*Egypt. J. Food. Sci.* **Vol. 47,** No. 1, pp. 91 - 103 (2019)

**Egyptian Journal of Food Science**
**http://ejfs.journals.ekb.eg/**

# Effect of Some Antioxidants on Rats Treated with Titanium Dioxide Nanoparticles

**Rehab Mohamed Ibrahim[1*], Marwa Farouk ElKady[2] and Amal Mahmoud Hassanein[1]**

*[1]Department of Special Food and Nutrition,Food Technology Research Institute, Agricultural Research Center ,Giza, Egypt*

*[2]Chemical and Petrochemical Engineering Department, Egypt-Japan University of Science and Technology, New Borg El-Arab City, Alexandria 21934, Egypt*

TITANIUM Dioxide Nanoparticles ($TiO_2$NPs) is used in the production of food colorants, some nutritional supplements and many other products on large scale. Many studies stated that the $TiO_2$NPs are more toxic than titanium dioxide ($TiO_2$) and as possibly carcinogenic to humans. The aim of study was carried out investigate on impacts of vitamin C and vitamin E on free radicals and antioxidant enzymes activities in male rats treated with $TiO_2$NPs. The obtained results showed that, there were no significant ($P \geq 0.05$) differences found in red blood cells (RBC), hematocrit (Ht), hemoglobin (Hb) and platelets count (PLT) after treated with titanium dioxide nanoparticles ($TiO_2$NPs) or natural antioxidants (vitamin E and C). The treatment with $TiO_2$NPs alone caused significant ($P \leq 0.05$ ) increase in kidney and liver functions, while the oral intake of V.C and V.E or V.C+V.E reduced this increase. Also, the treatment with $TiO_2$NPs increased the level of free radicals and decreased the activity of some antioxidant enzymes such as Superoxide dismutase (SOD),Glutathione-S-transferase (GST) and the content of Glutathione (GSH) in plasma. Meanwhile, V.C and V.E decreased the increased in free radicals and protected the activity of antioxidant enzymes in plasma of $TiO_2$NPs- treated rats.

**Keywords:** Titanium Dioxide Nanoparticles, Natural Antioxidants, Nano- toxicology.

## Introduction

Nanomaterial's are recently used in many applications, widespread due to their good physical and chemical properties. But, the potential deleterious effect of many nanoparticles exposure on the human body and health has become a very matter of concern (Sha et al., 2015). Food is an important route of human exposure to $TiO_2$NPs. At least 36% of the $TiO_2$ particles present in food are in nano-sized form. Candies, sweets, chewing gum and many other food products contain higher amounts of this $TiO_2$NPs with diameters less than 100 nm (Weir et al., 2012; Orazizadeh et al., 2014 and Shakeel et al., 2015). Absorption in gastrointestinal may be an important way for $TiO_2$NPs in the human body. Many earlier studies reported that nanoparticles of $TiO_2$ are more toxic than titanium dioxide ($TiO_2$) (Oberdörster, 2001; Fabian et al., 2008 and Zhao et al., 2009). In addition, The International Agency for Research on Cancer (IARC) has classified $TiO_2$ as possibly carcinogenic to humans (class 2B) (Baan et al., 2006). $TiO_2$NPs can easily enter the body and then cause toxic effect and pass through blood-heart and testicular barriers (Meena and Paulraj, 2012). Free radical formation as the main mechanism for toxicity. These free radicals subsequently lead to oxidative stress, cytotoxicity, damage in DNA, and causes tumor (Manke et al., 2013 and Khanna et al., 2015). Many last studies, investigated the toxic effects of $TiO_2$NPs on plants, animal and some strains of bacteria (Karlsson et al., 2008 and Atha et al., 2012). Vitamins A, C, E and carotenoids are the main natural antioxidants which derived from

*Corresponding author: Email: rehabmohamed_eg@yahoo.com ; drrehab2020@yahoo.com;
Cell Phone: (020) 1225707447

Received:17/6/2019; accepted:1/10/2019
DOI: 10.21608/EJFS.2019.13749.1009

the natural sources by dietary intake (Heistad, 2006). Vitamin C and vitamin E were used as antioxidants agents in many food supplements and classified as natural and essential elements in most biological systems. Vitamin C is easily available, inexpensive and non-toxic antioxidant, have a great role in reduction of toxic by most xenobiotics (Uchendu et al., 2012).Vitamin E is well known for its high health benefits, including antioxidant, anti-inflammatory and neuroprotective properties (Nesaretnam, 2008). Vitamin E has exhibit protective effects *in vivo* and *in vitro* against different pesticides (Zingg, 2015 and Sargazi et al., 2016). The objective of this study was to investigate the effect of vitamin C and E as an antioxidant agent on rats treated with titanium dioxide nanoparticles.

## Materials and Methods

*Materials*

Reduced glutathione; 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB); Sulfosalisilic acid (SSA); and Thiobarbituric acid (TBA) were obtained from Sigma- Aldrich. Co, Saint Louis, USA. Urea and Creatinine kits were obtained from Biosystems, Spain; Bilirubin kit was obtained from Diamond, Germany; AST, ALT kits were obtained from Quimica Clinica Aplicada, Spain; and ALP, ACP kits were obtained from Biodiagnostic, Egypt.

*Preparation of titanium dioxide nanoparticles using microwave assisted technology*

12 mL (0.4 mol) of titanium tetraisopropoxide (TTIP) was added drop wise to 40 mL NaOH solution with concentration of 5 *M*. After complete addition, the solution mixture was added inside a domestic microwave oven until complete dryness of yielded white powder. The microwave power was adjusted at 8 W to maintain the reaction mixture at 80 °C. The yielded white powder was washed with acidified distilled water (0.01*M*) to remove the remaining $Na^+$ ions. The solution was centrifuged at 2500 rpm and dried in an oven at 60 °C (Bregadiolli et al., 2017). The obtained particles were characterization using X-Ray diffractometer (Shimadzu 7000, USA) and electron microscopy (JEOL JEM-1230, Japan).

*Experimental design*

Experimental design: The design of the experiment was approved by the local committee in Alexandria University, Egypt, according to the protocol conforms to the guidelines of the National Institutes of Health (NIH). Forty-nine male albino rats (10 week old and 127±3.87g weight) obtained from the Institute of Graduate Studies and Research (IGSR), Alexandria University, were dwelled plastic cages (4 /cage) with saw dust bedding and maintained in an air-conditioned animal house at a controlled temperature 22 ± 2°C and relative humidity (60±10%) with a photoperiod of 12 hr light/12 hr dark (Childs et al., 2002) and feed on basic diet skim milk powder (37.5%) corn starch (30 %), corn oil (9 %), sucrose (13.5 %), cellulose (5 %), vitamins (1 %) and minerals (4%) based on the method of Walter (1981) and tap water was provided *ad libitum* (Childs et al., 2002). After two weeks of acclimatization, the animals were distributed into seven equal groups, 7 animals in each group.

Group 1: control (orally treated with 1% Tween 80 by gastric tube (0.5 ml daily /rat) according to Hassanein and El-Amir, (2017), Group 2: was daily oral intake of Ascorbic acid (V.C)100 mg/Kg BW in distilled water (El-Shafei and Saleh, 2016; Apaydin et al., 2017; Zhang et al., 2018). Groups 3 was daily orally treated with α-tocopherol (V.E)100 mg/Kg BW in corn oil (Apaydin et al., 2017), Groups 4: was daily orally $TiO_2$ nanoparticals ($TiO_2$NP) (150 mg/kg BW) in 1% Tween 80 (Wang et al., 2007a; Hassanein and El-Amir, 2017). Groups 5: daily orally $TiO_2$NP and V.C ( 150 mg $TiO_2$-100 mg V.C/ kg BW). Groups 6: daily orally $TiO_2$-NPs and V.E (150 mg) $TiO_2$NPs + 100 mg V.E/ kg BW), and Groups 7: daily orally $TiO_2$NPs, V.C and V.E (150 mg $TiO_2$NPs 100 mg V.E + 100 mg V.E/ kg BW). Animals were daily treated with the tested $TiO_2$NPs , V.C and V.E for 30 day.

*Feed and water intake*

Feed and water intake were recorded throughout the experimental period, and mean daily feed and water intake were determined.

*Body weight*

At first and end of the experimental period, body weight of tested rats was recorded.

*Hematological parameters*

At the end of the experimental period (after 30 days) rats were anesthetized with ether and sacrificed and the blood samples were collected in two tubes: one containing EDTA (anti-coagulant) and the other containing Heparin (anti-coagulant). Non coagulated blood by EDTA was tested shortly after collection by Particle counter (from ERMA INC.-Tokyo. Model PCE-210) for measuring total erythrocyte count (TEC) red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), platelets

count (PLT), red cell distribution width (RDW), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH). Total leukocyte counts (TLC) white blood cell count (WBC), lymphocytes, segmented neutrophils, monocytes and eosinophils.

*Blood biochemical parameters and enzyme activities*

The other part of heparinized blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 4000 rpm for 20 min, and was stored at -80°C until used for analyses.

*Kidney functions*

Kidney functions were evaluated by measuring the level of Urea, according to Chaney and Marbach (1962); Searcy et al., (1967); and Tabacco et al. (1979), Total Bilirubin concentration according to Kaplan et al. (1984) and Burtis et al. (1999), and Creatinine based on the method of Bartels & Böhmer (1971); and Fabiny & Ertingshausen (1971), in plasma.

*Liver functions*

Liver functions were determined by measuring the activities of liver enzymes such as Alanine Tansaminase (ALT) and Aspartate Ttransaminase (AST) according the method of Reitman and Frankel (1957), Acid Phosphatase (AcP) according to Kind and King (1954) and Alkaline Phosphatase (AlP) according to the method of Belfield and Goldberg (1971), in plasma.

*Antioxidant activity*

Plasma free radical was measured by thiobarbituric acid-reactive substances (TBARS) assay based on the method of Tappel and Zalkin (1959). Superoxide dismutase (SOD) and Glutathione S-transferase (GST) activities in plasma were determined according to the method of Misra & Fridovich (1972) and Habig et al. (1974), respectively. Meanwhile,the concentration of Glutathione reduced (GSH) was determined according to the method of Jollow et al. (1974).

*Statistical analysis*

The data were analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS, 2004. The differences among the means of all treatments at the significance level of $P \leq 0.05$. Correlations were calculated using Pearson's correlation coefficient.

## Results and Discussion

*Characterization of nano-$TiO_2$*

Titanium dioxide particles with diameter larger than 100 nm are considered biologically inert in both humans and animals (Gurr et al., 2005). However, adverse effects of $TiO_2$NPs have been uncovered (Li et al., 2010).

*X-Ray diffraction (XRD) of $TiO_2$NPs powder*

The X-Ray diffraction (XRD) pattern of $TiO_2$NPs powder was shown in Fig. 1. It was found that all of the peaks acquired in the XRD pattern of $TiO_2$NPs powder agreed totally with the standard rutile structure and the XRD pattern of $TiO_2$NPs of other previous studies. When the particle size of $TiO_2$ is less than 100 nm, ratable broadening in X-Ray diffraction lines will occur.

*Scanning electron microscopy (SEM) of $TiO_2$NPs*

Scanning Electron Microscopy (SEM) is a very helpful tool to explore morphology of nanoparticles powders. SEM images of the nanoparticles prepared via wet chemical route. It is unclouded that the nanoparticles apparent by SEM image consist of a number of crystallites with rutile structure. SEM images of the $TiO_2$NPs (Fig. 2) show that they are approximately in globular form and sizes are various (<100 nm).

*Changes in body weight and feed intake*

The effect of oral intake of V.E and V.C on initial and final body weight, body weight gain, feed intake and water intake of rats treated with $TiO_2$NPs were presented in Table 1. No significant differences ($P \geq 0.05$) were found in initial, final body weight and body weight gain between all treatments. Also, no significant differences ($P \geq 0.05$) were found among all groups in feed intake or water intake during the experimental period. These results showed that, there were no negative effects of $TiO_2$NPs on body weight or feed intake of rats. These results agree with Warheit and Donner (2015) who found that after rat's administration $TiO_2$, no opposite effects were observed on body weight or nutritional parameters. Also, there were no deaths associated with $TiO_2$ and no clinical or neurological observations were associated with exposure to $TiO_2$.

*Hematological analysis*

Data presented in Table 2 (A and B) showed the effect of V.E and V.C on hematological parameters of rats treated with $TiO_2$NPs. No significant ($P \geq 0.05$) changes were found in RBC, Hb% and PLT among all treated groups when compared with control. The treatment with

$TiO_2$NPs had no effect on Ht % compared with the control, Meanwhile, the treatment with V.E, V.C, V.C-$TiO_2$NPs and V.E-$TiO_2$NPs caused a significant increase (P≤0.05) in Ht % (Table 2A). On the other side, the treatment with $TiO_2$NPs significantly (P≤0.05) increased the WBC count than all other groups. The oral intact of V.C and V.E decreased with the increase in WBC of $TiO_2$NPs treated rats and the best effect was found after treatment with V.C and V.E together. Also, the treatment with $TiO_2$NPs alone caused a significant increase (P≤0.05) in Lymphocytes (%), Neutrophils (%), Monocytes (%) and Eosinophils (%) compared with the control and other treated groups, Moreover. the oral intake of V.C , V.E or V.C+V.E lowering the effect of $TiO_2$NPs. The oral intake V.C caused significant (P≤0.05) decreased in WBC count, Lymphocytes (%), Neutrophils (%), Monocytes (%) and Eosinophils (%) compared to control group. Also, treatment with V. E alone significantly decreased the WBC count, Neutrophils (%) and Monocytes (%) comparing with the control. The obtained results agree with those of Hassanein and El-Amir (2017) who reported that the $TiO_2$NPs induce toxicity, and significantly increase the total leukocyte count. Meanwhile, Duan et al. (2010) found that with higher $TiO_2$NPs doses for mice, the WBC count, PLT, MPV, MCV were significantly ($p < 0.05$) increased, while, RBC count, Hb % and Ht % were significantly ($p < 0.05$) decreased . We suggested that the decrease in RBC, Ht % and Hb % might be due to the suppression of erythropoiesis and hemosynthesis and to an increase in the rate of erythrocyte demolition in all hemopoietic organs.

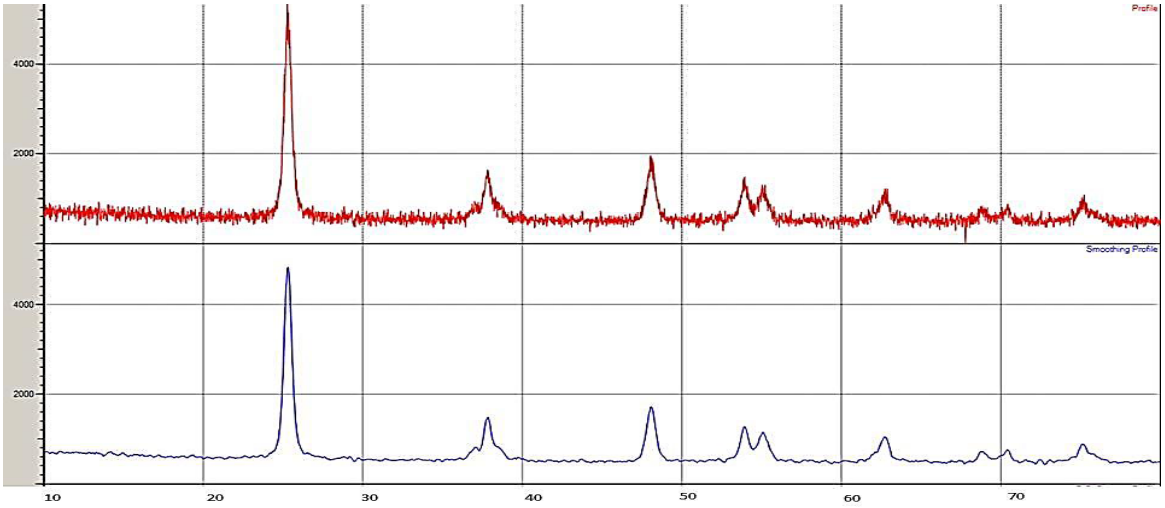


**Fig.1. X-Ray spectrum of $TiO_2$NPs .**

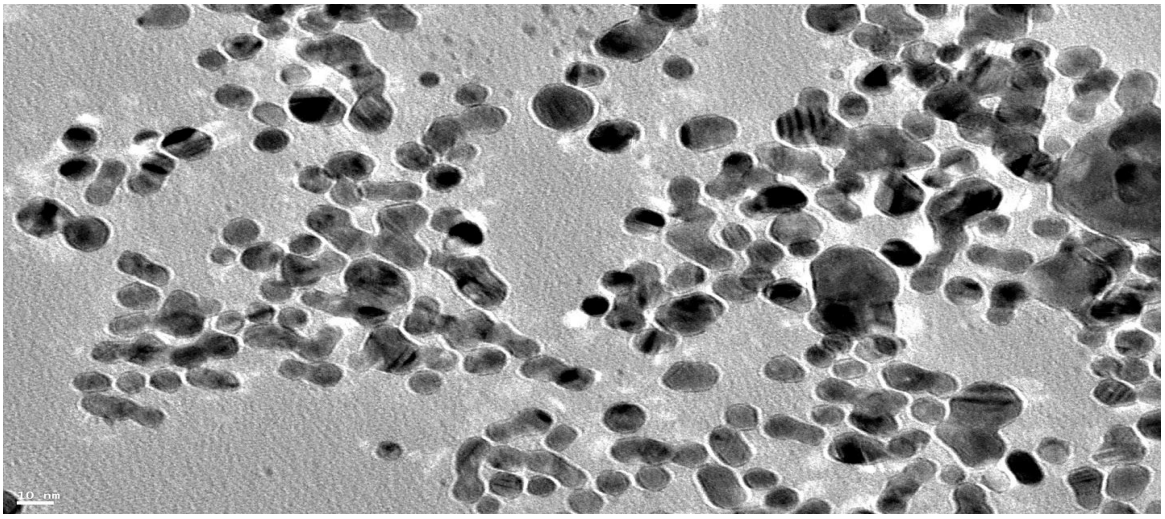


**Fig. 2. Electron microscope image of $TiO_2$NPs .**

**TABLE1. Effect of V.C and V.E on initial and final body weight, body weight gain, feed intake and water intake of rats treated with $TiO_2$ NPs.**

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | V.C | V.E | $TiO_2$NPs | $TiO_2$NPs+ V.C | $TiO_2$NPs + V.E | $TiO_2$NPs+ V.C+ V.E |
| Initial body weight (g) | 148±5.8[a] | 153±6.0[a] (3)* | 156±8.0[a] (5)* | 160±4.5[a] (8)* | 153±8.9[a] (3)* | 154±6.5[a] (4)* | 156±4.3[a] (5)* |
| Final body weight (g) | 169± 4.9[a] | 175± 3.5[a] (4)* | 1795.4±[a] (6)* | 1835.6±[a] (9)* | 1754.9±[a] (4)* | 177±6.2[a] (5)* | 1793.3±[a] (6)* |
| Body weight gain (g/ day) | 0.701±٠,٠217[a] | 0.733±0.0279[a] (5)* | 0.764±٠,٠371[a] (9)* | 0.766±٠,٠282[a] (9)* | 0.740±٠,٠194[a] (6)* | 0.766±0.0293[a] (9)* | 0.766±٠,٠54[a] (9)* |
| Feed intake (g/ day/kg BW) | 158±6.5[a] | 1672.9±[a] (5)* | 164±2.5[a] (4)* | 164±2.5[a] (3)* | 162±8.5[a] (2)* | 165±9.8[a] (4)* | 167±10.9[a] (6)* |
| Water intake (ml/ day/kg BW) | 75.3±3.31[a] | 76.9±4.33[a] (2)* | 78.2±3.31[a] (4)* | 76.3±3.10[a] (1)* | 77.1±2.67[a] (2)* | 76.6±2.60[a] (2)* | 77.3±3.64[a] (3)* |

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a common superscript letters (a) were significantly different, P≤0.05

**TABLE (2A) . Effect of V.C and V.E on hematological parameters of rats treated with $TiO_2$NPs**

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | V.C | V.E | $TiO_2$NPs | $TiO_2$NPs+ V.C | $TiO_2$NPs+ V.E | $TiO_2$NPs+ V.C+ V.E |
| RBC (x $10^6$/µl) | 6.20±٠,349[a] | 6.71±٠,104[a] (8)* | 6.46±٠,376[a] (4)* | 6.28±0.224[a] (1)* | 6.50±0.170[a] (5)* | 6.37±٠,343[a] (3)* | 6.48±0.277[a] (5)* |
| Hb (g/dl) | 10.90.35±[a] | 11.90.49±[a] (9)* | 11.30.75±[a] (4)* | 11.10.43±[a] (2)* | 11.90.52±[a] (9)* | 11.10.58±[a] (2)* | 11.10.64±[a] (2)* |
| Ht (%) | 30.80.85±[b] | 35.30.88±[a] (15)* | 33.60.53±[a] (9)* | 31.10.51±[b] (1)* | 33.70.42±[a] (9)* | 33.30.56±[a] (8)* | 33.30.77±[a] (8)* |
| PLT (×$10^3$/µl) | 3177.8±[a] | 3163.4±[a] (0)* | 3187.0±[a] (0)* | 3265.2±[a] (3)* | 3197.5±[a] (1)* | 3248.8±[a] (2)* | 3203.5±[a] (1)* |
| RDW (%) | 16.00.17±[b] | 17.00.38±[a] (7)* | 16.80.12±[ab] (5)* | 16.50.48±[ab] (3)* | 16.40.19±[ab] (3)* | 16.30.20±[ab] (2)* | 16.50.36±[ab] (3)* |
| MCV (fl) | 57.80.39±[c] | 63.20.72±[a] (9)* | 62.10.68±[a] (7)* | 59.80.78±[b] (3)* | 60.20.30±[b] (4)* | 59.50.31±[bc] (3)* | 59.40.65±[bc] (3)* |
| MCH (pg) | 18.90.31±[b] | 20.50.59±[a] (8)* | 20.30.69±[ab] (7)* | 19.70.53±[ab] (4)* | 19.80.26±[ab] (5)* | 19.90.34±[ab] (5)* | 20.10.41±[ab] (6)* |
| MCHC (%) | 32.8±0.35[b] | 34.50.35±[a] (5)* | 33.50.35±[bc] (2)* | 33.10.27±[b] (1)* | 33.70.44±[bc] (3)* | 33.60.38±[bc] (2)* | 33.50.35±[bc] (2)* |

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different, P≤0.05.
RBC = red blood cells; Hb = hemoglobin; Ht = hematocrit; PLT = platelets count; RDW = redcell distribution width; MCV = mean cell volume; MCH = mean corpuscular hemoglobin and MCHC = mean corpuscular hemoglobin concentration.*percentage of control group.

**TABLE ( 2B ). Effect of V.C and V.E on white blood cell count (WBC) and differential counts of rats treated with** $TiO_2$ NPs .

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **V.C** | **V.E** | **$TiO_2$NPs** | **$TiO_2$NPs+ V.C** | **$TiO_2$NPs + V.E** | **$TiO_2$NPs+ V.C+ V.E** |
| WBC ( $x10^3/\mu l$) | 8.800.378±$^{cd}$ | 8.340.397±$^{d}$ (-5)$^{*}$ | 8.240.402±$^{d}$ (-6)$^{*}$ | 11.600.365±$^{a}$ (32)$^{*}$ | 10.500.378±$^{b}$ (19)$^{*}$ | 10.020.384±$^{b}$ (14)$^{*}$ | 9.760.238±$^{bc}$ (11)$^{*}$ |
| Lymphocytes (%) | 43.01.58±$^{ab}$ | 40.01.14±$^{b}$ (-7)$^{*}$ | 41.62.02±$^{ab}$ (-3)$^{*}$ | 46.42.02±$^{a}$ (8)$^{*}$ | 45.21.36±$^{ab}$ (5)$^{*}$ | 44.81.93±$^{ab}$ (4)$^{*}$ | 44.01.79±$^{ab}$ (2)$^{*}$ |
| Neutrophils (%) | 46.00.17±$^{b}$ | 43.30.06±$^{c}$ (-6)$^{*}$ | 44.40.85±$^{c}$ (-4)$^{*}$ | 48.00.69±$^{a}$ (4)$^{*}$ | 47.10.49±$^{bc}$ (2)$^{*}$ | 47.10.62±$^{bc}$ (2)$^{*}$ | 46.80.44±$^{bc}$ (2)$^{*}$ |
| Monocytes (%) | 1.520.136±$^{ab}$ | 1.400.071±$^{b}$ (-8)$^{*}$ | 1.400.141±$^{b}$ (-8)$^{*}$ | 1.840.108±$^{a}$ (21)$^{*}$ | 1.720.136±$^{ab}$ (13)$^{*}$ | 1.680.156±$^{ab}$ (11)$^{*}$ | 1.600.141±$^{ab}$ (5)$^{*}$ |
| Eosinophils (%) | 1.400.152±$^{ab}$ | 1.200.158±$^{b}$ (-14)$^{*}$ | 1.320.128±$^{ab}$ (-6)$^{*}$ | 1.740.093±$^{a}$ (24)$^{*}$ | 1.600.152±$^{ab}$ (14)$^{*}$ | 1.500.158±$^{ab}$ (7)$^{*}$ | 1.460.129±$^{ab}$ (4)$^{*}$ |

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, P≤0.05.
*percentage of control group.

*Effect of $TiO_2$NPs, V.C and V.E on kidney functions of rats*

Exposure of $TiO_2$NPs from various ways results in their aggregation in the several organs tissues such as kidney, liver, spleen, and brain with possibility toxicological effects (Elgrabli et al., 2015).The effect of V.C and V.E on kidney functions of rats treated with $TiO_2$NPs is present in Table 3. The results showed that $TiO_2$NPs significantly (P≤0.05) increased the level of urea, bilirubin and creatinine, while the oral intake of V.C and V.E or V.C+V.E reduced this increased. These results agreed with Liu et al. (2009) who found that vitamin C and E exhibit a significant decreased in bilirubin value in compare with control, while the oral intake of V.C showed the lower value of creatinine than all other treatments. As kidney is a periodic target for toxic effects of xenobiotics, the kidney is found to be one of the main goal of $TiO_2$NPs precipitations, even in minimum exposure level of $TiO_2$NPs. Moreover, the subchronic toxicity of $TiO_2$NPs drove to chronic nephritis with several pathological lesions such as renal cell necrosis, proximal cell death, and renal fibrosis (Pujalté et al., 2011; Gui et al., 2011 and Hong et al., 2016). Newly, it was confirmed the main molecular mechanisms responsible for the renal toxicity from $TiO_2$NPs (Masoud et al., 2015). The reactive oxygen species (ROS) generated by $TiO_2$NPs may play a vital role in the mechanism of $TiO_2$NPs-induced toxicity (Masoud et al., 2015). Many earlier studies have revealed that antioxidant agents can reduce the toxicity of different metallic nanoparticles (Ibrahim et al., 2015, Escárcega-González et al., 2016 and Khalaf et al., 2016).

*Effect of $TiO_2$NPs, V.C and V.E on liver functions of rats*

The *in vivo* studies suggested that the nanoparticles can accumulate in several organs tissues such as the liver and kidneys tissues and can generate various inflammatory responses (Cemek et al., 2010). The effect of administration V.C and V.E on liver functions of $TiO_2$NPs-treated rats were presented in Table 4. The ALT and AST values were significantly (P≤ 0.05) increased in rats treated with $TiO_2$NPs alone, while, ALP and ACP values were significantly (P≤ 0.05) decreased compared with the control group. On the other hand, oral intake of each V.C and V.E alone or together reduced the change in liver enzymes after

treated with $TiO_2$NPs. No significant (P≥ 0.05) changes were found in healthy rats treated with V.C or V.E when comparing with control.The results of treatment with vitamin C and vitamin E alone or together did not differ significantly with most liver enzymes in $TiO_2$NPs -treated rats. From these results it can be concluded that the treatments with V.C and V.E protected the liver enzymes in $TiO_2$NPs -treated rats. This results in agree with (Jafari et al., 2018) who found a significant increase in the serum level of ALT, AST and ALP after oral intake of $TiO_2$NPs (100 mg/kg). Also,Wang et al. (2007b, c) found that the values of serum ALT, AST, LDH and BUN in mice treated with one dose (25, 80, and 155 nm, 5 g/kg) of $TiO_2$ particles were increased and pathological analysis revealed damage and injury of liver and kidney in mice. No significant changes were found in the lung, heart, ovary, or spleen. Earlier study found that the mice intraperitoneally receiving $TiO_2$NPs (5 nm with doses 5, 10, 50, 100, and 150 mg/kg/day) for 14 days, had inflammatory effects, harm to the kidneys, liver, and myocardium happened, and deranged in homeostasis of blood lipid and sugar (Ma et al., 2009) but when used $TiO_2$NPs ( size 80 and 110 nm ), in dose from 0 – 2592 mg/kg, occurred severe toxicity including negative conduct, loss of appetite, tremor, and lethargy (Chen et al., 2009).Wang et al. (2007a) reported that $TiO_2$ particles induced different pathological lesions in the kidneys and liver of female mice. Also, Jafari et al. (2018) reported that the oral administration of $TiO_2$NPs (100 mg/kg) for 60 consecutive days caused damage in animals liver. Our results suggested that the oral administration of vitamin C and E reduced the harmful effect of $TiO_2$NPs and may protect the liver and kidney tissues.

*Antioxidant activity*

Oxidative stress has been proposed as one of the main mechanisms participatory in genotoxicity due to $TiO_2$NPs (Kohen and Nyska, 2002). $TiO_2$NPs can elevate the obstetrics and accumulation of ROS, resulted indirect oxidative damage of DNA in cells and cause apoptosis in mammalian cells (Rahman et al., 2002). Treatment with $TiO_2$NPs alone significantly increased of TBARS and decreased the activity of antioxidant enzymes (SOD and GST) and the concentration of GHS in rats plasma. Meanwhile, the oral intake of V.C, V.E showed decreased the increase of TBARS and protected the activity of antioxidant enzymes in plasma of $TiO_2$NPs- treated rats. Vitamin C group showed the higher decrease in TBARS values compared with control and all other treated groups. Also, the treatment with V.C alone showed the higher activity of SOD and GST, and the higher GSH content compared with all other treatments. The oral intake of V.C and V.E together showed the best antioxidant agent in $TiO_2$NPs- treated rats compared with other $TiO_2$NPs- treated groups. These results agree with Morgan et al. (2018). They reported the $TiO_2$NPs caused a decrease in antioxidant enzymatic defense. The reduction in GSH level in $TiO_2$NPs- treated groups agree with that reported by Federici et al. ( 2007) and Rajapakse et al. (2012), They suggested that the oxidative stress after $TiO_2$ exposure can result in decreases in levels of hepatic malondialdehyde and an increase in reduced glutathione levels. The catalytic possessions of $TiO_2$NPs as one of the transition metal oxides are well documented to generate reactive oxygen species ROS (Jeon et al., 2013).

## Conclusion

$TiO_2$NPs are being used on a wide-ranging in food products and food colorants, nutritional supplements, water and beverages. According to many last studies the increased exposure to $TiO_2$ nanoparticles could cause inflammatory reaction, oxidative damage in DNA and serious damage to the liver, kidneys, lungs and myocardium. This study investigated the effect of natural antioxidants such as Vitamin C and E on $TiO_2$NPs-treated rats. The treatment with $TiO_2$NPs caused significant (P≤0.05) increased in kidney functions (urea, bilirubin and creatinine levels) and liver functions (ALT and AST) while the oral intake of V.C and V.E or V.C+V.E reduced this increase. Also, the treatment with $TiO_2$NPs increased the level of free radicals and decreased the activity of antioxidant enzymes. Meanwhile, V.C and V.E decreased the increased in free radicals (TBARS level) and protected the activity of some antioxidant enzymes (SOD and GST) in $TiO_2$NPs- treated rats. This study recommends banning the use of $TiO_2$NPs in food products because of its adverse effects on human health, and encourages the consumption of foods rich in vitamin C and E.

**TABLE 3. Effect of V.C and V.E on kidney function of rats treated with $TiO_2$ NPs .**

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | V.C | V.E | $TiO_2$ NPs | $TiO_2$ NPs | $TiO_2$ NPs | $TiO_2$ NPs+ V.C+ V.E |
| Urea ( mg/ dl ) | 19.1±1.63$^{b}$ | 18.5±1.05$^{b}$ (-3)* | 18.7±1.23$^{b}$ (-2)* | 23.1±1.16$^{a}$ (21)* | 21.5±0.74$^{ab}$ (13)* | 21.2±0.77$^{ab}$ (11)* | 21.1±0.50$^{ab}$ (10)* |
| Bilirubin ( mg/ dl) | 2.400.110±$^{ab}$ | 2.240.166±$^{b}$ (-7)* | 2.260.196±$^{b}$ (-6)* | 2.810.225±$^{a}$ (17)* | 2.700.177±$^{ab}$ (12)* | 2.630.100±$^{ab}$ (9)* | 2.530.116±$^{ab}$ (5)* |
| Creatinine (mg/dl) | 1.530.101±$^{bc}$ | 1.400.098±$^{c}$ (-9)* | 1.480.100±$^{bc}$ (-3)* | 1.910.119±$^{a}$ (25)* | 1.740.054±$^{ab}$ (14)* | 1.700.104±$^{ab}$ (11)* | 1.660.055±$^{abc}$ (8)* |

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a commonsuperscript letters (a, b,c) were significantly different, P≤0.05.
*percentage of control group.

**TABLE4. Effect of V.C and V.E on liver function of rats treated with $TiO_2$ NPs.**

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | V.C | V.E | $TiO_2$ NPs | $TiO_2$ NPs+ V.C | $TiO_2$ NPs+ V.E | $TiO_2$ NPs+ V.C+ V.E |
| AST (U/ml) | 41.31.04±$^{c}$ | 40.61.48±$^{c}$ (-2)* | 41.01.67±$^{c}$ (-1)* | 50.81.53±$^{a}$ (23)* | 47.41.29±$^{ab}$ (15)* | 44.61.63±$^{bc}$ (8)* | 43.11.55±$^{bc}$ (4)* |
| ALT (U/ml) | 37.01.11±$^{c}$ | 36.11.98±$^{c}$ (-2)* | 36.61.17±$^{c}$ (-1)* | 47.91.58±$^{a}$ (30)* | 43.51.19±$^{b}$ (18)* | 41.51.50±$^{b}$ (12)* | 39.51.11±$^{bc}$ (7)* |
| AlP (IU/L) | 29.61.59±$^{a}$ | 29.51.13±$^{a}$ (0)* | 29.61.53±$^{a}$ (0)* | 24.81.15±$^{b}$ (-16)* | 26.21.57±$^{ab}$ (-11)* | 27.31.15±$^{ab}$ (-8)* | 28.11.27±$^{ab}$ (-5)* |
| AcP (U/ L) | 25.5±1.20$^{a}$ | 25.3±1.15$^{a}$ (-1)* | 25.3±1.14$^{a}$ (-1)* | 21.2±1.33$^{b}$ (-17)* | 22.6±1.16$^{ab}$ (-11)* | 23.1±1.36$^{ab}$ (-9)* | 23.3±1.35$^{ab}$ (-9)* |

Values are expressed as means ± SE; n = 5 for each treatment group.

Meanvalues within a row not sharing a common superscript letters (a, b, c) were significantly different, P≤0.05.

AST = aspartate transaminase; ALT = alanine transaminase; AlP = alkaline phosphatase and AcP = acid phosphatase.
*percentage of control group.

**TABLE 5 . Effect of V.C and V.E on TBARS and antioxidant enzymes of rats treated with** TiO2 NPs .

| Parameters | Experimental groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **V.C** | **V.E** | **$TiO_2$ NPs** | **$TiO_2$ NPs+ V.C** | **$TiO_2$ NPs+ V.E** | **$TiO_2$ NPs+ V.C+ V.E** |
| TBARS (nmol/ml ) | 1.60±0.181bc | 1.38±0.124c (-14)* | 1.51±0.177bc (-6)* | 2.31±.175a (44)* | 1.98±0.191ab (23)* | 1.82±0.181abc (14)* | 1.70±0.251bc (6)* |
| SOD (U/ml) | 2.010.089±abc | 2.190.134±a (9)* | 2.150.142±ab (7)* | 1.320.177±d (-34)* | 1.690.076±c (-16)* | 1.790.078±bc (-11)* | 1.860.095±abc (-7)* |
| GSH (µlmol /ml) | 6.690.373±bc | 7.680.271±a (15)* | 7.500.391±ab (12)* | 5.170.3316±d (-23)* | 5.960.323±cd (-11)* | 6.080.323±cd (-9)* | 6.260.186±c (-6)* |
| GST (µlmol/min) | 0.7330.0788±abc | 0.8260.0500±a (13)* | 0.8060.0490±ab (10)* | 0.5590.0452±d (-24)* | 0.6320.0337±cd (-14)* | 0.6690.0405±bcd (-9)* | 0.6940.0145±abcd (-5)* |

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, P≤0.05.
TBARS = thiobarbituric acid reactingsubstance; SOD = superoxide dismutase; GSH = reduced glutathione andGST = glutathion–S transferase.
*percentage of control group.

**Acknowledgment**

The authors are grateful to Prof. Mokhtar Ibrahim Yousef, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Egypt, for his support during the biological study. Also, we would like to thank Dr. Mahmoud Ibrahim El-Sayed, Department of Dairy Technology Research, Food Technology Research Institute, ARC, Giza, Egypt for his help during writing and revision this article.

**References**

Apaydin, F.G., Bas, H., Kalender, S. and Kalender, Y. (2017) Bendiocarb induced histopathological and biochemical alterations inrat liver and preventive role of vitamins C and E, *Environmental Toxicology and Pharmacology,* **49**, pp.148–155.

Atha, D. H., Wang, H., Petersen, E. J., Cleveland, D., Holbrook, R. D., Jaruga, P. and Nelson, B. C. (2012)Copper oxide nanoparticle mediated DNA damage in terrestrial plant models, *Environmental Science & Technology*, **46,** *No.* 3, pp. 1819-1827.

Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F. andCogliano, V. (2006),Carcinogenicity of carbon black, titanium dioxide, and talc, *The Lancet Oncology*, **7,** *No.* 4, pp. 295-296.

Bartels, H. and Bohmer, M. (1971) Microestimation of creatinine,*Clinica Chimica Acta*, **32,** pp. 81-85.

Belfield, A. and Goldberg, D. M. (1971) Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine, *Enzyme*, **12,** pp. 561-573.

Bregadiolli, B. A., Fernandes, S. L. andGraeff, C. F. D. O. (2017),Easy and fast preparation of TiO2-based nanostructures using microwave assisted hydrothermal synthesis, *Materials Research*, **20,** No. 4, pp.912-919.

Burtis, C.A.; Ashwood, E.R. and Bruns, D.E. (1999) Serum total protein determination. In: Tietz textbook of clinical chemistry and molecular diagnostics, 3rd ed. AACC; 1915-1916.

Cemek, M., Aymelek, F., Büyükokuroğlu, M. E., Karaca, T., Büyükben, A. and Yilmaz, F. (2010),Protective potential of Royal Jelly against carbon tetrachloride induced-toxicity and changes in the serum sialic acid levels,*Food and Chemical Toxicology*, **48,** No.10, pp. 2827-2832.

Chaney, A. L. andMarbach, E. P. (1962) Modified reagents for determination of urea and ammonia,*Clinical*

*Chemistry*, **8,** No 2, pp.130-132.

Chen, J., Dong, X., Zhao, J. and Tang, G. (2009) *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection, *Journal of Applied Toxicology*, **29,** No 4, pp. 330-337.

Childs, A. C., Phaneuf, S. L., Dirks, A. J., Phillips, T. and Leeuwenburgh, C. (2002) Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2: Baxratio , *Cancer Research*, **62,** No16, pp.4592-4598.

Duan, Y., Liu, J., Ma, L., Li, N., Liu, H., Wang, J., Zheng, L., Liu, C., Wang, X., Zhao, X., Yan, J., Wang, S., Wang, H., Zhang, X. and Hong, F. (2010) Toxicological characteristics of nanoparticulateanatase titanium dioxide in mice, *Biomaterials*, **31,** No 5, pp.894-899.

Elgrabli, D., Beaudouin, R., Jbilou, N., Floriani, M., Pery, A., Rogerieux, F. and Lacroix, G. (2015) Biodistribution and clearance of $TiO_2$ nanoparticles in rats after intravenous injection, *PLoS One*, **10,** No 4, pp.e0124490.

El-Shafei, R. A. and Saleh, R. M. (2016), Pharmacological effects of vitamin C & E on diclofenac sodium intoxicated rats, *Biomedicine & Pharmacotherapy*, **84,** pp. 314-322.

Escárcega-González, C.E., Reynoso-Andeola, I.G., Jaramillo-Juárez, F., Martínez-Ruvalcaba, H., Posadas del Rio, F.A. (2016) The ginkgo biloba extract reverses the renal effects of titanium dioxide nanoparticles in adult male rats. *Biochemistry Research International*, 2, 1-9.

Fabian, E., Landsiedel, R., Ma-Hock, L., Wiench, K., Wohlleben, W. and Van Ravenzwaay, B. (2008) Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. *Archives of Toxicology*, **82** (3), 151-157.

Fabiny, D. L. and Ertingshausen, G. (1971) Automated reaction-rate method for determination of serum creatinine with the CentrifiChem. *Clinical Chemistry*, **17** (8), 696-700.

Federici, G., Shaw, B. J., & Handy, R. D. (2007). Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchusmykiss): Gill injury, oxidative stress, and other physiological effects. *Aquatic Toxicology*, **84** (4), 415-430.

Gui, S., Zhang, Z., Zheng, L., Cui, Y., Liu, X., Li, N. and Cheng, J. (2011) Molecular mechanism of kidney injury of mice caused by exposure to titanium dioxide nanoparticles. *Journal of Hazardous Materials*, **195,** 365-370.

Gurr, J.R., Wang, A.S.S., Chen, C.H. and Jan, K.Y. (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology,* 213, 66–73.

Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974) Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, **249** (22), 7130-7139.

Hassanein, K. M. and El-Amir, Y. O. (2017) Protective effects of thymoquinone and avenanthramides on titanium dioxide nanoparticles induced toxicity in Sprague-Dawley rats. *Pathology-Research and Practice*, **213** (1), 13-22.

Heistad, D. D. (2006) Oxidative stress and vascular disease: 2005 Duff lecture. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **26** (4), 689-695.

Hong, F., Wang, Y., Zhou, Y., Zhang, Q., Ge, Y., Chen, M., and Wang, L. (2016) Exposure to TiO2 nanoparticles induces immunological dysfunction in mouse testitis. *Journal of Agricultural and Food Chemistry*, **64** (1), 346-355.

Ibrahim, M.A., Khalaf, A.A., Galal, M.K., Ogaly, H.A.H.A. and Hassan, A.H.M. (2015) Ameliorative influence of green tea extract on copper nanoparticle-induced hepatotoxicity in rats, *Nanoscale Res. Lett.,* 10 (1), 1–9.

Jafari, A., Rasmi, Y., Hajaghazadeh, M. and Karimipour, M. (2018) Hepatoprotective effect of thymol against subchronic toxicity of titanium dioxide nanoparticles: Biochemical and histological evidences. *Environmental Toxicology and Pharmacology*, **58,** 29-36.

Jeon, J. M., Kim, W. J. and Lee, M. Y. (2013) Studies on liver damage induced by nanosized-titanium dioxide in mouse. *J. Environ Biol*, **34** (2), 283-287.

Jollow, D. J., Mitchell, J. R., Zampaglione, N. A. and Gillette, J. R. (1974) Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, **11** (3), 151-169.

Kaplan A, Rubaltelli FF, Hammerman C., Vilei M.T., Leiter C., Abramov A. Bilirubin. *Clin.Chem*. The C.V. Mosby Co. St Louis. Toronto. Princeton.1984, 1238-1241.

Karlsson, H. L., Cronholm, P., Gustafsson, J. and

Moller, L. (2008) Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical Research in Toxicology*, **21** (9), 1726-1732.

Khalaf, A.A., Zaki, A.R.,Galal, M.K., Ogaly, H.A., Ibrahim, M.A. and Hassan, A. (2016) The potential protective effect of a-lipoic acid against nanocopper particle– induced hepatotoxicity in male rats. *Human & Experimental Toxicology*, **36** (9), 881-89.

Khanna, P., Ong, C., Bay, B. and Baeg, G. (2015) Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials*, **5** (3), 1163-1180.

Kind, P. R. N. and King, E. J. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *Journal of Clinical Pathology,* **7** (4), 322.

Kohen, R., and Nyska, A. (2002) Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*, **30** (6), 620-650.

Li, N., Ma, L.,Wang, J., Zheng, L., Liu, J.,Duan, Y. *et al*. (2010) Interaction between nano-anatase TiO2and liver DNA frommice in vivo, *Nanoscale Res. Lett.* **5,** 108–115.

Liu, H., Ma, L., Zhao, J., Liu, J., Yan, J., Ruan, J. and Hong, F. (2009) Biochemical toxicity of nano-anatase TiO 2 particles in mice. *Biological Trace Element Research*, **129** (1-3), 170-180.

Ma, L., Zhao, J., Wang, J., Liu, J., Duan, Y., Liu, H., Li, N, Yan, J., Ruan, J., Wang, H& Hong, F. (2009) The acute liver injury in mice caused by nano-anataseTiO $_2$. *Nanoscale Research Letters*, **4** (11), 1275.

Manke, A., Wang, L., and Rojanasakul, Y. (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. *Bio Med Research International*. http://dx.doi.org/10.1155/2013/942916.

Masoud, R., Bizouarn, T., Trepout, S., Wien, F., Baciou, L., Marco, S. and Levin, C. H. (2015) Titanium dioxide nanoparticles increase superoxide anion production by acting on NADPH oxidase. *PloS one*, **10** (12), e 0144829.

Meena, R. and Paulraj, R. (2012) Oxidative stress mediated cytotoxicity of TiO2 nanoanatase in liver and kidney of Wistar rat. *Toxicological & Environmental Chemistry*, **94** (1), 146-163.

Misra, H. P., and Fridovich, I. (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, **247** (10), 3170-3175.

Morgan, A., Ibrahim, M. A., Galal, M. K., Ogaly, H. A., and Abd-Elsalam, R. M. (2018) Innovative perception on using Tiron to modulate the hepatotoxicity induced by titanium dioxide nanoparticles in male rats. *Biomedicine & Pharmacotherapy*, **103,** 553-561.

Nesaretnam, K. (2008) Multitargeted therapy of cancer by tocotrienols. *Cancer Letters*, **269** (2), 388-395.

Oberdörster, G. (2001) Pulmonary effects of inhaled ultrafine particles. *International Archives of Occupational and Environmental Health*, **74** (1), 1-8.

Orazizadeh, M., Fakhredini, F., Mansouri, E., and Khorsandi, L. (2014) Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats. *Chemico-biological Interactions*, **220,** 214-221.

Pujalté, I., Passagne, I., Brouillaud, B., Tréguer, M., Durand, E., Ohayon-Courtès, C. and L›Azou, B. (2011) Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells. *Particle and Fibre Toxicology*, **8** (1), 10.

Rahman, Q., Lohani, M., Dopp, E., Pemsel, H., Jonas, L., Weiss, D. G. and Schiffmann, D. (2002) Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. *Environmental health perspectives*, **110** (8), 797-800.

Rajapakse, K., Drobne, D., Valant, J., Vodovnik, M., Levart, A. and Marinsek-Logar, R. (2012). Acclimation of Tetrahymenathermophila to bulk and nano-$TiO_2$ particles by changes in membrane fatty acids saturation. *Journal of Hazardous Materials*, **221,** 199-205.

Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, **28** (1), 56-63.

Sargazi, Z., Nikravesh, M. R., Jalali, M., Sadeghnia, H. R. and Rahimi Anbarkeh, F. (2016). Apoptotic effect of organophosphorus insecticide diazinon on rat ovary and protective effect of vitamin E. *Iranian Journal of Toxicology*, **10** (2), 37-44.

SAS (2004) SAS Procedure Guide «Version 6.12 ed.» SAS Institute Inc., Cary.

Searcy, R. L., Reardon, J. E. and Foreman, J. A. (1967) A new photometric method for serum urea nitrogen determination. *The American Journal of Medical Technology.*, **33,** 15-20.

Sha, B., Gao, W., Cui, X., Wang, L., &Xu, F. (2015). The potential health challenges of $TiO_2$ nanomaterials. *Journal of Applied Toxicology*, **35** (10), 1086-1101.

Shakeel, M., Jabeen, F., Shabbir, S., Asghar, M. S., Khan, M. S. and Chaudhry, A. S. (2015) Toxicity of nano-titanium dioxide ($TiO_2$-NP) through various routes of exposure: a review. *Biological Trace Element Research*, **172** (1), 1-36.

Tabacco, A., Meiattini, F., Moda, E., &Tarli, P. (1979). Simplified enzymic/colorimetric serum urea nitrogen determination. *Clinical Chemistry*, **25** (2), 336-337.

Tappel, A. L., &Zalkin, H. (1959). Inhibition of lipide peroxidation in mitochondria by vitamin E. *Archives of Biochemistry and Biophysics*, **80** (2), 333-336.

Uchendu, C., Ambali, S. F. and Ayo, J. O. (2012). The organophosphate, chlorpyrifos, oxidative stress and the role of some antioxidants: a review. *African Journal of Agricultural Research*, **7** (18), 2720–2728.

Walter, C. L. (1981) The exposure of humans to nitrite. *Oncology*, **37** (4), 289-296. https://doi.org/10.1159/000225455

Wang, J. X., Li, Y. F., Zhou, G. Q., Li, B., Jiao, F., Chen, C. Y. and Chai, Z. F. (2007a) Influence of intranasal instilled titanium dioxide nanoparticles on monoaminergic neurotransmitters of female mice at different exposure time. *Zhonghuayu fang yixuezazhi [Chinese journal of preventive medicine]*, **41** (2), 91-95.

Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., Jia, G., Gao, Y., Li, B., Sun, J. and Li, Y. (2007b) Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. *Toxicology letters*, **168** (2), 176-185.

Wang, J. J., Sanderson, B. J. and Wang, H. (2007c). Cyto-and genotoxicity of ultrafine $TiO_2$ particles in cultured human lymphoblastoid cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **628** (2), 99-106.

Warheit, D. B. and Donner, E. M. (2015) Risk assessment strategies for nanoscale and fine-sized titanium dioxide particles: recognizing hazard and exposure issues. *Food and Chemical Toxicology*, **85,** 138-147.https://doi.org/10.1016/j.fct.2015.07.001.

Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K. and Von Goetz, N. (2012) Titanium dioxide nanoparticles in food and personal care products. *Environmental Science & Technology*, **46** (4), 2242-2250.

Zhang, M., Liu, W., Zhou, Y., Li, Y., Qin, Y., and Xu, Y. (2018) Neurodevelopmental toxicity induced by maternal PM2. 5 exposure and protective effects of quercetin and Vitamin C. *Chemosphere*, **213,** 182-196.

Zhao, J., Bowman, L., Zhang, X., Vallyathan, V., Young, S. H., 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. *Journal of Toxicology and Environmental Health, Part A*, **72** (19), 1141-1149.

Zingg, J. M. (2015). Vitamin E: a role in signal transduction. *Annual Review of Nutrition*, **35,** 135-173.https://doi.org/10.1146/annurev-nutr-071714-034347.

# تأثير بعض مضادات الأكسدة علي فئران التجارب المعاملة بجزيئات ثاني أكسيد التيتانيوم النانونية

**رحاب محمد إبراهيم[1] ، مروة فاروق القاضي[2] و آمال حسنين محمود[1]**

قسم بحوث الأغذية الخاصة والتغذية - معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية - الجيزة - مصر.

قسم الهندسة الكيماوية والبيتروكيماوية - الجامعة المصرية اليابانية - الإسكندرية - مصر.

يتم استخدام الجزيئات النانونية لثاني أكسيد التيتانيوم ($TiO_2NPs$) كمادة مبيضة لبعض الأغذية ، و كذلك في إنتاج المكملات الغذائية والعديد من المنتجات الأخري وذلك علي نطاق صناعي واسع. هذا. وقد ذكرت العديد من الدراسات أن الجزيئات النانونية لثاني أكسيد التيتانيوم ($TiO_2NPs$)هي أكثر سمية من الجزيئات العادية لهذا المركب. علاوة علي أنها ربما تكون مسرطنة للبشر. لذلك كان الهدف من هذه الدراسة هو بحث تأثير فيتامين ج (V.C) وفيتامين هـ (V.E) على الأصول الحرة وكذلك نشاط الإنزيمات المضادة للأكسدة في الفئران التي تم معاملتها بـ $TiO_2NPs$ . وقد أظهرت النتائج التي تم الحصول عليها أنه لم يتم العثور على اختلافات كبيرة في العد الكلي لخلايا الدم الحمراء (RBC) ، الهيموجلوبين (Hb) ، الهيماتوكريت (Ht) وكذلك في عدد الصفائح الدموية (PLT) في الفئران بعد معاملتها بالجزيئات النانونية لثاني أكسيد التيتانيوم ($TiO_2NPs$) أو مضادات الأكسدة الطبيعية (فيتامين ج وفيتامين هـ ). من ناحية أخري فقد تسببت المعاملة بـ $TiO_2NPs$ وحدها إلي زيادة ملحوظة في وظائف الكلى والكبد . في حين أن تناول فيتامين ج أو فيتامين هـ أو كلاهما أدى لخفض هذه الزيادة . كما أن تناول هذه الفيتامينات قد حافظ علي المعدلات الطبيعية لهذه الوظائف في الحيوانات غير المعاملة بـ $TiO_2NPs$. أيضًا ،وجد أن المعاملة بـ$TiO_2NPs$ وحدها قد أدي لزيادة معدلات تكون الأصول الحرة في بلازما الفئران . كما أحدث إنخفاضا معنويا في نشاط بعض الإنزيمات المضادة للأكسدة مثل Superoxide dismutase (SOD) و Glutathione-S-transferase (GST) وكذلك أحدث إنخفاض في تركيز الجلوتاثيون (GSH) في البلازما. وفي الوقت نفسه ، فإن المعاملة فيتامين ج أوفيتامين هـ أو كلاهما قد أحدثت إنخفاضا في معدلات تكون الأصول الحرة في الفئران المعاملة بـ $TiO_2NPs$ومع تحسينه لنشاط الإنزيمات المضادة للأكسدة.