

## Effects of Exposure to Titanium Dioxide Nanoparticles on Albino Rat Visual Cortex "Electron Microscopic Study"

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**Abstract:** Nanotechnology is the manipulation of matter on a near atomic scale to produce new structures, materials, and devices. It has become an important industry in the 21<sup>st</sup> century. Nanoparticles (NPs) are extremely small particle with large surface area. This property gives them different properties than its original forms. The extremely small size property of the NPs renders them more potentially dangerous with unexpected adverse health effects than their fine-sized counterpart. Our study aims to study the effect of exposure to TiO<sub>2</sub> NPs on the albino rat brain visual cortex. Thirty adult male albino rats were used in this study. The animals were divided into two main groups; control group, fifteen adult male rats, received 1 ml of 0.9% NaCl solution daily orally for seven days and experimental group, fifteen adult male rats, received 1 ml of TiO<sub>2</sub> NPs solution for seven days. Transmission electron microscope of the titanium treated rat visual cortex showed pyramidal cells with shrunken irregular nucleus and duplication of the nuclear membrane and their cytoplasm showed some inclusion bodies, swollen mitochondria, dilated rough endoplasmic reticulum and swollen Golgi apparatus. The dendrites and axonal bundles showed thinning and disintegration of myelin sheath. The Oligodendroglial cell showed small shrunken nucleus with peripherally clumping chromatin and dilated rough endoplasmic reticulum. In conclusion the exposure to TiO<sub>2</sub> NPs induced major degenerative changes in the albino rat visual cortex. So, we have to avoid exposure to these NPs as possible.

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**Key words:** titanium dioxide, nanoparticles, albino rat brain and visual cortex.

### 1. Introduction

Nanoparticles (NPs) are extremely small particle with large surface area. This property gives it a different properties than its original forms [De Belie *et al.*, 2004, Cassar *et al.*, 2007, Hashimoto, 2007]. The newly developed properties enhanced its use in industries as in paints, paper, cosmetics, sunscreens and so. According to the National Nanotechnology Initiative of America, the nanosized TiO<sub>2</sub> particles are among those most widely manufactured on a global scale [Liang *et al.*, 2009].

Unfortunately the extremely small size property of the NPs that gives it its unique properties, render them more potentially dangerous with an unexpected adverse health effects than their fine-sized counterpart. This fact resulted in developing a new science that is regarded as nanotoxicology. [Oberdorster *et al.*, 2005, Nel *et al.*, 2006, Demeestere *et al.*, 2008].

Titanium dioxide NPs (TiO<sub>2</sub>NPs) is a fine, white, crystalline, odorless, low-solubility powder. It is one of the most widely used NPs. However, the toxicological profile of TiO<sub>2</sub> NPs is not completely understood and several concerns have emerged on the potential undesirable effects of the TiO<sub>2</sub> NP

properties (Nel *et al.*, 2006, Sager *et al.*, 2008). The most dangerous of which is the carcinogenic potential of the inhaled TiO<sub>2</sub> NPs (Nel *et al.*, 2006 and Baan, 2007).

The minute size TiO<sub>2</sub> NPs enables it to translocate into the brain whatever the route of exposure. It was proved that these particles have the ability to accumulate in the brain. This translocated NPs induced numerical and structural changes in the neuronal architecture [Wang *et al.*, 2007, Cho *et al.*, 2010, Li *et al.*, 2010, Ma *et al.*, 2010]. Better understanding of this phenomenon requires the need of further studies (Iavicoli *et al.*, 2012).

Our understanding of the general and occupational health and safety aspects of NPs is still in its formative phase and greater effort is needed to understand how NPs interact with the human body [Yokel and MacPhail, 2011 and Clift *et al.*, 2011]. This experiment aims to study the effect of exposure to TiO<sub>2</sub> NPs on the albino rats brain visual cortex.

### 2. Materials and Method:

#### Experimental animals and design:

Thirty adult albino rats were used in this study. The average weight of the animal was 200

grams with 2 months of age. The animals were housed in the animal house of the Faculty of Medicine, Assiut University in a normal daily light and darkness cycle and fed with normal show and water. The rats were divided into two main groups:

1. **Control group:** fifteen male albino rats were received 1 ml of 0.9% NaCl solution daily orally for seven days.
2. **Titanium dioxide nanoparticles treated group:** fifteen male albino rats were received 1 ml of Titanium dioxide nanoparticle solution; where 0.9% NaCl solution were be used as a vehicle - in a dose of 1 gm daily via oral gavage for seven days according to Zhang *et al.*, (2010). All rats of the two groups were sacrificed for sampling after eight days from the starting of the experiment. For electron microscopy, 1mm X 1 mm of the brain visual cortex blocks were taken and put in cold 5% gluteraldehyde on cacodylate buffer solution pH 7.2 and were left for fixation before processing for electron microscopy. Gluteraldehyde-fixed epon embedded tissue samples were sectioned as 800 Å ultrathin sections, contrasted using lead citrate and uranyle acetate and examined using transmission electron microscope.

#### Stereological Procedures:

A number of non-overlapping diagrams were made by a camera Lucida (Leitz Wetzlar, Germany) at a magnification of x1000 times using a Leitz light research microscope. The diagrams exposed complete sectors of the visual cortices. These diagrams were drawn for stereological procedures.

A digitizing set consisted of Digitizer KD 3040 B connected to integer IBM compatible personal computer was used with a specially prepared program to measure lengths. The major diameter (a), which is the widest diameter, and minor diameter (b), which is the widest diameter, perpendicular over (a). The diameter of equivalent circle ( $D^*$ ) was calculated ( $D^* = a \cdot b$ ).  $\sqrt{\text{Schwartz - Saltikov}}$  correction procedure (appendix 1) was applied to obtain more reliable estimates of the true mean nuclear diameter ( $D^*$ ).

- 1- Estimation of the thickness of layer V rat visual cortex: in this parameter, the layer V thickness

from the diagrams is measured in cm. Then, the true magnification was estimated. The actual thickness in microns was calculated as follow:

The thickness in cm X 10000 / the true magnification.

- 2- Estimation of the numerical density ( $N_v$ ) of the pyramidal cells in layer V of rat visual cortex: the numerical density of the pyramidal cells was calculated as follow:

$$N_v = \frac{N / a}{D^* + t}$$

where:

(N) is the number of calculated cells.

(a) is the area in which the number of calculated cells were measured.

(D) is the corrected mean diameter of the nucleus. The corrected procedure for nuclear diameter was performed by using Schwartz and Saltikov correction table.

(t) : thickness of the section, which equals 1.0 micron.

#### Statistical Analysis:

The previously mentioned parameters were calculated for each animal group. The mean value and the standard deviation were calculated for each parameter. Unpaired student t-test was used to compare between the mean values of different groups. The level of significance ( $P$ ) was considered as follow:

- $P > 0.05$ , non-significant.
- $P < 0.05^*$ , significant.
- $P < 0.01^{**}$ , high significant.

#### 3-Results:

##### Stereological analysis

##### The thickness of layer V in rat visual cortex: (Tab. 1)

The mean thickness of layer V in rat visual cortex of the control group was  $923.63 \pm 3.69 \mu\text{m}$  and that of the titanium treated group was  $885.96 \pm 3.94 \mu\text{m}$ . The decrease in the thickness of titanium treated rat was highly significant ( $P = 1.36\text{E-}12$ ) when compared to the control group.

**Table (1):** The mean  $\pm$  SD of layer V thickness of the control and titanium treated rat visual cortex.

	Control N = 15	Treated N = 15	C. Vs T. P =
Mean Thickness	$923.63 \pm 3.69 \mu\text{m}$	$885.96 \pm 3.94 \text{Mm}$	1.36 E-12**

(N) number of the animals per group.

(\*\*) highly significant.

### Numerical Densities of pyramidal Cells in layer V in rat visual cortex Per Unit Volume (Tab. 2).

The mean of the numerical densities of the pyramidal Cells in layer V in control rat visual cortex Per Unit Volume was  $4967.3 \times 10^3 \pm 596.9 \times 10^3$

and that of titanium treated group was  $2662.5 \times 10^3 \pm 110.2 \times 10^3$ . The decrease in the mean value of the numerical densities of pyramidal cells of the treated group was highly significant when compared to the control group.

**Table (2): The mean  $\pm$  SD of the numerical densities of the pyramidal Cells in layer V in neonatal rat visual cortex the control and titanium treated groups.**

	Control N = 15	Treated N = 15	C. Vs T. P =
pyramidal Cells (Nv)	$4967.3 \times 10^3 \pm 596.9 \times 10^3$	$2662.5 \times 10^3 \pm 110.2 \times 10^3$	0.001**

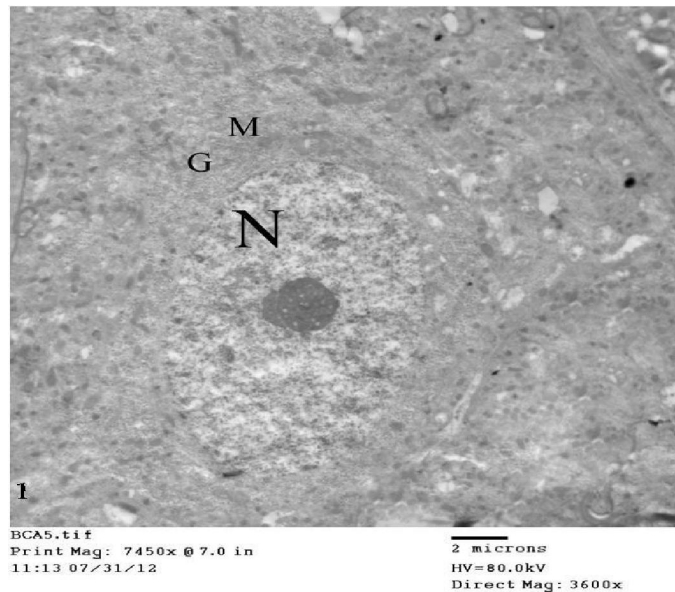
(N) number of the animals per group.

(\*\*) highly significant.

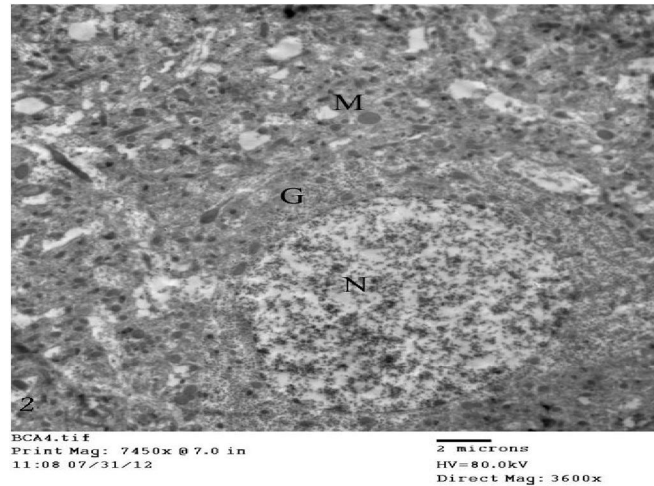
### Transmission Electron Microscope (TEM) examination:

TEM of the control rat visual cortex revealed that, the multipolar pyramidal cells with oval euchromatic nuclei and prominent nucleoli. Their cytoplasm contains numerous Nissle granules and small rounded mitochondria (Figs. 1, 2). The dendrites showed multiple mitochondria and Nissle granules (Fig. 3). Bundles of thick myelinated dendrites and axons were shown together with Oligodendroglial cells having a large rounded nucleus and scanty cytoplasm (Fig. 4).

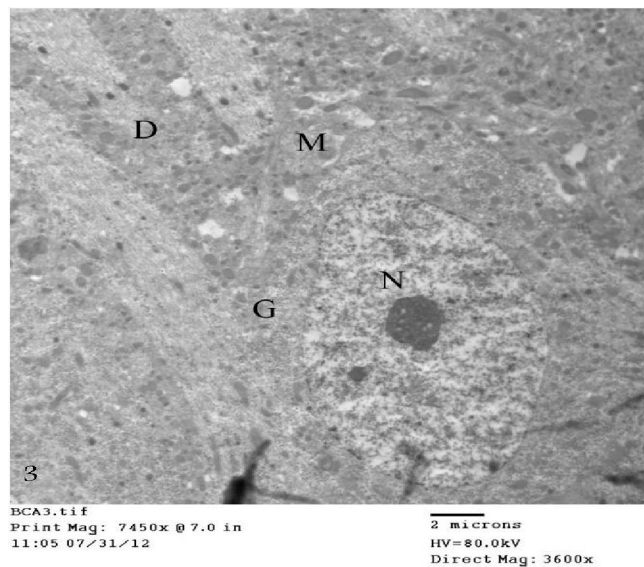
TEM of the titanium treated rat visual cortex showed pyramidal cells with shrunken irregular nucleus together with duplication of the nuclear membrane. The cytoplasm showed some inclusion bodies, swollen mitochondria, dilated rough endoplasmic reticulum and swollen Golgi apparatus (Figs. 5, 6, 7). The dendrites and axonal bundles have thin disintegrating myelin sheath. The Oligodendroglial cell showed small shrunken nucleus with peripherally clumping chromatin. Their cytoplasm show dilated rough endoplasmic reticulum (Fig. 8).



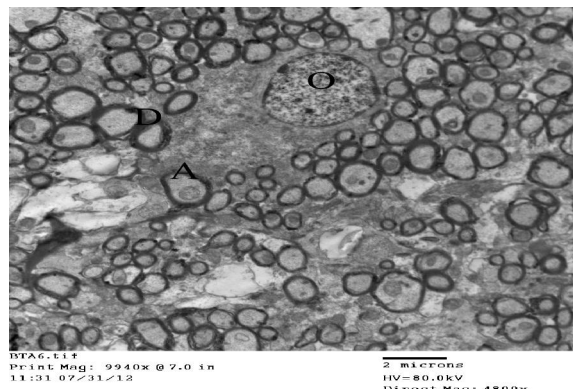
**Fig (1):** An electron micrograph demonstrating the pyramidal cell of the control rat visual cortex with oval nucleus (N) and prominent nucleolus, the cytoplasm has a numerous mitochondria (M) and Nissle granules (G).  
(Uranyl acetate & lead citrate x 3600)



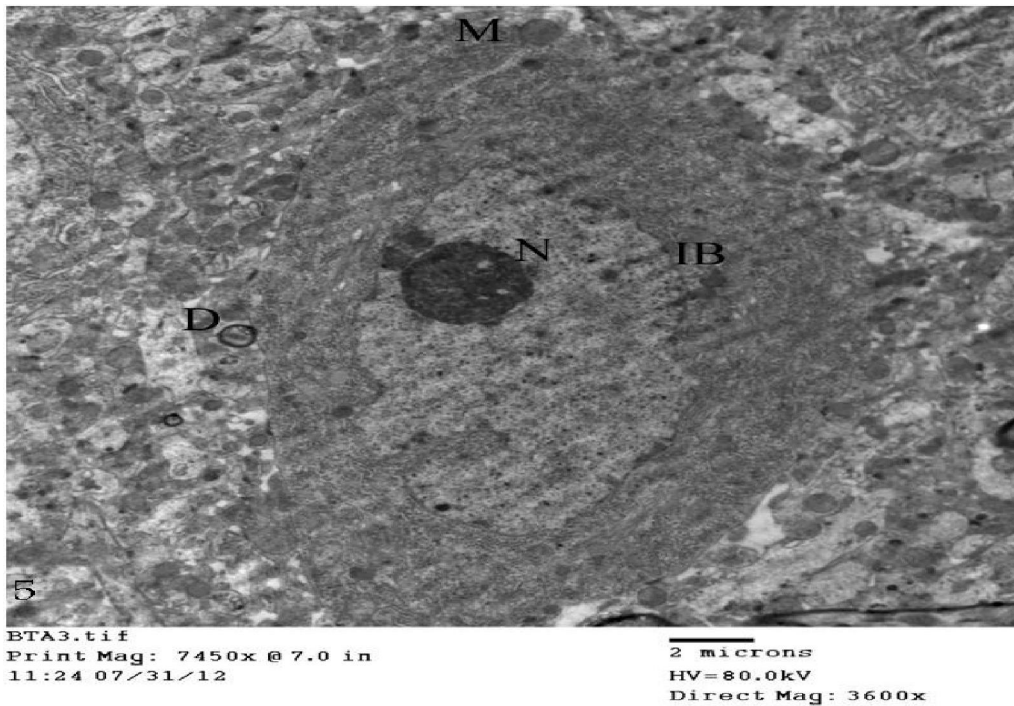
**Fig (2):** An electron micrograph demonstrating the pyramidal cell of the control rat visual cortex with euchromatic nucleus (N), the cytoplasm has a numerous mitochondria (M) and Nissle granules (G). (Uranyl acetate & lead citrate x 3600)



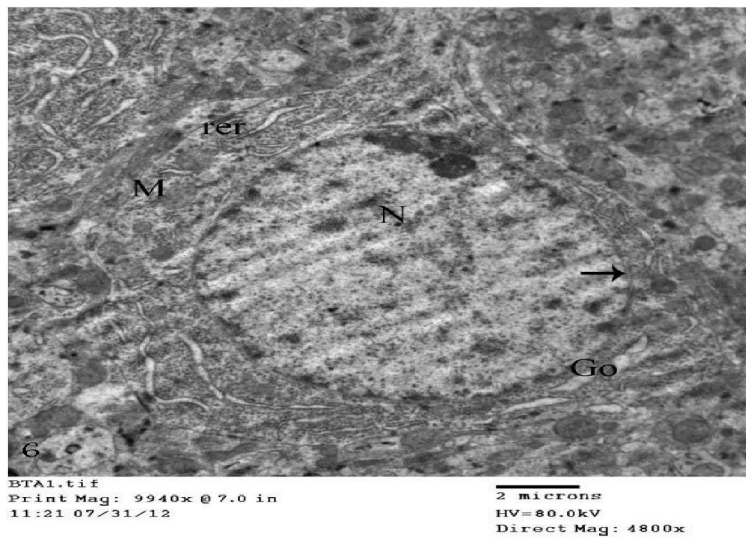
**Fig (3):** An electron micrograph demonstrating the pyramidal cell of the control rat visual cortex with oval nucleus (N) and prominent nucleolus, the cytoplasm has a numerous mitochondria (M), Nissle granules (G) and dendritic process with large number of mitochondria and Nissle granules (D). (Uranyl acetate & lead citrate x 3600)



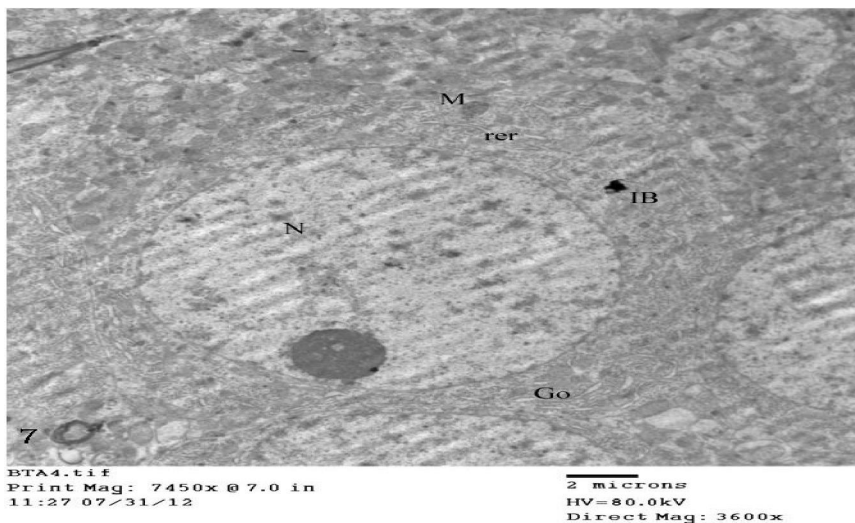
**Fig (4):** An electron micrograph demonstrating bundles of thickly myelinated dendrites (D) and axons (A) in the control rat visual cortex. Oligodendroglial cells (O) with rounded large nucleus and scanty cytoplasm. (Uranyl acetate & lead citrate x 4800)



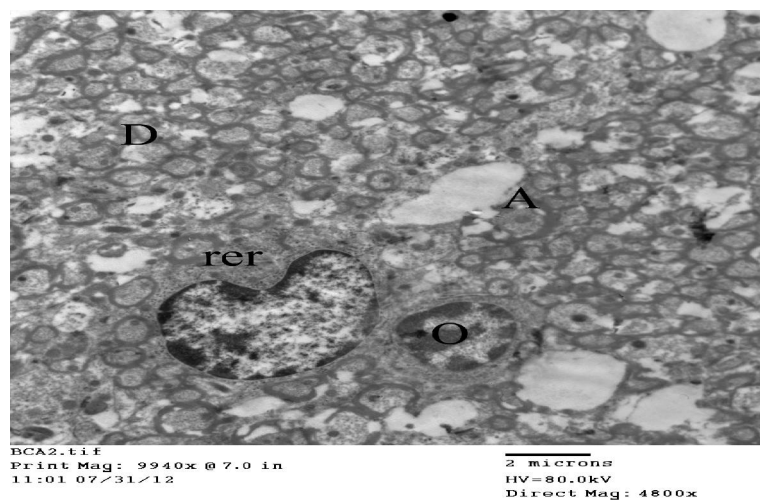
**Fig (5):** An electron micrograph demonstrating the pyramidal cell of the titanium treated rat visual cortex with shrunken, irregular nucleus (N), the cytoplasm has a few mitochondria (M), Nissle granules (G), inclusion bodies (IB) and thin myelinated dendrite (D). (Uranyl acetate & lead citrate x 3600)



**Fig (6):** An electron micrograph demonstrating the pyramidal cell of the titanium treated rat visual cortex with duplicated nuclear membrane (arrow), swollen mitochondria (M), dilated rough endoplasmic reticulum (rer) and Golgi bodies (Go). (Uranyl acetate & lead citrate x 4800)



**Fig (7):** An electron micrograph demonstrating the pyramidal cell of the titanium treated rat visual cortex with swollen mitochondria (M), dilated rough endoplasmic reticulum (rer) and Golgi bodies (Go).  
(Uranyl acetate & lead citrate x 3600)



**Fig (8):** An electron micrograph demonstrating bundles of thin myelinated, degenerated dendrites (D) and axons (A) in the treated rat visual cortex. Oligodendroglial cells (O) with a shrunken nucleus and dilated rough endoplasmic reticulum (rer).  
(Uranyl acetate & lead citrate x 4800)

#### 4. Discussion:

Nanotechnology is the manipulation of matter on a nearatomic scale to produce new structures, materials, and devices. It has become an important industry in the 21<sup>st</sup> century, and the U.S. National Science Foundation estimated it will grow into a trillion-dollar business, employing millions of workers worldwide, within the next decade (Castranova, 2011). TiO<sub>2</sub> is an example of a fine, white, crystalline, odorless, low-solubility powder which was considered to exhibit relatively low toxicity (Duan *et al.*, 2010). With regard to its

potential adverse health effects, several studies have defined TiO<sub>2</sub> as biologically inactive and physiologically inert in both humans and animals and thus as little risk to humans (Chen and Fayerweather, 1988, Bernard *et al.*, 1990 and Kang *et al.*, 2008). Other studies demonstrated several pathological effects on some organs such as the kidneys (Liang *et al.*, 2009), liver (Liang *et al.*, 2009 and Cui *et al.*, 2011), respiratory system (Moon *et al.*, 2010), immune system (Scuri *et al.*, 2010), skin (Yanagisawa *et al.*, 2009), reproductive system (Guo *et al.*, 2009) and nervous system (Wang

*et al.*, 2008). This investigation studied the ultrastructural changes in the albino rat visual cortex after exposure to TiO<sub>2</sub> NPs. The pyramidal cells of the TiO<sub>2</sub> NPs treated rats showed apoptotic changes in the form of nuclear shrinkage, irregularity and duplication of nuclear membrane. This is in commitment with the results of Wang *et al.* (2003) and Hu *et al.* (2011) who observed nuclear shrinkage and chromatin condensation in the neurons of the mouse hippocampus after treatment with TiO<sub>2</sub> NPs. Other studies demonstrated that TiO<sub>2</sub> NPs exposure can induce apoptosis or necrosis in neurons (Yu *et al.*, 2008) and Purkinje cells (Takeda *et al.*, 2009). Our study demonstrated also degenerated cytoplasm with few swollen mitochondria, dilated rER and few Nissle granules. Also the axons and dendrites were degenerated. These changes were observed by Hu *et al.* (2011). Oligodendroglia appeared shrunken with dilated rER and shrunken nucleus. Li *et al.* (2009) reported changes in microglia similar to our results. To understand the underlying mechanism for the brain injury of albino rat caused by exposure to TiO<sub>2</sub> NPs, the present study showed significant accumulation of TiO<sub>2</sub> NPs in the pyramidal cells of visual cortex in the form of multiple inclusion bodies. This indicates that TiO<sub>2</sub> NPs can easily cross the blood brain barrier and deposited in the brain tissue and damaging the brain integrity (Kwon *et al.*, 2008).

The previous ultrastructural changes were confirmed by statistical analysis which revealed significant reduction in the thickness of layer V and numerical density of the pyramidal cells in the visual cortex of treated rats.

In conclusion the exposure to TiO<sub>2</sub> NPs induced major degenerative changes in the albino rat visual cortex due to passage of these NPs through the blood brain barrier. So, we have to avoid exposure to these NPs as possible.

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