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CYTOTOXIC EFFECT OF TIO_2 NANOPARTICLES ON BREAST CANCER CELL LINE (MCF-7)

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ABSTRACT

The aim of this study was to evaluate the cytotoxic effects of TiO_2 Nanoparticles on MCF-7 Breast cancer cell line and human white blood cells (WBC) in vitro was examined. The cells were cultured in RPMI1640 liquid medium with 10٪ inactivated fetal bovine serum (FBS) and antibiotics; and were cultured with various concentrations of TiO_2 Nanoparticles (20 ,50 ,80 ,110 ,140 ,170 and 200 µg/ml) for 24, 48 and 72 hours and were analyzed using the MTT test.The absorbance was then determined by ELISA reader at 570 nm. The percentage growth inhibition was calculated. The data were analyzed using SPSS software. Results showed that TiO_2 Nanoparticles , had weakly inhibatory effect on MCF-7 cell line, after 24h incubation (from 0.58 to 3.00%). After 48h incubation, results showed significant effect on MCF-7 cell in concentrations of 140, 170 and 200 µg/ml compared with control, with 27.03, 41.30 and 56.33 percent of cell growth inhibition respectively($p<0.05$). Also, the TiO_2 Nanoparticles at 110 µg/ml and upper doses, suppressed the proliferation of cancerous MCF-7 cells by significant diffrence with control group, after 72h incubation ($p<0.05$). Maximum inhibitory effects were found at 200 µg/ml (84.58%). In the other hands, results showed significant effect on WBC cells in concentration of 200 µg/ml compared with control, after 72h with 25.59% of cell growth inhibition($p<0.05$). Overall, results showed the TiO_2 Nanoparticles dose and time-dependently suppressed the proliferation of MCF-7 cells.

KEY WORDS
Cytotoxic effect, Titanium Dioxide nanoparticles, MCF-7 cancer cell lines, MTT test

Published: 1 October 2016

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INTRODUCTION

Now a day cancer is the second most common cause of death in the world. Breast cancer is the most common malignancy among women and the most frequent cause of female cancer deaths after lung and stomach cancers. An estimated 40,430 breast cancer deaths (40,000 women, 430 men) are expected in 2014 [1]. According to reports of the Iranian Ministry of Health and Medical Education (MOHME), cancer is the third cause of death in following coronary heart disease and accidents [2]. Despite a marked decrease in mortality rate of breast cancer, especially developed countries, it is still a major challenge for health policy making in developing countries such as Iran. In Iran, breast cancer ranks first among female cancers comprising 24.4% of all malignancies in women [3].

Treatment of patients with breast cancer remains a clinical challenge due to the disappointing effects of most chemotherapies. The efficacy of chemotherapy is limited and patients have to suffer from serious side effects, some of which are life-threatening. Therefore, focus is now towards controlled and targeted drug delivery systems. Nanoparticles can provide a controlled and targeted way to deliver the encapsulated anticancer drugs and thus result in high efficacy with low side effects [4]. Hence we need to find new materials for treatment and cure. Nanoparticles and molecules are a potential alternative for treatment of disease because of their unique biological effect.

Titanium dioxide (TiO_2) nanoparticles has been increasingly employed in a variety of industrial applications including production of paper, plastics, cosmetics, and paints. Also the photocatalytic properties of TiO_2 have led to extensive research into its potential uses as a disinfectant, antibiotic, biological sensor, tumor cell–killing agent, and gene targeting device [5].

Thevenot et al. [6] was examined the non photocatalytic anticancer effect of TiO_2 on in vitro survival of several cancer and control cell lines. They confirmed that the cell viability depend on particle concentrations and suggested that TiO_2nanoparticles can be surface-engineered for targeted cancer therapy.

This paper deals with the influence of the TiO_2 nanoparticles on MCF-7 cell line and white blood cells growth inhibition, at various concentrations and for various treatment periods *in vitro*. MCF-7 cell line was selected since breast cancer is one of the most common tumors found worldwide. Furthermore, in the primary malignancy of the breast this cell line has been widely used as the human breast cancer model cell line in the development of new anti-tumor medicines. The aim of the study is to use *in vitro* studies to evaluate the cytotoxicity activity of TiO_2 nanoparticles. This further helps to find the appropriate anti-cancer medicine for *in vivo* studies.

MATERIALS AND METHODS

Suspensions preparation and Characterization of TiO_2nanoparticles

TiO_2Nanoparticles, with partial size 10-25 nm, were purchased from P25-DEGUSSA company, Germany (Nano-Rahpouyane Mahan Iranian company).Purified TiO_2Nanoparticles were characterized for Morphology, Particle size, zeta potential and polydispersity index (PDE). Morphology was determined by Scanning Electron Micrograph (SEM), particle size (Photon Correlation Spectroscopy), Zeta potential and PDE using zeta sizer (Delsi Nano,C, Beckman Coulter). TiO_2anoparticles were suspended in ultrapure water, sonicated (1min) and diluted in cell culture medium to optimize suspension stability.

Evaluation of in vitro cytotoxic activity of the TiO_2nanoparticles on cell lines

The cytotoxicity of TiO_2Nanoparticles on MCF-7 cell line and white blood cells was determined by the MTT assay. Cells were seeded in 96-well tissue culture plates. Stock solutions of TiO_2Nanoparticles (5 mg/ml) were prepared in sterile distilled water and diluted to the required concentrations (20, 50,80,110, 140, 170and 200 µg/ml) using the cell culture medium.

Cell cultures

MCF-7 cancer cell line were purchased from Pasteur Institute of Iran (NCBI C135). The cells was grown and maintained in a humidified incubator at 37 °C and in 5% CO_2 atmosphere. RPMI-1640 medium (SIGMA) was supplemented with 0.01 mg/ml heat inactivated Fetal Calf Serum (FCS), 100 units/ml penicillin, and 100 µg/ml streptomycin (ALL FROM INVITROGEN GIBCO) was used for cell cultures. Upon reaching confluency, the cells were passaged. After being harvested from sterile T75 culture flasks, the cells were counted using a hemocytometer and cell viability was determined by trypan blue exclusion. Three thousand cells from log phase cultures were seeded in 100 µl of RPMI medium supplemented with 10% fetal bovine serum per well of 96-well flat-bottom culture plates. Cells were incubated with the TiO_2Nanoparticles solution for a defined time (72 hours). Proliferative response and cell death of the TiO_2Nanoparticles treated cells were determined using MTT assay and cell death ELISA Cell Viability Assay, respectively.

MTT Cell viability assay

Growth of tumoral cells quantitated by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl- 2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide (MTT) to a blue formazan product. At the end of 24, 48 and 72 hours incubation, the medium in each well was replaced by MTT solution. 20 µl of MTT solution (5 mg/ mL) in phosphate buffer saline (1/10 of total volume in a well) was added to wells. Then, the plates were incubated for 4 hours under 5% CO_2 and 95% air at 37°C. MTT reagent was removed and the formazan crystals produced by viable cells were dissolved in 100 DMSO and gently shaken. The absorbance was then determined by ELISA reader at 570 nm. The percentage growth inhibition was calculated using following formula,

% cell inhibition = 100- [(At-Ab)/(Ac-Ab)] × 100

Where, At = absorbance value of test compound, Ab = Absorbance value of blank and

Ac = Absorbance value of control.

The effects of TiO_2Nanoparticles were expressed by IC50 values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells).

Statistical Analysis

The data are expressed as mean ± standard deviation (SD) for at least three independent determinations in triplicate for each experimental point. The data were analyzed using IBM SPSS Statistics 20 software. For all the measurements, one-way ANOVA followed by Duncan's New Multiple Range Test ($P \leq 0.05$) was used to assess the statistically significance of difference between control and treatments.

RESULTS

Cell growth inhibition Analysis of TiO_2Nanoparticles (MTT Assay)

In this study, the growth of human MCF-7 cell line were treated with various concentrations of TiO_2 nanoparticles ranging from 20 to 200 µg/ml, and the cell growth inhibition was measured by the MTT assay.The experimental data are summarised in [Table 1] with data presented as mean values ± standard deviations.

Results showed that cell growth inhibition was induced in atime-dependent manner after 24, 48 and 72h exposure of human MCF-7 cell line to nanoparticles using the MTT assay, andas the concentration of TiO_2 nanoparticles is increased from 20 to 200 µg/ml, the cell viability decreased, in a dose-dependent manner.

After 24 h, the various concentrations of TiO_2 nanoparticles had not significant suppression on the proliferation of MCF-7 cell line. The cell growth inhibition of MCF-7 cell line significantly decreased after 48h exposure to TiO_2nanoparticles of concentrations 140, 170 and 200 ($P< 0.05$) with 27.03, 41.30 and 56.33 percent of inhibition respectively.Also, after 72h incubation, the MTT assay of TiO_2Nanoparticles

showed significant effect on MCF-7 cell in concentration of 80 µg/ml and upper doses compared with control and the value of cell growth inhibition percentage were 67.15, 71.89, 74.60, 83.44 and 84.58, respectively(P<0.05). The highest cytotoxicity of this solution against MCF-7 cell was obtained 84.58 percent of cell growth inhibition by 200 µg/ml after 72h[Table 1].

The IC50 value for TiO_2nanoparticles after 24h is 163.7 µg/ml, after 48h is 7.16 µg/ml and after 72h is6.73µg/ml. As seen, when the time of incubation increases, the IC_{50}values decreases (Table 1).
Overall, after exposure to TiO_2, a time- and dose-dependent significant increase in cell growth inhibition, with MTT test [Fig.1] was observed.

The white blood cell viability for TiO_2 nanoparticles of 200 µg/ml after 24, 48 and 72h incubation are given in Figure 2. Results showed that white blood cell viabilitywere73.94, 63.33 and 74.40% after 24, 48 and 72h incubation, respectively [Fig. 2].After48 h incubation with TiO_2 nanoparticles had shown more suppression effect on the viability of white blood cells as a comparison with the control group (63.33 %) (P<0.001) .

Table 1: Cytotoxicity activity of TiO_2 NPs against MCF-7 cell line and white blood cells at different concentrations by MTT assay. Values represent the mean of three experiments.

Concentrations of TiO_2NPs (µg/ml)	Absorbance	Inhibition (%)	IC_{50} (µg/ml)	SEM	P-value
After 24h incubation					
Control of WBC	0.454 ±0.017	-		0.017	-
WBC	0.358 ±0.018	26.064 ±0.046		0.033	0.066
Control	0.155 ±0.012	-		0.008	-
20	0.153 ±0.001	0.580 ±0.141		0.100	0.819
50	0.152 ±0.037	0.620 ±0.504		0.365	0.924
80	0.151 ±0.025	0.858 ±0.456		0.322	0.864
110	0.152 ±0.010	1.095 ±0.272		0.192	0.828
140	0.151±0.034	1.411 ±0.495		0.350	0.890
170	0.151±0.023	1.885±0.419	163.7	0.296	0.841
200	0.152 ±0.017	3.003±0.224		0.158	0.811
After 48h incubation					
Control of WBC	0.271 ±0.010	-		0.007	-
WBC	0.187±0.006	36.674±0.004**		0.002	0.001
Control	0.097 ±0.005	-		0.0004	-
20	0.089 ±0.016	14.938 ±0.245	7.166	0.173	0.475
50	0.089 ±0.015	15.300 ±0.236		0.167	0.463
80	0.085 ±0.012	21.754 ±0.194		0.137	0.252
110	0.085 ±0.006	22.605 ±0.088		0.062	0.074
140	0.082 ±0.004	27.038 ±0.070*		0.049	0.029
170	0.074 ±0.001	41.307 ±0.009**		0.006	0.0006
200	0.066 ±0.013	56.332 ±0.194*		0.137	0.033
After 72h incubation					
Control of WBC	0.380 ±0.009	-		0.006	-
WBC	0.294 ±0.074	25.597 ±0.164		0.116	0.599
Control	0.335 ±0.069	-		0.049	-
20	0.205 ±0.004	41.726 ±0.086	6.730	0.061	0.081
50	0.197 ±0.039	44.577 ±0.158		0.111	0.114
80	0.137 ±0.028	67.154 ±0.040*		0.028	0.014
110	0.120 ±0.008	71.899 ±0.041*		0.029	0.028
140	0.111 ±0.007	74.606 ±0.051*		0.036	0.034

170	0.086 ±0.004	83.443 ±0.038*		0.027	0.025
200	0.082 ±0.002	84.585 ±0.024*		0.017	0.025

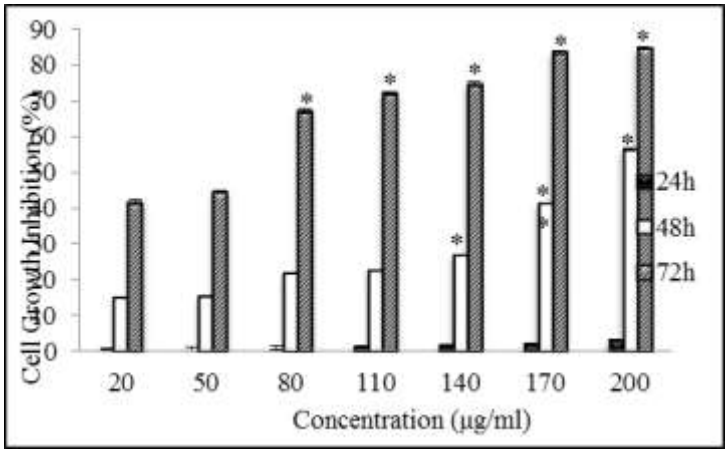

Fig. 1: Effect of different concentrations of TiO_2 NPsagainst MCF-7 cell line in 24, 48 and 72 h

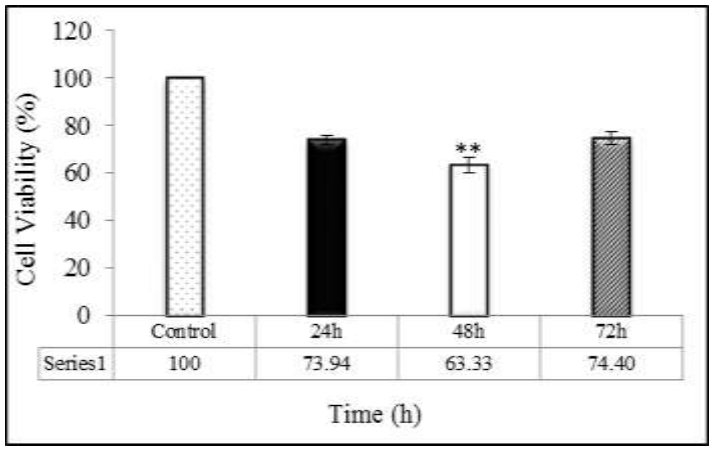

Fig. 2: Effect of TiO_2NPs (200 µg/ml)on viability ofwhite blood cell in 24, 48 and 72

DISCUSSION

TiO_2 nanoparticles are widely used for industrial and medical applications [7, 8]. Since nanoparticles can interact with cell membranes and intracellular organelles in a manner not totally understood, there are increasingly concerns about the adverse health effects of TiO_2 and other nanoparticles. The use of *in vitro* assays of cellular viability in different cancer cell lines is essential for evaluating the potential toxicity of the nanoparticles to discovery of cancer medicins [9]. In this study, we employed toxicity studies based on MTT method. It was seen that TiO_2nanoparticles induced a massive toxicity in MCF-7 cell line at 72 hours. Also, the present study showed that TiO_2 nanoparticles may be an alternative chemotherapeutic agent for treatment of cancer and with higher nanoparticle concentrations and longer exposure times the percentage of cell growth inhibition increased.

The mechanism of cytotoxicity induced by TiO_2 nanoparticles is also of scientific interest to researchers. For example Lozano et al. [9] reported this could be due to either the presence of small aggregates formed by TiO_2nanoparticles, which could have greater interaction with the cell causing its death.

Also some researchers reported that TiO_2 particle-mediated tissue toxicity is potentially via particle:cell interactions, presumably related to the surface properties of the TiO_2 particles [10]. It has been reported in several *in vivo* studies that TiO_2 can migrate in the bloodstream via binding to plasma proteins, through the lymphatic system after phagocytosis by macrophages, or to the bone marrow via monocytes [11, 12]. The specific interactions between TiO_2 particles and proteins are not well understood. Studies have revealed that TiO_2 particles have a net negative charge (at pH = 7) [13] and also bind preferentially to amino acids containing –OH, -NH, and $–NH_2$ in their side chains [14]. These findings indicate that TiO_2 particles may react with cell membrane proteins and contribute to cell:particle interaction.

There are several studies on mechanism of toxicity of nanomaterials which have shown that oxidative stress, lipid peroxidation and reaction of DNA with ROS has an important role on DNA damage, destruction of membrane and finally cell death [15, 16, 17, 18]. Nanomaterials play an significan trole in DNA damage, membrane destruction and finally cell death via oxidative stress and lipid per-oxidation [19]. which can develop effective circumstances to destroy tumor with least side effects [20]. Among nanomaterials, TiO_2 is a biocompatible agent which causes inflammation in nano-domain [19], and leads to cell toxicity by super-oxide, H_2O_2 and free hydroxyl radical formation in mammalians [20]. Increasing free radicals due to induction of oxidative stress activates necrosis and apoptosis reactions and finally leads to cell death [18, 21]. TiO_2 is a biocompatible material which in nano size causes some inflammatory effects [15]. Some evidence showed that nano-TiO_2 causes H_2O_2 and hydroxyl free radical formation which result to cell toxicity in mammals [19, 20, 21]. This nanoparticle interacts with water molecules in cell medium and using electron capture pathway, produces free radicals specially ROS [22]. The exact mechanism of free radical production by TiO_2 has not determined yet, but several studies have shown that this crystals aggregate in mitochondria and cause some defect in electron chain and destruct its function. This leads to more and more production of free radicals.

Rezaei-Tavirani et al. [23] findings support that TiO_2 nanoparticles has a high effect on the breast cancer cell line. There exists a wide variety of literature on cytotoxicity induced by TiO_2. These include fibroblast and epithelial cells [24, 25], kidney cells [26], neuroblast cells [25] and endothelial cells [27]. All of these studies relate this toxicity to ROS production. Some studies consider synergistic effect of UV radiation and TiO_2 nanoparticles on several cells such as CHO [28], glioma [29] and HeLa [30]. These studies relate the enhanced cytotoxicity to the capability of this nanoparticle to react with water molecule in cell medium and yield ROS via electron capture pathway.

Several authors have investigated the cytotoxic effects of TiO_2 nanoparticles on white blood cells. In particular, the cytotoxic effects of P25 Degussa TiO_2 nanoparticles were studied on human peripheral blood lymphocytes by king et al [21] that reported a dose- and time-dependent reduction in cell viability. In this investigation cell viability decreased from 73.94 to 63.33% after 24 to 48h in a time-dependent, but after 72h the cell viability rebounded to 74.40%. Also in a subsequent study [31], the same researchers found that P25 Degussa TiO_2 nanoparticles caused apoptosis of human peripheral blood lymphocytes. Similar effects were also observed in macrophage cell lines, particularly a decrease in cell viability and an increase in apoptosis [32].

In conclusion, this study represents a novel observation on influence of TiO_2 particles on cancer cell deeth. These interesting results suggest that TiO_2 nanopparticles may provide a degree of toxicity based on the interactions between the time and it's concentrations. However, more studies are needed to elucidate the effects of TiO_2nanoparticles, and to clarify the mechanisms of the TiO_2 nanoparticles toxicity on cells, with the aim of developing new strategies for the treatment of cancer and other illness.

CONFLICT OF INTEREST
There is no conflict of interest

ACKNOWLEDGEMENTS
None

FINANCIAL DISCLOSURE
None

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