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Hematological Changes and Oxidative Stress Induction of Titanium Dioxide Nanoparticles in Male Mice after Intraperitoneal Injection of Different Doses for 28 Days: Study of Organ's Responsibility

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Abstract

Titanium dioxide nanoparticles (TiO_2NPs) are supposed to be the most widely engineered nanoparticles (NPs) that are used in the world. The study evaluated the toxicity of TiO_2NPs (12 - 64 nm) in male mice by intraperitoneal injection at five doses of either control (0), 2.5, 5.0, 10.0, and 20.0 mg/kg of body weight for 28 d. Hematological changes and oxidative stress indicators like Catalase (CAT), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Glutathione reductase (GR), Lipid peroxidation (LPO), Glutathione (GSH) content as well as Carbonyl protein (CP) were explored. Present results showed significant differences ($P < 0.05$) in all hematological measurements at the end of the study for treated groups, respect to their control. TiO_2NPs caused dose-dependent oxidative stress in lung, liver, spleen, kidney, heart, and muscle samples of the treated animals, concerning the control group. Noticeable important increases ($P < 0.05$) of malondialdehyde (MDA) levels were represented in all treatments of samples as following: heart > muscle > spleen > liver > kidney > lung, with respect to their controls. Also, noticeable important increases were recorded for GPx activity and CP level. However, significant declines were noted in CAT, and GST activities, and GSH content in treated animals, concerning control. Moreover, excess of such CP, MDA, and other biomarker alterations may provide a fingerprint of potential toxic effects of TiO_2NPs during long-term exposure scenario. Thus, extensive application of such NPs raises the consideration about biosafety.

Keywords

Titanium dioxide nanoparticles, Mice, Hematology, Oxidative stress, Intra-peritoneal injection

Introduction

Titanium dioxide (TiO_2) is considered the most abundantly produced NMs and used in food, paints and in personal care products [1-2]. It may be proper to the greatest briefly engineered nanoparticles (NPs; 1-100 nm) in the worldwide. It has broad spectrum as bio-medical ceramic and orthopaedic implants [3]. Due to these properties, TiO_2NPs are progressively used in endoprosthesis and supports in bone tissues re-establishments [4-5]. However, nano-toxicological investigations exist critical role for the harmless and sustainable enlargement of the evolving and recognized nanomaterials (NMs) e.g. TiO_2NPs [5]. Actually, TiO_2NPs could likewise induce risky/cytotoxic effects. For example, it induced DNA double strand breaks in bone marrow cells following oral administration in rats [6]. Zhang et al. [7] detected that, TiO_2NPs enhanced pro-inflammatory

gene expression in proosteoblast cells (MC3T3-E1). Wang et al. [8] showed that, TiO_2NPs have potential toxic effects in some organs, where they caused damage to the knee joints in rabbits.

Moreover, TiO_2NPs can become heightened and harmless in different body parts after entering the body through numerous pathways, e.g. administration via the abdominal cavity or inhalation [9-10]. It can be toxic to numerous of cell, e.g. human lymphoblastoid cells and hepatoma cells [11-12]. It can induce a critical stress response in glial cells of mouse brains, resulting in neuron injury and dysfunction [13]. The persistence degree of neuron cell lines exposed to TiO_2NPs significantly decreased in a typical time and dose-dependent manner [14]. Several studies demonstrated mechanisms of NPs toxicity. It may impose genotoxic effects through change the configuration of molecular composite and porousness of the cell membrane and induce oxidative stress [15-17]. In oxidative stress, reactive oxygen species (ROS), e.g. hydroxyl radicals, are generated and cause DNA oxidation as noted for 8-hydroxygaunisine (8-OHdG), resulting in mistakes and alterations in DNA repetition [18-19]. Moreover, ROS could make inflammation and joint feed-forward relations between oxidative stress and inflammation, resulting in DNA damage and cell apoptosis [20-21]. Nevertheless, the complete regular data on the toxicity of TiO_2NPs remains limited. Goals of most investigators was to disclose the effect and the principal mechanism of TiO_2NPs exposure on human health.

In spite of this increase in the prolonged use of engineered NMs and the profits of such use of engineered NPs to society, there is a little knowledge concerning their likely toxicological effects on human as well as environmental health and safety [22-26]. Physical and chemical properties of NMs are predictable to impose significant effects on the performance and properties of macromolecules, cells and body parts [27].

Due to their particularly small size and unique physical properties, the behavior of NMs in the environment, uptake, distribution and impacts within living organisms are likely to be different when compared to their conventional forms [28]. For example, gold NPs (AuNPs) are far smaller than the diameter of a common cell and thus has an opportunity to pass in the human body during production, transportation, storage and consumption. The same properties make them to be useful for different applications, despite they are harmful and toxic to the environment and organisms [29]. Just, various metallic NPs have been displayed to cause severe effects both *in vitro* and *in vivo* [30-31]. Some NMs have been established for their toxicity to humans and other organisms either upon contact or after persistent environmental exposure [24]. Thus, there is an urgent need to fill up the gaps in our accepting and for research and regulatory activities to ensure these compounds do not pose a significant hazard to human and environmental health. This is vital to ensure the sustainability of the industry. TiO_2NPs are very reactive and may be toxic due to their properties, especially larger surface area [32]. They can damage human and animal cells by increasing oxidative stress mechanism. Biosafety of this material needs to be estimated. Some reviews have suggested that the smaller-scale NPs had a greater inflammogenic effect than larger particles. Induction of ROS, free radicals, oxidative stress, damage and apoptosis are common observations in a wide variety of cell types exposed to TiO_2NPs *in vivo* and *in vitro*. Oxidative stress consequences in changes in the production of superoxide dismutase (SOD) or antioxidant defense enzymes. The liver is an active organ for detoxication and TiO_2NPs can enter liver cell. Humans are ever more exposed to TiO_2 via inhalation, dermal or oral exposure, thus keeping in view the potential health hazards of TiO_2NPs on humans. Established on animal assessments, International Agency for Research on Cancer (IARC) classified TiO_2 as a group 2B carcinogen (possibly carcinogenic to humans) [33]. Also, the French agency for food, environmental and occupational health and safety (ANSES) banned the use of TiO_2 as a food additive (E171) due to its genotoxic potential [34]. While numerous research sites noted that, TiO_2NPs can induce adverse effects including DNA damage and chromosomal damage, findings are contradictory [6, 35]. The study aimed to investingate the adverse impacts of different intraperitoneal doses of TiO_2NPs for 28 d on male mice using haematological studies as well as oxidative stress enzyme activity assays.

Materials and Methods

Chemical and reagents

Titanium dioxide nanoparticles (TiO_2NPs) were supplied by Nano-Tech Lab., Dream Land, 6th October City, Egypt. Nanoparticles (NPs) were achieved for reliable characterization techniques. Chemicals: thiobarbituric acid (TBA) and sodium azide (NaN_3) were supplied by LOBA CHEMIE PVT. Ltd, Mumbai-400005, India. Phosphate buffer, sodium phosphate monobasic; dibasic and potassium phosphate monobasic; dibasic were supplied by J.T. BAKER Chem. Co, Phillipsburg, N.J. 08865. Trichloro acetic acid (TCA; Cl_3C_3COOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were obtained from Research Lab. Fine Chem. Indust., Mumbai 400002, India. Ethylene diamine tetra acetic acid disodium salt (EDTA), ethanol (C_2H_5OH), 1-Chloro 2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), Tris-HCl: 2-amino-2-hydroxy methyl-propane-1, 3-diol, β-nicotinamide adenine dinucleotide reduced form (β-NADPH), oxidized glutathione (GSSG), and bovine serum albumin (BSA) were attained from Sigma Chem. Co. P.O. Box 14508 St. Louis MO 63178, USA.

Characterization of NPs

An aliquot of prepared TiO_2 was achieved for visualization on Scanning Electron Microscopy (SEM) (JOEL, JSM 5300) with high resolution at an accelerating voltage of 120 Kev. The sample was coated on a copper grid and scanned for its size and shape. X-ray Electron Dispersive Analysis (EDA) was accomplished by using X-ray Oxford detector unit (model 6647, England) equipped with SEM (JOEL, JSM 5300) to scan the purity of the prepared NPs. Titanium ions (Ti2+) were firm in the used solutions of TiO_2NPs by dynamic light scattering (DLS) (DTS Nano v 5.2; Malvern Zeta sizer Nano ZS, Malvern Instruments, UK). Nanoparticles (NPs) suspension was sonicated by using sonicator bath at ambient conditions for 20 min at 40 W.

Animal rearing

Healthy male mice (mean; 30.0 ± 3.0 g) were obtained from Institute of Public Health, Alexandria University, Egypt. They were allocated to plastic cages covered with metal grids and allowed to acclimate for 2 weeks under laboratorial conditions, before divided into experimental groups. The mice were provided with free water and food ad libitum. Damanhour University Animal Health Care (DU-AHC) committee approved the animal caring ethics (DMU-2020-0026). Guideline of animal care was surveyed according to the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1987).

Dosage procedures

Oxidative stress and hematological alterations in mal mice were investigated. The animals were randomly divided into four group and an additional control group, with 10 mouse/group. Suspension of TiO_2NPs was intraperitoneal injected (i.p) at dosage: 2.5, 5.0, 10.0 and 20.0 mg /kg b.w once a day for 28 d. Vehicle (citrate solution) was injected into mice of the control group. The animals were observed every day, and no animal died during the study. On the 28th d, the animals were anesthetized with 2% phenobarbital (60 ml/kg, i.p) and dissected for sampling procedures. Blood samples were collected from heart by using heparinized syringe and specific blood tubes. They were subjected for complete blood count (CBC). On the other hand, liver spleen, heart, kidney and muscles were taken and stored at -20 °C until analysis.

Hematological measurements

One ml of blood from treated animals was collected in sterilized tube with 0.5 µl of EDTA as an anticoagulant. For CBC analysis, the samples were subjected on cell counter instrument (Medonic serial no.14641-Swdish) and the data were presented at software program.

Biochemical quantifications

Sample preparation

An aliquot (0.5 g, each tissue) was homogenized with cold-saline solution (1:10 w/v) and then centrifuged at 5000 rpm and 4 °C for 15 min. Supernatant was used as a source for Catalase (CAT), Glutathione-S-transferase (GST), Glutathione peroxidase (GPx), Glutathione reductase (GR), while homogenate was used for Lipid peroxidation (LPO) and Glutathione (GSH) content.

LPO

Thiobarbituric acid reactive substances (TBARS) were used as an index of LPO according to Rice-Evans et al. [36] through quantification of malondialdehyde (MDA) content. An aliquot (250 µl) of each tissue homogenate was mixed with 1 ml of 15% (w/v) trichloroacetic acid (TCA) in 25 mM HCl C.m and 2ml of 0.37% thiobarbituric acid (TBA). The mixture was boiled for 10 min, quickly cooled, and immediately centrifuged at 5000 rpm for 5 min. The absorbance was determined at 535 nm. MDA was quantified by using an extinction coefficient of 156 mM-1 and expressed as mM g-1 tissue.

GSH

Principle of the used method depends on reduction of 5, 5′-dithiobis 2- nitrobenzoic acid (DTNB) with GSH to produce a yellow complex which was measured at 405 nm [37]. Enzyme source (500 µl) was mixed with the same volume of 500 mM TCA, followed by centrifugation at 3000 rpm for 15 min. An aliquot (500 µl) was well mixed with 1 ml of each 100 mM PBS buffer, pH 7.4 and 1 mM DTNB. After 10 min, the absorbance was measured at 405 nm against blank. GSH concentration was expressed as nM g^{-1} tissue.

CAT

The enzyme activity was assayed independent on decrease of absorbance at 240 nm in association with hydrogen peroxide (H_2O_2) consumption [38]. The reaction mixture consisted of 1 ml of 12.5 mM H_2O_2 (substrate), 2 ml of 66.7 mM phosphate buffer, pH 7.0 and enzyme source. The activity was expressed as U mg^{-1} tissue. The unit of CAT is the amount of enzyme which liberates half the peroxide oxygen from hydrogen peroxide solution of any concentration in 100 µl at 25°C.

GST

The activity was determined by the spectrophotometric method of Habig and Jakoby [39] by using 1-Chloro, 2-4 dinitrobenzene (CDNB). Enzyme source was mixed with 500 µl of potassium phosphate buffer (50 mM; pH 6.5). The incubation was done at 25°C for 5 min, followed by mixing with 100 µl of 0.2 M CDNB and 150 µl of 10 mM GSH. After 1 min, the change of absorbance was recorded every 30 s for 6 min at 340 nm. The enzyme activity was expressed as nM mg^{-1} min^{-1}.

GPx

The enzyme activity was measured according to Flohe and Gunzler [40] by mixing phosphate buffer solution (100 mM), EDTA (50 mM), sodium azide (250 mM), H_2O_2 (10 mM) and enzyme in a cuvette. The change in absorbance was followed every 3 s for 40 s at 340 nm. Enzyme activity was expressed as mUGPx mg^{-1} protein. One unit of GPx is defined as the amount of enzyme necessary to oxidize 1µmol of β-NADPH per min.

GR

The activity of GR was assayed independent on the decrease in the absorbance during β-NADPH oxidation [41]. In each cuvette, 0.1M potassium phosphate buffer, 3.4 mM EDTA, pH 7.6, 30 mM oxidized glutathione (GSSG), 0.8 mM β-NADPH and 1.0% of bovine serum albumin (BSA) were mixed by inversion. Then, 100 µl of the enzyme was added. The absorbance was followed at 340 nm for approximately 5 min. Enzyme activity was expressed as U mg^{-1} protein. One unit will reduce 1.0 µM of GSSG per min at pH 7.6 at 25 °C.

Carbonyl protein (CP)

An aliquot of frozen tissue was mixed with 5% ice-cold sulfosalicylic acid (1:20 w/v) and then centrifuged at 13.000 rpm for 15 min. The supernatant was discarded and 0.5 ml of 2, 4-dinitrophenylhydrazine (10 mM) solution was added to the pellets. The samples were kept at room temperature for

1h with vigorous vortex every 15 min, then 0.5 ml of 20% TCA solution was added and the tubes were re-centrifuged as described above. The supernatant was again discarded and the excess of 2, 4-dinitrophenylhydrazine was removed by washing the pellet three times with 1 ml of ethanol: ethyl acetate (1:1 v/v), followed by hearty vortex and re-centrifuging as defined before. Finally, the pellets were liquefied in 6 M of guanidine chloride and incubated for 15 min at 37°C. The maximum absorbance at 360-370 nm was verified and the find CP content was estimated by using the extinction coefficient of 22 mM^{-1} [42].

Total protein assay

Protein level was determined according to the method of Lowry et al. [43]. BSA was used as a standard.

Statistical analysis

All data presented as mean ± SE were subjected to analysis of variance (ANOVA) and means were compared to significance by Student-Newman Keuls at the probability of 0.05 [44].

Results

TiO_2NPs characterization

The examined NPs exhibited characteristic spherical shape with size ranged from 12 to 64 nm as illustrated in SEM image (Figure 1a). In addition, EDA pattern for elemental analysis is plotted in Figure 1b achieving the dominance of TiO_2 (100.0%) respect to total count dividing into Ti (27.06%) and O (72.94%). The average of zeta size of TiO_2NPs in vehicle solution ranged from 90.0 to 105.0 nm as checked in DLS (Figure 1c).

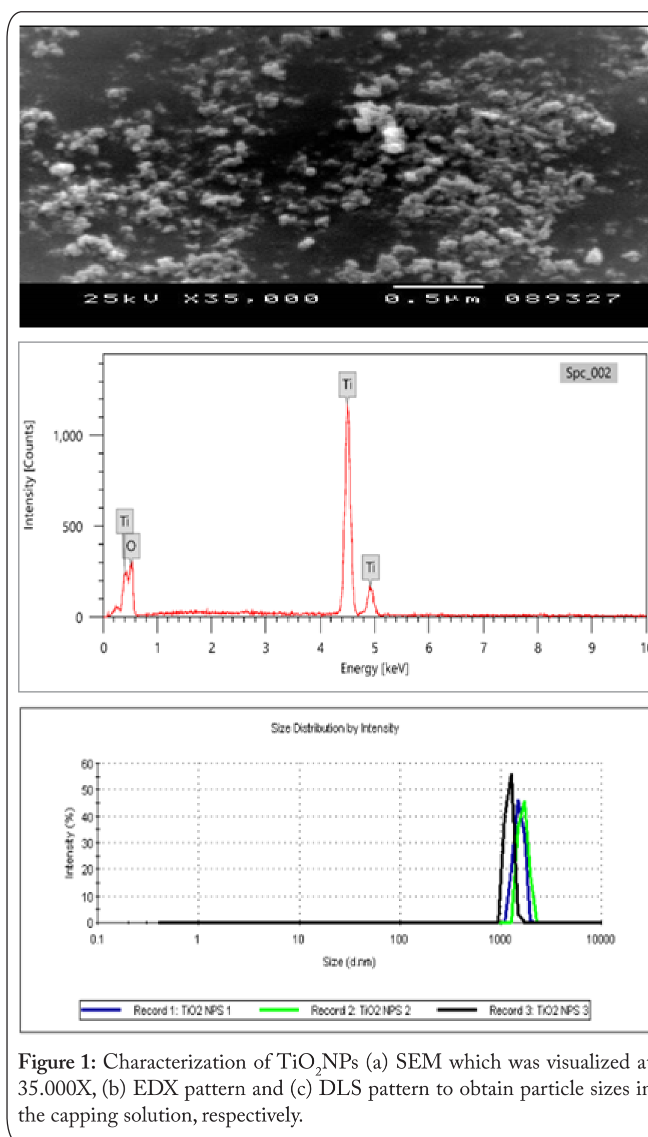

Figure 1: Characterization of TiO_2NPs (a) SEM which was visualized at 35.000X, (b) EDX pattern and (c) DLS pattern to obtain particle sizes in the capping solution, respectively.

Hematological changes

The data among CBC of treated and control animals are presented in Table 1. White blood cells (WBCs) exhibited levels in the treated animals greater than control with mean values: 12.53, 12.33, 13.73, and 16.80 × 103µl for treatments: 2.5, 5.0, 10.0 and 20.0 ppm, respectively, respect to control (7.78 × 103µl). In addition, TiO_2NPs doses significantly increased RBCs levels with mean values: 5.70, 6.35, 7.33 and 6.18 × 103µl for the same treatments, respect to control (2.30 × 103µl). In the same manner, hemoglobin and hematocrit significantly increased in all treated animals, respect to control. However, mean corpuscular hemoglobin (MCH) significantly was lower than the control. Platelets (PLT) significantly increased in the treated animals at levels: 278.3, 290.0, 317.7, and 332.0 × 103µl for the above treatments, respect to control (183.0 × 103µl). lymphocytes (LYM), monocytes (MON) and granulocytes (GAR) increased with increasing dose, where the high dose (20 mg/kg) exhibited the highest LYM (13.60 x 103 µl), respect to control (5.43 x 103µl). While, the highest one of MON (1.27 x 103µl) was induced by the same dose, respect to control (0.87 x 103µl). The same patter was observed for GAR levels in a decreasing order.

Oxidative stress responses

LPO

All treatments exhibited MDA levels greater than control (Figure 2). In heart tissue, dose (10 ppm) exhibited the greatest increase in MDA level (7.02 mM g^{-1} tissue) (Figure 2a). This value represented 3.5-folds of control (2.11 mM g^{-1} tissue) or % of control (358.83%) (Figure 2b). However, dose (20 ppm) exhibited the greatest increase (11.03 mM g^{-1} tissue) arising % of control (566.71%). In spleen tissue, MDA levels were 4.68 and 7.18 mM g^{-1} tissue, representing % of control (192.85 and 429.80%) for doses 10 and 20 ppm, respectively, respect to control (1.68 mM g^{-1} tissue). Regarding muscle tissue, MDA levels were 5.90 and 7.91 mM g^{-1} tissue for the same doses, representing % of control (311.52 and 417.99%). Lung tissue showed the least values of MDA level (3.08 and 5.18 mM g^{-1} tissue), respect to control (1.71 mM g^{-1} tissue) representing % of control (198.67 and 306.38%). MDA levels displayed the order: heart > muscle > spleen > liver > kidney > lung with mean values: 9.03, 6.90, 5.93, 5.68, 5.35 and 4.13 mM g^{-1} tissue, respectively.

Table 1: Changes in CBC levels of daily i.p administered male mice with different doses of TiO_2NPs for 28 days.

Parameter	Unit	Dose (ppm)				
		2.5	5.0	10.0	20.0	Control
WBC	X103µl	12.53b ±0.09	12.33b ±0.09	13.73b ± 0.08	16.80a ±0.07	7.78c ±0.15
RBC	X106µl	5.70b ±0.09	6.35ab ±0.08	7.330a ± 0.07	6.18ab ±0.09	2.30c ±0.23
HGB	g/dL	7.93b ±0.13	8.90ab ±0.11	10.67a ±0.09	9.90ab ±0.10	4.33c ±0.53
HCT	%	25.63c ±0.08	32.10b ±0.06	33.33b ±0.06	40.77a ±0.05	15.0d ±0.14
MCV	FI	40.97a ±0.27	41.90a ±0.27	44.83a ±0.25	33.17a ±0.34	36.9a ±0.31
MCH	Pg	14.20b± 0.07	14.50b ±0.07	14.80b ±0.07	15.50b ±0.07	35.0a ±0.03
MCHC	g/dL	26.27c ±0.05	29.40b ±0.04	31.70b ±0.04	36.33a ±0.03	30.73b ±0.04
PLT	X103µl	278.3b ±0.03	290.0b ±0.11	317.7a ±0.09	332.0a ±0.14	183.0c ±0.05
LYM	X103µl	8.10c ±0.09	9.13bc ±0.08	10.30b ±0.07	13.60a ±0.05	5.43d ±0.13
MON	X103µl	0.97ab ±0.14	1.03ab ±0.13	1.10ab ±0.12	1.27a ±0.10	0.87b ±0.15
GRA	X103µl	1.60d ±0.09	1.90c ±0.08	2.33b ±0.07	3.50a ±0.04	1.33d ±0.53

-Each reading represents means±S.D of 3 observations; WBC: White blood cell count; RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, LYM= Lymphocytes, MON= Monocytes; and GRA= Granulocytes.

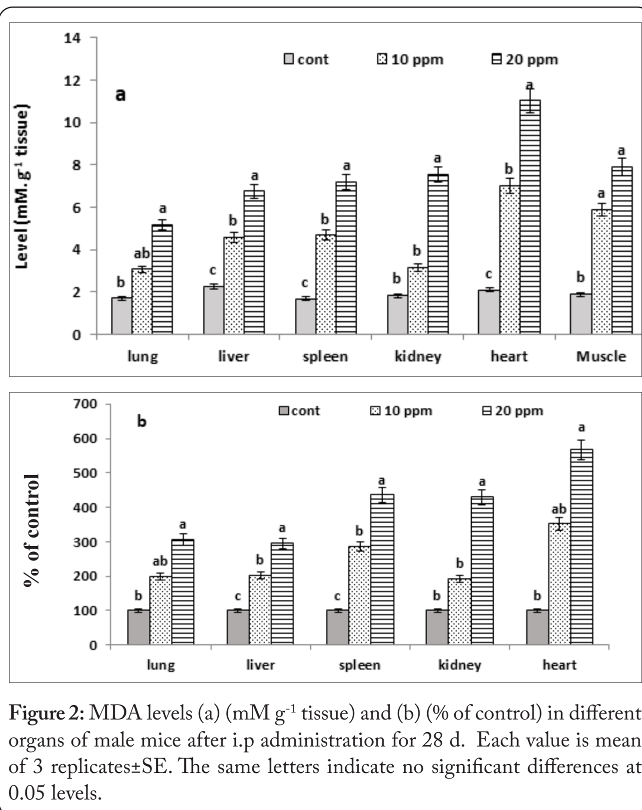

Figure 2: MDA levels (a) (mM g^{-1} tissue) and (b) (% of control) in different organs of male mice after i.p administration for 28 d. Each value is mean of 3 replicates±SE. The same letters indicate no significant differences at 0.05 levels.

GSH

All treatments exhibited significant declines in GSH content in homogenates of the selected organs, respect to control (Figure 3a). Dose, 20 ppm exhibited significant decreases in GSH content than dose 10 ppm, where GSH levels were 6.02 (4.71-folds), 7.23 (4.52-folds), 5.58 (4.35-folds), 15.53 (1.89-folds), 13.25 (2.06-folds), and 7.64 nM mg^{-1} protein (4.52-folds), respectively, in lung, liver, spleen, kidney, heart, and muscle samples, respect to their control. Slight decreases were induced after 10 ppm administration arising the folds: 2.20, 1.40, 2.02, 1.45, 1.36 and 3.21-folds for organs described above, respect to their controls.

CAT

Activities of CAT decreased in all organ homogenates for doses 10 and 20 ppm, respect to control (Figure 3b). In lung homogenate, no significant difference was obtained for dose 20 ppm (1.83 U mg^{-1} protein), respect to control (1.88 U mg^{-1} protein), while dose 10 ppm exhibited significant decrease (0.90 U mg^{-1} protein) (2.0-folds), in lung homogenate, respect to control. Also, significant decreases were induced in heart homogenate (1.19 and 1.22 U mg^{-1} protein) for doses: 10 and 20 ppm, respect to control (2.39 U mg^{-1} protein). Also, significant decreases (1.20 and 1.63 U mg^{-1} protein) were induced in spleen homogenate after administration (10 and 20 ppm), respect to control (2.52 U mg^{-1} protein). No significant differences were obtained in muscle, kidney and liver samples, and respect to their control (untreated groups).

GST

Significant declines in GST activity were induced in all organs after 10 and 20 ppm administration of TiO_2NPs (Figure 3c). Dose, 20 ppm exhibited significant declines in enzyme activity as follows: kidney, lung, muscle, liver, spleen, and heart arising mean values: 1.77 (8.38-folds), 2.59 (3.03-folds), 5.84 (8.44-folds), 7.40 (1.77-folds), 7.47 (3.46-folds), and 7.61 nM mg^{-1} protein min^{-1} (4.89-folds), respectively, respect to their control. While, dose 10 ppm exhibited significant decrease in lung (3.91 nM mg^{-1} min^{-1}) equivalent to 2.01-folds, followed by liver (8.20 nM mg^{-1} min^{-1}) (1.59-folds), kidney (9.35 nM mg^{-1} min^{-1}) (1.59-folds), and spleen (10.69 nM mg^{-1} min^{-1}) (2.42-folds), respectively. The greatest activity was found in muscle (15.4 nM mg^{-1} min^{-1}) (3.18-folds), followed by heart (13.23 nM mg^{-1} min^{-1}) (2.81-folds), respect to their control that did not exceed 49.26 and 37.21 nM mg^{-1} min^{-1}.

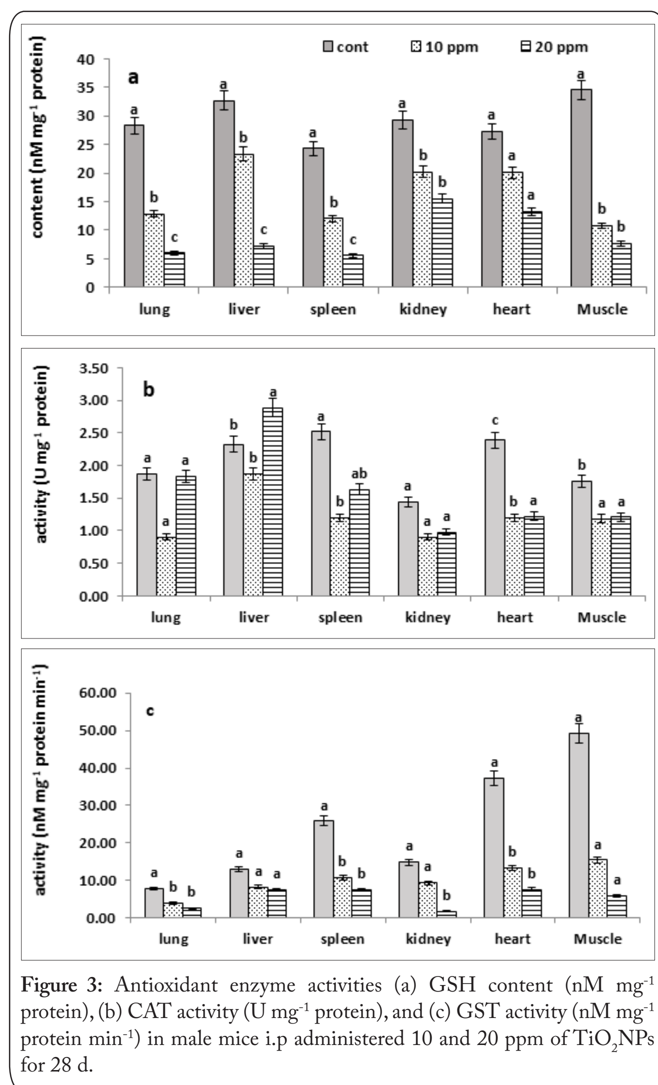

Figure 3: Antioxidant enzyme activities (a) GSH content (nM mg^{-1} protein), (b) CAT activity (U mg^{-1} protein), and (c) GST activity (nM mg^{-1} protein min^{-1}) in male mice i.p administered 10 and 20 ppm of TiO_2NPs for 28 d.

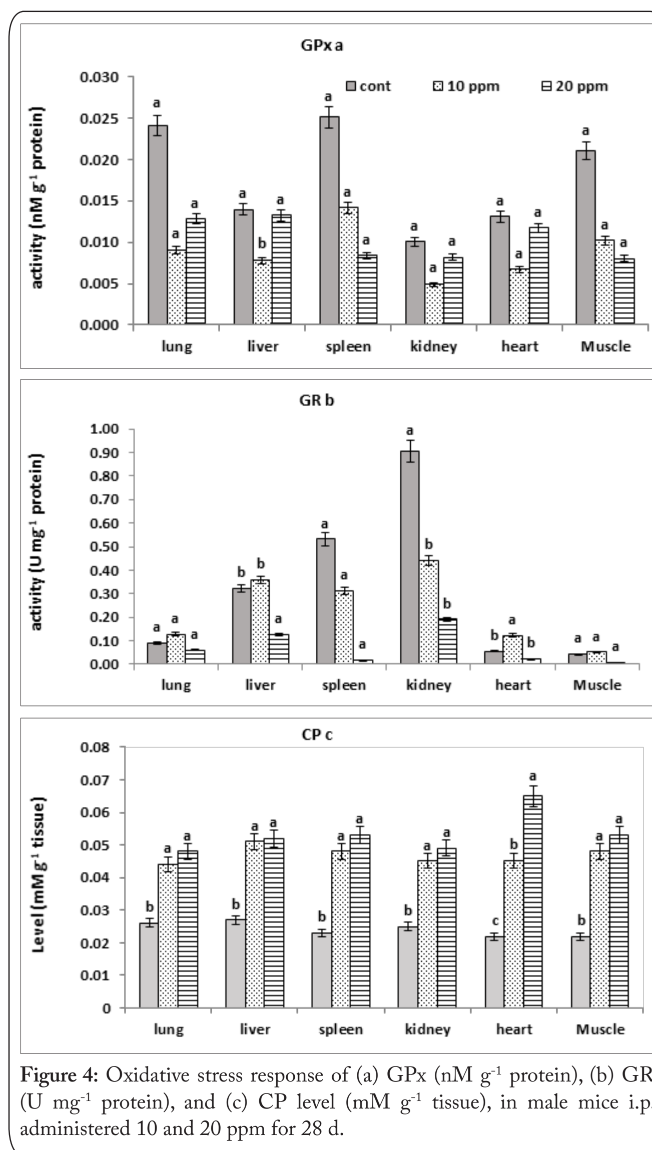

Figure 4: Oxidative stress response of (a) GPx (nM g^{-1} protein), (b) GR (U mg^{-1} protein), and (c) CP level (mM g^{-1} tissue), in male mice i.p. administered 10 and 20 ppm for 28 d.

GPx

Significant decreases were recorded in GPx activities in tissue homogenates of the organs after 10 and 20 ppm administration (Figure 4a). The greatest decreases 0.005 and 0.008 UGPx mg^{-1} protein were found in kidney after administration of 10 and 20 ppm, followed by muscle (0.010 and 0.008 UGPx mg^{-1} protein), and heart (0.007 and 0.012 UGPx mg^{-1} protein), respect to their controls: 0.010, 0.021 and 0.013 UGPx mg^{-1} protein, respectively. Dose 10 ppm displayed the highest activity (0.014 UGPx mg^{-1} protein) in spleen, followed by dose 10 ppm in lung and liver (0.013 UGPx mg^{-1} protein), respect to their controls: 0.025, 0.024 and 0.014 UGPx mg^{-1} protein, respectively.

GR

Significant alterations were noted in GR activity in the organs of administered rats with 10 and 20 ppm of TiO_2NPs (Figure 4b). Dose 20 ppm exhibited the greater decrease in activity than dose 10 ppm. The least activity (0.010 U mg^{-1} protein) was noted in muscle, followed by heart and spleen (0.020 U mg^{-1} protein), respect to their controls: 0.040, 0.060 and 0.530 U mg^{-1} protein, respectively. Dose 10 ppm exhibited the highest activity (0.44 U mg^{-1} protein) in kidney, surveyed by liver and spleen (0.31 U mg^{-1} protein), respect to their controls: 0.910, 0.320, and 0.530 U mg^{-1} protein, respectively. While, the smallest activity (0.05 U mg^{-1} protein) was noted in muscle, surveyed by heart (0.12 U mg^{-1} protein), and lung (0.13 U mg^{-1} protein), respect to their controls.

CP

Significant increases in CP levels were noted in the organs of administered rats for 28 d with TiO_2NPs at 10 and 20 ppm (Figure 4c). Dose 20 ppm exhibited the greater values than dose 10 ppm, where the greatest level (0.065 mM g^{-1} tissue) was noted in heart, followed by spleen and muscle (0.053 mM g^{-1} tissue), liver (0.052 mM g^{-1} tissue), and kidney (0.049 mM g^{-1} tissue), respect to their controls: 0.022, 0.023, 0.022 and 0.025 mM g^{-1} tissue, respectively. The least level (0.048 mM g^{-1} tissue) was noted in lung. No significant differences were obtained for dose 10 ppm in the above mentioned organs, where CP level ranged from 0.044 to 0.051 mM g^{-1} tissue, respect to their controls.

Discussion

As documented in the literature, TiO_2NPs are extensively used in many areas of the industry and environment, with some revelations for risk arising from NPs exposure. Therefore, the potential effects on human and mammals should be explored in more detail [45]. The present work conducted that, TiO_2NPs are able to make haematological alterations, inhibit the antioxidant enzymatic and non-enzymatic parameters after oxidative stress induction and may cause damage or death cells in the exposed mice. Also, they are capable to enter into the cytoplasm and nucleus prompting toxic manifestations in the animals [46]. There are numerous investigations in the literature sites show the adverse health impacts of NPs on the biological targets in mammals after different routes of administration. The results presented that, significant generation of ROS was made up and consecutively antioxidant enzyme activities altered. This finding was documented, where there has been considerable indication in the previous investigations on ROS increase and antioxidant system failure after exposure to metallic NPs e.g. CuO, TiO_2, ZnO and Al_2O_3 in the experimental animals [47-49]. Nanoscale of TiO_2 may cause particular and different toxicity than conventional TiO_2 fine particles [50]. The physico-chemical properties of TiO_2NPs change than their bulk form due to high surface-to-volume ratio, where % of atoms on these surfaces makes them more reactive [32]. In fact, impacts of TiO_2NPs on different animal's models, followed by different routes of administration have been demonstrated [11, 51-53]. For example, the inhaled TiO_2NPs caused enhanced pulmonary toxicity and translocation of the particles compared with their bulk form [54-56].

Hematological measurements are normally achieved to identify the hematological toxicity of different chemicals. For example, Xu et al. [57] stated that, 14 d after intravenous (i.v) administration of TiO_2NPs (645 mg/kg) in mice, no significant hematological toxicity could be observed. However, data of biochemical quantifications and histological detections directed that, TiO_2NPs action might encourage much grades of damage in the organs of mice. However, Shakeel et al. [58] revealed significant differences in all the haematological parameters of rats exposed to TiO_2NPs for 28 d by subcutaneous injection (s.c), respect to control group. Altitudes of MCH, MCV, HCT, PLT, and WBCs significantly enlarged related with the control. In fact, WBCs play a critical role in the body defense system, but fluctuations in their count are due to the non-specific response of immune system against the stress conditions. The data of present study are in accordance with the above mentioned, where PLT showed significant increases. This increase is due to proficient use in modulating and supporting immune response and inflammatory reactions. In addition, decrease of RBCs and enhancement of MCH and MCV was independent on the effect of TiO_2NPs and depletion in PLT, where NPs induce severe damage in PLT. In the present work, significant increase in the LYM count for all doses of NPs was induced, respect to control. This finding is in accordance with that obtained by the above authors, where high dose of TiO_2NPs (150 mg/kg b.w) for 28 d induced significant increase in LYM of rats. Also, increase in MCV may be in line for to interruption in mitotic phase and DNA destruction is considered main cause that can stimulate this process.

Lipid peroxidation (LPO) processes are able to modify the arrangement and utility of membrane lipid resulting in cell damage and death [59]. The present data are in accordance with that obtained by Carri et al. [60], who noted that TiO_2NPs led to extreme providing of $O^{\cdot -}$ causing enhanced LPO and oxidative stress. Also, level of MDA enlarged in liver, gill, and brain tissues of carps exposed to TiO_2NPs for 8 d [61]. Ma et al. [62] showed that LPO increased in brain of mice which daily injected with TiO_2NPs into abdominal cavity for 14 d. In another investigation, Shakeel et al. [58] noted that different doses of TiO_2NPs significantly increased liver and blood levels of MDA in treated rats for 28 d. In fact, induction of LPO might be associated with the oxidation of molecular oxygen to yield superoxide radicals. This reaction also produces hydrogen peroxide (H_2O_2) that result in the production of MDA by activating peroxidation of unsaturated fatty acids in the cellular membrane. The hydroxyl radical can activate LPO that is a free radical chain reaction resulting in loss of membrane structure and function [63-65]. Additionally, some lipid oxidative biomarkers e.g. MDA was elevated in TiO_2 production workers, respect to control group [66]. As documented in the literature, Xiong et al. [67] noted that, there is a link between cytotoxicity of TiO_2NPs and particle size, because smaller particles have more specific surface area, which might captivate more biomolecules in the environment. The biological effects might associate with particle size, and surface area [68].

In the present study, decreased activity of CAT in the exposed mice is in accordance with that obtained by Shakeel et al. [58], where significant declines in CAT activity of rats were observed after s.c injection with TiO_2NPs for 28 d. This is due to marked decline in SOD activity after NPs dosage resulting in accumulation of H_2O_2. Accumulated H_2O_2 is known to inhibit CAT activity [69]. Also, significant elevated levels of MDA and GSH were observed in rats received TiO_2NPs (150 mg/kg b.w) for 28 d by s.c injection. However, significant declines were induced in the activities of CAT, SOD and GST [58].

Formation of CP was patterned in this finding as an index of oxidation process for biomolecules in TiO_2NPs-exposed mice. The obtained data revealed the induction of CP in all analyzed tissues. Generally, the present data propose that long-term exposure to such materials may give rise to greater stages irreversible modification such as carbonylation of protein, similarly with that obtained by McDonagh et al. [70]. Gupta et al. [71] also maintained the relationship between CP creation and ROS formation with the up-regulation of the letter in toxicants-exposed organisms, demonstrating the association of ROS in stimulation the protein modification.

Bio-distribution studies indicated that, TiO_2NPs mostly accumulate in liver and their elimination is slow [50-51, 72, 73]. Their clearance of small-sized NPs is very difficult from the liver, where NPs (25-80 nm) which orally administered at

5 mg/kg retained for long-time and induced liver damage in mice [11]. In addition, Fabian et al. [72] investigated tissue distribution of TiO_2NPs (20-30 nm) after single dose (5 mg/kg) as i.v administration in rats. Levels of NPs were highest in the liver and retained for experiment's period (28 d). The particles had not been entirely cleared from the liver and spleen within the observation period representing that NPs can accumulate in these organs after long-term exposure. On the other hand, different doses: 324, 648, 972, 1296, 1944 and 2592 mg/kg of TiO_2NPs (80-100 nm) by i.p. administration in mice resulted in high accumulation in spleen, liver, followed by kidney and lung in a decreasing order [74].

Nanoparticles (NPs) e.g. TiO_2NPs usually enter the body through dermal, ingestion and inhalation routes. Suspension of TiO_2 (5000 mg/kg b.w) was orally administered for 14 d, where it accumulated in liver, spleen, kidney and lung tissues [75]. Also, Shiuohara et al. [76] noted that, 94, 2.0, 0.17 and 0.023% of cation, Ti were detected in liver, spleen, lung and kidney after 6 h-intratracheal injection with TiO_2NPs. Also, inhalation rates (2, 10 and 50 mg/m^3 of TiO_2NPs) for 6 h/daily for 5 d, resulted in accumulation values: 118.4, 544.9 and 1635 µg, respectively. However, after 16-d recovery TiO_2NPs burdens declined to 25.0, 144.5, and 295 µg in the lung [77]. On the other hand, Oberdorster et al. [78] stated that, retention half-times were 117 d for individuals exposed to fine particles (250 nm), and 541 d for those exposed to NPs (20 nm). Also, Han et al. [79] showed that significant increase of Ti content in liver and kidney tissues with time may be associated with extended half-life time of TiO_2. This concept was noted as Ti has slowly clearance and low translocation of the particles which may incompletely clarify that Ti was greater in lung matched to liver and kidney. Such this persistence in lung tissue triggered continuous destruction to the respiratory system. Also, accumulation of Ti in the kidney may cause kidney damage and reduces the execration rates.

For example, exposure to TiO_2NPs can effect on the cardiovascular system. The effects hinge on the amounts, dose of exposure, mechanism and transfer route, duration of exposure and target organ [80]. Some studies stated that, TiO_2NPs have negative effects on the cardiovascular system. For example, amplified expression of inflammatory cytokines such as TNF-α, INF-g and IL-8 in blood after uptake of NPs was noted [81-82]. Also, after 90 d of i.v administration of TiO_2NPs (0, 2, 10 and 50 mg/kg), heart arrhythmia manifested in reduced activity of lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (α-HBDH) and creatine kinase (CK) [80]. On the other hand, Roursgaard et al. [83] established that, inflammation in the lung was induced after TiO_2NPs treatment in mice by intratrached instillation.

On the other hand, Jia et al. [84] investigated the mechanism of liver toxicity for TiO_2NPs. They stated that, great doses of TiO_2NPs may induce swelling of hepatocytes with obvious vacuoles in cells and nuclear condensation in hepatocytes, apoptosis and necrosis of hepatocytes in liver tissue. This concept was previously documented by Cui et al. [9], Duan et al. [85] and Cui et al. [86] who suggested that, TiO_2NPs generate excess ROS and reduce the antioxidant capacity of the cells through damaging the mitochondria causing expression disorders of protective genes. Therefore, TiO_2NPs may induce oxidative stress and disrupt orders of neurochemical metabolism in brain tissue ending to neurotoxic effects in the central nervous system [87]. On the other hand, exposure of pregnant mice to a single dose of TiO_2NPs for 14 d increased sensitivity of inflammatory response in F1 generation [88-89].

Conclusion

Extensive applications of TiO_2NPs raise the consideration about biosafety for environment and humans. The present work reveals the ability of these NPs to induce haematological alterations and oxidative stress in some species of mammals. The toxic effect posed to different organs e.g. lung, heart, kidney, liver, spleen and muscles. Moreover, excess MDA, and CP and antioxidant system parameters alterations may provide fingerprint of potential toxic effects of TiO_2NPs, especially during long-term exposure scenario. Thus, human exposure to such substances should be minimized to down rough of safety margins.

Ethics approval and consent to participate

Applicable.

Consent for publication

Not applicable.

Availability of Data and Materials

All data generated or analysed during this study are included in this published article.

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Compliance with Ethical Standards

The authors declare that they have no conflict of financial or non-financial interests to disclose in any material discussed in this article.

Authors' Contributions

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

Supplementary Data

No supplementary data are provided.

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