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Structural, Optical and Cytotoxic Behavior of Titanium Dioxide Nanoparticles and its Nanocomposites with Zinc Oxide

Allah Ditta Khalid[1], Naeem-ur-Rehman[2], Sadia Saghar Iqbal[1], Saeed Ahmed Buzdar[2], Mushtaq Ahmad[3]

[1]Department of Physics, The University of Lahore, Lahore, Pakistan.

[2]Institute of Physics, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

[3]Department of Physics, COMSATS University Islamabad, Park Road, Islamabad.

Corresponding Email: naeem.rehman@iub.edu.pk

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Abstract:
Different metallic oxide nanoparticles showed cytotoxicity against various types of cancerous cells. The cytotoxicity mostly depends on the reactive oxygen species (ROS) generation. For that purpose, TiO_2 and ZnO nanoparticles have been synthesized by chemical route. Additionally, the ZnO was deposited on TiO_2 to prepare nanocomposites ZnO@TiO_2 .The Prepared nanoparticles (NPs) were characterized for their structural, morphological, optical and cytotoxicity behaviour by using techniques by X-ray diffraction (XRD), scanning electron microscopy (SEM), UV–Visible spectroscopy and ROS generation .Their structural properties indicate the anatase phase of TiO_2 while ZnO formed has shown hexagonal wurtzite structure. The SEM images showed that TiO_2 NPs are formed in very small and uniform spherical morphology while ZnO formed in the form of small nanorods. Both types of morphology are visible in the ZnO@TiO_2 nanocomposites as well. The optical energy band gap values show the reduction in the band gap of TiO_2 when it's composite has been made with ZnO nanorods. The anticancer activity was checked through ROS generation, MTT assay was used for this purpose. The results show almost uniform ROS generation in case of TiO_2 and ZnO nanostructures but it shows a reduction in ROS generation in case of ZnO@TiO_2 nanocomposites. This result may be related to the less available surface area for the individual particles of one type for ROS generation.

Keywords: Titanium dioxide, Nanocomposites, Band gap, ROS generation, MTT assay, anticancer activity.

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Introduction:

In current development of nanotechnology, more attention has been provided to metal oxides nanoparticles due to their unique optical electrical and biomedical applications. Among these TiO_2

and ZnO are one of the important nanocomposites owing their physical and chemical properties which make them applicable in photo catalysis, solar cells , batteries and anticancer activity. Cancer is abnormality of tissues, which spreads to all over the body and mostly leads to the death. Nanotechnology is one of the famous and promoting researches in the field biology and medicine. The nanoparticles have large surface and volume ratio. Among these nanoparticles metal oxides nanoparticles have biological applications like antibacterial and antitumor.Titanium dioxide (TiO_2) is most attractive due to its various applications among metal oxides. TiO_2 is present in three structures anatase, rutile and brookite. Metallic titanium (Ti), its oxide and alloys have been broadly applied as insert material in orthopaedics and dentistry, due to their unique characteristics; they are biocompatible, good resistor to decay, less dense, and more strength. The element Zinc (Zn) is a recognized as a trace element which creates the bones in humans, in recent times it revealed hopeful osteoblast production with action of alkaline phosphatise. Zinc oxide also has greater specific surface area so it generally used as a antibacterial and anticancer agent. Due to its greater antibacterial and anticancer action it also used with coating of biological materials. Titanium coated with zinc oxide (ZnO-Ti) reduces the growth of bacteria both germs positive and germs negative TiO_2 NPs have been recognized to produce Reactive Oxygen Species (ROS) which property uses as antibacterial and anticancer activity. AycaTas et al. synthesized the ZnO and TiO_2NPs via solution combustion method and their anticancer activity was performed on HeLa and L-99 cells. The cell viability was checked by MTT assay. They concluded that uncoated TiO_2 NPs were more active in cancer cells then its composite with ZnO NPs. The reduction of cytotoxicity was also reported when nanocomposites (SiO_2@TiO_2) were exposed on human alveor A549 cells. It can be due to influence of inter-particle interaction and nano particle-membrane interaction. The same results were also reported when a titanium dioxide/ nitrogen doped grapheme quantum dot nanocomposites were exposed on breast cancer cells (NDA-MB-231). The reduction of cytotoxicity is due to increasing the size of particles. The size of particles is important factor of cytotoxicity. In present work, we investigated the anatase TiO2 and its effect with ZnO nanocomposites on its structural, optical and anticancer activity. Their anticancer activity was checked on Human liver cancer cells (HepG2) through generation of reactive oxygen species (ROS) as a novelty.

Experiment:

The TiO_2, ZnO are separately synthesized by the sol. gel, and co-precipitation methods, respectively. Additionally, TiO_2@ZnO nanocomposites have been deposited using two step synthesis approach, i.e., sol. gel and co-precipitation methods. Initially, the TiO_2 nanoparticles are deposited using sol-gel method by using Titanium Isopropoixide ($C_{12}H_{28}O_4Ti$), Nitric acid (HNO_3) +Distilled water and ethanol as precursors. A mixture of ethanol (20ml) and Titanium isopropoxide (20ml) was prepared by stirring followed by the addition of 24ml distilled water into the mixture. In order to maintain the pH of the solution, 10ml of 30% HNO_3 was added drop wise into the solution. The mixed solution was stirred for 4 hours while maintaining a constant temperature of 60^{o}C. As a result a gel is formed which was then put into the Oven at 80^{o}C for 10 hours for drying purpose. This dried gel was then grinded to convert it into powder form followed by an annealing at 500^{o}C for 2 hours to get good crystalline of the TiO_2 nanostructures. Further the ZnO nanoparticles were synthesized by co-precipitation method. For the purpose, 2.725 g of $ZnCl_2$ was added into distilled water to make solution of 20ml. then a sodium hydroxide solution by adding 4.8 g of (NaOH) into 30ml of distilled water. It was added drop wise into the $ZnCl_2$ solution with continuous stirring at 45^{o}C. After mixing the solution was stirred for 2 hours which resulted in the formation of white precipitates. These ZnO precipitates were then separate out from the solution by centrifuge machine and washed with a mixture of ethanol and distilled water several times. These particles were then place in a drying oven at 100^{o}C for 24 hours followed by the grinding process.

In order to prepare ZnO and TiO_2 nanocomposites, the as prepared TiO_2 nanoparticles were added into the $ZnCl_2$ solution and repeated the same procedure as mentioned above for the synthesis of ZnO nanoparticles.

The structure of the synthesized ZnO, TiO_2 and ZnO@TiO_2 nanocomposites has been studied by the Bruker D8 Discover X-ray Diffract meter. The morphology of the particles have been analysed by VEGA3 TESCAN scanning electron microscopy. The optical nature has been studied by taking the absorbance spectra from UV-Visible spectrophotometer. In vitro activity of synthesized NPs has been tested on HepG2 cells (Human liver cancer cells). For the purpose, MTT-based colorimetric assay was used to measure the number of feasible cells. The activity of samples was

determined by measuring the absorbance light of wavelength at 570 nm, which is proportional to the number of living cells.

RESULTS AND DISCUSSIONS:

For morphology of the synthesized nanostructures, every material has been scanned for SEM images and shown in Figure (1). We can see in the figure 1(a) that TiO_2 nanoparticles are formed in the shape of small circular particles with average size up to 100 nm in diameter. This morphology is uniform and has been seen for all TiO_2 particles in the sample. In the second micrograph Figure 1(b) the morphology of ZnO nanostructures has depicted. These nanostructures are seen to have columnar growth pattern with a non-uniform rod length. The average diameter of these nano-rods is found to be around 40 nm. There have been so many broken and agglomerated nanostructures are also visible in the ZnO micrograph. The SEM image for the nano-composite sample shows various nanostructures spreading on a non uniform big agglomerated particle. In Figure 1(c), it can be seen that it contain small particles along with the nano-rods, which suggest that TiO_2 and ZnO particles have maintained their individual shape in the prepared nanocomposites as well as compared to when they prepared in the single sample form.

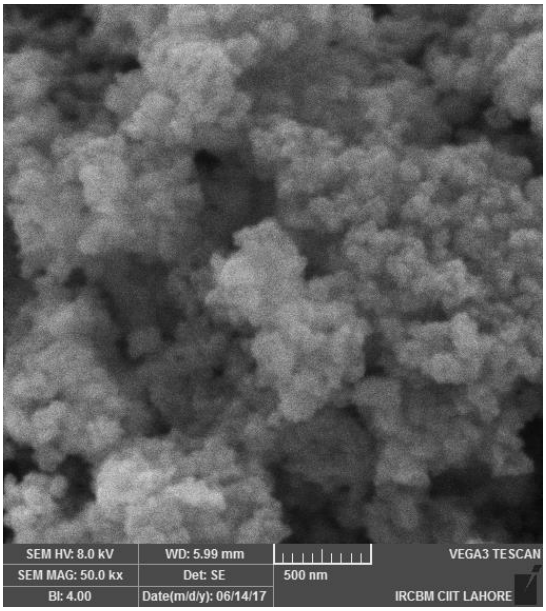


Fig. 1(a): SEM image of TiO_2 nanoparticles.

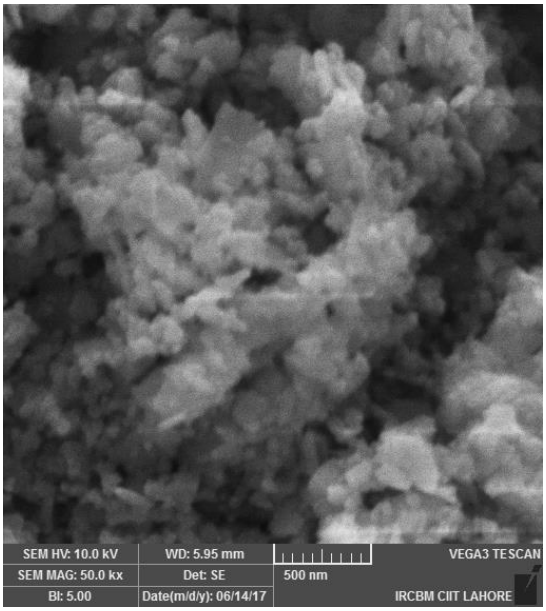


Fig. 1(b): SEM image of ZnO nanoparticles.

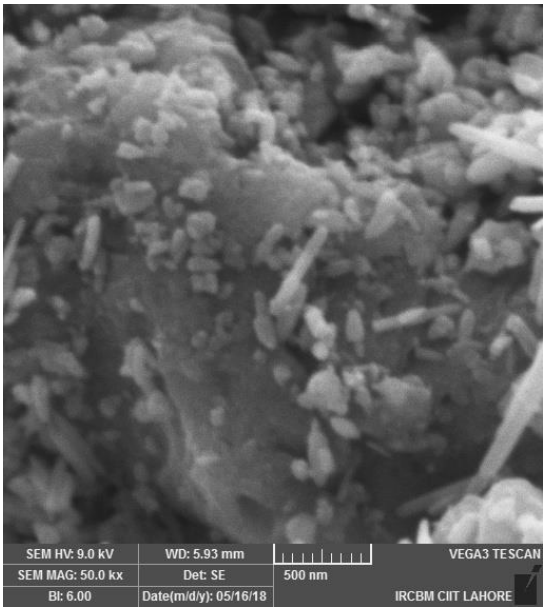


Fig. 1(c): SEM image of ZnO@TiO_2 nanoparticles.

1. The x-ray pattern for TiO_2, ZnO and then ZnO@TiO_2 nanocomposites are shown in the Figure (2) The black curve in the figure that corresponds to the TiO_2 nanoparticles demonstrates the anatase type of crystal structure as referenced by the JCPDS card no. 21-1272. The characteristic diffraction peaks for anatase TiO_2 crystal structure are exhibited at 25.40°, 38.06°, 48.19° and 53.90° that are corresponding to the (101), (004), (200) and (211) planes, respectively. The second curve plotted in red corresponds to the ZnO nanoparticles that have been synthesized separately. It can be seen that this XRD spectrum exhibits the hexagonal wurtzite structure of ZnO has been in good agreement with the JCPDS card no. 36-1415. The structure has confirmed by the characteristic peaks at angles 31.70°, 34.45°, 36.19°, 47.50° and 56.60° indexed to (100), (002), (101), (102) and (110) planes, respectively. When ZnO nanoparticles are on the TiO_2 particles then both structures exhibit their characteristic peaks in the third curve confirming that in the ZnO@TiO_2 nanocomposites both the structures have maintained their individual identity. In addition, the crystallite sizes of each sample are estimated using Scherer's formula and the values are tabulated in the table1. The average particle size of TiO_2 and ZnO generally remain same in both cases, i.e., individually and in composite form.

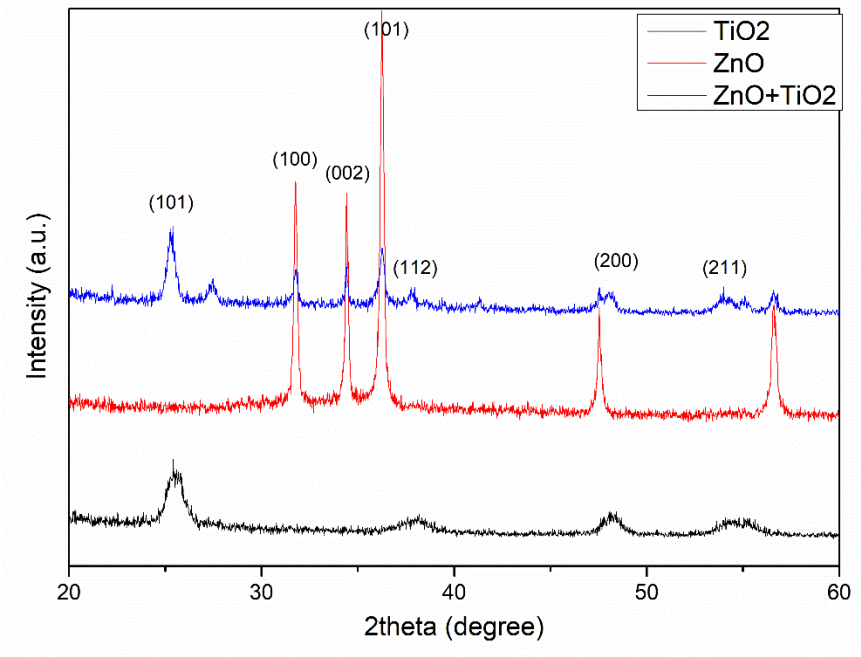
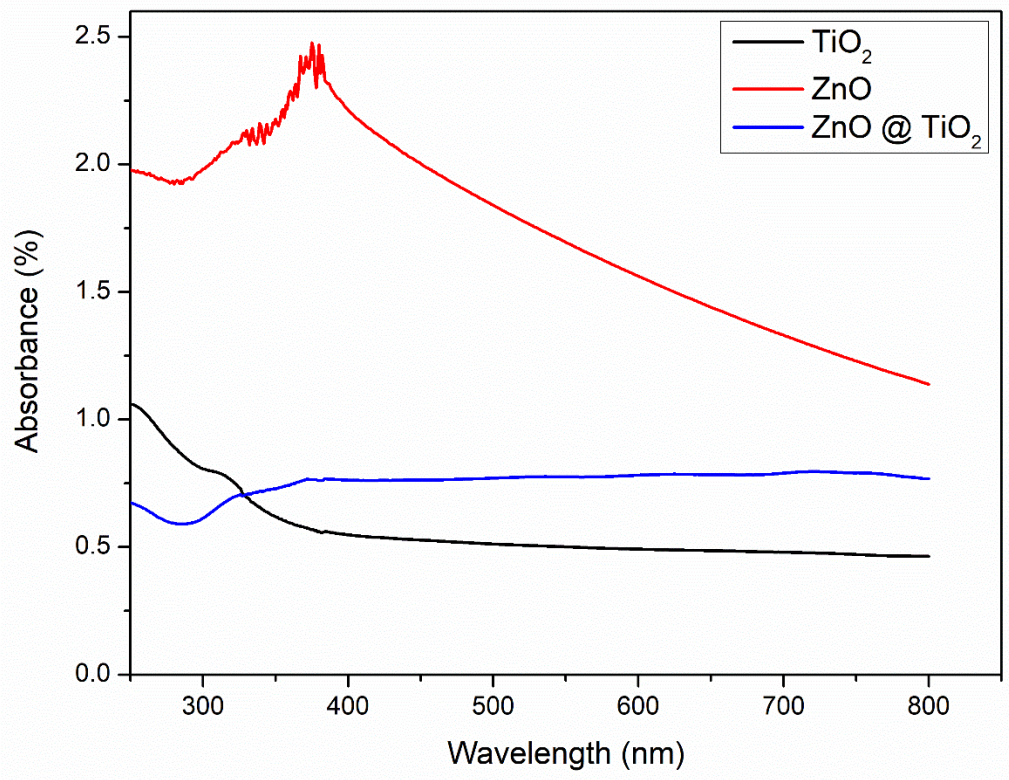


Fig. (2): X-ray diffraction patterns of TiO_2, ZnO and their nanocomposites

2. UV-Vis absorption measurements describe the optical properties of TiO_2; ZnO and ZnO@TiO_2 samples are shown in Figure (3). In the figure it can be seen that ZnO shows higher absorbance in comparison to TiO_2 in the visible region but with a decreasing trend toward higher wavelengths. Furthermore, when ZnO attached to the TiO_2 to from nanocomposites, it again causes the rise in overall absorbance of the sample.



3. To calculate the band gap of all material samples, Tauc relation have been used and is plotted in the Figure (4). The estimated band gap energies of TiO_2 and ZnO nanostructures were 3.78ev and 2.85eV, respectively. But when these two materials were merged to make nanocomposites its band gap energy value becomes 3.68ev which is less to the TiO_2 that was the host material in the composite. Obviously the band gap of ZnO@TiO_2 was narrowed down as compared to uncoated TiO_2. The similar results were also seen in other groups such as Fan et al. reported that TiO_2 reduces its band gap with graphene oxide. The band gap reduction of TiO_2 was also reported with its nanocomposites of Ag-TiO_2/rGO. The same results were reported when TiO_2 was made composites with Fe. The band gap is associated of electronic transition between valance band and conduction band. Reduction of band gap increases photo activity of

$ZnO@TiO_2$ which can be described to introduce new impurity level between valance and conduction band of TiO_2, from that impurity electrons are promoted to conduction band. Therefore $ZnO@TiO_2$ has narrow band gap (3.78ev) then pure TiO_2 (3.68ev) which results increase absorption in visible region.

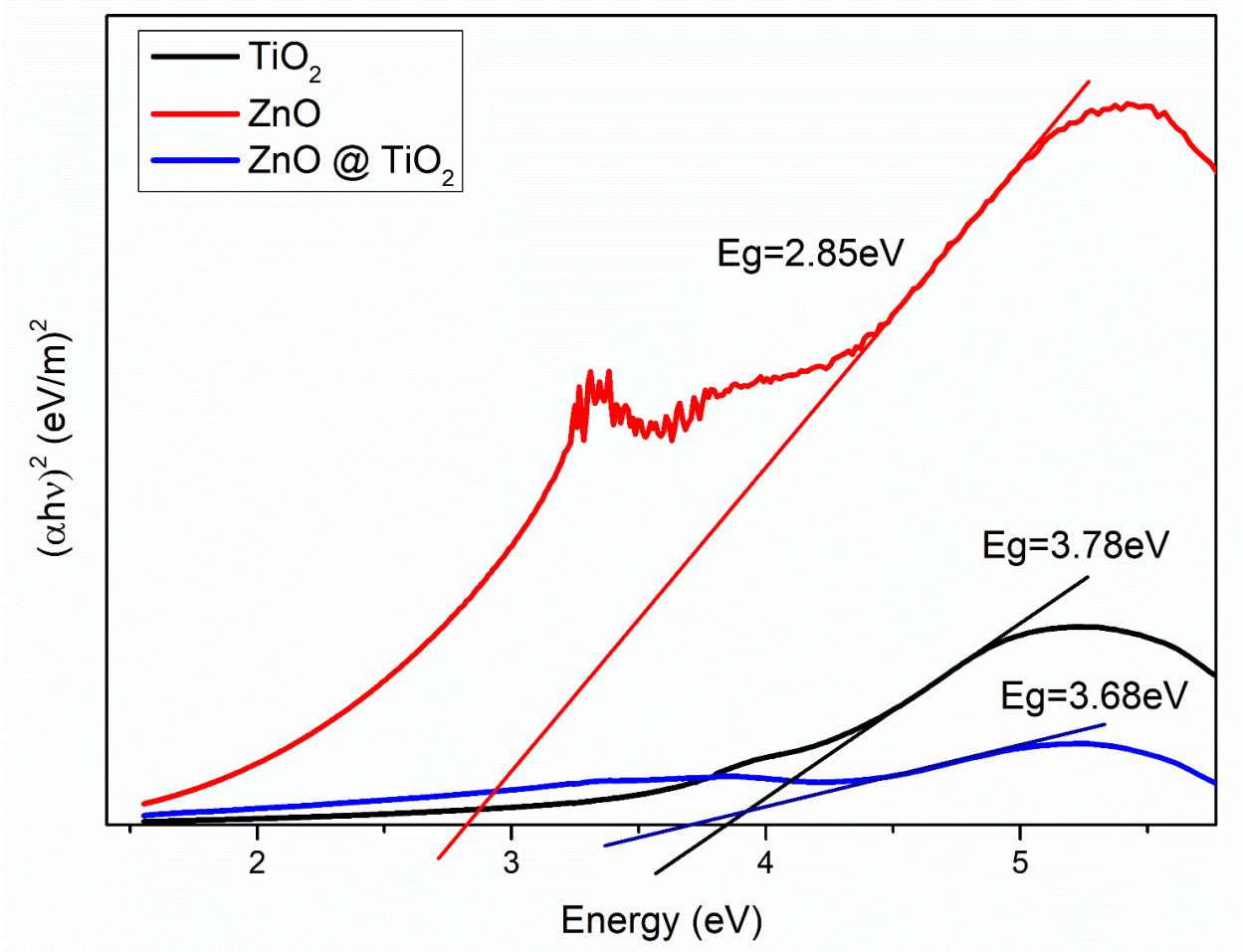


Fig. (4): Plot of $(\alpha h\upsilon)^2$ versus photon energy $(h\upsilon)$ of TiO_2, ZnO and $ZnO@TiO_2$ nanocomposites,

4. The results for the toxicity have been presented in Figure (5), where the absorption of light by the living cells is shown in case of untreated sample (UT), and TiO_2, ZnO and $ZnO@TiO_2$ nanocomposites. Metal oxide nanoparticles also showed their cytotoxicity against cancer cells through cellular oxidative stress.The cytotoxicity of each sample (TiO_2, ZnO and $ZnO@TiO_2$ NPs) may make sure their potentials. There was considerable difference in cell viability of all samples groups of NPs. However cell feasibility $ZnO@TiO_2$ was greater than naked samples. The up-regulation of intracellular reactive oxygen species (ROS) can produce oxidative stress

which may direct to apoptosis[6]. It has earlier been confirmed that metal oxide nanoparticles can carry apoptosis in human cancer cells by ROS generation. To clarify the mechanism the effectiveness TiO_2, ZnO and ZnO@TiO_2 NPs, ROS-generation assay was performed in HepG2 cells. Intracellular ROS levels were confirmed by detecting the population percentage of live cells.

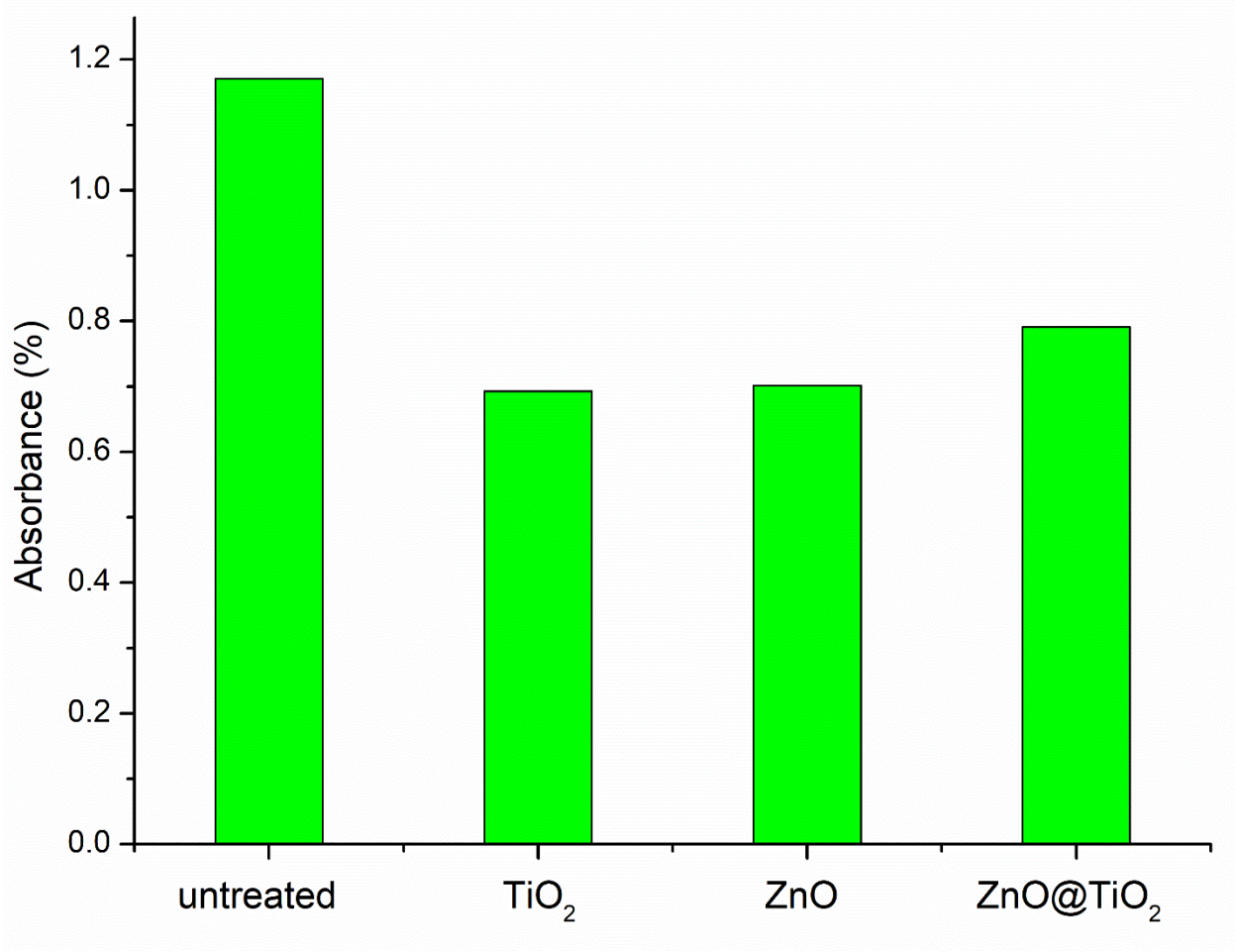


Fig. (5): Anticancer activity of TiO_2, ZnO NPs and ZnO-TiO_2nanocomposites in HepG2 cells

5. The population percentage of the composite ZnO@TiO_2 was significantly greater than those of Individual TiO_2 and ZnO NPs. The higher ROS level of the individual TiO_2 and ZnO compared with the composite ZnO@TiO_2 NPs may explain the improved cytotoxicity on HepG2 cells. The individual TiO_2 and ZnO exhibited a larger ROS level compared with the ZnO@TiO_2 NPs particles which may be due to their greater cellular contact and cytotoxicity

stroke mechanisms. The toxicity of nanoparticles also depends on important parameters for example crystalline nature, size and composition. The size of nanoparticles changes surface area which leads to adverse biological properties. So size of particle is one of the important parameter which affects the toxicity. It is also expected that the decreasing the cytotoxicity of ZnO@TiO_2 composites is due to increasing the size of particles. This decrease in cytotoxicity has been also observed in case when the oxide nanoparticles of one kind are covered with the second one, i.e., core shell structure, due to the less exposed surface area that may responsible for ROS generation. Similar effects of toxicity reduction has been seen when SiO_2 and ZrO_2 were used as composite materials with TiO_2. The cytotoxicity of TiO_2 and ZnO NPs was also larger than that of composites sample as shown in Figure (5). In gathering, in the case of in vivo applications, it is anticipated that individual TiO_2 and ZnO NPs may make stronger in vivo anticancer behaviour via ROS generation in tumour tissues. The cytotoxicity of their composites decreases due to increasing size of particles.

Conclusion:

In conclusion, the individual ZnO and TiO_2 nanoparticles and their nanocomposites have been formed by using co precipitation and sol-gel methods, respectively. It was observed that the crystallite size remain almost same for the particles in individual and nanocomposites form. The TiO_2 nanoparticles were formed in spherical form which ZnO got the rod shape. The optical band gap of TiO_2 NPs was 3.78eV and for ZnO it value found to be 2.85eV. When TiO_2 is formed with ZnO, then energy band gap value reduced to 3.68ev in comparison with pure TiO_2. It was also observed that the cell viability was more in composite then pure samples which concluded that anticancer activity of composite materials was decreased. ROS was mainly responsible for the damage of cell membranes of cancer cells and it is dependent on the ions production. The reduction in the free Ti and Zn ions in nanocomposites was the main reason for the weakened toxicity. The composite formation of ZnO and TiO_2 might have effectively reduced the exposed area for ROS generation and ultimately reducing the cytotoxicity of the nanomaterials for HepG2 cancerous cells.

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