Original Article

Toxic Effect of Titanium Dioxide Nanoparticles on the Lung of Adult Male Albino Rat and Possible Protective Role Of β-Carotene: Light and Electron Microscopic Study

Walaa A. Rashad and Ola A. Abdelwahab

Department of Human Anatomy and Embryology, Faculty of Medicine, Zagazig University, Egypt.

ABSTRACT

Background: Titanium dioxide nanoparticles (TiO2 NPs) are commonly used in cosmeceutical, biomedical, pharmaceutical and industrial fields but its adverse effects are also increasing alarmingly. β-Carotene is an antioxidant that can guard against the toxicity induced by TiO2 NPs.

Aim of the Work: To study the histological alterations in the normal lung tissue of adult albino rats after administration of TiO2 NPs and to assess the potential protective effect of β-Carotene.

Materials and Methods: four groups of adult male albino rats were used in this work; treated group received a dose of 300 mg/kg of TiO2 NPs was administered intraperitoneally daily for 14 days. Along with one protected group received 10mg/kg β-carotene by gavage for 7 days before and another 14 days with 300 mg/kg TiO2 NPs administration, in addition to one control group and one group received only β-carotene. Tissue samples from the lung were stained with hematoxylin and eosin (H&E) and toluidine blue, ultrastructural study and morphometric analysis were also conducted.

Results: The treated group revealed marked histological alterations appeared as vacuolated cytoplasm of pneumocytes type II and loss of lamellar bodies' characteristic pattern in addition to dilated congested pulmonary blood vessels. Morphometric results showed thickening of the interalveolar septa and increased numbers of macrophages and pneumocytes type II in comparison to the control group. In the protected group, β-Carotene co-administration with TiO2 NPs greatly preserved the normal lung structure.

Conclusion: It can be concluded from this study that supplements of β-carotene can reduce the detrimental effect of TiO2 NPs on lung tissues via antioxidant mechanism.

Received: 24 May 2020, **Accepted:** 24 July 2020

Key Words: Beta carotene; nanotoxicology; lung injury; TiO2 nanoparticles.

Corresponding Author: Walaa A. Rashad, MD, Department of Human Anatomy and Embryology, Faculty of Medicine, Zagazig University, Egypt, **Tel.**: +20 1033037730, **E-mail:** dr_wa_anatomy@yahoo.com

ISSN: 1110-0559, Vol. 44, No.2

INTRODUCTION

Nanoscience has prospered a lot in the last decade with the rapid advance of nanotechnology and its widespread applications[1]. More than 2800 nanoparticle based applications were found to be available in various commercial areas, for example; medicine, electronics, energy and materials[2]. Nanoparticles are commonly used in the everyday life such as food additives, water treatment, disease diagnosis, implants and clothing. Consequently, researchers and scientists raised new concerns regarding the possible effects of nanoparticles on health and environment[3,4].

Titanium dioxide nanoparticles (TiO2 NPs) are ones of the most widely used and highly manufactured nanoparticles in the world[5]. TiO2 is a well-known semiconductor and a versatile compound that exists in three crystalline forms, rutile, brookite and anatase which can be activated with ultra violet light only owing to its high band gap energy[6]. The yearly production of TiO2 NPs in 2002 was approximately 3000 tons per annum, and is estimated to increase to 2.5 million metric tons annually by 2025[7]. TiO2 NPs have important role in various industries; such as industrial pigments owing to the high catalytic, optical and electrical properties. They are also used as photocatalyst in environmental purging, in purifing the water, in sunscreen creams, destruction of cancer cells, filteration of gases, health equipments, food colorants, ceramics, paper and plastics[8,9]. It is reported that in the manufacture of sunscreen and cosmetic products approximately 50% of TiO2 NPs are used[10].

The skin and the respiratory tract were recorded to be the main areas of exposure. Previous studies showed an inflammatory response in the pulmonary airways in a size-dependent way after intra-tracheal administration of TiO2 NPs to mammals[11,12]. It was also recorded that TiO2 NPs change the cell membrane integrity, destruct the nucleus and DNA by initiating inflammatory mechanisms, apoptosis and generation of the oxygen free radicals, and also alteration of the cell functions[13].

Epidemiological data suggest that antioxidants may have a beneficial effect on many diseases. Among dietary supplements, antioxidants including carotenoids represent ones of the largest categories. They are natural pigments; β-carotene is the most prominent among more than 600

 DOI: 10.21608/ejh.2020.28716.1281

different compounds that have been recognized up till now. β-carotene is a common constituent of carrots, apricots and green vegetables. It has been proved to be a powerful antioxidant and has the strongest provitamin A activity of all carotenoids. It deactivate harmful free radicals which are formed as a result of various stressors and during normal cellular activity[14,15,16,17].

MATERIALS AND METHODS

Animals

40 healthy adult male albino rats aged 6–8weeks (150–200 g) were used. They were brought from the Animal House, Faculty of Medicine, Zagazig University, Egypt. Rats were housed in a room with controlled light and temperature permitting free access to food and water. All procedures of the experiment were performed according to the pertinent guidelines and protocols of the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC committee), approval number ZU-IACUC/3/f/13/2020.

Chemicals

TiO2 NPs is white odorless fine nanopowder with particle size 26-56nm, 35-65 m2/g surface area, ≥99.5% purity and CAS no. is 634662. It was purchased from Sigma-Aldrich chemical company Egypt. β-carotene is a red orange powder, code no. c9750 was purchased from Sigma-Aldrich chemical company, Egypt. Phosphate buffer (PBS), dissection set, 10% formal saline, alcohol, xylene and paraffin wax for preparation to light microscopic examination, toluidine blue and H&E were purchased from faculty of medicine, Zagazig University. For transmission electron microscope; 2.5% glutaraldehyde, sodium phosphate buffer, 2% osmium tetraoxide, gradual ethanol series, epoxy resin, uranly acetate and lead citrate were purchased from Faculty of science, Zagazig University.

Experiment protocol

The rats were randomly divided into 4 groups each contained 10 rats: control group the rats were given intraperitoneal (i.p.) injection of 2 ml saline, daily for 14 days. β-carotene group rats were received 10mg/kg β-carotene by gavage once daily for 21 days[18]. Treated group rats were received 300 mg/kg TiO2 NPs by i.p. injection daily for 14 days[19]. Protected group rats were received 10mg/kg β-carotene daily by gavage for 7 days prior and 14 days together with i.p. injection of 300 mg/kg TiO2 NPs[19]. The solution for injection of TiO2 nanoparticles was prepared with distilled water while that for βC was diluted in corn oil. At the end of the experimental period, rats were anesthetized with i.p. injection of sodium thiopental and the extent of pulmonary toxicity was evaluated by light and electron microscopic examination of the obtained lung tissue samples.

Histological examination

Lung tissue samples from all rats were carefully dissected. Some of the specimens were immersed immediately in 10% buffered formalin (pH 7.2) for 48 hours then processed and embedded in paraffin wax to prepare sections of 5µm thickness stained with hematoxylin and eosin (H&E). Other specimens were post fixed in 1% osmium tetroxide, then were processed and prepared as semithin sections stained by 1% toluidine blue. Both were examined and photographed by light microscope in the anatomy department unit, faculty of medicine, Zagazig University. The remaining specimens were processed into ultrathin sections (80–90 nm) were stained with uranyl acetate 5% for 15 minutes and lead citrate for 8 minutes then examined and photographed by transmission electron microscope (JEOL JEM-2100; Boston, united states, Faculty of Agriculture, electron microscope research unit, Al-Mansoura University, Egypt. For assessment of the morphology and the particle size of TiO2 NPs, the powder of the particle was suspended in water and sonicated at 40 W for 20 minutes and TiO2 suspensions were dropped on carbon-coated copper T grids then dried before the measurement. Then photographed by transmission electron microscope (JEOL JEM-2100; Boston, united states, Faculty of agriculture, electron microscope research unit, Al-Mansoura University, Egypt (Figure 1a,b).

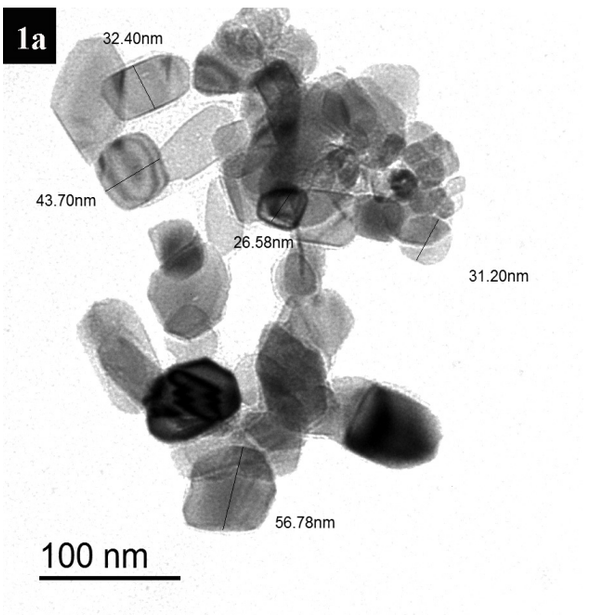

Fig. 1a: TEM micrograph of titanium nanoparticles. The morphological characteristics of TiO2 nanoparticles are shown, having a wide range of size from 26 nm to 56 nm. The particle shape is spherical, rounded or crystal shape. Some particles are electron dense while others are electron lucent; some particles are aggregated while others are separated.

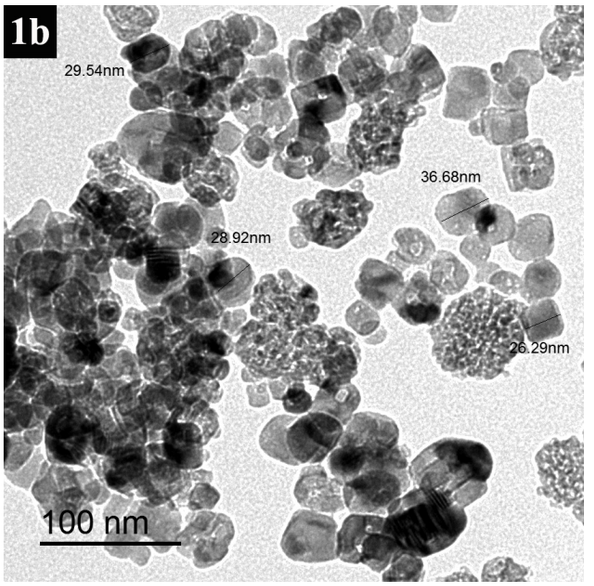

Fig. 1b: Another TEM micrograph of titanium nanoparticles. The morphological characteristics of TiO2 nanoparticles are shown, having a wide range of size from 26 nm to 36 nm. The particle shape is spherical, rounded or crystal shape. Some particles are electron dense while others are electron lucent. Some particles are aggregated while others are separated.

Morphometric analysis

Non-overlapping fields from all groups were analyzed after being randomly selected. Number of macrophages and pneumocytes type II from toludine blue photomicrographs (X 1000 magnification) were counted using Image J analyzing software. Also, the thickness of the interalveolar septum was measured using the same program[20].

Statistical analysis

Statistical analysis was done for the number of macrophages and pneumocytes type II and the thickness of the interalveolar septum using SPSS software (version 19). All the values of the experiment were represented as mean ± Standard Deviation. Data were analyzed by analysis of variance (ANOVA) followed by Posthoc test to assess the differences among groups.

RESULTS

Light microscopic examination

Lung sections from all groups were stained with H&E and toluidine blue. Specimens from the control group showed the normal histologic features of the lung. The alveolar sacs were seen clearly. A lot of alveoli separated by thin interalveolar septa and lined with pneumocytes type I that appeared as flat cells with densely stained nuclei and thin cytoplasm were observed. The cuboidal pneumocytes type II were also seen as cells with large rounded nuclei. The bronchioles were intact without inflammatory cell infiltration (Figures 2,3). In β-carotene (βC) group, the pattern of histology of the lung tissue was the same as the control group.

In the treated group: 300 mg/kg TiO2 NPs were administrated intraperitoneal daily for 14 days. Sections stained with H&E and toluidine blue stains showed thickening of the interalveolar septa and their infiltration with inflammatory cells and macrophages and narrowing of the alveolar sacs. The pulmonary blood vessels were dilated, thickened, congested and about to rupture (Figures 7,8).

In Protected group: 10mg/kg β-carotene were administrated by gavage for 7 days prior and 14 days with i.p. 300 mg/kg TiO2 NPs, the lung tissue preserved its normal architecture. But some areas with thick interalveolar septa were also noticed (Figures 13,14).

Electron microscopic examination

In the control group pneumocytes type II cells were slightly cuboidal containing vesicular euchromatic nucleus and slightly convex free cell surface with few short scattered microvilli on it and numerous lamellar bodies in the cytoplasm (Figure 4). The air–blood barrier was formed of cytoplasm of capillary endothelial cells, fused basal lamina and attenuated pneumocyte type I cytoplasm which contained flattened nucleus (Figure 5). Interstitial macrophage appeared as cell containing nucleus, rough endoplasmic reticulum and some electron dense granular bodies (Figure 6). While the findings in β-carotene (βC) group were similar to the control group.

Examination of ultrathin sections from the treated group showed large number of pneumocytes type II, some of them showed irregular outline, irregular nucleus with lost apical microvilli, many cytoplasmic vacuoles, lost lamellar bodies' specific pattern and disturbed architecture of many of them. There were many interstitial cells with irregular outline. Also there were areas with thick interalveolar septa and infiltration with the characteristic granular mast cells (Figures 9,10). The air–blood barrier of this treated group was formed of cytoplasm of capillary endothelial cells, thick irregular fused basal laminae and swollen irregular cytoplasm of pneumocyte type I (Figure 11). Macrophages appeared as cells with irregular outline and irregular heterochromatic nucleus and its cytoplasm contained numerous electron dense granular bodies and numerous vacuoles (Figure 12). On the other side, preservation of the normal lung histological architecture was apparent in the protected group (Figures 15,16).

Morphometric and statistical results

In comparison with the protected group the mean numbers of both alveolar macrophages and pneumocytes type II were significantly increased in the treated group. However, the protected group wasn't significantly changed from the control group (Figures 17,18) and (Tables 1,2). Also there was a significant increase in the mean thickness of the interalveolar septa in the treated group compared to the other two groups (Figure 19) and (Table 3).

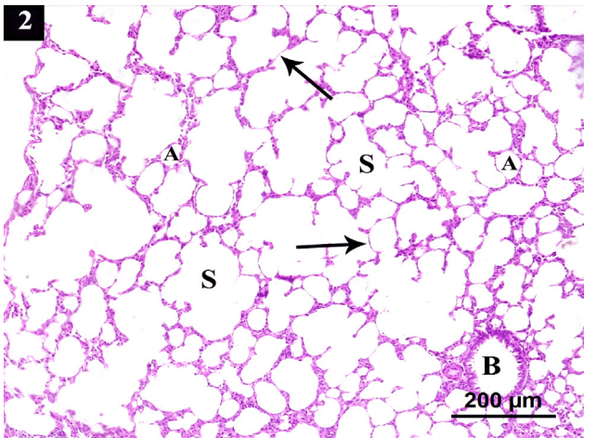

Fig. 2: H&E stained lung tissue section at 100x magnification from the control group showing the alveoli (A) and intervening thin interalveolar septa (thin arrow). Notice the normal alveolar sacs (S) and the intact bronchiole (B).

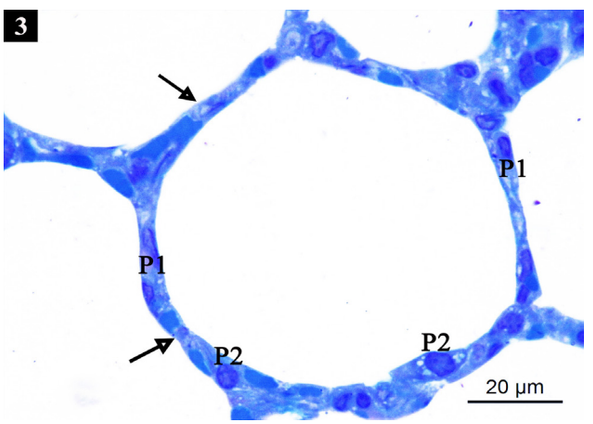

Fig. 3: Toluidine blue stained lung tissue section at 1000x magnification from the control group showing the flattened pneumocytes type I (P1) and the cuboidal pneumocytes type II (P2) lining the alveoli. Notice the thin interalveolar septa (thin arrow).

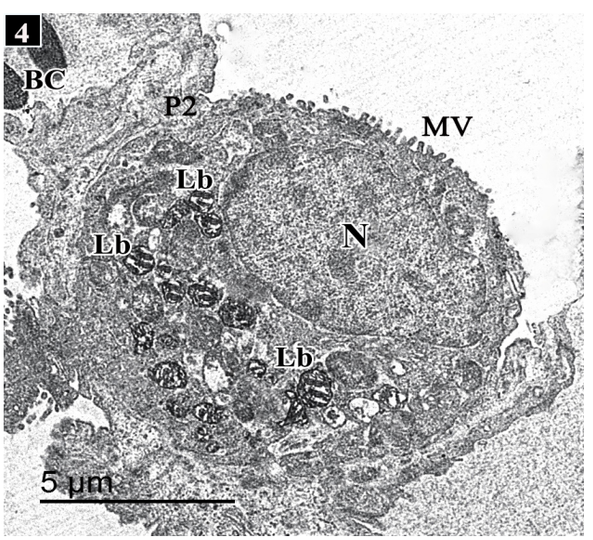

Fig. 4: TEM of a section in a rat's lung from the control group at 1200x magnification showing pneumocytes type II (P2) having convex luminal border with apical microvilli (MV) and euchromatic rounded nucleus (N). Many lamellar bodies (Lb) in the cytoplasm are also observed. Notice the blood capillary (BC).

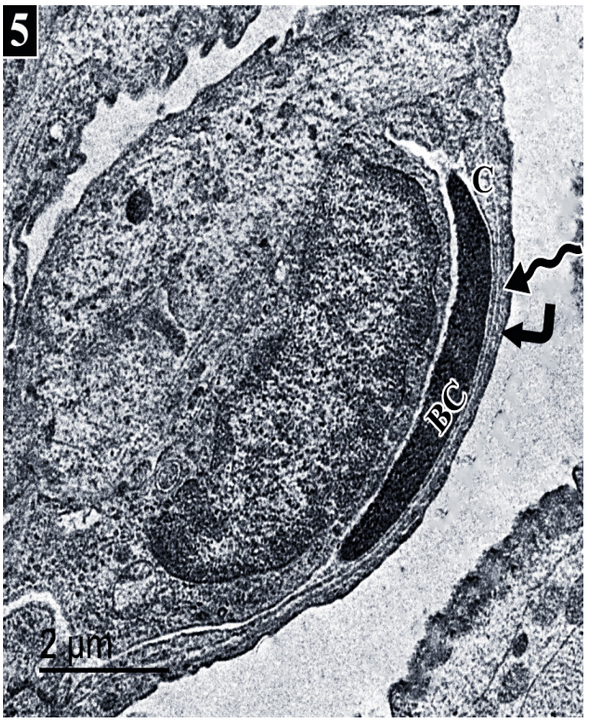

Fig. 5: TEM of a section in a rat's lung from the control group at 2000x magnification showing the air–blood barrier which is formed of attenuated cytoplasm of pneumocyte type I (right angled arrow), fused basal lamina (wavy arrow) and cytoplasm (C) of capillary endothelial cells (BC).

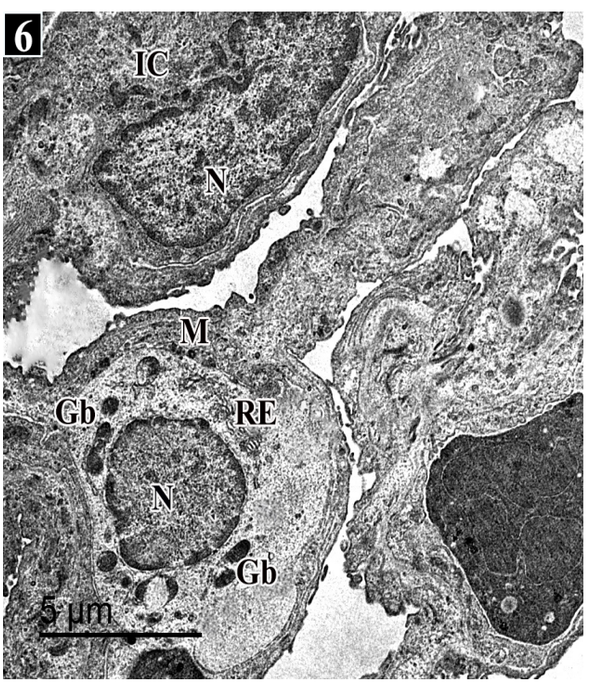

Fig. 6: TEM of a section in a rat's lung from the control group at 1000x magnification showing an interalveolar septum with single blood capillary. There is an interstitial macrophage (M) containing a nucleus (N), rough endoplasmic reticulum (RE) and some electron dense granular bodies (Gb). Notice the presence of an interstitial cell (IC) with eccentric nucleus (N).

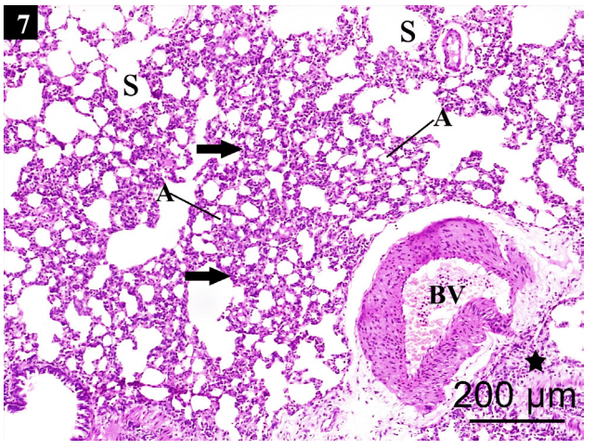

Fig. 7: H&E stained lung tissue section at 100x magnification from the treated group showing thick interalveolar septa (thick arrow) separating the alveoli (A) with narrowing of the alveolar sacs (S). Notice the thick dilated congested blood vessel (BV) which is about to rupture and the surrounding perivascular inflammation (star).

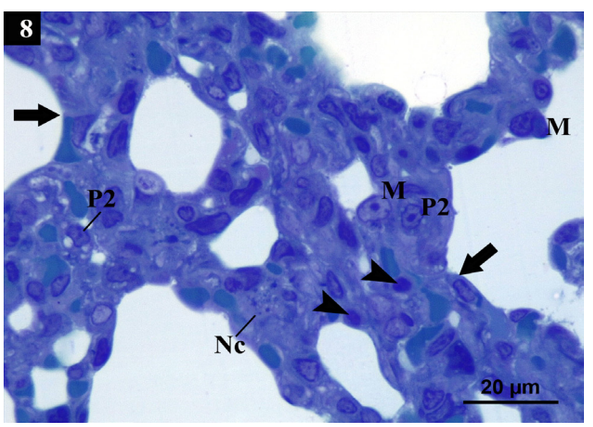

Fig. 8: Toluidine blue stained lung tissue section at 1000x magnification from the treated group showing the thick interalveolar septa (thick arrow), multiple pneumocytes type II (P2) and macrophages (M). Notice the inflammatory cellular infiltration (arrowhead) and the necrotic debris (Nc).

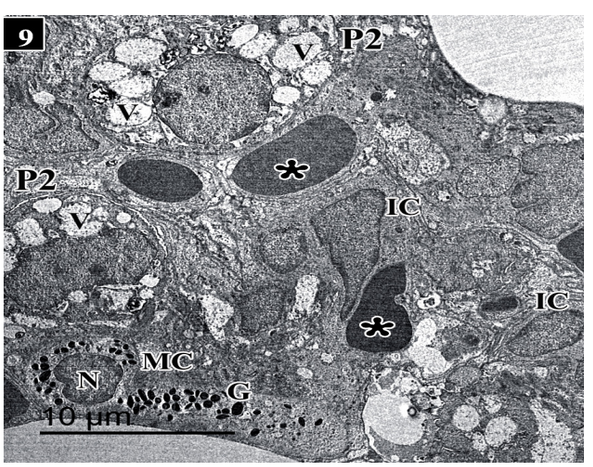

Fig. 9: TEM of a section in the rat's lung from the treated group at 600x magnification showing many interstitial cells (IC) and pneumocytes type II (P2) with multiple vacuoles (V). Notice the presence of mast cell (MC) containing a nucleus (N) and the characteristic granules (G) in the cytoplasm. Notice also extravastion of RBCs (asterisk) into the interstitium.

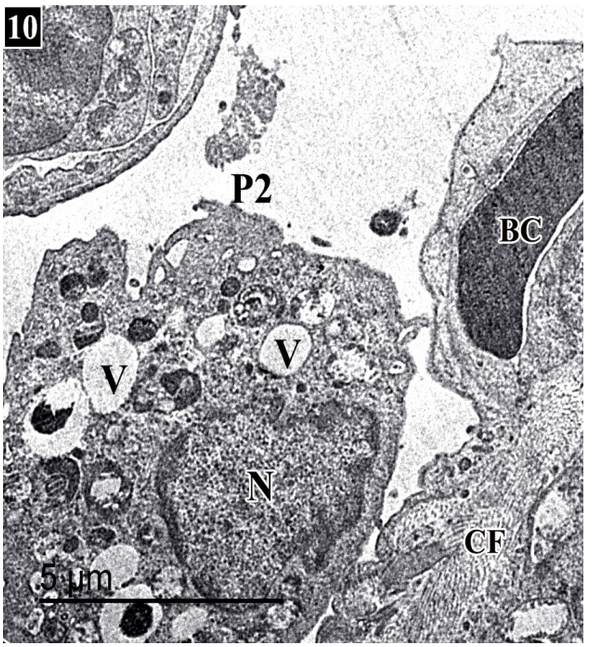

Fig. 10: TEM of a section in a rat's lung from the treated group at 1500x magnification showing pneumocyte type Π (P2) having irregular outline and irregular nucleus (N) containing clumbs of heterochromatin. Numerous vacuoles (V) are noticed in the cytoplasm. Notice also the presence of collagen fibers (CF) and blood capillary (BC).

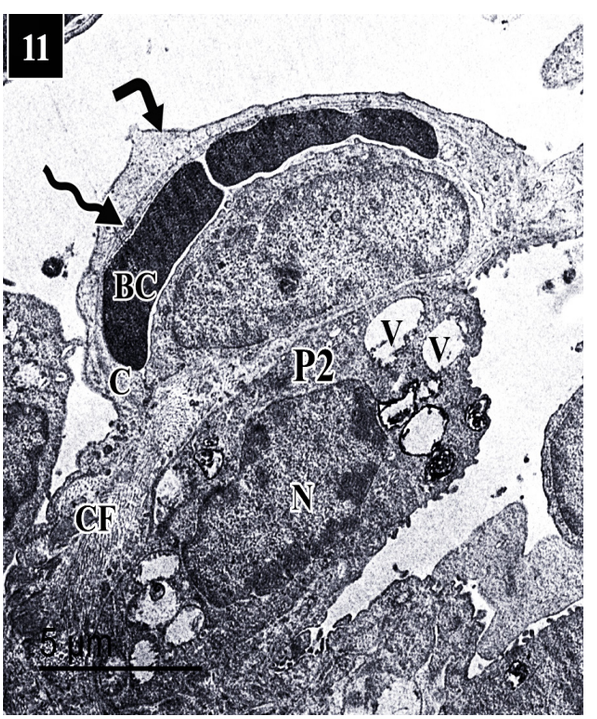

Fig. 11: TEM of a section in a rat's lung from the treated group at 1000x magnification showing the air–blood barrier which is formed of the swollen irregular cytoplasm of pneumocyte type I (right angled arrow), thick irregular fused basal lamina (wavy arrow) and cytoplasm (C) of capillary endothelial cells (BC). Notice also pneumocyte type Π (P2) with irregular outline and irregular heterochromatic nucleus (N) and its cytoplasm shows also numerous vacuoles (V). Notice also the presence of collagen fibers (CF).

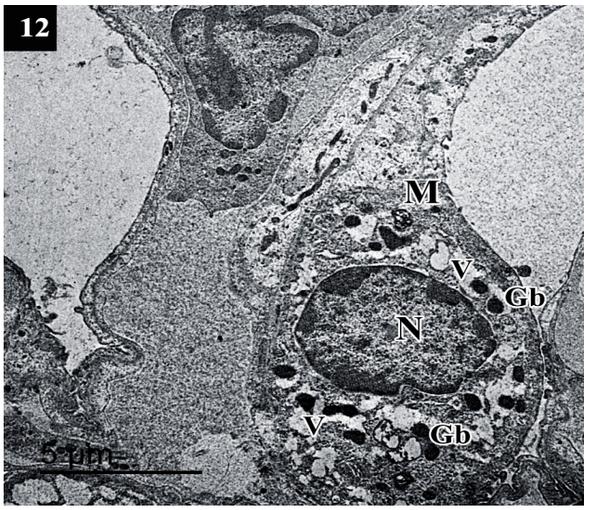

Fig. 12: TEM of a section in a rat's lung from the treated group at 1000x magnification showing a macrophage (M) with irregular outline and irregular nucleus containing clumbs of heterochromatin (N). Its cytoplasm shows numerous electron dense granular bodies (Gb) and numerous vacuoles (V).

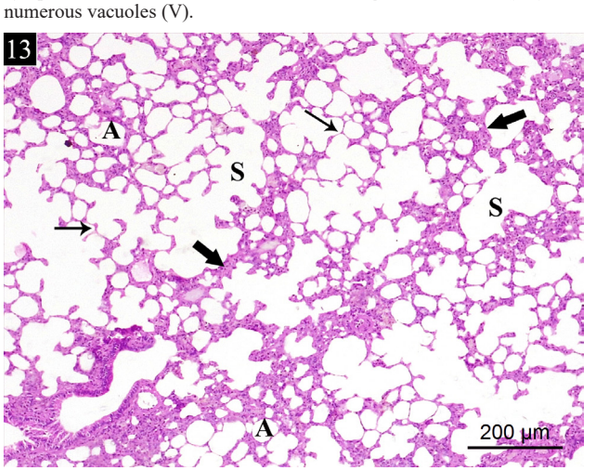

Fig.13: H&E stained lung tissue section at 100x magnification from the protected group showing areas of thick interalveolar septa (thick arrow) and other areas with thin interalveolar septa (thin arrow) separating the alveoli (A). Notice the alveolar sacs (S).

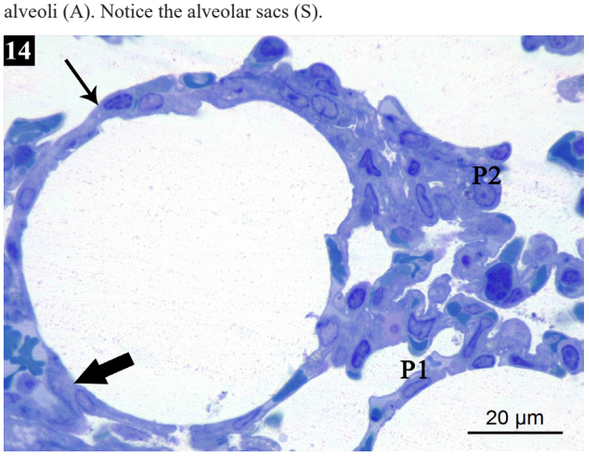

Fig. 14: Toluidine blue stained lung tissue section at 1000x magnification from the protected group showing areas of thick interalveolar septa (thick arrow) and other areas with thin interalveolar septa (thin arrow). Notice flattened pneumocytes type I (P1) and the cuboidal pneumocytes type II (P2) lining the alveoli.

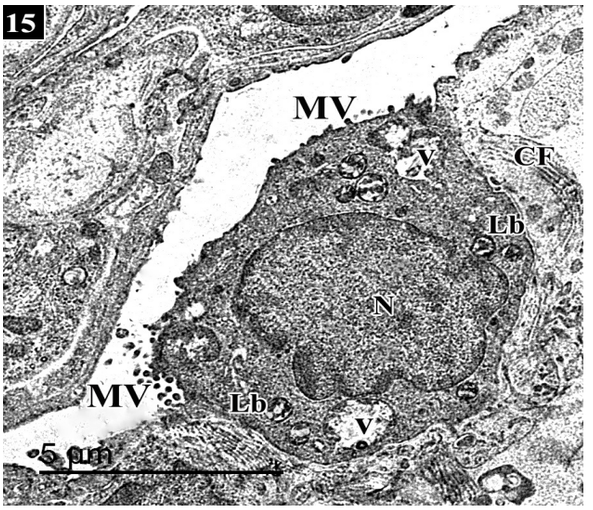

Fig. 15: TEM of a section in the rat's lung' from the protected group at 1500x magnification showing pneumocyte type II (P2). It has a slightly convex luminal border with some apical microvilli (MV). The nucleus (N) is slightly rounded and euchromatic. There are some lamellar bodies in the cytoplasm (Lb) and some absorbed lamellar bodies leaving empty vacuoles (V). Notice also the presence of collagen fibers (CF).

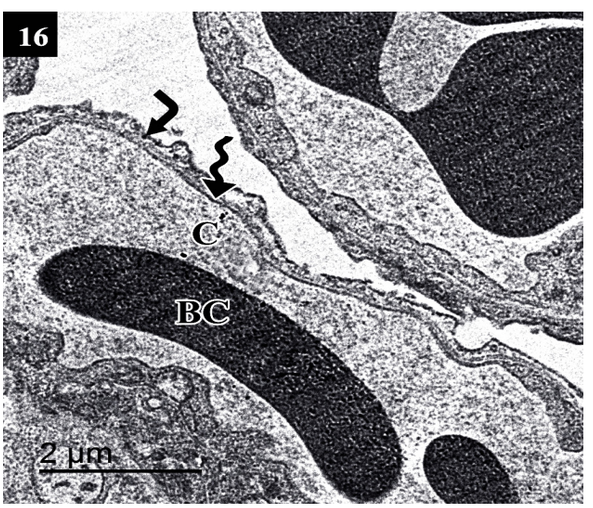

Fig. 16: TEM of a section in a rat's lung from the protected group at 2500x magnification showing the air–blood barrier which is formed of attenuated cytoplasm of pneumocyte type I (right angled arrow), fused basal lamina (wavy arrow) and cytoplasm (C) of capillary endothelial cells (BC).

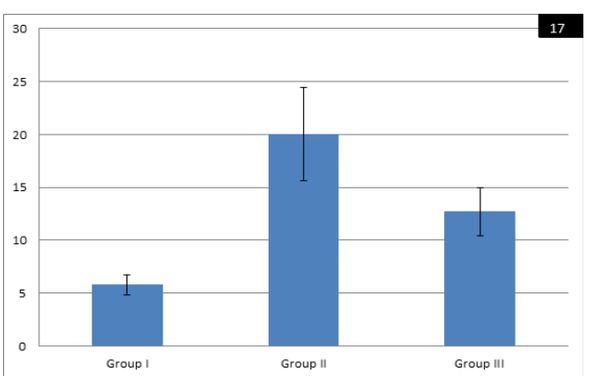

Fig. 17: Bar chart showing the number of macrophages in the different groups.

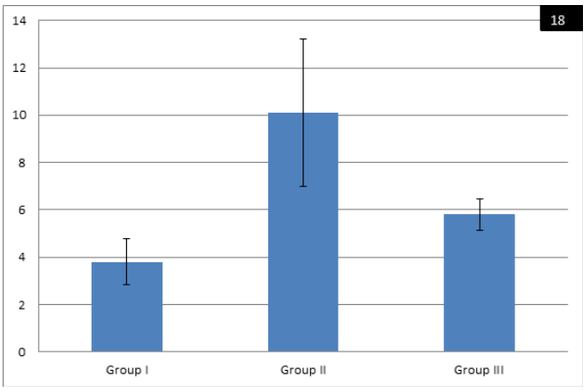

Fig. 18: Bar chart showing the number of pneumocytes type II in the different groups.

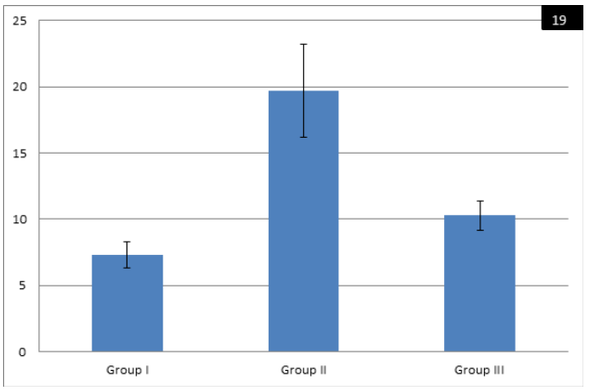

Fig. 19: Bar chart showing the thickness of the interalveolar septum (µm) in the different groups.

Table 1: Statistical analysis of number of macrophages

Parameter	control group	treated group	protected group	F	*P*
	Mean ± SD	Mean ± SD	Mean ± SD		
number of macrophages	5.8 ± 0.97	20[a] ± 4.4	12.7[ab] ± 2.3	48.23	<0.001**

a: significant from the control group b: significant from group II **: highly significant one-way ANOVA test

Table 2: Statistical analysis of number of pneumocytes type II

Parameter	control group	treated group	protected group	F	*P*
	Mean ± SD	Mean ± SD	Mean ± SD		
number of pneumocytes type II	3.8 ± 0.97	10.1[a] ± 3.1	5.8[ab] ± 0.67	25.16	<0.001**

a: significant from the control group b: significant from group II **: highly significant one-way ANOVA test

Table 3: Statistical analysis of thickness of the interalveolar septum (µm)

Parameter	control group	treated group	protected group	F	*P*
	Mean ± SD	Mean ± SD	Mean ± SD		
thickness of the interalveolar septum (µm)	7.3 ± 0.98	19.7[a] ± 3.5	10.3[ab] ± 1.1	77.4	<0.001**

a: significant from the control group b: significant from group II **: highly significant one-way ANOVA test

DISCUSSION

TiO2 NPs are ones of the most commonly used nanoparticles. They are added to many foodstuffs, including ice cream, cheeses, skimmed milk, sauces and as coating on chewing gum and sweets[21,22]. Its content in sweets, particularly in white-coated products, chocolate, candy and chewing gum is very high when compared to other products reaching up to 2.5 mg Ti/g of food[23]. It was estimated that a child can consume TiO2 NPs even 2–4 times more per 1 kg of body weight /day than an adult person.

In 2006, TiO2 was categorized as a carcinogen (group 2B) that can causes harmful effects to human. Furthermore, it is considered as a chemical carcinogen because it was grouped as D2A by WHMIS (the Workplace Hazardous Materials Information System)[24]. So it was of great importance to understand the toxicity of TiO2 NPs in humans very well to help the assessment of the risk and how to get benefit from its applications in a safe way[25]. This was the cause of spotting light on pulmonary toxicity of TiO2 NPs in this study.

Furthermore, this work studied the ameliorative effect of β-carotene against the pulmonary toxicity of TiO2 NPs. It was proved that βC has many biological and pharmacological activities including: anti-epilepsy, radio-protective, antioxidant and cardiovascular protection. So it was a great value to benefit from the antioxidant property of βC to protect from the oxidative stress caused by the toxic TiO2 NPs[26,27].

Rat was the animal model in this study because it was found that it developed considerable inflammatory responses in the lung after being exposed to TiO2. This agrees with Bermudez *et al*., 2004[28] who reported that there was significant difference between different animal species in their pulmonary response after being exposed to TiO2 particles. They found that mice or hamsters developed a less severe pulmonary inflammatory response than rats. This was proved after studying different lung parameters such as histopathologic alterations.

The results of this study showed thickening of the interalveolar septa and increase in the number of

pneumocytes type II with vacuolation of cytoplasm which were the most prominent changes in this study. Irregularity of the nuclei of pneumocystes type II and lost lamellar bodies characteristic pattern, interstitial haemorrhage, dilatation and congestion of the pulmonary vessels and increased number of macrophages were also observed in this study.

These results agree with Chen *et al*., 2009[29] who found thickening of the alveolar septa and interstitial pneumonia. Also this agrees with the results of previous studies which revealed degenerated and thickened epithelial cells of the lung and macrophages loaded with particles in the alveoli[28,30]. The significant increase in the mean thickness of interalveolar septa recorded by the morphometric study in this work was attributed to the infiltration with inflammatory cells in the septa and increase of collagen fibers[31].

In the present study, increased numbers of mast cells and alveolar macrophages which play an important role in the process of inflammation was observed. This is in accordance with Nel *et al*., 2006, Wang *et al*., 2009 and Li *et al*., 2013[4,32,33] who stated that TiO2 can deposit in the alveoli and infiltrate the interstitium of the lung leading to an inflammatory response in the lung and its injury. This pulmonary inflammatory response was attributed to the production of ROS (Reactive Oxygen Species) as a mechanism of occurence to that characteristic effect of TiO2 NP toxicity especially in case of exposure to UV rays or light.

This was confirmed by Sun *et al*., 2012[34] who found that TiO2 NPs markedly accumulated and augmented production of ROS in the lung of mouse after administration by intra-tracheal route and increasing the period of exposure. Also, other authors stated that some factors such as TNF-α, nuclear factor erythroid 2 (Nrf 2) and heme oxygenase are increased as a compensatory action to the oxidative stress caused by exposure to TiO2[35,36].

Vacuolation of cytoplasm of pneumocytes type II observed in this study was due to release of lamellar bodies and absence of their uptake by pneumocytes type II, in this case surfactant was released in interstitium and is phagocytosed by macrophages. This agrees with Crim and Simon, 1988[37] who attributed the impaired function of pneumocytes type II to the injurious effect caused by the released reactive oxygen metabolites. Besides, the observed significantly increased pneumocytes type II indicates cellular hyperplasia which suggests the proliferation trial of type II cells for regeneration of the injured epithelium[38].

Dilated congested thick walled blood vessels that were about to rupture was also a finding in this study. It occurred as a result of injury to epithelial cells by the nanoparticle. This was confirmed by Dodd and Jha, 2008[39] who reported that nanoparticles can activate macrophage, neutrophil and epithelial cells, which can produce ROS that attach to the epithelial cells in vessel wall causing their injury and lead to hemorrhage in alveolar wall and spaces[40].

In this study, the pathological changes observed in the treated group weren't markedly observed in the protected group; this means that β-carotene had a significant protection to the lung against TiO2 NPs toxicity. An explanation for this is that β-carotene can enter the cells rapidly because it is a lipophilic substance that passes easily through biological membranes. Via the provitamin A activity of β-carotene, it can protect lipoprotein and cellular membranes against oxidative damage, this is another possible explanation for this protective effect[41,17].

CONCLUSION

It can be concluded from this study that β-carotene may be considered a beneficial medication that can guard against pulmonary toxicity induced by titanium nanoparticles.

ACKNOWLEDGMENTS

Many thanks to all members of Anatomy Department, Animal House Department and Scientific Medical Research Center Faculty of Medicine, Zagazig University.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Gonc DM, Alves and Girard D: Titanium dioxide (TiO2) nanoparticles induce neutrophil influx and local production of several pro-inflammatory mediators in *vivo*. International Immunopharmacology (2011) 11(8): 1109–1115.
2. Chusuei, CC, Wu CH, Mallavarapu S, Hou FY, Hsu CM, Winiarz JG, Aronstam RS and Huang YW: Cytotoxicity in the age of nano: The role of fourth period transition metal oxide nanoparticle physicochemical properties. Chem. Biol. Interact (2013) 206: 319–326.
3. Oberdorster G, Oberdorster E and Oberdorster J: Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. (2005) 113: 823–839.
4. Nel A, Xia T, Madler L and Li N: Toxic potential of materials at the nanolevel. Science, (2006) 311: 622–626.
5. Wu J, Sun J and Xue Y: Involvement of JNK and P53 activation in G2/M cell cycle arrest and apoptosis induced by titanium dioxide nanoparticles in neuron cells. Toxicology Letters (2010) 199(3): 269–276.
6. Markowska-Szczupak A, Ulfig K and Morawski AW: "The application of titanium dioxide for deactivation of bioparticulates: an overview. Catalysis Today (2011) 169(1): 249–257.
7. Robichaud CO, Uyar AL, Darby MR, Zucker LG and Wiesner MR: Estimates of upper bounds and trends in nano-TiO2 production as a basis for exposure assessment. Environ Sci Technol. (2009) 43:4227-4233.
8. Mital GS and Manoj T: A Review of TiO2 Nanoparticle. Chinese Sci Bull (2011) 56: 1639- 1657.

9. Gandamalla D, Lingabathula H and Yellu NR: Cytotoxicity evaluation of titanium and zinc oxide nanoparticles on human cell lines. Int. J. Pharm. Sci. (2017) 9:240-246.
10. Hu R, Gong X, Duan Y, Li. N, Che Y, Cui Y *et al*. Neurotoxicological effects and the impairment of spatial recognition memoryin mice caused by exposure to TiO2 nanoparticles. Biomaterials (2010) 31: 8043-8050.
11. Bermudez E, Mangum JB and Asgharian B *et al*.: Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol Sci. (2002)70(1):86–97.
12. Renwick LC, Brown D, Clouter A *et al*.: Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types. Occup. Environ. Med. (2004) 61(5):442–447.
13. Kang SJ, Kim BM, Lee YJ, Hong SH and Chung HW: Titanium Dioxide Nanoparticles Induce Apoptosis Through the JNK/p38-Caspase-8-Bid Pathway in Phytohemagglutinin-Stimulated Human Lymphocytes. Biochem. Biophysi. Res. Communi. (2009) 386: 682-687.
14. Colditz G, Manson J, Stampfer M, Ronser B, Willett W and Speizer F: Diet and risk of clinical diabetes in women. Am. J. Clin. Nutr. (1992) 55: 1018-1023.
15. Pryor WA, Stahl W and Rock CL: Beta carotene: from biochemistry to clinical trials. Nutr. Rev. (2000) 58: 39-53.
16. Stahl W and Sies H: Antioxidant activity of carotenoids. Mol Aspects Med. (2003) 24:345–51.
17. El-Demerdash FM, Yousef MI, Kedwany FS and Baghdadi HH: Cadmium induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and b-carotene. Food Chem. Toxicol. (2004) 42:1563–71.
18. Lyama T, Takasuga A, and Azuma M: beta-carotene accumulation in mouse tissue and a protective role against lipid peroxidation. Int J Vitam Nutr. (1996) 66:301-305.
19. Jiaying X, Hongbo S, Magaye R, Hongsheng Y, Lissy L, Baobo Z, Cui Y, Aiguo W and Jinshun Z: Acute Toxicity of Intravenously Administered Titanium Dioxide Nanoparticles in Mice. (2013) PLOS ONE, 8.
20. Soliman, ME: Evaluation of Time Dependent Changes of the Rat's Lung in Experimentally Induced Diabetes Mellitus: Light and Electron Microscopic Study. Egypt. J. Histol. (2010) 33(1): 45 – 54.
21. Peters RJ, van Bemmel G, Herrera-Rivera Z, Helsper HP, Marvin HJ, Weigel S, Tromp PC, Oomen AG, Rietveld AG and Bouwmeester H: Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. J Agric Food Chem. (2014) 62:6285–6293.
22. Dudefoi W, Moniz K, Allen-Vercoe E, Ropers M-H, Virginia K: Impact of food grade and nano-TiO2 particles on a human intestinal community. Food Chem Toxicol. (2017) 106:242–249.
23. Bachler G, von Goetz N and Hungerbuhler K: Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO2) nanoparticles. Nanotoxicology (2015) 9:373–380.
24. Baan RA: Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: Recent evaluations by an iarc monographs working group. Inhal. Toxicol. (2007) 19(1): 213-228.
25. Bessa M J, Costa C, Reinosa J, Pereira C, Fraga S, Fernandeez J, Banares M A and Teixeira JP: Moving into advanced nanomaterials. Toxicity of rutile TiO2 nanoparticles immobilized in nanokaolin nanocomposites on HepG2 cell line. Toxicology and applied pharmacology (2017) 316:114-122.
26. Schweiggert R M, Kopec R E, Villalobos-Gutierrez M G, Hogel J, Quesada S, Esquivel P, Schwartz S J and Carle R: Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. Br J Nutr British Journal of Nutrition (2014) 111: 490-498.
27. Karmakar A, ZHANG Q and ZHANG Y: Neurotoxicity of nanoscale materials. Journal of Food and Drug Analysis, (2014) 22: 147-160.
28. Bermudez E, Mangum JB, Wong BA *et al*.: Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci. (2004) 77(2):347–57.
29. Chen J, Dong X, Zhao J, and Tang G: In *vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection," Journal of Applied Toxicology (2009) 29 (4): 330–337.
30. Kwon S, Yang YS, Yang HS, Lee J, Kang MS, Lee BS, Lee K, Song CW: Nasal and pulmonary toxicity of titanium dioxide nanoparticles in rats. Toxicol. Res., (2012) 28: 217–224.
31. Fukuda Y, Mochimaru H, Terasaki Y, Kawamoto M and Kudoh S: Mechanism of structural remodeling in pulmonary fibrosis. Chest Jul. (2001) 120(1): 41S-43S.
32. Wang J, Fan Y, Gao Y, Hu Q and Wang T: TiO2 nanoparticles translocation and potential toxicological effect in rats after intraarticular injection. Biomaterials (2009) 30, 4590–4600
33. Li B, Ze Y, Sun Q, Zhang T, Sang X, Cui Y, Wang X, Gui S, Tan D, Zhu M *et al*.:Molecular mechanisms of nanosized titanium dioxide-induced pulmonary injury in mice. PLoS One, (2013) 8(2): e55563.

34. Sun Q, Tan D, Zhou Q, *et al*.: Oxidative damage of lung and its protective mechanism in mice caused by long-term exposure to titanium dioxide nanoparticles. J. Biomed. Mater. Res. (2012) 100A: 2554–2562.

35. Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, Couillin I, Tschopp J: Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β. Proc. Natl. Acad. Sci. USA, (2010) 107: 19449–19454.

36. Scherbart AM, Langer J, Bushmelev A, van Berlo D, Haberzettl P, van Schooten FJ, Schmidt AM, Rose CR, Schins, RP and Albrecht C: Contrasting macrophage activation by fine and ultrafine titanium dioxide particles is associated with different uptake mechanisms. Part. Fibre Toxicol. (2011) 8: 31.

37. Crim C and Simon RH: Effects of oxygen metabolites on rat alveolar type II cell viability and surfactant metabolism. Lab. Invest. Apr. (1988) 58(4):428-437.

38. Hinata N, Takemura T, Ikushima S, Yanagawa T, Ando T, Okada J, Oritsu M, and Koike M: Phenotype of regenerative epithelium in idiopathic interstitial pneumonias. J. Med. Dent. Sci. (2003) 50(3):213-224.

39. Dodd N, Jha AN.: Titanium dioxide induced cell damage: A Proposed of the carboxyl radical .Mut Res; (2008) 660: 79–82.

40. Mohammadi, F., Sadeghi, L., Mohammadi, A., Tanwir, F., Yousefi Babadi, V., & Izadnejad, M.: The effects of Nano titanium dioxide (TiO2NPs) on lung tissue. Bratisl Lek Listy, (2015) 116(6), 363–367.

41. Pavia SAR and Russell RM: b-carotene and other carotenoids as antioxidants. J. Am. Coll Nutr. (1999) 18: 426–33.

الملخص العربى

التأثير السام لجزيئات ثاني أكسيد التيتانيوم النانونية على رئة ذكر الجرذ الأبيض البالغ والدور الوقائى المحتمل للبيتا كاروتين: دراسة مجهرية ضوئية والكترونية

ولاء عبد الحليم رشاد، علا على عبد الوهاب

قسم التشريح الآدمى وعلم الأجنة - كلية الطب - جامعة الزقازيق

الخلفية: تستخدم جسيمات ثاني أكسيد التيتانيوم النانونية عادة فى المجالات الطبية الحيوية والدوائية والصناعية، لكن آثارها الضارة بدأت تتزايد أيضًا بشكل مثير للقلق. كما يعتبر البيتا كاروتين مضادا للأكسدة ويمكن أن يحمي من الأثار السمية التي تسببها جسيمات ثاني أكسيد التيتانيوم النانونية.

الهدف من الدراسة: هودراسة التغيرات النسيجية في أنسجة الرئة الطبيعية في الجرذان البيضاء بعد تناولها جسيمات ثاني أكسيد التيتانيوم وتقييم تأثير الحماية المحتمل للبيتا كاروتين.

المواد والطرق المستخدمة: في هذه الدراسة تم استخدام ٤٠ فأر وتقسيمهم الي ٤ مجموعات. مجموعة معالجة تم اعطاؤها جرعة ٣٠٠ مجم/ كغم من جسيمات ثاني أكسيد التيتانيوم النانوية بالحقن البريتوني يوميًا لمدة ١٤ يومًا ومجموعة محمية واحدة تلقت ١٠ مجم / كجم من البيتا الكاروتين عن طريق الفم لمدة ٧ أيام قبل ولمدة ١٤ يوم بعد اعطاء ٣٠٠ مجم/ كغم من جسيمات ثاني أكسيد التيتانيوم النانوية إلى جانب مجموعة ضابطة واحدة ومجموعة واحدة تلقت فقط ١٠ مجم / كجم من البيتا الكاروتين. ثم تم صبغ عينات من نسيج الرئة بصبغة الهيماتوكسيلين والأيوسين وصبغة التوليدين الأزرق كما تم إجراء دراسة البنية التحتية والتحليل الاحصائي.

النتائج: وقد أظهرت النتائج حدوث تغيرات نسيجية ملحوظة في المجموعة المعالجة مثل تفرغ سيتوبلازم النوع الثاني من خلايا الأكياس الرئوية وفقدان النمط المميز للهيئات الصفيحية وتمدد واحتقان الأوعية الدموية الرئوية كما أظهرت النتائج المورفومترية سماكة الحواجز مابين الحوبصلات الهوائية وزيادة أعداد كلا من البلاعم والخلايا الرئوية من النوع الثانى مقارنة بالمجموعة الضابطة. وقد حافظ اعطاء البيتا كاروتين مع جسيمات ثاني أكسيد التيتانيوم النانوية بشكل كبير على بنية الرئة الطبيعية في المجموعة المحمية.

الاستنتاج: يمكن أن نستنتج من هذه الدراسة أن المكملات الغذائية للبيتا كاروتين يمكن أن تقلل من التأثير الضار لجسيمات ثاني أكسيد التيتانيوم النانوية على أنسجة الرئة عبر آلية مضادات الأكسدة.