell line, was seeded out in 25 cm^2 plastic tissue-culture flasks (Nunc Wiesbaden Germany) individually, in Minimum Essential Medium (MEM) with Hanks salts, containing 10% fetal bovine serum (FBS) and 2 mM L-glutamine along with some non-essential amino acids and were incubated for 24 hours for proper attachment to the substratum. Cells were maintained at 37^o C in a moist environment as a sub-confluent monolayer and were routinely sub-cultured two or three times weekly. The cell culture with 70-80% confluence was harvested using 0.25% trypsin [24-25].

HepG2 cells were kept at a 24 well plate, incubated at 37 ^{o}C and 5% CO_2 [26]. Individual plate was used for each experiment; results were verified by repeating them three times. In our previous published data, it has been investigated that concentration of 250 µg/ml ALA along with 80 J/cm^2 at a wavelength of 635 nm light dose have shown excellent cytotoxicity and photocytotoxicity after 24 hour of time of spam for rhabdomyosarcoma (RD) cell line [27]. In the current study 24 well plates have been used, and were arranged in 6 columns each column consists of 4 wells. This arrangement was used for different sample preparations e.g. (0-1000 µg/ml of TiO_2).

Atif et al. [27] has also reached similar results by using the same kind of experimental procedure for different cell line. Cells were incubated for 24 hours, and were examined under inverted microscope. The Naphthalocyanine-reconstituted LDL NPs for cancer (*in vivo/in vitro*) imaging and treatment of HepG2 was used as reference. L. Song et al. [28] have claimed excellent results by displaying the confocal images approach. The results of microscopic images in our previous paper are verified in this experiment [29]. We suggest in published data that ZnO may be used as biomarker with green fluorescence emitted by ZnO nanoparticles (wavelength 488, 514 of Light). Cytotoxicity of the ZnO, ZnO Complex Nanoparticles was determined by Neutral Red Assay using cultured HepG2 cell in 96 well plates.

4. Results and discussion

In first step of current work author focused the localization of ZnO NPs and TiO_2 NPs in HepG2 cellular model. The corresponding figures are shown in Fig. 4 and 5 respectively. For Fig. 4, ZnO NPs are dispersed in PBS as well as ethanol. For cell culturing model 96 well plates were selected. For each sample/concentration 4 wells in each row were marked and last two columns were selected as controlled (without labeling of ZnO NPs disperses solution). Fig.4 shows that influence of rising trend/pattern of ZnO absorbance (a. u) (Optical density) by increasing the concentration of ZnO NPs in HepG2 Cellular model. Same frame of work was conducted in given reported data [30].

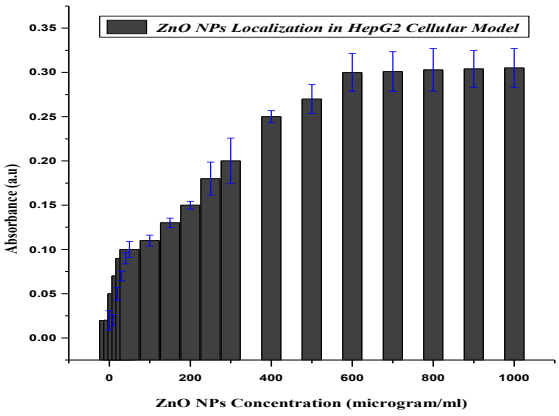

Fig. 4 Zinc Oxide Nanoparticles absorbance in Hepatocellula r (HepG2 Cell) Model. Each data point corresponds to mean ±σ (n=4).

But in this article comparative toxicity of ZnO NPs as well as TiO_2 NPs were investigated and compared. The strangeness in the plot is shown in Fig. 5.

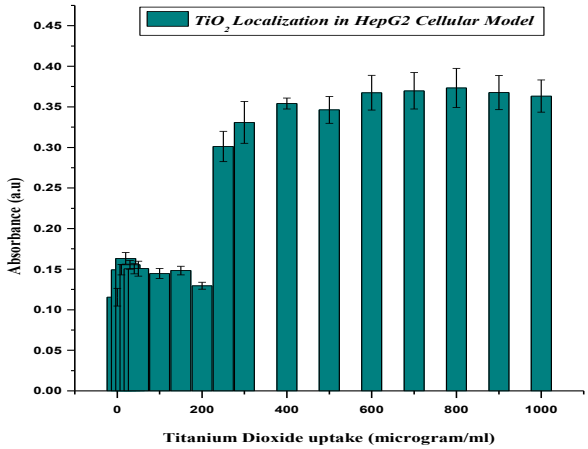

Fig. 5. Titanium Oxide Nanoparticles uptake in Hepatocellular (HepG2 Cell) Model . Each data point corresponds to mean ±σ (n=4).

After 200 µg/ml of TiO_2 solution sudden rise in absorbance/optical density were recorded. Uptil 600 µg/ml concentration of titanium dioxide significant uptake of said disperse solution were found. In given experiment 600µg/ml were considered as optimal dose of TiO_2 concentration. Same nature of work was already done for multiple malignant cellular models [30-57]. Basically author is interested the actual mechanism of cell killing model, either biotoxicity involved or due to morphology of nanomaterials cells were being necrosed. Results were confirmed by applying reactive oxygen species (ROS) test. After counting ROS fluorescence, we are assured that up to 95% of cells were being necrosed due to chemical reactions instead of mechanical stress/trauma due to morphology (size, shape) of said nanoparticles.

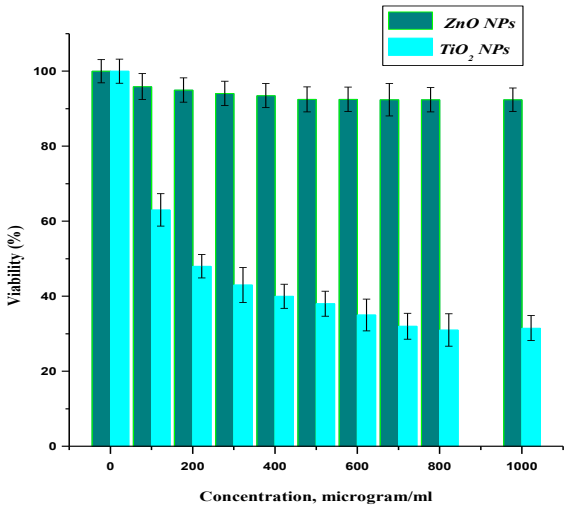

Fig. 6. Comparison of Cellular Viability (%) of ZnO NPs (µg/ml) and TiO_2 NPs (µg/ml) treated HepG2 cells for different Concentrations. Each data point corresponds to mean ±σ (n=3).

Fig. 6 compares the cytotoxicities for different treatment arms regarding ZnO NPs (µg/ml) and TiO_2 NPs (µg/ml). It is cleared from figure that countable difference between losses in cell viability was recorded. 5% loss in cell viability was examined when HepG2 cells were exposed to 600µg/ml of ZnO NPs. At said optimal dose of TiO_2 NPs loss in cell viability increases to 70%. This implies that TiO_2 NPs is about 10 times more toxic that ZnO NPs which can be used as photosensitizing agent for cancer treatment. It is under debate that which malignancy/cancerous disease is the most target of TiO_2. TiO_2 is precious material for cancerous treatment. In the light of experimental results, Titanium dioxide nanoparticles may be found effective in several studies and can be suitable agent for further use *in vitro* as well as *in vivo* model.

5. Conclusion

Hydrothermal and Sol-Gel technique were used for growth of ZnO NPs and TiO_2 NPs respectively. The morphology and structural analysis were confirmed by applying SEM and X-ray diffraction technique. The biotoxocity of said nanoparticles were tested in the hepatocellular (HepG2 cell) model. Optimal time and concentration of mentioned nanopartilces were optimized 22 hours and 600µg/ml. It was concluded that this nanoformulation could be used for biological applications, e.g., targeted therapy, chemotherapy etc. due to marvelous biotoxicity.

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