

# Study Of TiO<sub>2</sub> Nanoparticles Induction For Biological System

**Hassan Tuhmaz Hamad**

**Lames Majid Hammed**

## **Abstract:**

### **Background:**

The biological activity of NPs strongly depends on physicochemical parameters but these are not routinely considered in toxicity screening, such as dose metrics which is studied by many researchers. Titanium dioxide nanoparticles (nano-TiO<sub>2</sub>) are extensively used in the cosmetics, pharmaceutical, and paint industries as a coloring material due to its high stability, anticorrosion and photocatalytic properties

**Aim:** This study represents the study of TiO<sub>2</sub> nanoparticles induction for biological systems.

**Method:** Curve fitting in matlab program is used to study the effect of parameters of the system on the cell levels with existence TNPs.

### **Results:**

The Consequences showed that nanoparticles induce human embryonic kidney cells (HEK-293) the generation of reactive oxygen species (ROS) followed by significant depletion of glutathione levels and increased lipid peroxidation.

From the results, we observe that the behavior of can be predicated by estimating the equations which described the variation of culler effecting by increased the TNPs concentration at different exposure times.

**Keywords:** TiO<sub>2</sub> nanoparticles; Cell viability; HEK-293.

**Thi-Qar University, College of medicine**

**Introduction:**

The development in nanotechnology transports advantages in diverse areas of our lives such as engineering, information-technology and medicine. The small size and high surface-volume ratio endowed them with an active group or essential toxicity [1-5]. With the rapid development of nanotechnology, a variety of engineered nanoparticles (NPs) are being produced. Nanotoxicology has become a hot topic in many fields, as researchers attempt to elucidate the potential adverse health effects of NPs. The biological activity of NPs strongly depends on physicochemical parameters but these are not routinely considered in toxicity screening, such as dose metrics [1].

Titanium dioxide nanoparticles (nano-TiO<sub>2</sub>) are widely used in the cosmetics, pharmaceutical, and paint industries as a coloring material due to its high stability, anticorrosion and photocatalytic properties [6-10]. TiO<sub>2</sub> NPs have also been shown to produce reactive oxygen species (ROS) leading to the toxicity [9, 11-12]. TiO<sub>2</sub> nanoparticles besides having photo-catalytic activity also induced oxidative DNA damage. At the nano level, TiO<sub>2</sub> NPs at a given size can agglomerate in different sizes and structures. Influence of the agglomeration of TiO<sub>2</sub> NPs on pulmonary toxicity has been investigated by others [9,12]. The objective of the present study is to introduce the cytotoxicity and induction of apoptosis by TiO<sub>2</sub> nanoparticles in HEK-293 (theoretically) with following objectives: (1) To estimate whether nanoparticles are capable of inducing cytotoxicity and genotoxicity in human embryonic kidney cells, (2) To perceive

the link between nanoparticles induced oxidative stress and cell apoptosis.

**Theory:**

The nanoparticles were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD) and dynamic light scattering (DLS). TNPs (50 µg/ml) was dissolved in distilled water and ultrasonicated for 30 min to make the homogeneous suspension. MTT (tetrazolium salt) assay was applied to evaluate the effect of TNPs on HEK-293 cells viability by measuring the uptake and reduction of tetrazolium salt to an insoluble formazan dye by cellular microsomal enzymes [1]. *Catalase assay*: The activity of catalase enzyme was measured and described [2]. *GPx assay*: The activity of glutathione peroxidase (GPx) was measured using amount of (µg) protein of the cell lysates. *LPx assay*: Lipid peroxidase in microsomes was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method as described by Varshney and Kale [5] and is expressed in terms of formation of malonaldehyde (MDA) per mg protein.

**Method:**

Curve fitting, also known as regression analysis, is used to find the "best fit" line or curve for a series of data points. Most of the time, the curve fit will produce an equation that can be used to find points anywhere along the curve. In some cases, you may not be concerned about finding an equation. Instead, you may just want to use a curve fit to smooth

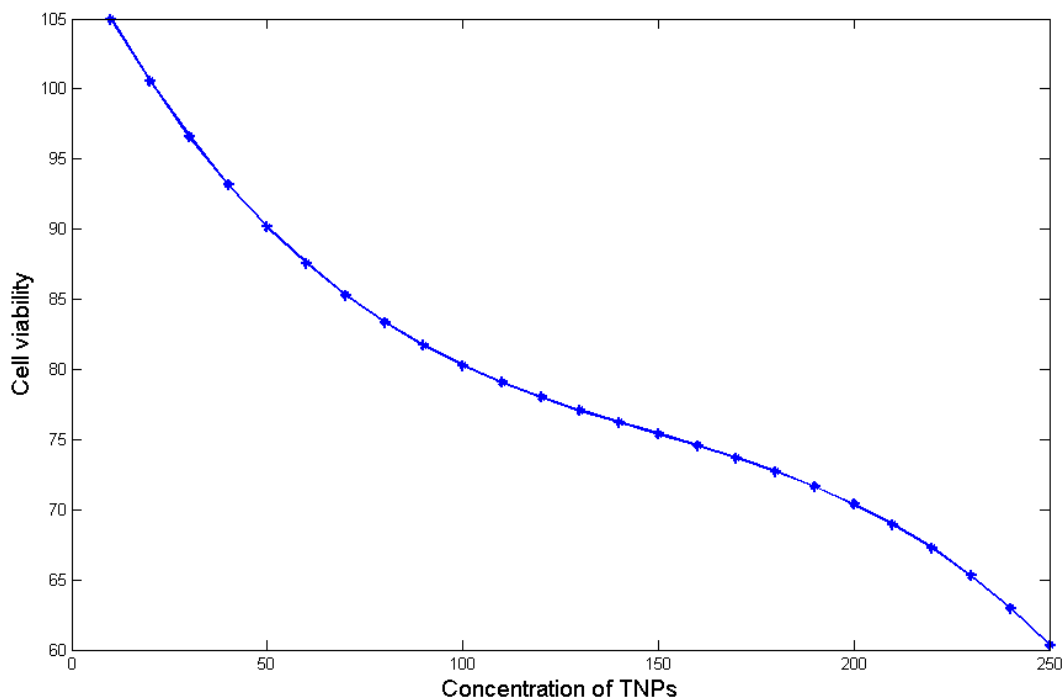
the data and improve the appearance of your plot.

**Results and discussions:**

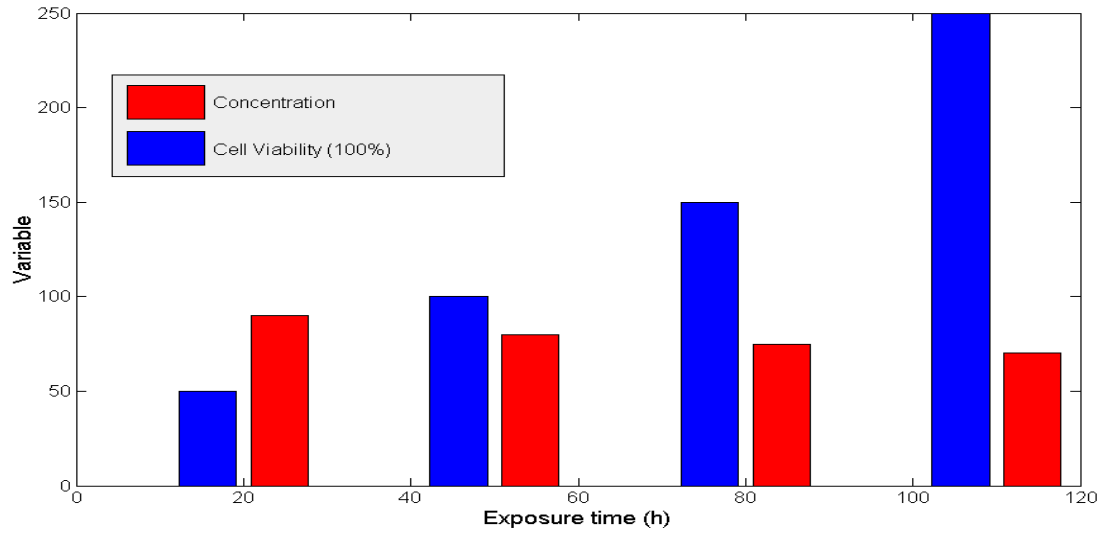
The variable indicates to the y-axes ,i.e., the level of study for such as SOD activity , Catalase activity , GPx activity, and LPx activity

Figure(1) shows cell viability of HEK-293 cells vs. concentration of (TNPs), Figure(2) represents cell viability of HEK-293 cells after exposure to 50, 100 and 250 mg/L of TNPs for 10, 60 and 120 h, Figure(3)shows cell apoptosis HEK-293 cells the nanoparticles(the total

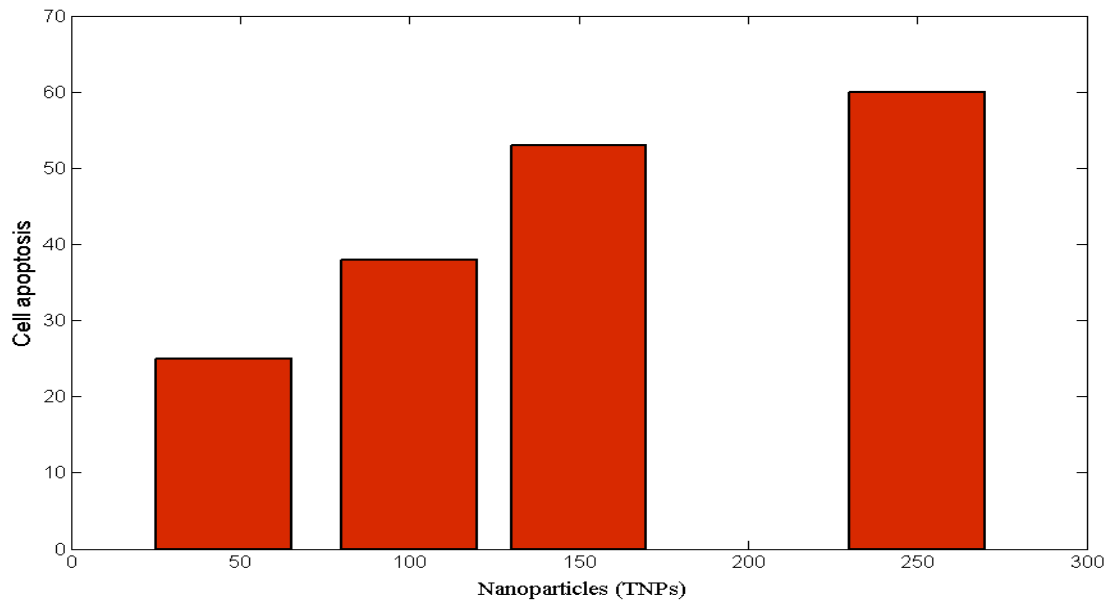
apoptotic cells population of HEK-293 cells in percentage), Fig(4) refers to the cell viability of HEK-293 cells after exposure to 50, 100 and 250 mg/L of TNPs for 10, 60 and 120 h and Figure(5) shows the antioxidative enzyme level nanoparticles (TNPs) treated HEK-293 cells. (A) SOD activity, (B) Catalase activity,(C) GPx activity (Cellular GPx level exhibited also a dose-dependent decrease), (D) LPx activity (Cellular SOD level exhibited a dose-dependent decrease. Compared to control, the SOD levels were decreased after exposure to respective dose of TNPs).



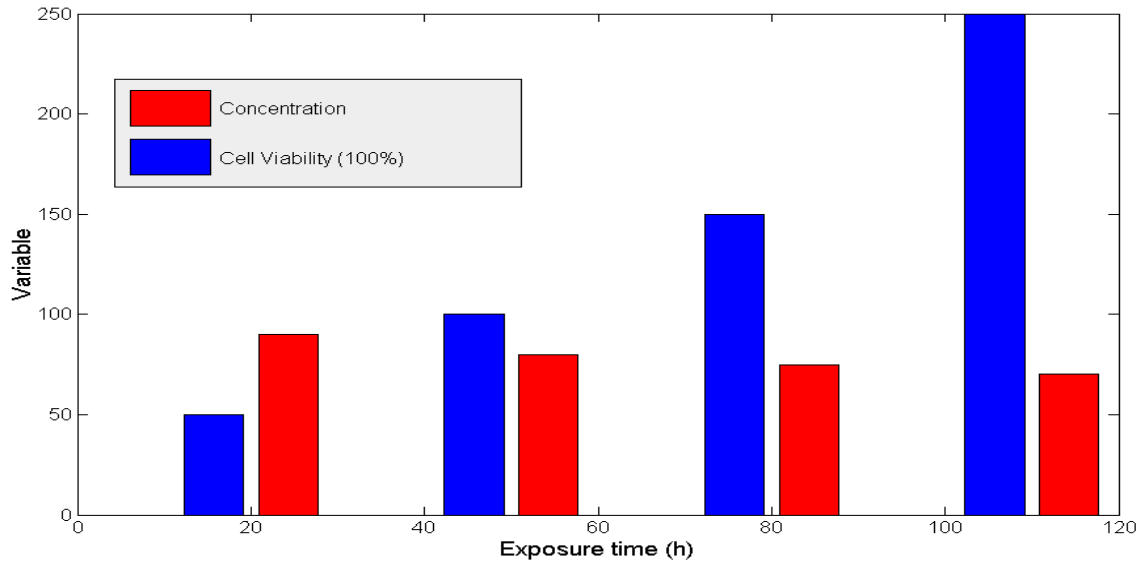
**Fig. 1.** Shows cell viability of HEK-293 cells vs. concentration of (TNPs).



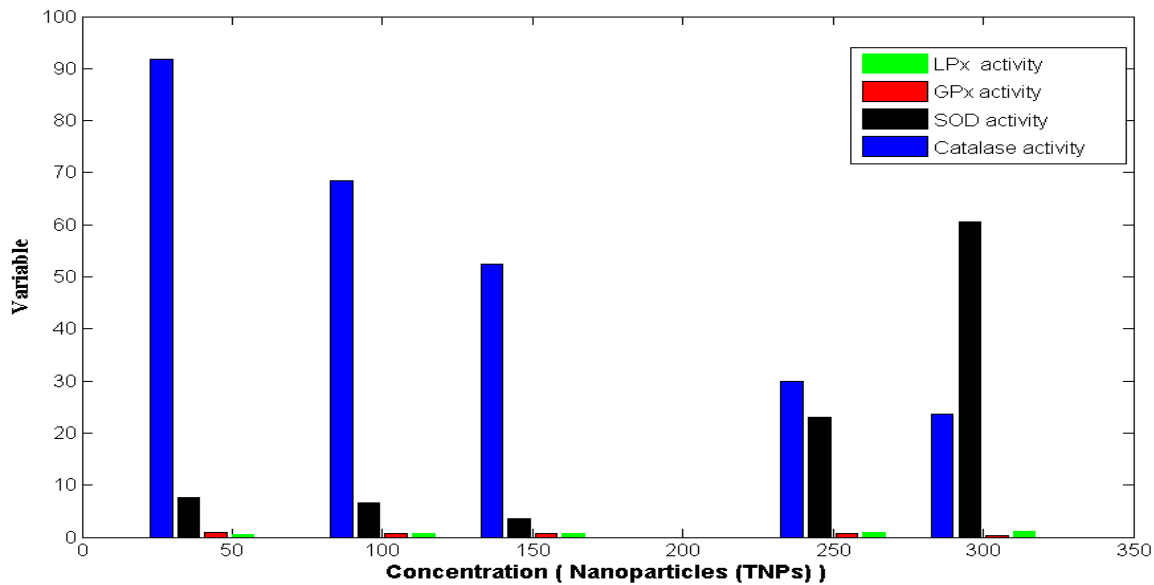
**Fig. 2.** Shows cell viability of HEK-293 cells after exposure to 50, 100 and 250 mg/L of TNPs for 10, 60 and 120 h.



**Fig. 3.** Shows cell apoptosis HEK-293 cells the nanoparticles (the total apoptotic cells population of HEK-293 cells in percentage).



**Fig. 4.** Shows cell viability of HEK-293 cells after exposure to 50, 100 and 250 mg/L of TNPs for 10, 60 and 120 h.



**Fig. 5.** Shows the antioxidative enzyme level nanoparticles (TNPs) treated HEK-293 cells. (A) SOD activity (Unit/mg protein), (B) Catalase activity (nKat/mg protein), (C) GPx activity (nM of NADPH oxidised/min/mg protein), (D) LPx activity (nM MDA/ mg protein).

## Conclusions:

From this study we find that the behavior of can be predicated by estimating the equations which are described the variation of culler effecting by increased the TNPs concentration at different exposure times.

TNPs increase cell population in a dose dependent manner. GPx activity (Cellular GPx level exhibited also a dose-dependent decrease) and LPx activity (Cellular SOD level exhibited a dose-dependent decrease. Compared to control, the SOD levels were decreased after exposure to respective dose of TNPs).

## References

- 1- Bhattacharya, K.; Davoren, M.; Boertz, J.; Schins, R.P.; Hoffmann, E.; Dopp, E. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in humanlung cells. *Part. FibreToxicol.* **2009**, *6*, 17.
- 2- Geiser M, Rothen-Rutishauser B, Kapp N, Schuerch S, Kreyling W, Schulz H, Semmler M, Im HV, Heyder J, Gehr P. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environtal Health Perspectives.* 2005; 113:1555-60.
- 3- Ramovatar Meena<sup>1</sup>, Ruchita Pal<sup>2</sup> , Surya Narayan Pradhan<sup>1</sup>, Madhu Rani and R. Paulraj, Comparative study of TiO<sub>2</sub> and TiSiO<sub>4</sub> nanoparticles induced oxidative stress and apoptosis of HEK-293 cells , *Adv. Mat. Lett.* 2012, 3(6), 459-465.
- 4- Chithrani BD, Chan WCW. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Letters.* 2007; 7:1542-50.
- 5- GurrJR,Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology.* 2005; 213, 66–73.

6- Noël, A.; Maghni, K.; Cloutier, Y.; Dion, C.; Wilkinson, K.J.; Hallé, S.; Tardif, R.; Truchon, G. Effects of inhaled nano-TiO<sub>2</sub> aerosols showing two distinct agglomeration states on rat lungs.

*Toxicol. Lett.* **2012**, *214*, 109–119.

7- Noël, A.; Cloutier, Y.; Wilkinson, K.J.; Dion, C.; Hallé, S.; Maghni, K.; Tardif, R.; Truchon, G. Generating nano-aerosols from TiO<sub>2</sub> (5 nm) nanoparticles showing different agglomeration states.

Application to toxicological studies. *J. Occup. Environ. Hyg.* **2013**, *10*, 86–96.

8- Noël, A.; Charbonneau, M.; Cloutier, Y.; Tardif, R.; Truchon, G. Rat pulmonary responses to

inhaled nano-TiO<sub>2</sub>: Effect of primary particle size and agglomeration state. *Part. Fibre Toxicol.* **2013**, *10*, 48.

9- Wang, J.; Fan, Y.; Gao, Y.; Hu, Q.; Wang, T. TiO<sub>2</sub> nanoparticles translocation and potential toxicological effect in rats after intraarticular injection. *Biomaterials* **2009**, *30*, 4590–4600.

10- Li, B.; Ze, Y.; Sun, Q.; Zhang, T.; Sang, X.; Cui, Y.; Wang, X.; Gui, S.; Tan, D.; Zhu, M.; Molecular mechanisms of nanosized titanium dioxide-induced pulmonary injury in mice. *PLoS One* **2013**, *8*, e55563.

11- Soto K, Garza KM, Murr LE: Cytotoxic effects of aggregated nanomaterials. *Acta Biomaterials*. 2007; 3:351-8.

12- Park S, Lee YK, Jun, M, Kim KH, Chung N, Ahn EK, Lim Y, Lee KH. Cellular toxicity of various inhalable metal

nanoparticles on human alveolar epithelial cells. *Inhalation Toxicology*. 2007; 19:59–65.

# دراسة تحفيز الجسيمات النانوية $TiO_2$ لأنظمة بايولوجية

لميس ماجد حميد

حسن طهماز حمد

كلية الطب / جامعة ذي قار

## الخلاصة:

**الخلفية العلمية:** يعتمد النشاط البيولوجي للـ NPs بشدة على العوامل الفيزيائية والكيميائية، لكن هذه العوامل لا تؤخذ بشكل روتيني في فحص السمية، مثل مقاييس الجرعة التي يدرسها العديد من الباحثين. تستخدم جزيئات ثاني أكسيد التيتانيوم ( $nano-TiO_2$ ) على نطاق واسع في صناعات مستحضرات التجميل والأدوية والطلاء كمادة تلوين بسبب ثباتها العالي ومقاومة التآكل وخصائص التحفيز الضوئي الهدف: تمثل هذه الدراسة دراسة تحريض الجسيمات النانوية  $TiO_2$  للأنظمة البيولوجية.

**الطريقة:** يستخدم منحنى برنامج mat lab لدراسة تأثير معلمات النظام على مستويات الخلايا مع وجود TNPs.

**النتائج:** أظهرت النتائج أن الجسيمات النانوية تحفز خلايا الكلى الجنينية البشرية-HEK (293) على توليد أنواع الأكسجين التفاعلية (ROS) تليها استنفاد كبير لمستويات glutathione وزيادة بيروكسيد الدهون. نلاحظ أنه يمكن التنبؤ بسلوك المعادلة من خلال تقدير المعادلات التي تصف تباين التأثير الناتج عن زيادة تركيز TNPs في أوقات التعرض المختلفة.

**الكلمات المفتاحية:** الجسيمات النانوية  $TiO_2$  ؛ بقاء الخلية ؛ HEK-293.