doi.org/10.36721/PJPS.2020.33.3.SUP.1199-1207.1

Genotoxic and cytotoxic of biosynthesized titanium dioxide nanoparticles against CaCO2 and MDA-MB-231 cell lines

Magdah Ganash
Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract: Till now there is a general lack of information concerning the genotoxic and cytotoxic effects of nanoparticles. Titanium dioxide nanoparticles (TiO_2NPs) were synthesized using *Trichoderma harzianum* and characterized by Fourier Transmission Infrared spectroscopy, Ultra-Violet visible spectroscopy and Transmission Electron Microscope. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay revealed the concentration-dependent cytotoxic effects of TiO_2NPs in a concentration range of 7.8 to 500 µg/ml. The reduction in proliferation of CaCO2 and MDA-MB-231 cell lines was observed in a concentration dependent manner which became more clear especially at higher (500 µg/ml) concentrations of TiO_2NPs. The anti-proliferative effect of TiO_2NPs treatment was more potent on CaCO2 than MDA-MB-231 cells, where the IC_{50} was 124 and 107 µg/ml respectively. Exposure of CaCO2 cell line to 7.80, 15.60, 31.25, 62.50, 125, 250 and 500 µg/ml of TiO_2NPs showed an increase by 1.10, 1.54, 1.83, 2.64, 3.76, 4.76 and 5.21 fold respectively in SOD activity with respect to untreated control. The effect of low concentrations of TiO_2NPs up to 62.5 µg/ml was weakly induced the release of LDH, while followed these concentrations become high. DNA "laddering" pattern in CaCO2 cells treated with TiO_2NP is one of the reasons for cell death.

Keywords: Biosynthesis, titanium dioxide nanoparticles, cytotoxicity, genotoxicity.

INTRODUCTION

Biosynthesis methods of nanoparticles are a promising alternative compared to chemical methods, during the last and current years, nanoparticles biosynthesis methods including microorganisms as well as plant extracts (Abdel-Ghany *et al*., 2013; Benakashani *et al*., 2016; Abdel-Ghany *et al*., 2018). There are several metals nanoparticles were synthesized sush as silver, copper, gold, titanium and others, however titanium dioxide nanoparticles (TiO_2NPs) are between the top five NPs utilized in consumer products, their annual production in the USA alone is evaluated at 3800-7800 tonnes and is continuously rising in production (Shi *et al*., 2013; Boland *et al*., 2014; Carrouel *et al*., 2020; Ziental *et al*., 2020). Green methods for the production of TiO_2NPs have been progressed with using many sources involving yeasts such as *Saccharomyces cerevisae* (Jha *et al*., 2009), fungi (Rajakumar *et al*., 2012), bacteria such as *Lactobacillus sp.* and *Bacillus mycoides* (Jha *et al*., 2009; Órdenes-Aenishanslins *et al*., 2014) and plant extracts (Rajakumar *et al*., 2012).

Several applications of TiO_2 involve air, water and surface cleaning were reported (Pan *et al*., 2010). Titanium is selected for desalinization plants due to its potent resistance to corrosion by salt water. For non-reactive nature of titanium pins when contacting bone and flesh, its recommended in surgical proceses as well as other medical applications (Prasad *et al*., 2007). Mondal *et al*. (2013) and Gao *et al*. (2014) also stated that TiO_2 is used in biomaterials due to of its good stability, microbiocdal and anticorrosive activities. Therefore, several scientific papers concentrated towards synthesis of nanoform of TiO_2. TiO_2NP is an important product in industrial field that is excessively used as an additive in cosmetics, medicinal drugs, and food colorants (Jin *et al*., 2008; Weir *et al*., 2012; Órdenes-Aenishanslins *et al*., 2014; Sohal *et al*., 2020) as well as agriculture sector (Faraz *et al*., 2020).

Green synthesis of TiO_2NPs were accomplished utilizing extract of *Aspergillus flavus* as a reducing and capping agent which evidenced to be idealistic antibacterial compound against *E. coli* (Rajakumar *et al*., 2012), *S. aureus, E. coli, Salmonella typhimurium* and *Klebsiella pneumoniae* (Hassan *et al*., 2012). Recently, antimicrobial properties of TiO_2NPs was reported (Subhapriya and Gomathipriya 2018). Using of TiO_2NPs are based on their toxic properties contra pathogens like bacteria (Rajakumar *et al*., 2012) and mites (Marimuthu *et al*., 2013). Furthermore, biocompatibility studies on TiO_2NPs have been achieved and their biocidal/photocatalytic activity on aquatic biofilms has been evaluated *in vivo* and *in vitro* (Dhandapani *et al*., 2013). Liu *et al*. (2013) propose that interactions of chemicals or physical factors with TiO_2NPs may consequence in an elevate in toxicity or adverse effects, such as photo-catalytic activity of TiO_2NPs that inhibit the fungal colonization of wood specimens for long periods when compared to untreated ones (Filpo *et al*., 2013).

From the available demonstration mentioned by Chen *et al*. (2014), TiO_2NPs stimulate genotoxicity mainly through creating oxidative stress in cells. Also, in human monoblastoid and bronchial epithelial cells, the TiO_2NPs

*Corresponding author: e-mail: mganash@kau.edu.sa

stimulate apoptosis mainly by destabilizing the lysosomal membrane and lipid peroxidation (Zhao *et al*., 2009). Helinor *et al*. (2009) indicated that TiO_2NPs responsible for DNA harm and induction of cell death that was due to it stimulated the oxidative stress, TiO_2NPs were generated of reactive oxygen species (ROS) and decreasing cellular compounds responsible for antioxidants like vitamin E and glutathione. DNA damage and apoptosis induced by TiO_2 have also been elucidated in human lymphocytes, U937 human monoblastoid cells, A549 alveolar epithelial cells, NRK-52E normal rat kidney cells, and A431 human epidermal cells (Barillet *et al*., 2010; Shukla *et al*., 2011). Cytotoxicity and DNA damage induced by TiO_2NPs have been investigated also by Saquib *et al*. (2012) using human amnion epithelial cells, as an *in vitro* model , cells exposed to TiO_2NPs exhibited significant decreasing in catalase activity and glutathione concentration. And more recently, Biola-Clier *et al*. (2017) focused on the genotoxicity of TiO_2NPs and their negative imbact both major DNA repair mechanisms: base excision repair and nucleotide excision repair. Furthermore,Vevers and Jha (2008) have reported the correlation between TiO_2NPs and enhancing DNA damage in presence of UV light in rainbow trout gonadal tissue cells. Whereas, in the goldfish skin cells, the TiO_2NPs caused DNA damage in the absence of UV light. The cytotoxicity effect of TiO_2NPs on human colon carcinoma cells in the existence of UVA light was dose and time-dependent (Zhang and Sun 2004).The current research aim to biosynthesis of TiO_2NPs, its characterization and role in cytotoxicity against cancer cells.

MATERIALS AND METHODS

Myco-biosynthesis of TiO_2NPs using Trichoderma harzianum

Trichoderma harzianum fungal strain was kindly provided by culture collection unit of the Regional Center for Mycology and Biotechnology(RCMB). *T. harzianum* was grown in 250 ml Erlenmeyer flask containing 100 mL modified malt extract-peptone (MGYP) medium adjusting at pH 6.8, the culture was grown with continuous shaking on a rotary shaker (150 rpm) at 28°C for 72 hrs. After 72 hrs, fungal balls of mycelia were separated from the culture broth by centrifugation (4000 rpm) at 4 °C for 10 mins and then fungal mycelia were washed with sterile distilled water. The harvested fungal biomass (15 g wet weight) was resuspended in 100 ml sterile Milli-Q-Water in 250 ml Erlenmeyer flask and again kept on shaker (150 rpm) at 28°C for 62 hrs. After incubation the cell free filtrate was obtained which was added to TiO_2 salt in concentration 500 µg/ml. The entire mixture was put into shaker (150 rpm) at 28°C and the reaction allowed for a period of 48 hrs (Abdel-Ghany 2013).

TiO_2NPs Characterization

The biotransformation TiO_2NPs were collected periodically and monitored for characterization using UV-visible spectrophotometer, particle size analyzing system of Transmission Electron Microscopy (TEM), X-ray diffractive spectroscopy (XRD) and Fourier Transform Infrared Spectroscopy (FTIR).

CaCO2 and MDA-MB-231 cell lines

Colon adenocarcinoma cell line CaCO2 and Mammalian cell lines MDA-MB-231were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

Chemicals

Dimethyl sulfoxide (DMSO), MTT and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Belgium).

Cell line propagation

The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37 ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week (Mosmann, 1983).

Cytotoxicity of TiO_2NPs against CaCO2 and MDA-MB-231 cell lines using viability assay

For antitumor assays, the tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hrs. Six dilutions of TiO_2NPs were then added into 96-well plates (three replicates) .Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 hrs, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 ml of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hrs. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 mins. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the TiO_2NPs and ODc is the mean optical density of untreated cells. The relation between surviving cells and TiO_2NPs concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA) (Mosmann, 1983).

DNA fragmentation protocol

Treated MDA-MB-231 cell line (*breast cancer carcinoma cells*) by different concentrations of TiO_2NPs were lysed in NTE buffer (100 mM NaCI, 40 mM Tris-HCI, 20 mM EDTA, pH 7.4) containing 0.5% SDS and 0.2 mg/ml proteinase K., then the cells were vigorously, grinded, centrifuged and collected in one tube. After overnight incubation at 37°C, DNA was extracted twice with phenol-chloroform and precipitated by ethanol. Then dissolved in TE buffer and digested for 2 hrs with 0.1 mg/ml RNAse A (Klaus *et al*., 1994). DNA fragmentation was analyzed on a 1.8% Agarose gel in the presence of 0.5 µg/ml ethidium bromide using electrophoresis.

Lactate dehydrogenase (LDH) and oxidative enzymes detection

For measuring LDH releasing, 24-well cell culture plate was inoculated with 1.5×105 cells per well and incubated for 12 hrs.The collected supernatant added to a black 96-well culture plate (200 µl per well) after cells were exposed to different levels of TiO_2NPs for 48 hrs. LDH cytotoxicity assay kit as per the manufacturer's instructions was used to measure LDH release. At wavelength 450 nm, the absorbance in each well was assayed using an enzyme-linked immunoassay instrument (Nakagawa *et al*., 2005). Oxidative enzymes catalase (CAT) and superoxide dismutase (SOD) in cell homogenates were determined by methods of Aebi (1984) and Oyanagui (1984), respectively.

STATISTICAL ANALYSIS

Three independent replicates of each treatment were applied to calculate the results as mean ± SD (standard deviation). Statistical analyses of data were carried out by computer using SPSS ver. 22.0 software.

RESULTS

The extracellular biosynthesis TiO_2NPs by *T. harzianum* was carried out in the current study. The TEM image shows that the TiO_2NPs are roughly spherical with an average size 53.52 nm (fig. 1). All TiO_2NPs detected by TEM were ranged in size more than 20 and less than 100 nm (Fig. 2). The observed absorption of UV-Visible spectrum for TiO_2NPs produced by *T. harzianum* at about 380 nm in the broad peak (fig. 3). In the current study, FTIR spectrum was measured in a range of 400-4000/nm (fig. 4). The peaks at 555.14 nm characteristic of Ti–O bending mode of vibration which confirms the formation of metal oxygen bonding. Many peak were showed in fig. (4) including peaks at 3055.10/nm, 1312.11/nm and at 3400 to 3300/nm.

The reduction in proliferation of CaCO2 and MDA-MB-231 cell line was observed in a concentration dependent manner which became more clear especially at higher (500 µg/mL) concentrations of TiO_2NPs (table 1 and figs. 5a&b). An initial viability decline was observed on CaCO2 and MDA-MB-231 cells upon addition of 15.6 µg/ml of TiO_2NPs, followed by a consistent proliferation but slow decline at 31.25 to 62.5 µg/ml and a strong decline at 250 to 500 µg/mL. MTT assay data have revealed that TiO_2NPs are effective antiproliferative agents on CaCO2 and MDA-MB-231 cells. The anti-proliferative effect of TiO_2NPs treatment for all concentration was more potent on CaCO2 than MDA-MB-231 cells, where the IC_{50} was 124 and 107 µg/ml respectively (table 1). In the present study, TiO_2NPs seem to have triggered oxidative stress in CaCO2 and MDA-MB-231 cell line which is evident from the changes in SOD and CAT levels (fig. 6). There was a rise in SOD and CAT levels with respect to control. Exposure of CaCO2 cell line to 7.80, 15.60, 31.25, 62.50, 125, 250 and 500 µg/ml of TiO_2NPs showed an increase by 1.10, 1.54, 1.83, 2.64, 3.76, 4.76 and 5.21 fold respectively in SOD activity with respect to untreated control. The same trails were observed in the enzyme activity of MDA-MB-231 cell line.

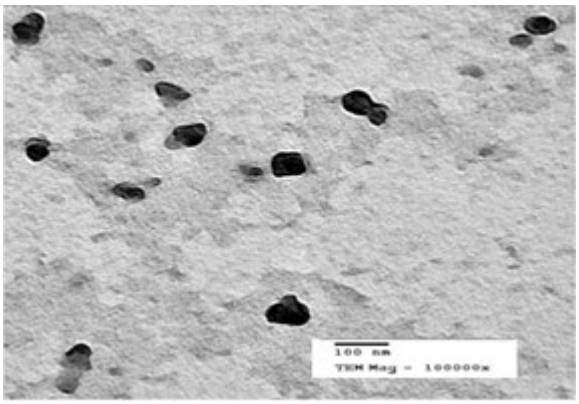

Fig. 1: TEM micrograph of biological synthesized TiO_2NPs at 100 nm scale bar.

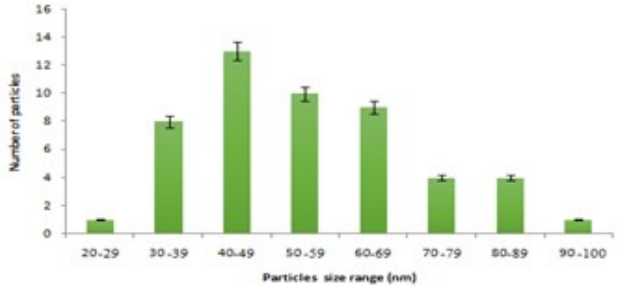

Fig. 2: Particle size distribution and range of biological synthesized TiO_2NPs

The effect of low concentrations of TiO_2NPs up to 62.5 µg/ml was weakly induced the release of LDH, while followed these concentrations the LDH releasing was high. Treatment with TiO_2 reduced the LDH activity in the supernatant in a dose-dependent manner (fig. 7).

The DNA fragmentation in the present study was verified by extracting DNA from CaCO2 cells treated with various

Table 1: Cytotoxicity of TiO_2NPs against CaCO2 cell line MDA-MB-231 cell line.

Sample conc. (µg/ml)	CaCO2 cell line		MDA-MB-231 cell line	
	Viability %	Inhibitory %	Viability %	Inhibitory %
500	15.92±0.74	84.08	12.38±0.16	87.62
250	34.16±0.91	65.84	29.45±0.53	70.55
125	49.73±1.35	50.27	43.82±1.97	56.18
62.5	73.24±2.82	26.76	65.41±1.35	34.59
31.25	90.67±0.51	9.33	89.26±0.52	10.74
15.6	98.46±0.28	1.54	97.18±0.39	2.82
7.8	100±0.0	0.00	100±0.0	0.00
0	100±0.0	0.00	100±0.0	0.00
IC_{50}	124 ± 4.3 µg/ml		107± 2.9 µg/ml	

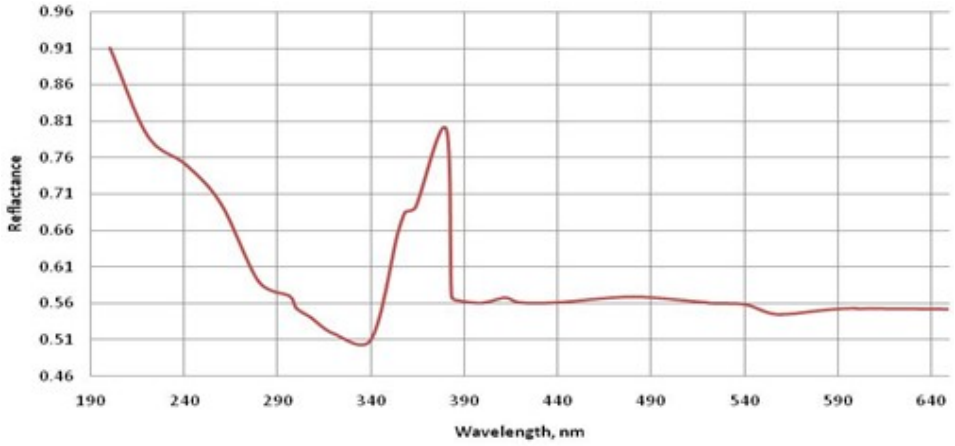

Fig. 3: UV-Spectrum Peak Value of biological synthesized TiO_2NPs.

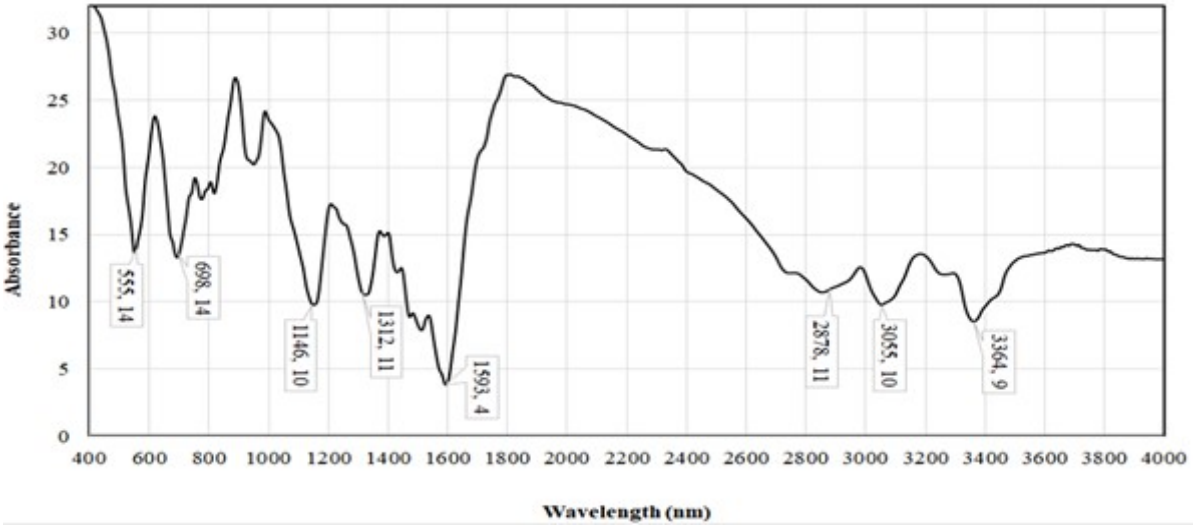

Fig. 4: FTIR spectra of biological synthesized TiO_2NPs.

concentrations of TiO_2NPs followed by detection in the agarose gel. Fig. 8 clearly indicates that the DNA laddering pattern in CaCO2 cells treated with TiO_2NPs is one of the reasons for cell damage.

In the current study TiO_2NPs exhibited good activity against *E. coli* and *B. subtilis* compared with non-nano TiO_2 form at the same used concentration. However, the best activity was found against bacteria with synthetic antibiotic used (fig. 9). Furthermore the addition of TiO_2NPs to antibiotic created synergistic action of antibacterial activity with fold increase 17 % and 12% against *B. subtilis* and *E. coli* respectively, although *B. subtilis* was more sensitive to TiO_2NPs than *E. coli*.

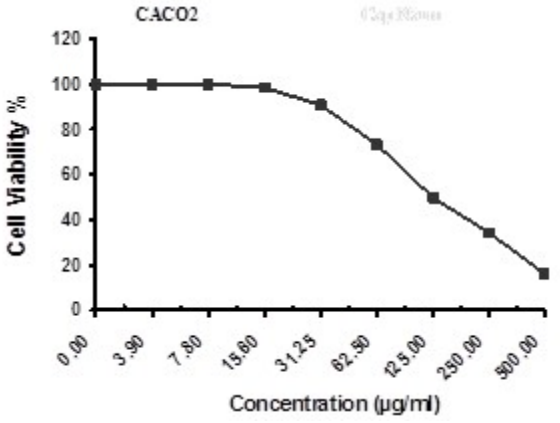

Fig. 5a: Cytotoxicity of TiO_2NPs against CaCO2 cell line.

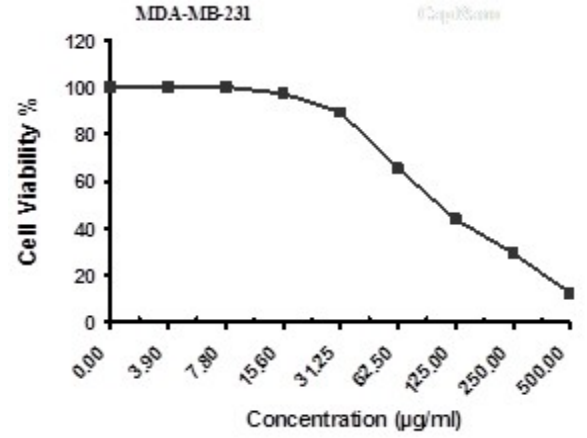

Fig. 5b: Cytotoxicity of TiO_2NPs against MDA-MB-231 cell line.

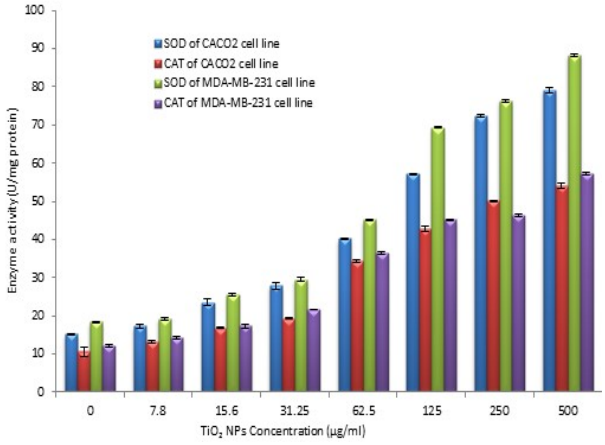

Fig. 6: Oxidative enzymes of CaCO2 and MDA-MB-231 cell line under different concentration of TiO_2NPs. Bars mean standard error.

DISCUSSION

Biosynthesis of TiO_2NPs by fungi as well as other microorganisms or plants considered safe methods and

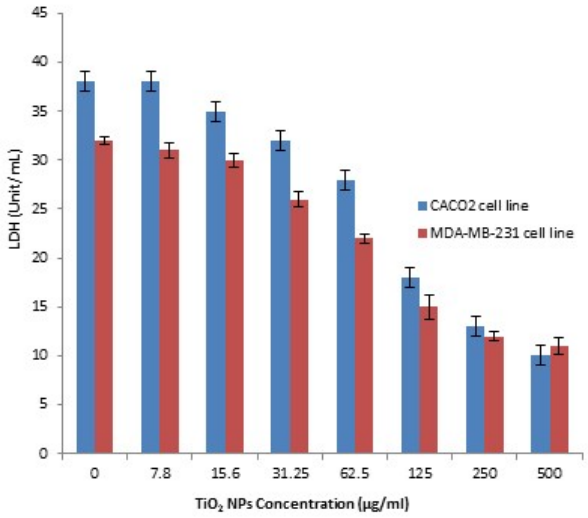

Fig. 7: Lactate dehydrogenase release from CaCO2 and MDA-MB-231 cells under different concentration of TiO_2NPs.

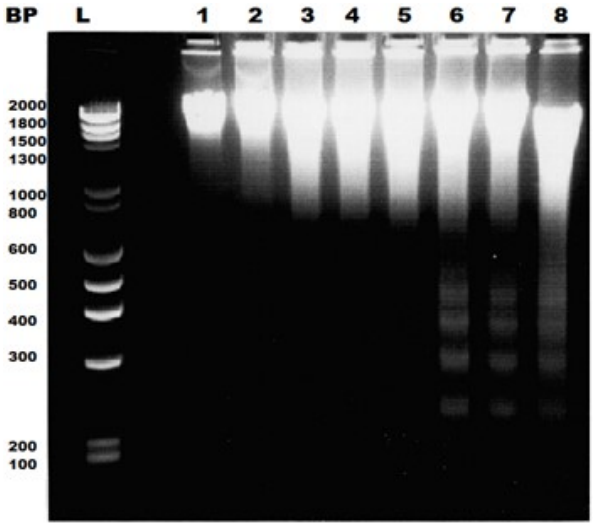

Fig. 8: Effect of TiO_2NPs on DNA fragmentation of MDA-MB-231 Cells. Lane M, 1 kB ladder; lane 1, control; lanes 2-8, TiO_2NPs (7.8, 15.6, 31.25, 62.5, 125, 250 and 500 µg/ml respectively).

alternative for chemical methods in the current years. In this present work, TiO_2NPs were synthesized using *T. harzianum* with size less than 100nm. Similar results were also reported by others (Babitha and Korrapati 2012; Rajakumar *et al*., 2012; Khan *et al*., 2015; Srinivasan *et al*., 2019). Recently, *T. harzianum* was used as a source for titanium oxide nanoparticles (TiO_2NPs) synthesis (Farhat *et al*., 2018), where its particles ranged from 2.0 to 16.0 nm. At the same time the UV-Visible spectrum for TiO_2NPs was similar to result of (Órdenes-Aenishanslins *et al*., 2014). FTIR spectrum reveals the information about the interaction between the functional groups of the biosynthesized agent and the nanoparticles formation. Several peaks positions were detected by

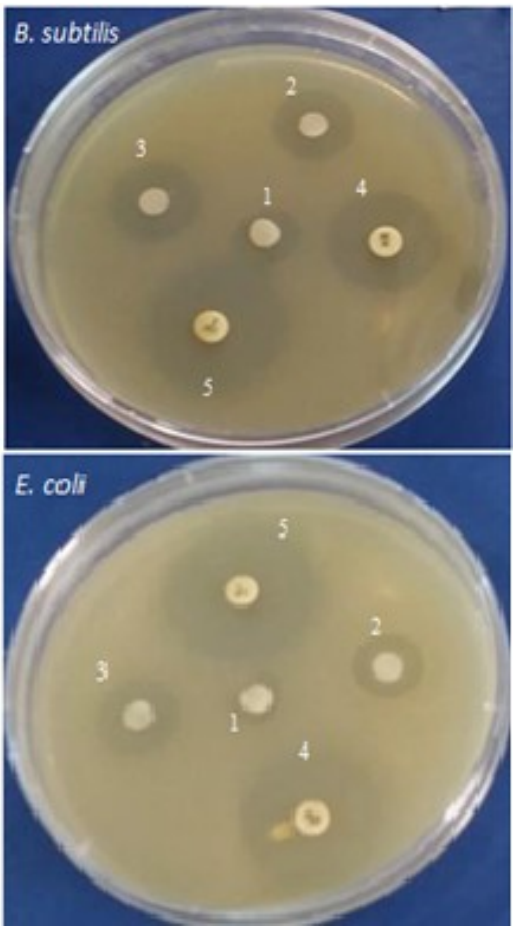

Fig. 9: Antimicrobial activity of TiO_2NPs and their synergistic effect with *Amoxicillin* against *B. subtilis* and *E. coli* (1, TiO_2 125 µg/ml; 2, TiO_2NPs 125 µg/ml; 3, TiO_2NPs 250 µg/ml; 4, *Amoxicillin;5, Amoxicillin* + TiO_2NPs.

FTIR, its indicated, presumably, some metabolites of *T. harzianum* acted as reducing and capping agents for the biological synthesis of TiO_2NPs. The detected peaks that characterized the formed TiO_2NPs were also discussed earlier, where Abdul *et al*. (2016) mentioned that the intense peak between 800 and 450/cm describes the Ti-O stretching bands. The peak at 2878.11/nm indicated the secondary amines. These results are in agreement with Rosi and Kalyanasundaram (2018) who stated that the peak around 2885/nm indicated the secondary aminesduring green synthesis of TiO_2NPs. The peak at 3055.10/nm appeared due to the -OH stretching. The peak at 1312.11/nm indicated the C-O stretching represents aromatic ester, peak at 1593.40 N-H bending represent amine group. Peak at 3400 to 3300 indicated the presence of N-H stretching group, may represent aliphatic primary amine at 3364.6/nm in the current study. All these signals can be attributed to the presence of biomolecules bound to the TiO_2NPs produced by *T. harzianum.* Effect of TiO_2NPs on proliferation of CaCO2 and MDA-MB-231 cell line reflect the dose dependent cytotoxicity of TiO_2NPs. These observation was recorded by Li *et al*. (2008). Sharply decreasing of CaCO2 and MDA-MB-231 cells at high concentrations 250 to 500 µg/mL TiO_2NPs, but slow reductin was observed at low and moderate concentrations. This data suggests that at low concentrations, despite initially responding, the CaCO2 and MDA-MB-231 cells can recover from the anti-proliferative effects of the TiO_2NPs where a robust reduction is observed at higher concentrations. The major mechanism of nanoparticle toxicity is related to oxidative stress (Reeves *et al*., 2008). In the current study, both SOD and CAT levels showed a dose dependent increase in their activity. Rise in SOD and CAT activities was also recorded in human erythrocytes exposed to TiO_2NPs (Khan *et al*., 2015) which are consistent with current results. Inducing lactate dehydrogenase (LDH) release from CaCO2 and MDA-MB-231 cells elucidated the cytotoxic effects of TiO_2NPs. From the obtained results, LDH releasing was sensitive to high concentration of TiO_2NPs, suggesting that TiO_2NPs induced damage of cells at rise concentration. Many studies reported that LDH release from cells is a marker of cell damage and death (Rose *et al*., 1993; Lai *et al*. 2001, 2008). Zaqout *et al*. (2012) suggest that TiO_2NPs bind to LDH, and consequently, TiO_2NP-induced toxicity could be underestimated by the LDH activity assay. DNA fragmentation of MDA-MB-231 Cells at different concentrations of TiO_2NPs was confirmed on the agarose gel. The deposition of metal particles inside the nucleus could affect the DNA and cell division. Genotoxic studies of TiO_2NPs revealed dose-dependent DNA damage, chromosomal aberrations and errors in chromosome segregation and formation of sister chromatic exchanges (Lu *et al*. 1998). Recently, many studies elucidated the genotoxicity of TiO_2NPs (El-Said *et al*., 2014; Wang *et al*., 2015; Baranowska-Wójcik *et al*., 2020). Recently Biola-Clier *et al*. (2020) observed DNA damage A549 human pulmonary alveolar epithelial cells that exposer to TiO_2NPs. Wang *et al*. (2015) TiO_2NPs can induce cytotoxicity, significant DNA damage, and apoptosis of A549 cells, suggesting that exposure to TiO_2NPs could cause cell injury. DNA fragmentation was induced by TiO_2NPs in human lymphocytes at a concentration of 25 mg/ml (Ghosh *et al*., 2013). Jugan *et al*. (2012) evaluated genotoxicity of varying sizes of NPs in A549 human lung carcinoma cells and found that they were genotoxic when assayed with the Comet assay. TiO_2NPs induced significant oxidative DNA damage in HepG2 cells measured with the Fpg-Comet assay even at 1 mg/ml (Shukla *et al*., 2013). The interactions of NPs with cells resulted in the generation of ROS, and the resultant oxidative stress may cause DNA fragmentation (Shukla *et al*., 2011; Hariharan *et al*., 2020).

Bacteriocidal of TiO_2NPs against *E. coli* and *B. subtilis* was reported in the current study compared with non-nano

TiO_2 form. However its antifungal activity was also reported in another studies, therefore TiO_2NPs may applied in medicinal field. The obtained results agree with numerous studies (Ahmad and Sardar 2013; Gupta *et al*., 2013).

Results of Sara *et al*. (2016) and Waseem *et al*. (2020) demonstrated antifungal and antibacterial activities of TiO_2NPs. Earlier the antimicrobial activity of TiO_2NPs are exploited in medical devices, in order to prevent biofilm formation and sepsis (Arora *et al*., 2010) beside, enhancing the long-term antibacterial ability and toxicity reduction of antibiotics (Yuehong *et al*., 2019; Zietal *et al*., 2020). The mechanisms of antimicrobial activity of TiO_2NPs was elucidated by Rajakumar *et al*. (2012) who stated that TiO_2NPs are capable of dissolving the outer membranes of bacteria. Synergistic action between TiO_2NPs and antibiotic was observed in the obtained results. These notes were recently mentioned by Bakri *et al*. (2020), where the antimicrobial activity by plant extract or antibiotics was enhanced and reported by the addition of nanoparticles.

CONCLUSION

The present study deals with the ecofriendly biologically synthesis of TiO_2NPs using *T. harzianum*. The biosynthesized showed anticancer and antibacterial activity.

REFERENCES

Abdel-Ghany TM (2013). *Stachybotrys chartarum*: A novel biological agent for the extracellular synthesis of silver nanoparticles and their antimicrobial activity. *Indones. J. Biotechnol.,* **18**(2): 75-82.

Abdel-Ghany TM, Abdel Rhaman M, Shater A, Abboud MA and Alawlaqi MM (2013). Silver nanoparticles biosynthesis by *Fusarium moniliforme* and their antimicrobial activity against some food-borne bacteria. *Mycopath*, **11**(1): 1-7.

Abdel-Ghany TM, Aisha M, Al-Rajhi H, Al Abboud MA, Alawlaqi MM, Magdah G, Helmy EAM and Mabrouk AS (2018). Recent advances in green synthesis of silver nanoparticles and their applications: About future directions: A Review, *Bio Nano Sci*, **8**(1): 5-16.

Abdul K, Dilip KB and Meda KK (2016). Synthesis and characterization of controlled size TiO_2 Nanoparticles via Green Route using *Aloe vera* extract. *IJSR*, **5**(11): 1913-1916.

Aebi H (1984). Catalase *in vitro*. *Method Enzymol.*, **105**: 121-126.

Ahmad R and Sardar M (2013). TiO2 nanoparticles as an antibacterial agents against *E. coli*. *Int. J. Innov. Res. Sci. Eng. Technol.,* **2**(8): 3569-3574.

Arora H, Doty C, Yuan Y, Boyle J, Petras K and Rabatic B (2010). Titanium dioxide nanocomposites. Nanomaterials for the life sciences (Series Nr. 8) Weinheim. Wiley-VCH, pp.1-42.

Babitha S and Korrapati PS (2013). Biosynthesis of titanium dioxide nanoparticles using a probiotic from coal fly ash effluent. *Mater Res Bull*, **48**(11): 47384742.

Bakri MM, Medhat A El-Naggar, Helmy EA, Mona S Ashoor and Abdel Ghany TM (2020). Efficacy of *Juniperus procera* constituents with silver nanoparticles against *Aspergillus fumigatus* and *Fusarium chlamydosporum. Bio Nano Sci.*, **10:**62–72

Baranowska-Wojcik E, Szwajgier D, Oleszczuk P and Winiarska-Mieczan A (2020). Effects of titanium dioxide nanoparticles exposure on human health – A Review. *Biological Trace Element Re.*, **193**: 118-129.

Barillet S, Simon-Deckers A, Herlin-Boime N, Mayne-L'Hermite M, Reynaud C, Cassio D, Gouget B and Carriere M (2010). Toxicological consequences of TiO_2, SiC nanoparticles and multi-walled carbon nanotubes exposure in several mammalian cell types: an *in vitro* study. *J Nanopart Res*, **12**(1): 61-73.

Biola-Clier M, Bea Dl, Caillat S, Libert S, Armand L, Herlin-Boime N, Sauvaigo S, Douki T and Carriere M (2017). Comparison of the DNA damage response in BEAS-2B and A549 cells exposed to titanium dioxide nanoparticles. *Mutagenesis*, **32**(1): 161-172.

Biola-Clier M, Gaillard J, Rabilloud T, Armengaud J and Carriere M (2020). Titanium dioxide nanoparticles alter the cellular phosphoproteome in A549 Cells. *Nano Materials,* **10**(2): 185.

Boland S, Hussain S and Baeza-Squiban A (2014). Carbon black and titanium dioxide nanoparticles induce distinct molecular mechanisms of toxicity Nanomed. *Nano Biotechnol*, **6**(6): 641-652.

Carrouel F, Viennot S, Ottolenghi L, Gaillard C and Bourgeois D (2020). Nanoparticles as anti-microbial, anti-inflammatory, and remineralizing agents in oral care cosmetics: A review of the Current Situation. *Nano Materials*, **10**(140).

Dhandapani P Maruthamuthu S and Rajagopal G (2012). Bio-mediated synthesis of TiO_2 nanoparticles and its photocatalytic effect on aquatic biofilm. *J Photochem Photobiol B*, **110**: 43-49.

El-Said KS, Ali EM, Kanehira K and Taniguchi A (2014). Molecular mechanism of DNA damage induced by titanium dioxide nanoparticles in toll-like receptor 3 or 4 expressing human hepatocarcinoma cell lines. *J Nano Biotechnol,* **12**: 48.

Faraz A, Faizan M, Fariduddin Q and Hayat S (2020). Response of titanium nanoparticles to plant growth: Agricultural perspectives. *Sustainable Agriculture Reviews,* **41**: 101-110.

Filpo GD, Palermo AM, Rachiele F and Nicoletta FP (2013). Preventing fungal growth in wood by titanium dioxide nanoparticles. *Int. Biodeter Biodegr.*, **85**: 217-222.

Farhat MG, Haggag WM, Thabet MS and Mosa AA (2018). Efficacy of silicon and titanium nanoparticles biosynthesis by some antagonistic fungi and bacteria for controlling powdery mildew disease of wheat plants. *IJAT,* **14**(5): 661-674.

Gao A, Hang R, Huang X, Zhao L, Zhang X, Wang L, Tang B, Ma S and Chu PK (2014). The effects of titania nanotubes with embedded silver oxide nanoparticles on bacteria and osteoblasts. *Biomaterials*, **35**(13): 4223-4235.

Ghosh M, Chakraborty A and Mukherjee A (2013). Cytotoxic, genotoxic and the hemolytic effect of titanium dioxide (TiO_2) nanoparticles on human erythrocyte and lymphocyte cells *in vitro*. *J. Appl. Toxicol.*, **33**(10): 1097-110.

Gupta K, Singh RP, Pandey A and Pandey A (2013). Photocatalytic antibacterial performance of TiO_2 and Ag-doped TiO_2 against *S. aureus. P. aeruginosa* and *E. coli. Beilstein J. Nanotechnol.*, **4**: 345-51.

Hariharan D, Thangamuniyandi P, Jegatha Christy A, Vasantharaja R, Selvakumar P, Sagadevan S, Pugazhendhi A and Nehru LC (2020). Enhanced photocatalysis and anticancer activity of green hydrothermal synthesized nanoparticles. *J. Photoch. Photobio. B*, **202**: 111636.

Hassan MS, Amna T, Mishra A, Yun SI, Kim HC and Kim HY (2012). Fabrication, characterization and antibacterial effect of novel electrospun TiO2 nanorods on a panel of pathogenic bacteria. *J. Biomed. Nanotechnol.*, **8**(3): 394-404.

Helinor JJ, Gary RH, Frans MCh, SheonaP, Steve H and Vicki S (2009). Cytotoxic effects of titanium dioxide nanoparticles on rat embryo fibroblast REF-3 cell line *in vitro*. *Toxicol,* **6**: 33.

Chen T, Jian Y and Yan L (2014). Genotoxicity of titanium dioxide nanoparticles. *J. Food Drug Anal.,* **22**(1): 95-104.

Jha AK, Prasad K and Kulkarni AR (2009). Synthesis of TiO_2 nanoparticles using microorganisms. *Colloids Surf B*, **71**(2): 226-229.

Jin CY, Zhu BS, Wang XF and Lu QH (2008). Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. *Chem. Res. Toxicol*, **21**(9): 1871-1877.

Jugan ML, Barillet S and Simon-Deckers A (2012). Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells. *Nano Toxicology,* **6**(5): 501-13.

Khan K, Alim HN and Masood A (2015). Comparative study of the cytotoxic and genotoxic potentials of zinc oxide and titanium dioxide nanoparticles. *Toxicol. Rep.,* **2**(1): 765-774.

Lai JCK , Lai MB, Sirisha J, Vikas VD, Alok B, Christopher KD and Solomon WL (2008). Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. *Int. J. Nanomed.,* **3**(4) 533-545.

Li SQ, Zhu RR, Zhu H, Xue M, Sun XY, Yao SD and Wang SL (2008). Nanotoxicity of TiO_2 nanoparticles to erythrocyte *in vitro*. *Food Chem. Toxicol.,* **46**(12): 3626-3631.

Liu K, Lin X and Zhao J (2013). Toxic effects of the interaction of titanium dioxide nanoparticles with chemicals or physical factors. *Int. J. Nanomedicine,* **8**: 2509-2520.

Lu PJ, Ho IC and Lee TC (1998). Induction of sister chromatid exchanges and micronuclei by titanium dioxide in Chinese hamster ovary-K1 cells. *Mutat. Res.,* **414**(1-3): 15-20.

Lutfana Banu A, Soundhari C and Karkuzhali S (2018). Green Syntheis of titanium dioxide nanoparticles using *Lawsonia inermis* leaf extract and its antibacterial activity. *World J. Pharm. Res.,* **7**(7): 180-190.

Marimuthu S, Rahuman AA, Jayaseelan C, Kirthi AV, Santhoshkumar T, Velayutham K, Bagavan A, Kamaraj C, Elango G, Iyappan M, Siva C, Karthik L and Rao KVB (2013). Acaricidal activity of synthesized titanium dioxide nanoparticles using *Calotropis gigantean* against *Rhipicephalus microplus* and *Haemaphysalis bispinosa*. *Asian Pac. J. Trop. Dis.,* **6**(9): 682-688.

Mondal D, Nguyen L, Oh IH and Lee BT (2013). Microstructure and biocompatibility of composite biomaterials fabricated from titanium and tricalcium phosphate by spark plasma sintering. *J. Biomed. Mater. Res.*, **101**(5):1489-1501.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Aapplication to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**(1-2): 55-63.

Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K and Yamagata H (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature,* **434** (7033): 652-658.

Ordenes-Aenishanslins NA, Luis Alberto Saona, Vicente Maria Duran-Toro, Juan Pablo Monrás, Denisse Margarita Bravo and Jose Manuel Perez-Donoso (2014). Use of titanium dioxide nanoparticles biosynthesized by *Bacillus* mycoides in quantum dot sensitized solar cells. *Microb. Cell Fact.*, **13**: 90.

Oyanagui Y (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Biochem.*, **142**(2): 290-296.

Pan X, Ramirez IM, Mernaugh R and Liu J (2010). Colloids and Surfaces B: *Biointerfaces*, **77**: 82-89.

Prasad K, Jha AK, Kulkarni AR (2007). *Lactobacillus* assisted synthesis of titanium nanoparticles. *Nanoscale Res Lett,* **2**: 248-250.

Rajakumar G, Rahuman AA, Priyamvada B, Khanna VG, Kumar DK, Sujin PJ. (2012). *Eclipta prostrata* leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles. *Mater Lett*, **68**: 115-117.

Rajakumar G, Rahuman AA, Roopan SM, Khanna VG, Elango G, Kamaraj C, Zahir AA and Velayutham K (2012). Fungus-mediated biosynthesis and characterization of TiO_2 nanoparticles and their activity against pathogenic bacteria. *Spectrochim Acta Part A.*, **91**: 23-29.

Reeves JF, Davies SJ, Dodd NJF and Jha AJ (2008) Hydroxyl radicals ($^{+}$OH) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells*: Mutat. Res.,* **640**(1-2): 113-122.

Rose K, Goldberg MP, Choi DW. (1993). Cytotoxicity in murine neocortical cell culture. *Methods Toxicol.*, **1A**: 46-60.

Rosi H and Kalyanasundaram S (2018). Synthesis, characterization, structural and optical properties of titanium-dioxide nanoparticles using *Glycosmis cochinchinensis* Leaf extract and its photocatalytic evaluation and antimicrobial properties. *WNOFNS*, **17**: 1-15.

Schulze-Osthoff K, Walczak H, Dröge W and Krammer PH (1994). Cell Nucleus and DNA Fragmentation Are Not Required for Apoptosis. *J. Cell Biol.*, **127**(1): 15-20.

Saquib Q, Abdulaziz A, Al-Khedhairy, Maqsood A Siddiqui, Faisal M Abou-Tarboush, Ameer Azam and Javed Musarrat (2012). Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells. *Toxicol. In vitro,* **26**: 351-361.

Sara A, Iftikhar A, Saima T, Furqan A, Ahmed S and Sikander KS (2016). Synthesis and characterization and antibacterial activity of titanium dioxide nanoparticles. *Fuuast. J. Biol*, **6**(2): 141-147.

Shi H, Ruth M, Vincent C and Zhao J (2013). Jinshun Zha Titanium dioxide nanoparticles: A review of current toxicological data. *Part Fibre Toxicol.*, **10**(1): 15.

Shukla RK, Kumar A and Gurbani D (2013). TiO_2 nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. *Nanotoxicology*, **7**(1): 48-60.

Shukla RK, Kumar A, Pandey AK, Singh SS and Dhawan A (2011). Titanium dioxide nanoparticles induce oxidative stress-mediated apoptosis in human keratinocyte cells. *J. Biomed. Nanotechnol.*, **7**(1): 100-101.

Shukla RK, Sharma V, Pandey AK, Singh S, Sultana S and Dhawan A (2011). ROS mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicol. In vitro,* **25**(1): 231-241.

Sohal IS, DeLoid GM, O'Fallonc KS, Gainesd P, Demokritou P and Bello D 2020. Effects of ingested food-grade titanium dioxide, silicon dioxide, iron (III) oxide and zinc oxide nanoparticles on an *in vitro* model of intestinal epithelium: Comparison between monoculture vs. amucus-secreting co culture model. *NanoImpact,* **17**:100209.

Srinivasan M, Venkatesan M, Arumugam V and Natesan G (2019). Green synthesis and characterization of titanium dioxide nanoparticles (TiO_2NPs) using *Sesbania grandiflora* and evaluation of toxicity in zebrafish embryos. *Process Biochem.,* **80**: 197-202.

Subhapriya S and Gomathipriya P (2018). Green synthesis of titanium dioxide (TiO_2) nanoparticles by *Trigonella foenum-graecum* extract and its antimicrobial properties. *Microb. Pathog.,* **116**: 215-220.

Yuehong Li, Yue Yang, Ruiyan Li, Xiongfeng Tang, Deming Guo, Yun'an Qing and Yanguo Qin (2019). Enhanced antibacterial properties of orthopedic implants by titanium nanotube surface modification: A review of current techniques. *Int. J. Nanomedicine,* **14**: 7217-7236.

Vevers WF and Jha AN (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells *in vitro. Ecotoxicology,* **17**(5): 410-420.

Wang Y, Haiyan C, Jiaping Z, Fengjuan L, Jinju W, Mianhua C and Qingdai L (2015). Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. *Environ. Sci. Pollut. R.*, **22**(7): 5519.

Waseem A, Krishna K and Shivani S (2020). Green synthesis of titanium dioxide (TiO_2) nanoparticles by using *Mentha arvensis* leaves extract and its antimicrobial properties. *Inorg. Nano-Met. Chem.,* DOI: 10.1080/24701556.2020.1732419.

Weir A, Westerhoff P, Fabricius L, Hristovski K and Von Goetz N (2012). Titanium dioxide nanoparticles in food and personal care products, *Environ Sci Technol,* **46**: 2242-2250.

Zaqout MS, Sumizawa T, Igisu H, Wilson D, Myojo T and Ueno S (2012). Binding of titanium dioxide nanoparticles to lactate dehydrogenase. *Environ Health Prev. Med.*, **17**(4): 341-345.

Ziental D, Czarczynska-Goslinska B, Dariusz T Mlynarczyk, Glowacka-Sobotta A, Stanisz B, Goslinski T and Sobotta L (2020). Titanium Dioxide Nanoparticles: Prospects and Applications in Medicine. *Nanomaterials,* **10**(2): 387.

Zhang AP and SunYP (2004). Photocatalytic killing effect of TiO_2 nanoparticles on Ls-174-t human colon carcinoma cells. *World J. Gastroenterol.,* **10**(21): 3191-3193.

Zhao J, Bowman L, Zhang X, Vallyathan V, Young SH, Castranova V and Ding M (2009). Titanium dioxide (TiO_2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/bid and mitochondrial pathways. *J. Toxicol. Environ. Health*, **72**(19): 1141-11511.