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Research Article

Cytotoxic activity of zinc oxide/titanium dioxide nanoparticles on prostate cancer cells

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ABSTRACT

Prostate cancer is caused by uncontrolled growth of cells in the prostate gland. The aim of this study was to determine the cytotoxic activity of titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles (NPs) and TiO_2 + ZnO nanocomposite (NC) in human prostate cancer cell line (DU-145) and healthy mouse fibroblast cell line (L-929). In the study, TiO_2 and ZnO NPs and TiO_2 + ZnO NC were synthesized. Cytotoxic activities of NC and NPs was then analyzed in human prostate cancer cell line (DU-145) and healthy mouse fibroblast cell lines (L-929) using the MTT method. TiO_2, ZnO and TiO_2 + ZnO IC_{50} values were determined in DU-145 and L-929 cell cells (n = 6). TiO2 + ZnO NC in the Du-145 cell line was found as the most active, having statistically significant (***p < 0.0001, **p < 0.001 and **p < 0.01).

Keywords: Prostate cancer, Du-145, L-929, TiO_2/ZnO.

Çinko oksit/titanyum dioksit nanopartiküllerin prostat kanser hücreleri üzerinde sitotoksik aktivitesi

ÖZ

Prostat kanseri, prostat bezindeki hücrelerin kontrolsüz büyümesinden kaynaklanır. Bu çalışmanın amacı, insan prostat kanseri hücre hattında (DU-145) ve sağlıklı fare fibroblast hücre hattında (L-929) titanyum dioksit (TiO_2) ve çinko oksit (ZnO) nanopartikülleri (NPs) ve TiO_2 + ZnO nanokompozit (NK) 'in sitotoksik aktivitesini belirlemektir. Çalışmada TiO_2 ve ZnO NPs ve TiO_2+ZnO NK sentezlenmiştir. NK ve NPs' lerin sitotoksik aktiviteleri, daha sonra MTT metodu kullanılarak insan prostat kanser hücre hattı (DU-145) ve sağlıklı mouse fibroblast hücre hatlarında (L-929) analiz edilmiştir. DU-145 ve L-929 hücre hücrelerinde TiO_2, ZnO ve TiO_2+ZnO IC_{50} değerleri belirlenmiştir (n = 6). Du-145 hücre hattında Ti02 + ZnO NK, istatistiksel öneme sahip olarak en aktif olarak bulunmuştur (*** p < 0.0001, **p < 0.001 ve ** p < 0.01).

Anahtar Kelimeler: Prostat kanseri, Du-145, L-929, TiO_2/ZnO.

1. INTRODUCTION

Cancer is a serious health problem and it is one causes of deaths in the world.[1] Prostate cancer is the second most common type of cancer in men after lung cancer and is the fifth most common type of cancer worldwide.[2] The onset of prostate cancer is characterized by a relatively slow growth, low rate of progression, high incidence rate, presence of tumor markers, and detectable preneoplastic lesions.[3] Surgery is often successful for organ-limited prostate cancer. However, most prostate cancer patients after few years of treatment developed tumor regeneration and shorten their survival when tumor recurrence.[4] One of methods that use in treatment of prostate cancer is chemotherapeutic drugs. Etoposide, paclitaxel, vinblastine, mitoxantrone and estramustine some drugs like; can use in chemotherapy. However, these chemotherapeutic drugs have many side effects

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such as toxic, deaths, paralysis, blood clotting, leukocyte decline, difficulty breathing and fatigue. Therefore, in the prolonged treatment of prostate cancer, the discovery of new anti-cancer compounds is important to improve quality of life and to eliminate these side effects. The aim of anticancer drug development studies is to discover artificial and natural compounds that can be used in cancer treatment, as well as to reduce toxicity and maximize efficacy. In recent years, scientists have focused on the field of nanotechnology, which has made significant progress in developing pharmaceuticals for cancer treatment. Nanoparticles (NPs) are used as drug carriers that target the drug directly to the tumor area and in the same time maintaining healthy tissue. Compared to conventional chemotherapeutic drugs, nano-carriers have many advantages and offer various advantages as drug carriers.[5,6] Various inorganic NPs such as iron oxide, porous silica, graphene oxide and TiO_2 NPs have been used for successful drug delivery and treatment in cancer treatment.[7] TiO_2 and ZnO have many important properties. Some of these properties are biocompatibility, low toxicity, high chemical stability and specific photocatalytic and sonocatalytic. Therefore, even in the field of potential multimodal treatment agents, photodynamic and sonodynamic therapy methods used in the treatment of cancer have received great interest.[8] TiO_2 can produce highly reactive radical oxygen species under ultraviolet (UV) light irradiation and cause cancer cell death.[9] Based on its unique properties, TiO_2 NPs also play an important role in the drug delivery field of drugs used in the chemotherapeutic treatment.[10] ZnO is a semiconductor material which has gained considerable scientific interest due to its wide range of applications such as biomedical, optical, electronic and optoelectronics.[11] ZnO NPs offer biocompatibility advantages for use in biomedical applications where nanomaterials prepared using high colloidal dispersion in water and toxic chemicals are unsuitable.[12] In this study, we prepared a complex of TiO_2 + ZnO, and evaluated the cytotoxicity activity properties of this complex in human Du-145 and mouse L-929 cell lines.

2. MATERIALS AND METHODS

2.1. Synthesis of TiO_2, ZnO NPs and TiO_2 + ZnO nanocomposite (NC)

TiO_2, ZnO NPs and TiO_2 + ZnO NC were prepared as in our previous study.[13] In a typical synthesis step to prepare zinc oxide NPs, zinc nitrate hexahydrate and ascorbic acid were dissolved in distilled water at 1:0.3 molar ratios and heated on a hot plate at 300°C until a brown precipitate formed. The precipitate was crushed finely and calcined at 400°C.[14] The resulting powder was white and consisted of ZnO NPs. TiO_2 NPs were prepared using a similar process using titanium isopropoxide and glycine as the precursors.[15] To prepare NC, a suspension with water was prepared in a 1:1 weight ratio from the previously prepared ZnO and TiO_2 particles. The solution was sonicated for 1 h with a probe sonicator to prepare a high stability suspension. Afterwards, solution was heated at 100°C to obtain a dry powder, which is the composite of the samples. All samples were ultrasonicated 60 minutes to break any possible aggregation of NPs in a Probe Sonicator(Sonics & materials INC, USA). Stability of the samples was determined by measuring their zeta potantial values (Malvern Zetasizer Nano Z).

2.2 Cell Culture

Du-145 and L-929 cell lines were maintained in DMEM medium containing, 10% fetal bovine serum (FBS), penicillin and streptomycin (1%). Cells were incupated at 37°C, 5% CO_2 and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

2.3. Cytotoxic effect of TiO_2 + ZnO NC on Du-145 and L-929 cell lines

The cytotoxicity activity of TiO_2 and ZnO NPs and TiO_2 + ZnO NC in the Du-145 and L-929 cell lines was performed by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay according to Skehan's method.[16] The cells were then trypsinized and seeded in 96-well plates (Corning, USA) in 0.1 mL of complete culture medium at a density of 1 x 10^5 cells per well and allowed to bind for 24 h. 1 µl of test substance at concentrations ranging between 1-100 µM were added into each well containing the cells. The plates were incubated at 37°C with an internal atmosphere of 5% CO_2. After 24, 48 and 72 h incubation, 10 µl/well MTT (5 mg ml^{-1} dissolved in PBS) was added to the cells in the plates and incubated at 37°C for 2 h. The supernatant was carefully withdrawn from each well and 100 µl of DMSO was added to each well to dissolve formazan crystals. After mixing with a mechanical plate mixer for 15 min, the absorbance of plates were recorded at 570 nm on a microplate reader (Bio-Tek, USA). All these drug doses were tested in parallel in three replicates. Control samples were run with 1% sterilized water.

2.4. Statistical analysis

SPSS (Statistical Package for Social Sciences, ver: 25.0) program was used to evaluate the data in the study.

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All experiments were run in triplicate and the results expressed as mean ± SEM. The data were analyzed using one-way ANOVA and differences were considered significant (*p < 0.05, **p < 0.01 and **p < 0.0001).

3. RESULTS AND DISCUSSION

3.1. Characterization of TiO_2, ZnO NPs and TiO_2 + ZnO NC

It has been assumed that, under pure electrostatic interaction, a suspension that exhibits a zeta potential within ± 15 mv is considered unstable and tends to aggregate, from ± 15 mv to ± 30 mv it would be predominantly stable, and above ± 40 mv it would be well stabilized.[17] The zeta potential of synthesized NPs are given in Table 1 and are within the acceptable range for stability.

Table 1. Zeta potential of TiO_2, ZnO NPs and TiO_2 + ZnO NC

Nanoparticle	Zeta potential, mV
TiO_2	33
ZnO	29
TiO_2 + ZnO	24

3.2. Cytotoxicity activities of TiO_2 + ZnO on DU-145 and L-929 cell lines

Nanotechnology is a new technology that provides new uses for a wide range of biological and biomedical applications. Scientists are directed to this area because the size of the so-called nanoparticle is less than 100 nm.[18] Due to their superior targeting capabilities and efficacy, NPs are becoming increasingly important in modern cancer therapy. It has also begun to outperform traditional cancer treatments such as chemotherapy, radiation and surgery.[19] TiO2 and ZnO NPs are widely used metal oxide NPs because of their superior biomedical and biological properties.[20] ZnO NPs show high biocompatibility. Bulkier forms are generally considered safe by the FDA. Zinc is an important co-factor in many cellular mechanisms. Therefore, it plays an important role in maintaining cellular homeostasis; therefore, ZnO shows biocompatibility.[12] ZnO NPs exhibit selective cytotoxicity to cancer cells in vitro compared to many other NPs.[21] TiO_2 NPs are environmentally friendly, relatively stable, exhibiting excellent biocompatibility because they are smaller than cellular organelles.[22] These properties make TiO_2 NPs an excellent candidate for biomedical applications such as cancer treatment.[23] In this study, we evaluated the cytotoxic activity of TiO_2 and ZnO and TiO_2+ZnO NC in DU-145 and L-929 cells. The aim of this process is to utilize the synergistic effects of these NPs used in biomedical applications. In our study, Figure 1-3 show changes in cell inhibition for 24, 48 and 72 h versus increasing concentrations of DU-145 cell lines. Compared to the control group (DU-145 cell without test substance), TiO_2 + ZnO treated DU-145 prostate cancer cells showed significantly decreased tumor survival rate after 24 h, 48 h and 72 h of incubation (***p < 0.0001, **p < 0.001 and **p < 0.01). Cell survival rate in group after 24h, 48h and 72 h of incubation were significantly decreased than those in the control group. The survival rate of tumor cells was significantly reduced with TiO_2 + ZnO NC treatment time. TiO_2 + ZnO NC on DU-145 cells were the most active for 72 h of incubation. In addition, TiO_2 + ZnO and IC_{50} values for 24 h, 48 h and 72 h were 3.78 ± 2.08 µM, 3.12 ± 0.65 µM and 1.38 ± 0.79 µM respectively (Table 2). We also tried to determine the cytotoxic activity of these molecules in the L-929 cells for health control purposes. We have found that these NPs are more active in the L-929 cell line than in the control group (L-929 cell without test substance). IC_{50} values of TiO_2 + ZnO after 24 h, 48 h and 72 h incubation were 24.44 ± 1.09 µM, 20.63 ± 1.44 µM and 19.36 ± 3,04 µM in L-929 cells, respectively (Table 2).

Table 2. Comparison of IC50 values between TiO_2+ZnO NC, ZnO and TiO_2 NPs on Du-145 and L-929 cells after 24 h, 48 h and 72 h of incubation

	Du-145 cell line IC_{50} (µM ± SD*)		
	24 h	48 h	72 h
TiO_2 + ZnO	3.78±2.08	3.12±0.65	1.38±0.79
ZnO	70.14±3.41	62.19±2.26	22.43±5.02
TiO_2	11.68±0.69	4.99±1.08	1.12 ± 0.16
	L-929 Cell Line IC_{50} (µM ± SD*)		
	24 h	48 h	72 h
TiO_2 + ZnO	24.44±1.09	20.63±1.44	19.36±3.04
ZnO	67.27±2.78	63.56±3.09	53.63±1.08
TiO_2	19.14±0.87	15.00±1.13	13.45±0.25

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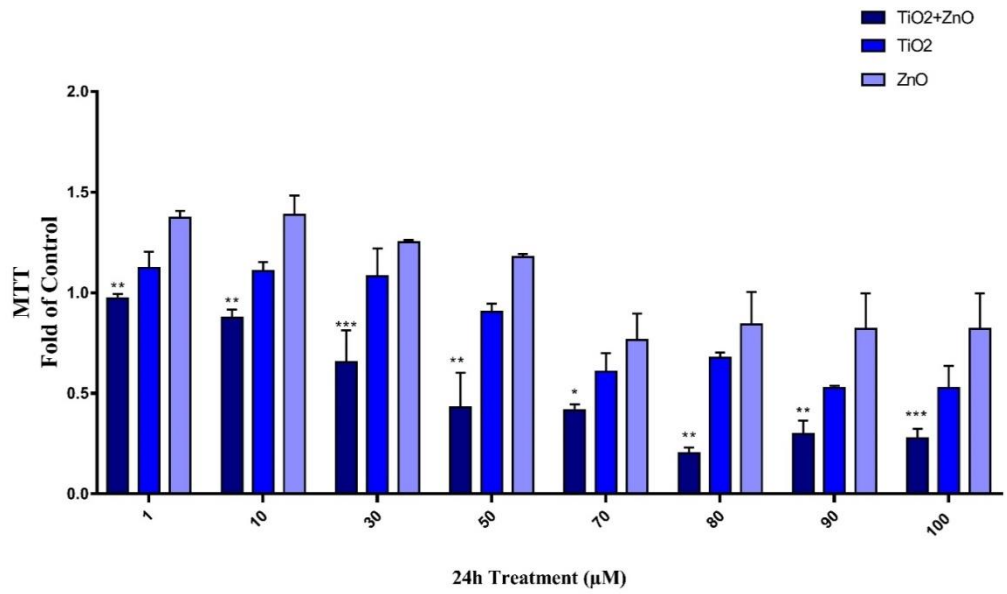

Figure 1. Cytotoxicity activity study of TiO2 + ZnO, TiO2 and ZnO in Du-145 cells. Du-145 cells were treated with these molecules for 24 h in a concentration range of 1 to 100. These molecules were compared with control. Represents the mean ± SEM of three separate experiments (***p< 0.0001 , **p < 0.001 and **p < 0.01).

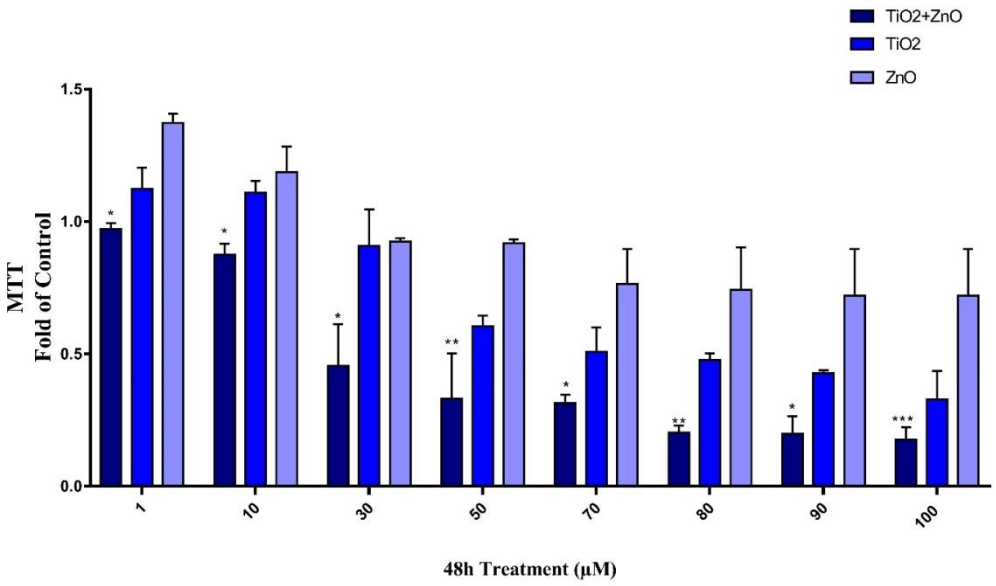

Figure 2. Cytotoxicity activity study of TiO_2 + ZnO, TiO_2 and ZnO in Du-145 cells. Du-145 cells were treated with these molecules for 48 h in a concentration range of 1 to 100. These molecules were compared with control. Represents the mean ± SEM of three separate experiments (***p < 0.0001 , **p < 0.001 and **p < 0.01).

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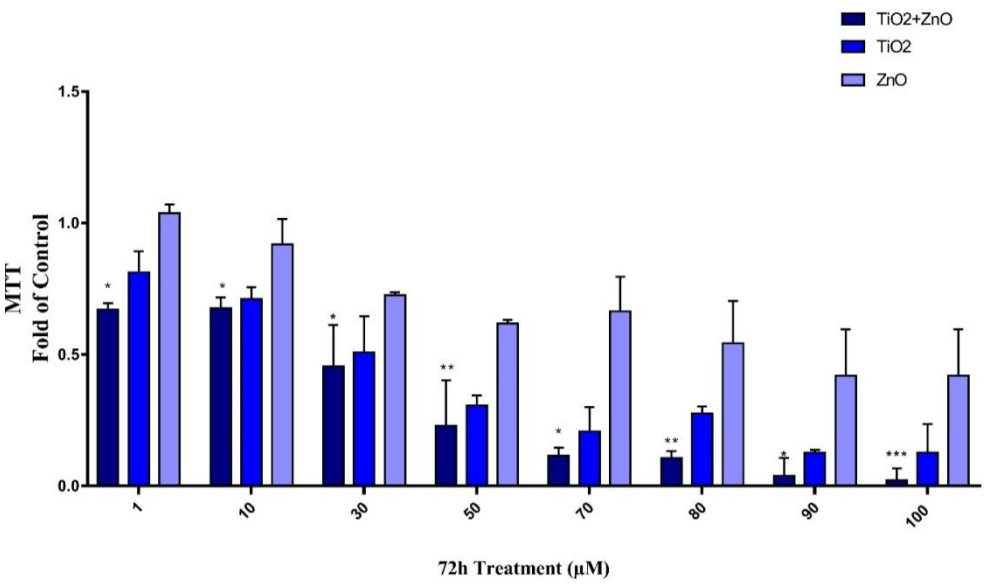

Figure 3. Cytotoxicity activity study of TiO_2 + ZnO, TiO_2 and ZnO in Du-145 cells. Du-145 cells were treated with these molecules for 72 h in a concentration range of 1 to 100. These molecules was compared with control. Represents the mean ± SEM of three separate experiments. (***p < 0.0001, **p < 0.001 and **p < 0.01).

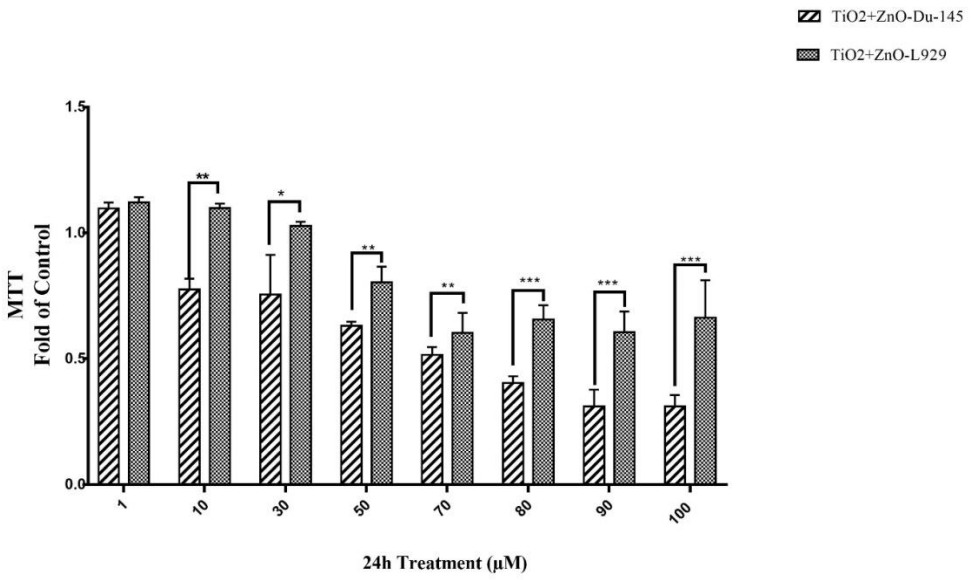

Figure 4. Cytotoxicity study of TiO_2 + ZnO on Du-145 and L-929 cells. Du-145 and L-929 cells were treated with these drugs for 24 h in a concentration range of 1 to 100 TiO_2 + ZnO. TiO_2 + ZnO, which is more active than TiO_2 and ZnO, were compared with Du-145 cells and L-929 cells. Represents the mean ± SEM of three separate experiments (***p < 0.0001 , **p < 0.001 and **p < 0.01).

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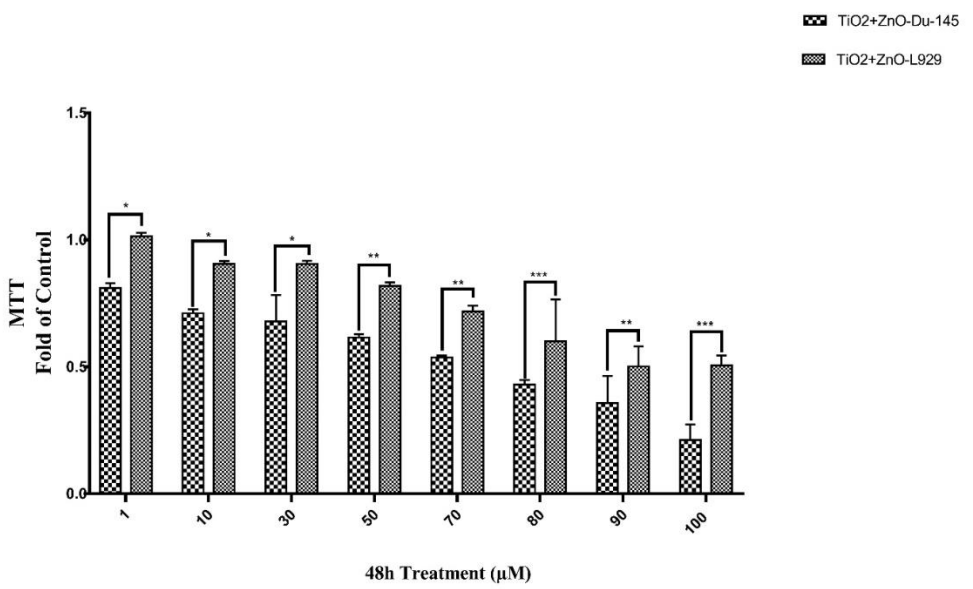

Figure 5. Cytotoxicity study of TiO_2 + ZnO on Du-145 and L929 cells. Du-145 and L-929 cells were treated with these drugs for 48 hours in a concentration range of 1 to 100 TiO_2 + ZnO. TiO_2 + ZnO, which is more active than TiO_2 and ZnO, were compared with Du-145 cells and L-929 cells. Represents the mean ± SEM of three separate experiments (***p < 0.0001 , **p < 0.001 and **p < 0.01).

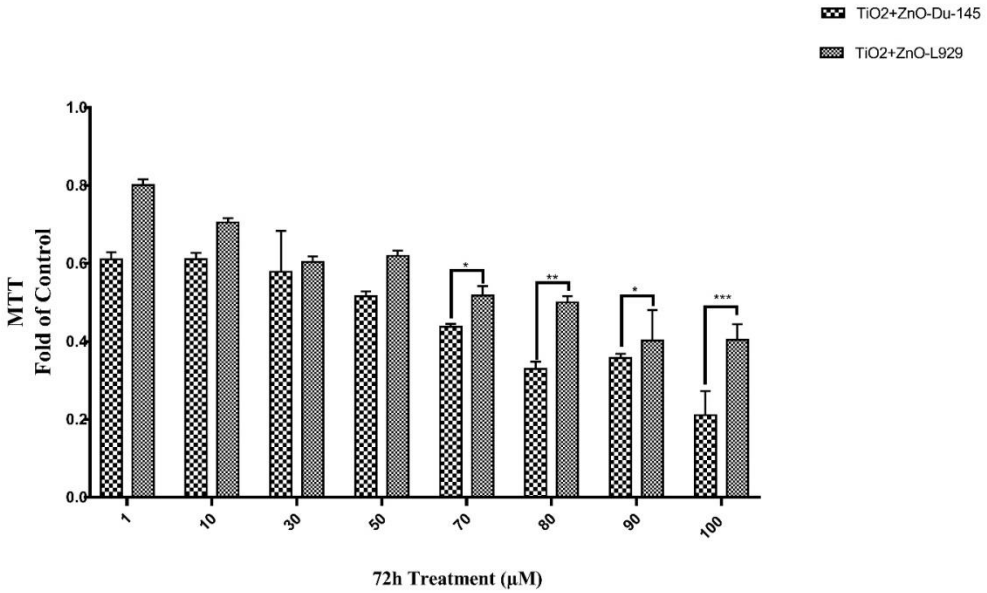

Figure 6. Cytotoxicity study of TiO_2 + ZnO on Du-145 and L-929 cells. Du-145 and L-929 cells were treated with these drugs for 72 h in a concentration range of 1 to 100 TiO_2 + ZnO. TiO_2+ ZnO, which is more active than TiO_2 and ZnO, were compared with Du-145 cells and L-929 cells. Represents the mean ± SEM of three separate experiments (***p < 0.0001 , **p < 0.001 and **p < 0.01).

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Cytotoxic activity of TiO_2 + ZnO was high after 72 h in the L-929 cell line. However, we found that TiO_2 + ZnO cytotoxic activity was more active in DU-145 cells compared to L-929 cells after 24 h, 48 h, and 72 h incubation (Figures 4-6). We found this difference statistically significant. (***$p < 0.0001$, **$p < 0.001$ and **$p < 0.01$). As a result, we noted that the synergistic effect of NPs showed more cytotoxic activity in cancer cells. In our previous study in human cervical cancer (HeLa) cells, we found similar results.[13] Fadoju and co-workers support the results of our study.[24]

4. CONCLUSIONS

In summary, TiO_2 + ZnO NC was synthesized successfully. The synthesized NPS and NC maintained their stability and are within the acceptable range for stability. This study demonstrates the possibility of using TiO_2 + ZnO to inhibit the growth of DU-145 cancer cells with therapeutic treatments. Healthy L-929 cells were used as the control of DU-145 cells. The cytotoxic activity of TiO_2 + ZnO molecule was highest in DU-145 cancer cells after 72 h incubation. Therefore, we found that the synergistic effect of TiO_2 and ZnO NPs was higher in the cancer cell. By utilizing this synergistic effect, TiO_2 + ZnO NC based drugs can be developed.

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Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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