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Cytotoxic Effect of Titanium Dioxide Nanoparticles Exposed to Thermal Radiation

A.F.Saad[1], Yasser A. Selim[2,*], Mona Hamada [1], Mohammed Elywa[1]
[1] Physics Department, Faculty of Science, Zagazig University, Zagazig, 44519, Egypt.
[2] Faculty of Specific Education, Zagazig University, Zagazig, 44519, Egypt.
* Correspondence , Email: y2selem@yahoo.com

ABSTRACT :Through this work, green tea extract—an environmentally friendly reducing and capping agent—has been used to manufacture titanium dioxide nanoparticles (TiO_2.NPs). Therefore, we investigated in this work how TIO_2 nanoparticles could be harmful to the growth of HuH-7 cells (human Hepatocellular cancer cell line). The cytotoxic effect and the UV–Vis (ultraviolet–visible) spectra of TIO_2 (NPs) have been investigated following exposure to a 250W infrared radiation lamp for varied durations, ranging from 2.5 to 20min. The MTT test was used to measure the number of viable cells, which was used to assess the cytotoxic effect. The survival curve of each tumour cell line following treatment with the designated substance is obtained by plotting the relationship between remaining cells and drug concentration. Using visual representations of the dose response curve for each conc., the 50% inhibitory concentration (IC_{50}), or the dosage needed to produce harmful effects in 50% of undamaged cells, was calculated. According to the current analysis, infrared radiation (IR) is treated as thermal radiation and has the same effect as annealing temperature. As a result, the IC_{50} values of HuH-7cells for the 5-FU Standard, the control (TIO_2.NPs prior to irradiation) and TIO_2.NPs following exposure to 20m IR-irradiation, respectively, were 12.8.8±3.2 µg/m, 60.43±7.9 µg/mL, and 80.1±21.3 µg/mL. The IC_{50} values for the 5-FU Standard, TIO_2.NPs before irradiation, and TIO_2.NPs after exposure to 20m IR-irradiation were 65,1±9.8 µg/mL, 64,7±12.48 µg/mL, and 98,6±20.2 µg/mL, respectively, in normal WI38 cells.

KEYWORDS: TIO_2, IR -irradiation, cytotoxic

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I. INTRODUCTION

Nanoparticles give a range of beneficial properties for biology and novel analytical techniques in the field of life sciences **(Bruchez M et al.,1998) (Cui Y, Wei Q et al., 2001) (Colvin VL, 2003)** . Amorphous, rutile, brookite, and anatase are just a few of the crystal formations in which nano-sized titanium dioxide (TiO_2) can be found. It is an odorless, nonflammable nanoparticle **(Kuroda, A. and Ogino, K ,1994) (Dunford R et al., 1997) (Yuda J et al.,1997)** . Moreover, it has been demonstrated that titanium dioxide can effectively kill cancer cells using photocatalytic chemistry **(Halliwell Band Aruoma OI, 1991)**. Reactive oxygen species are produced as a result of UV light photoexcitation, which is the mechanism. Again, the chemical is less helpful for deeply rooted tissues because of the modest depth at which UV radiation penetrates. Knowing how steric stabilisation works, which helps keep nanoparticles stable in biological systems, is one of the most significant characteristics of these tiny particles. Furthermore, this offers a chance to implement drug delivery systems (DDS) built around nanoparticles **(Mahmoodi NO et al., 2016)**. Controlling the surface properties and particle size of nanoparticles is essential for their use in the administration of medication. Anticancer drugs, carrier proteins, and ligands are produced by targeted drug delivery systems. Unlike ligands, which target nanoparticles, antibodies attached to nanoparticles can precisely target cancer cell receptors **(Khare V et al., 2014)**. Since nanoparticulates are small and may bind target ligand to particle surfaces, they are a far better delivery method than free chemotherapeutic medicines **(Khare V et al.,2014).**They are currently employed as agents in radiotherapy for cancer and photodynamic therapy. This lessens the drug's adverse effects and increases its efficacy **(Smith L et al., 2012).** Paper, plastic, paint, colorants, sunscreen, cosmetics, and, most recently, a number of cancer treatments and drug delivery systems are just a few of the products that include titanium dioxide **(Lomer MCE et al., 2002).**

The main foods that individuals consume to obtain flavonoids include tea, fruits, wine, and vegetables. Most recent studies have concentrated on the impact of flavonoids on human health. Though some flavonoids might have antiviral properties, numerous flavonoids have demonstrated antioxidative activity, the capacity to scavenge free

radicals, the potential to prevent coronary heart disease, the capacity to protect the liver, and the potential to combat cancer. Additionally, flavonoids function in plant systems as growth regulators and antioxidant defence mechanisms (**Shashank Kumar and Abhay K. Pandey, 2013**). Flavonoids can scavenge harmful active oxygen species like O-2, H_2O_2, and OH by giving electrons to guaiacol-type peroxidases (GuPXs) for the detoxification of H_2O_2 generated under stressful situations.

Most studies investigating the effects of solar radiation on human health have concentrated on UV radiation. This is surprising because the majority of solar energy that reaches the earth's surface is attributed to infrared radiation (**Schroeder P et al., 2008**). The IR-A band (780–1400 nm), which accounts for about 30% of solar energy overall, contains the majority of the infrared radiation. IR-B (1400–3000 nm) and IR-C (3000 nm to 1 mm) are in opposition to one another (**Schroeder P and Pohl C, 2007).** It was originally believed that heat was the only factor influencing effects mediated by infrared radiation. There's been increasing data recently suggesting that IR-A has waveband-specific effects.

II. EXPERIMENTAL PROCEDURE

-Green synthesis of TiO_2 NPs1.2

In a standard process, 150 millilitres of boiling distilled water was used to dissolve green tea extract before synthesizing TiO_2.NPs. Next, green tea extract was mixed with 0.01 mL of titanium tetraisopropoxide (TTIP) and heated to 70 °C for five minutes. After adding ammonium hydroxide (NH_4 OH) solution gradually and stirring continuously for 30 minutes, the solution's pH was adjusted to basic (pH 9.0), neutral (pH 7.0), and acidic (pH 5.0). A yellow solution with white precipitates was the result. To get TiO_2 (NPs), the precipitate was centrifuged at 8500 rpm for 10 minutes. It was then repeatedly washed with distilled water and allowed to dry at room temperature (**A. F. Saad, Yasser A. Selim et al., 2023**).

2.2- IR- irradiation

An IR lamp with a power of 250 W was used for IR exposure, and it was placed one centimeter away from the source output. The TiO_2 (NPs) integrated infrared exposure durations varied from 2.5 to 20 minutes.

2.3- In vitro toxicity study

2.3.1 - **Evaluation of Cytotoxic Effects of certain Chemical compound**

I- Mammalian cell lines:

Normal human lung fibroblast cells (WI 38) and human hepatocellular cancer cell line (HuH-7) were obtained from the American Type Culture Collection. (ATCC, Rockville, MD).

II- **Chemicals Used:**

We purchased from Sigma foetal bovine serum, dimethyl sulfoxide (DMSO), trypan blue dye, MTT, and DMSO. (St. Louis, Mo., USA).

We bought RPMI-1640, DMEM, HEPES buffer solution, gentamycin, L-glutamine, and 0.25% Trypsin-EDTA from Lonza (Belgium).

2.3.2- Cell line Propagation:

A growth medium consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated foetal bovine serum, 1% L-glutamine, HEPES buffer, and 50 g/mL gentamycin was utilised for WI 38 cells.

In RPMI-1640 medium, HuH-7 cells were cultivated with the addition of 50 g/mL gentamycin and 10% inactivated foetal calf serum.

Then, all cells were subcultured twice a week while being kept at 37°C in a humid environment with 5% CO_2.

I-Cytotoxicity evaluation using viability assay:

For the cytotoxicity experiment, 1 X 10^4 cells were planted in each well of 96-well plates together with 100 L of growth medium. Following a 24-hour seeding period, fresh medium was added that contained different concentrations of the test material. A multichannel pipette was used to dispense confluent cell monolayers into 96-

well flat-bottomed microtiter plates (Falcon, NJ, USA), to which successive two-fold dilutions of the chemical component under investigation were added. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for period of 24 hours. Three wells were used for each test material concentration. Control cells were grown without the test substance and with or without DMSO. The experiment demonstrated that the small amount of DMSO (maximum 0.1%) in the wells had no effect.

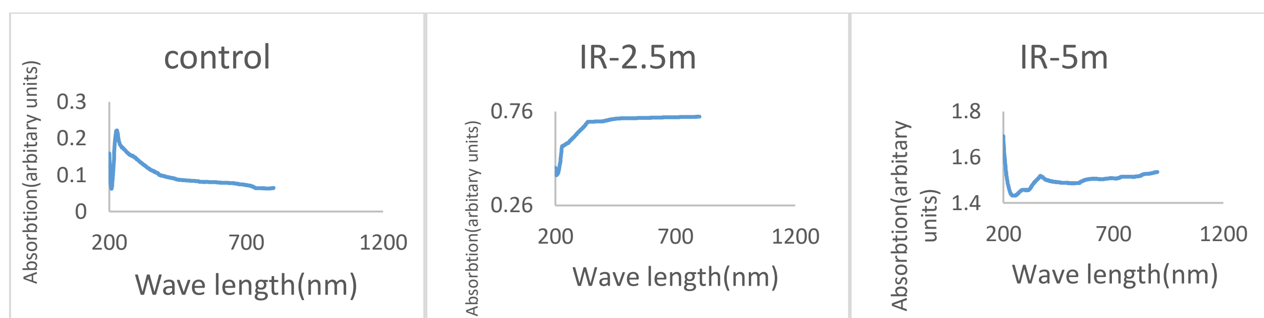
Using Corning® 96-well tissue culture plates, the cancer cell lines were plated at 5 x10^4 cells/well for the antitumor assays. The plates were then incubated for a full day. The chemicals were added to 96-well plates to produce twelve concentrations of each of the drugs under investigation. (three duplicates). A control consisting of six vehicle controls containing medium or 0.5% DMSO was conducted for every 96-well plate.

The viable cell yield following incubation was determined using a colorimetric method. After a 24-hour incubation period, the MTT test was utilised to determine the quantity of viable cells.
In a nutshell, 10L of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) was added to each well, includ ing the untreated controls, and the RPMI 1640 medium without phenol red was substituted with 100 l of new cultur e medium for 96-well plates. After that, the 96-well plates were incubated for four hours at 37°C and 5% CO2. 50 l of DMSO was added to each well after an 85 l aliquot of the media was removed, and the wells were then meti culously mixed using a pipette before being incubated for ten days at 37 °C. The optical density at 590 nm was then measured using a microplate reader to calculate the number of live cells. (SunRise, TECAN, Inc., USA). Using the formula $[(OD_t/OD_c)]$x100%, the percentage of viability was determined. OD_t represents the mean optical density of the wells treated with the tested sample, whereas OD_c represents the mean optical density of the cells that were left untreated.

The link between surviving cells and drug concentration is shown in order to calculate the survival curve of each cancer cell line after treatment with the specified material. Both the 50% inhibitory concentration (IC_{50}) and the cytotoxic concentration (CC_{50}), which are the concentrations required to produce toxic effects in 50% of intact cells, were estimated using Graphpad Prism software (San Diego, CA, USA) **(Mosmann, T, 1983) (Gomha, S.M et al., 2015).**

III. RESULTS AND DISCUTION

After being exposed to various doses of infrared radiation, measurements of the UV-visible spectra (in the absorption mode) were made on the control non-irradiated and irradiated samples of TiO_2 (NPs), using UV-Vis spectroscopy throughout the range of 200–800 nm. The irradiated sample's absorption grew progressively until it reached its maximum value of 1.519 at wavelength 368 nm after 5 min of radiation. Additionally, the sample with the largest peak emerged first, and as we increased the exposure to infrared light, we saw a decrease in absorption, as seen in **Figure** 1(a,b).



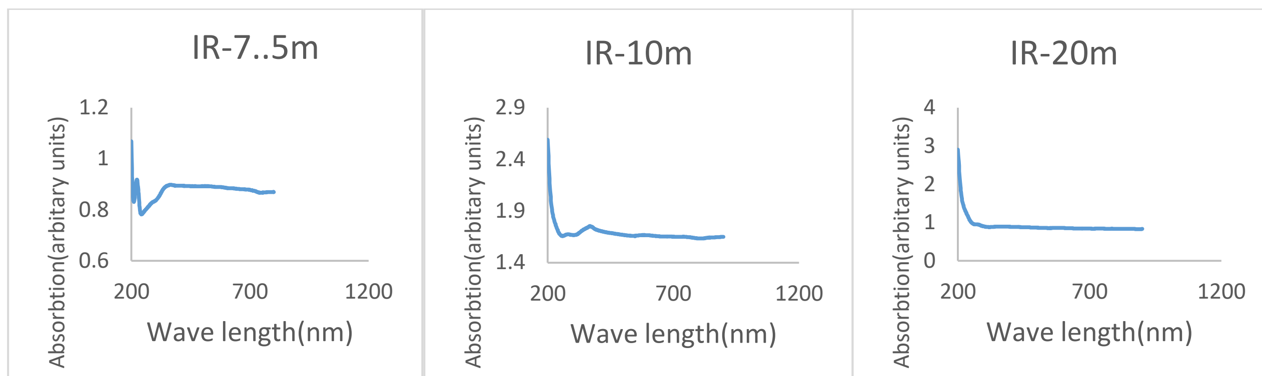


Figure 1,a. UV–visible spectra of the control and IR-radiation-exposed to TiO2 (NPs) for exposure periods of **2.5-20** min

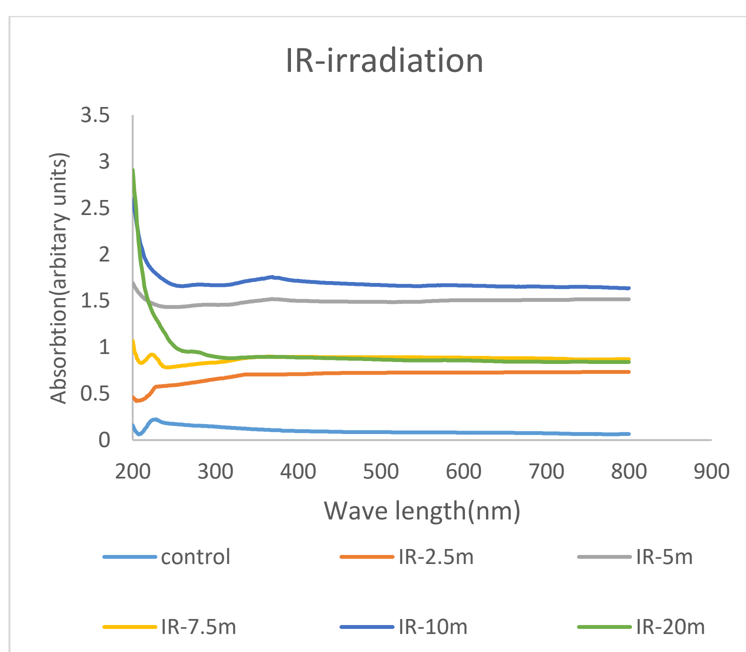
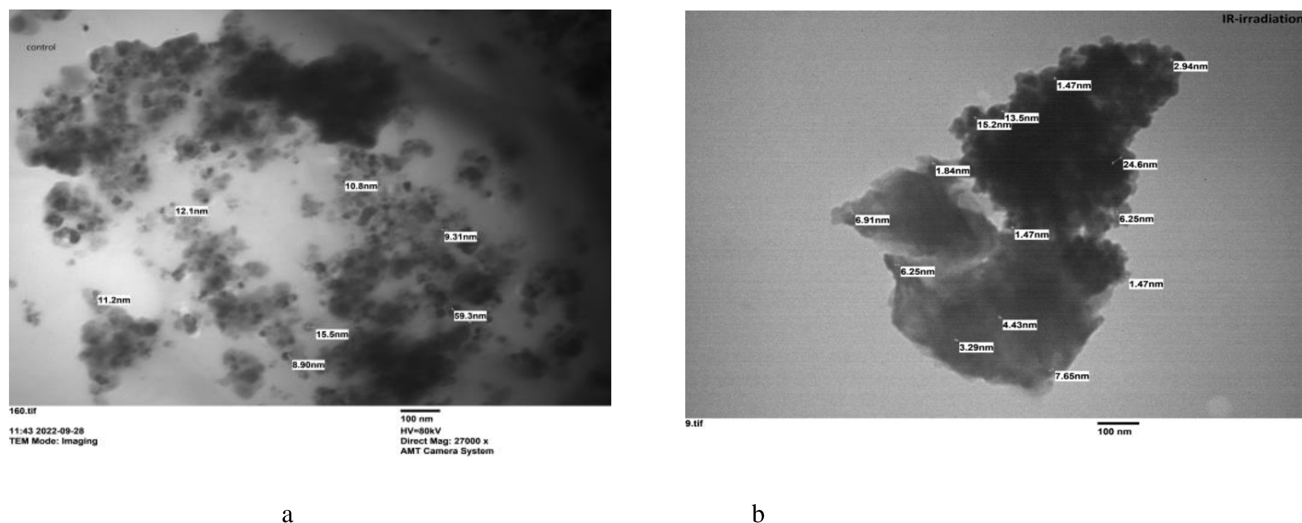


Figure 1. UV–visible spectra of the control and IR-radiation-exposed to TiO2 (NPs) for exposure periods of **2.5-20** min

3.1- TEM analysis

TEM analysis can be used to understand the crystalline characteristics and size of TiO_2 (NPs) before and after irradiation. The analysis was carried out using JEOL (JEM-100CX) Electron Microscope and the images at different magnification (**100** nm) are shown in **Figure 2**.



a b

Figure 2. HR-TEM micrograph of TiO_2 (NPs), (a) .control , (b). TiO_2 (NPs) with IR-irradiation.

3.2- HR-SEM analysis

Figure.3 displays a SEM image of the fabricated TiO2NPs' surface morphology. (SEM). The SEM revealed that the TiO_2NPs were uniformly dispersed and cylinder-shaped.

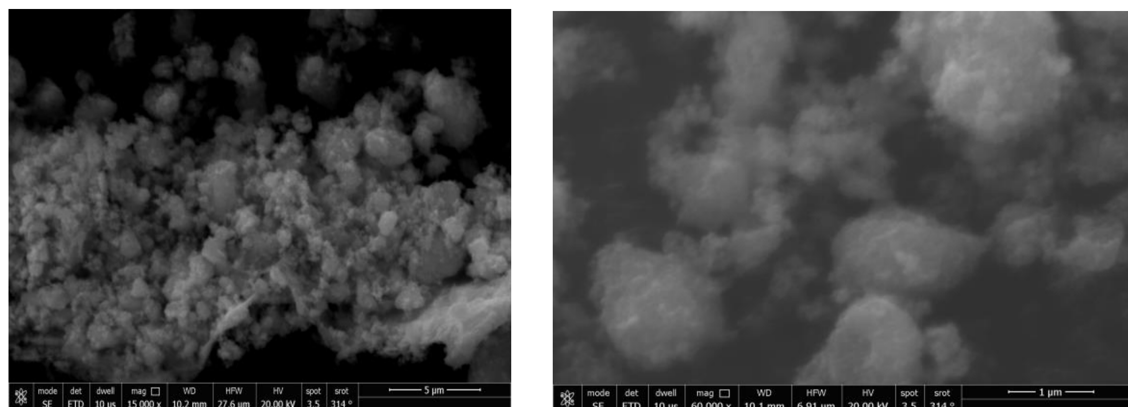


Figure 3. SEM images of synthesized TiO_2NPs

3.3- **Cytotoxic assay**

Cancer is considered to be the second leading cause of mortality worldwide. It is a malignant condition resulting from uncontrolled cell growth. Hepatocellular cancers are the deadliest of the several types of cancer and have the highest death rates **(WHO, 2018).** Creating new anticancer medications with lower side effects and more selectivity and efficacy is one of the most difficult fields of contemporary scientific research **(Ali, R et al., 2012).** The cytotoxicity of the evaluated materials (TIO_2.NPs) was investigated both before and after irradiation. Based on the MTT assay results shown in Figures 4, 5 and Table 1. TIO_2 was exposed to an infrared lamp. Since infrared radiation (IR) has the same impact as annealing temperature, an increase in annealing temperature resulted in an increase in the average size of synthetic TiO_2NPs. At annealing temperatures, synthesized TIO_2.NPs aggregated because of the nanoparticles' high surface energy **(C. Weiwei et al., 2014) (S. Bakardjieva et al., 2005) (L. Tesfaye Jule, K et al., 2021)**. The values of IC_{50} in case of HuH-7cells at IR-irradiation showed 12.8±3.2, 60.43±7.9 and 80.1±21.3 µg/mL for the 5-FU Standard, the control and TIO_2.NPs after exposure to 20m IR-irradiation respectively as shown on Table 1, **Figure** 6. While IC_{50} of normal WI38 cells at IR -irradiation showed

65.1±9.18, 64.7±12.84 and 98.9±20.2 μg/mL for the 5-FU Standard, the control and TIO_2.NPs after exposure to 20m IR-irradiation respectively. Where IC_{50} of TIO_2.NPs after exposure to 2.5m IR-irradiation is 53.4±7.6 μg/mL and 55.5±5.1 μg/mL for HuH-7cells and WI38 cells respectively. This means that the result of less exposure time to IR-radiation is closed to the result of the 5-FU Standard and the controlled. Unlike UV radiation, infrared radiation lacks the energy to cause electronic transitions. Non-ionizing radiation includes visible light, infrared, microwaves, radio waves, and even near ultraviolet light. In these circumstances, thermal ionization may result even from non-ionizing radiation if enough heat is deposited to bring temperatures to ionization energy levels **(Alton, GD, 1988).** However, the increasing of the exposure time of IR -irradiation makes heat to the particles and effect on the poly phenolic acid and make aggregation to the molecules.

Table 1: Titanium nanoparticle effect on cell inhabitation of Hepatocellular cancer and WI- 38 cell line after IR-irradiation.

IR-Exposure(min)	**Sample Concentration (µg/ml)**									
	100	**500**	**250**	**125**	**62.5**	**31.25**	**15.6**	**8.7**	**3.9**	***IC_{50} (µg/ml)***
	Evaluation of cytotoxicity against HuH-7 cell line **% Inhibition**									
5-FU Standar	**97.34**	**93.83**	**88.59**	**81.46**	**69.84**	**58.89**	**49.79**	**35.6**	**21.8**	***12.8± 3.2***
control	**81.89**	**75.79**	**70.85**	**55.68**	**47.74**	**14.74**	**5.78**	**0.00**	**0.00**	***60.43 ±7.9***
2.5	**28.79**	**26.76**	**24.59**	**21.89**	**18.74**	**0.00**	**0.00**	**0.00**	**0.00**	***53.4 ±7.6***
5	**29.89**	**26.99**	**25.89**	**23.86**	**16.65**	**0.00**	**0.00**	**0.00**	**0.00**	***57.2 ±3.7***
7.5	**34.86**	**31.89**	**30.73**	**23.86**	**17.79**	**0.00**	**0.00**	**0.00**	**0.00**	***60.2±3.12***
10	**37.89**	**35.79**	**33.86**	**24.36**	**17.19**	**13.81**	**0.00**	**0.00**	**0.00**	***70.7 ±15.2***
20	**25.67**	**33.73**	**31.76**	**19.36**	**12.12**	**8.81**	**0.00**	**0.00**	**0.00**	***80.1±21.3***
	Evaluation of cytotoxicity against WI-38 cell line **% Inhibition**									
5-FU Standar	**95.88**	**85.89**	**73.86**	**63.89**	**50.89**	**25.85**	**10.95**	**3.88**	**0**	***65.1± 9.1***
control	**95.09**	**86.79**	**71.79**	**63.79**	**51.86**	**28.89**	**10.55**	**4.89**	**0**	***64.7± 12.***
2.5	**30.89**	**26.86**	**25.57**	**23.87**	**18.15**	**0.00**	**0.00**	**0.00**	**0**	***55.5 ±5.1***
5	**29.13**	**26.89**	**24.86**	**23.89**	**16.69**	**0.00**	**0.00**	**0.00**	**0**	***58.2 ±3.8***
7.5	**34.24**	**31.88**	**29.83**	**29.79**	**14.89**	**0.00**	**0.00**	**0.00**	**0**	***64.7±3.1***
10	**39.87**	**34.75**	**31.89**	**20.36**	**14.89**	**13.71**	**0.00**	**0.00**	**0**	***104.3±33.***
20	**28.69**	**34.67**	**31.79**	**17.76**	**9.19**	**7.81**	**0.00**	**0.00**	**0**	***98.9±20.2***

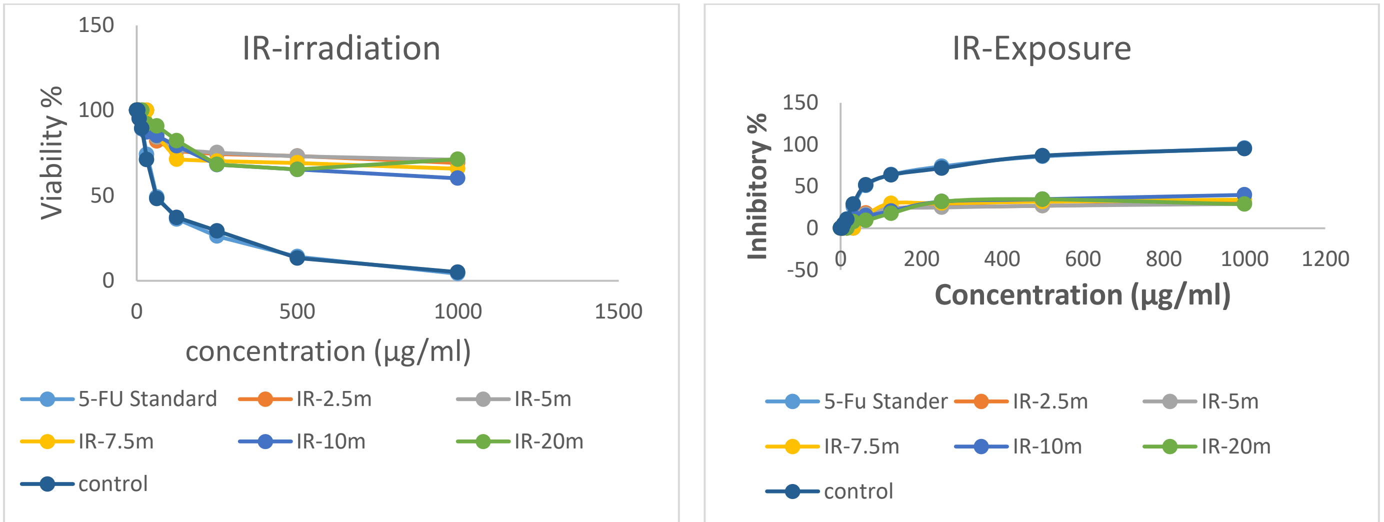


Figure 4. Titanium nanoparticle effect on cell viability and inhabitation of WI-38 cell line after IR-irradiation.

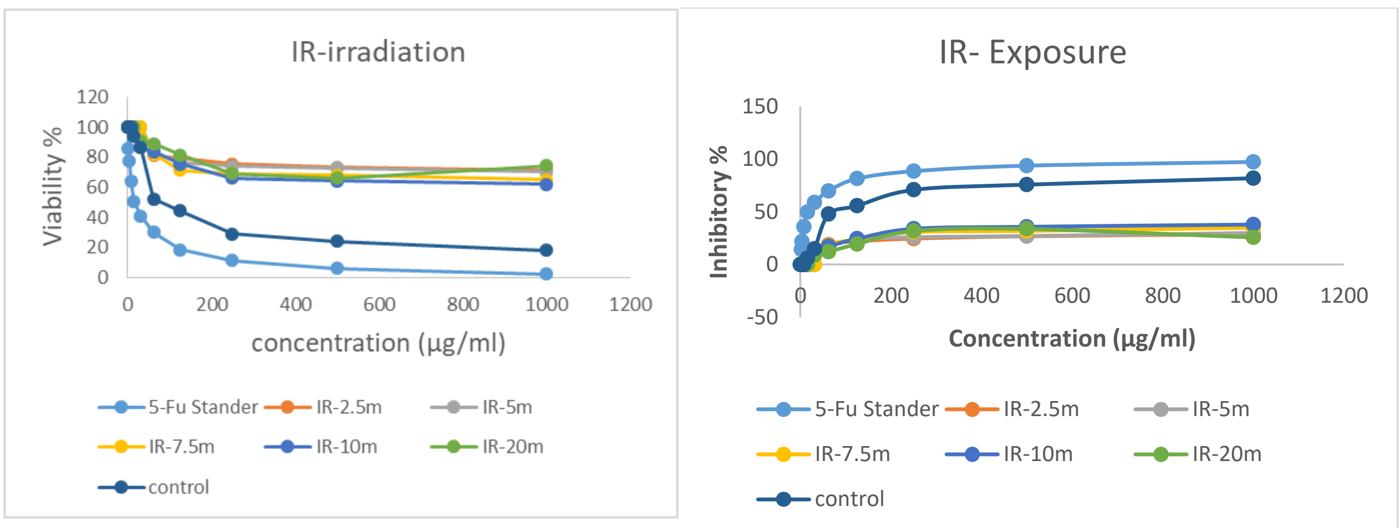


Figure 5. Titanium nanoparticle effect on cell viability and inhabitation of Hepatocellular cancer cell line after IR-irradiation.

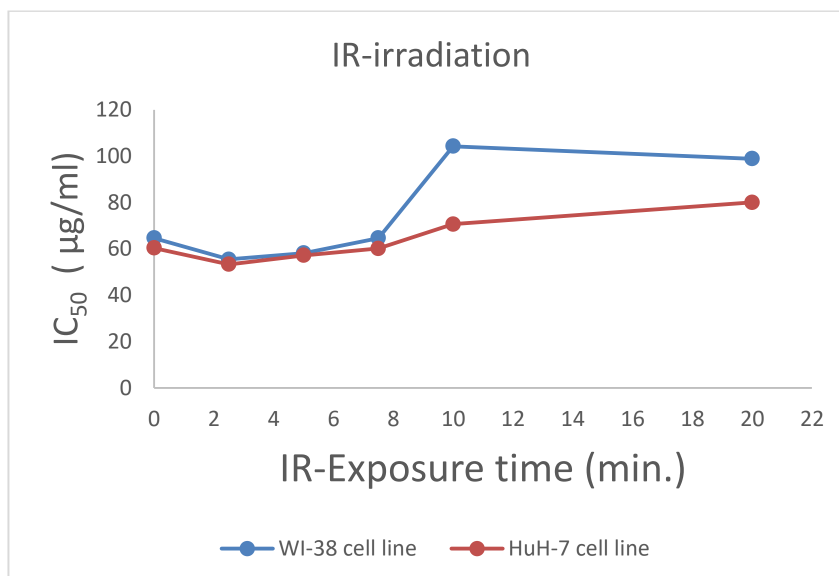


Figure 6. Relation between IC_{50} of TIO_2 and IR-exposure time

IV. Conclusion

- The TiO_2 nanoparticles in this work were synthesised using a green synthesis approach, and they were synthesised successfully at three different pH levels: basic (pH 9.0), neutral (pH 7.0), acidic (pH 5.0). Utilizing green tea extract produces particles in the nano-size range that can be further utilized in numerous biological applications while also being time- and environmentally-friendly.

- The increasing of the exposure time of IR -irradiation makes heat to the particles and effect on the poly phenolic acid and make aggregation to the molecules.

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