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Toxicokinetics of Titanium Dioxide (TiO₂) Nanoparticles After Intraperitoneal Injection in Male Mice

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Abstract

In the present study, male albino mice were used to estimate the effects of titanium dioxide nanoparticles (TiO₂) suspension used in two doses (150, 600 mg/kg) through intraperitoneal route. The results revealed a significant difference ($p \leq 0.05$) among the control and experimental groups in all haematological parameters, including a significant increase in White Blood Cell (W.B.C) count, Mean Cell Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC), and Mean Cell Haemoglobin (MCH). Also, the results showed a significant decrease in Red Blood Cell (R.B.C.) count and Haemoglobin (Hb). Biochemical tests included AST and ALT and showed a significant elevation in all exposed groups, while ALP was decreased after fourteen and thirty days of exposure. In the case of kidney function, creatinine was increased in all groups during the experiment, whereas uric acid was increase in many cases and recorded the highest mean value after fourteen days of exposure to the dose of 150mg/kg and after thirty days of exposure to the dose of 600mg/kg. Level of urea was decreased in the fourteen-days and thirty-days treatment groups, while its mean values after using the two doses did not change significantly after one day. Cholesterol level was decreased after thirty days, recording the lowest mean value at 600mg/kg, whereas the level of HDL was significantly ($p \leq 0.05$) decreased and that of LDL significantly increased. The study of bioaccumulation demonstrated that the TiO₂ NPs are accumulated mainly in the spleen, followed by the liver and kidneys of mice, respectively. Also, the doses used caused histological alterations such as changes in the congested dilated portal tract, with heavy inflammatory cells infiltration and dilated central venule in the liver, along with glomerular congestion, tubular congestion, atrophy, chronic inflammatory cells infiltration, and dilated tubules with flat atrophied lining epithelium in the kidneys. The histologic alterations observed may represent an indication of different degrees of organ injury due to the toxicity of TiO₂ NPs, resulting in an inability to deal with accumulated residues from the metabolic and structural disturbances caused by these nanoparticles.

Keywords: Toxicity, TiO₂, nanoparticles (NPs), biochemical test, bioaccumulation.

الحركية السمية لدقائق ثنائي اوكسيد التيتانيوم النانوية بعد الحقن البريتوني في ذكور الفئران البيض

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الخلاصة

تم استخدام في هذه الدراسة ذكور الفئران البيض لتقدير تأثير معلق يحتوي على جزيئات ثاني اوكسيد التيتانيوم النانوية باستخدام جرعتين (150 , 600) ملغم/كغم عن طريق الحقن البريتوني, بحيث كشفت الدراسة

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ان هنالك فروقات معنوية ($p \leq 0.05$) بفحوصات الدم بين المجاميع المعرضة للدقائق ومجاميع السيطرة التي تشمل زيادة كبيرة في خلايا الدم البيضاء (W.B.C); متوسط حجم الخلية (MCV); متوسط تركيز الهيموغلوبين (MCHC); متوسط الهيموغلوبين في الخلية (MCH). وايضا انخفاض معنوي بنسبة كريات الدم الحمراء (R.B.C) و الهيموغلوبين (Hb). اما الفحوصات الكيموحيوية لوحظت زيادة معنوية بنسبة الانزيمات (ALT,AST) مترامنا مع انخفاض معنوي لانزيم (ALP) بمجموعتان المعرضة لمدة اربعة عشر يوم والمعرضة لمدة ثلاثون يوم. في حالة وظائف الكلى ، زاد الكرياتينين في جميع المجاميع المعرضة في التجربة ، وفي كثير من الحالات يزداد حمض اليوريك حيث سجل اعلى نسبة بعد اربعة عشر يوماً من التعرض للجرعة (150 ملغم/كغم) وبعد ثلاثين يوماً من التعرض للجرعة (600 ملغم/كغم). اما تراكيز اليوريا فقد قلت في المجاميع المعرضة لاربعة عشر يوماً ومجموعة المعرضة لثلاثون يوماً وسجلت فروقات غير معنوية بعد يوم واحد. هنالك انخفاض بنسبة الكوليسترول بعد ثلاثون يوماً حيث سجل اقل نسبة بعد التعرض للجرعة (600 ملغم/كغم) ولوحظ انخفاض بنسبة الدهون عالية الكثافة وارتفاع بنسبة الدهون قليلة الكثافة. دراسة التراكم الحيوي تشير الى ان جزيئات ثنائي اوكسيد التيتانيوم النانوية التي تم حقنها قد تراكمت في الكبد والطحال والكلية بالتتابع للفران المعرضة ، وهذه الجرعة المستخدمة تسببت في حدوث تغيرات نسيجية تكون على شكل احتقان وتوسع في الوريد البوابي الكبدي مع ارتشاحات الخلايا الالتهابية وكذلك توسع في الاوردة الكبدية المركزية الدقيقة ، اما التغيرات النسيجية الكلوية تكون على شكل احتقان وضمور في الكبيبات والنيبيبات الكلوية ، وارتشاحات الخلايا الالتهابية المزمنة للنيبيبات والكبيبات الكلوية مع توسع وضمور تسطحي للظهارة البطانة للنيبيبات الكلوية، هذه التغيرات التي لوحظت قد تكون مؤشرا على اصابات بدرجات متفاوتة لتلك الاعضاء بسبب سمية جزيئات ثنائي اوكسيد التيتانيوم النانوية ، مما يؤدي الى عدم القدرة على التعامل مع المخلفات المتراكمة الناتجة عن العمليات الايضية اضافة الى الاضطرابات التركيبية الناجمة عن هذه الجسيمات النانوية

Introduction

In the last decade, nanotechnology has received an increased attention from the media and scientific communities for its remarkable potential, ranging from optical properties, flexibility, reactivity, and increased strength. Hence, nanomaterials have been widely used in electronics, cosmetics, drug delivery and antibacterial materials [1]. The global use, production and disposal of TiO_2 NPs certainly lead to their release into the environment and may apply harm to organisms and ecosystems [2]. Titanium dioxide (TiO_2) nanoparticles, which are a main ingredient in sunblock, absorb UV light and effectively shield skin from unsafe UV light exposure, in addition to their remarkable antimicrobial activities and light-weight characteristics. Regardless of the commercial benefits, their introduction to the nature may cause hazardous biological impacts [3]. Animal exposure to TiO_2 NPs can be estimated by measuring chemical substance levels in various body tissues and organs along with biochemical parameters that change according to the type of exposure and dose. These measurements are known as biomarkers or biological markers. Thus it was important to study translocation of nanoparticles after intraperitoneal injection to systemic sites, which is considered as a precarious step in toxic kinetics [4]. Titanium dioxide nanoparticles with higher doses injected into the abdominal cavity of mice can significantly induce liver injury by modify mRNA and protein expressions of several inflammatory cytokines, in addition to changing histopathology, causing apoptosis of liver cells, and damaging liver function [5]. Nanoparticles larger than six nanometers in diameter cannot be excreted by the kidneys and can accumulate in specific organs such as the liver and spleen, until clearance by the mononuclear phagocyte system takes place [6]. Moreover, a recent research presented the results of TiO_2 nanoparticles injection into the abdominal cavity of mice for 14 consecutive days. When the dose was increased, the coefficients of the liver, kidneys, and spleen were increased steadily, whereas the coefficients of the lung and brain decreased regularly and those of the heart had slight changes, with alterations in the serum biochemical parameters [7]. Likewise, nephrotoxicity and pathological changes of the kidneys were also resulted from exposure to the same type of NPs [8]. In this study, we investigate the toxicity of titanium dioxide nanoparticles on blood parameters, in addition to liver and kidney function and lipid profiles. The accumulation of TiO_2 in a particular part of the body in addition to renal and hepatic tissue changes were also studied.

Materials and methods

Animals: Adult male albino mice at an age of four to six weeks and an average weight of 25 ± 10 g were obtained from the Iraqi Center for Genetics and Cancer Research. The healthy mice were housed in polypropylene cages under special maintenance conditions of 12 ± 3 hours light / dark cycle, temperature of $25 \pm 5^\circ\text{C}$, and 60 ± 10 relative humidity. Food and water were available and libitum. The animals were treated and housed in the animal house of the Iraqi Center for Genetics and Cancer Research / Al- Mustansiriyah University.

Equipment and chemicals: dissection tools, EDTA K3 tube, micropipette, eppendorf tubes, plastic container, syringes, de-ionized distilled water, eosin stain ,ethanol , formaldehyde, hematoxylin stain ,ketamine 10%, paraffin wax, titanium dioxide nanoparticle ,xylene (Xyl-M2) and xylazine.

Instruments: Atomic absorption flame emission spectrophotometer NovAA 350 (Analytic Jena company), Cobas c111 (Roche company), Mindray BC-3000 PLUS (Shenzhen Mindray company), Scanning probe microscope SPM AA3000 (Angstrom Advanced In company).

Preparation of TiO_2 : In the current study, we used two different doses of titanium dioxide nanoparticles (150 and 600)mg/kg. Titanium dioxide nanoparticles (Anatase form 58.00 nm) was purchased from Areej Al-furat Company (a chemicals company in Baghdad, Iraq) and characterized by scanning probe microscopy (Figure-1). It had a white powder appearance with nearly spherical morphology. The suspension was prepared by taking a specific amount of nanoparticles with the addition of de-ionized distilled water under sonication for one hour.

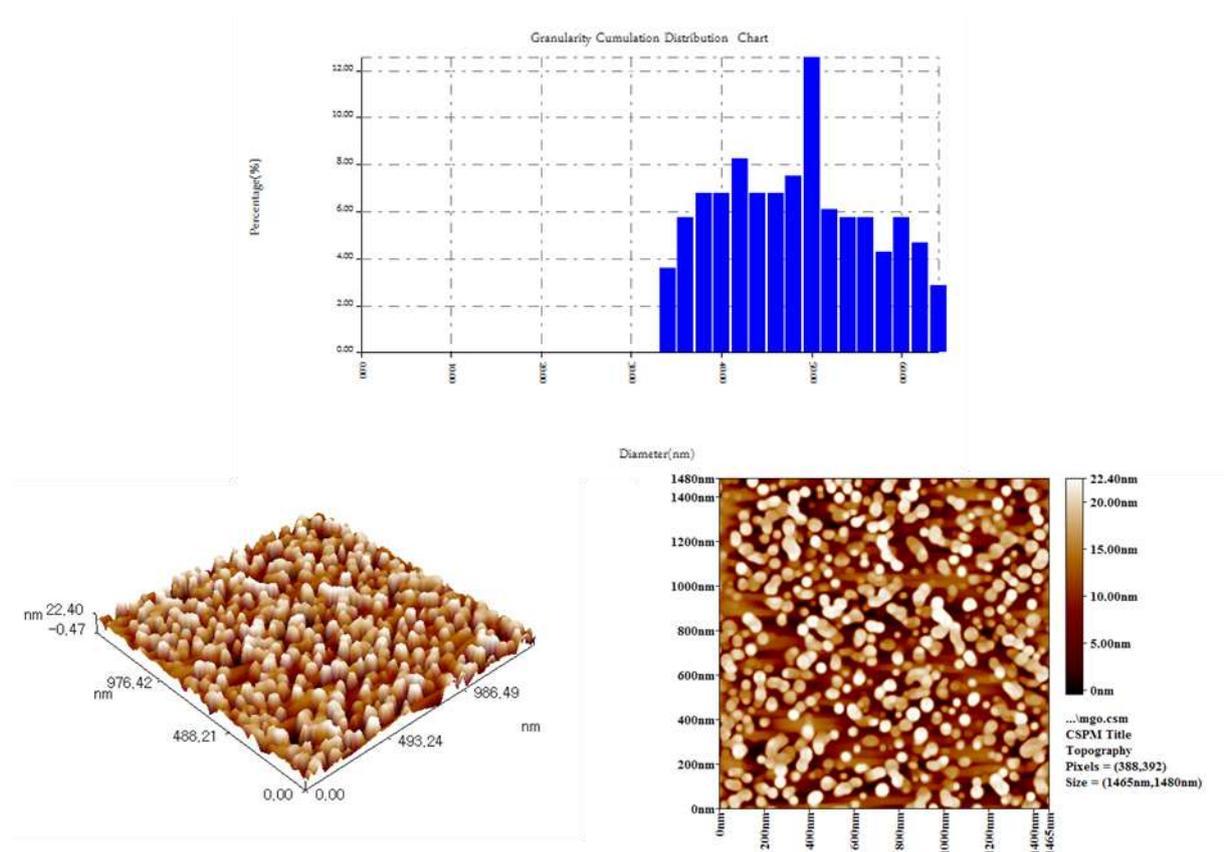


Figure 1- Characterization of titanium dioxide nanoparticles

Experimental study: The mice were divided according to exposure time into three groups, each containing sixteen mice. The first group was exposed to TiO_2 NPs for one day, while the second and third groups were exposed for fourteen and thirty days, respectively. The animals were injected intraperitoneally every two days with 0.1 ml of the NPs. The two doses given to each group were selected according to previous papers [9, 10]. In particular, preliminary experiments were performed

with different concentrations before deciding for these two doses, which were selected based on their acute and nontoxic effects as well as the absence of visible symptoms or mortality in the animals.

Working steps: One day after the last treatment, blood samples were collected from the animals by heart puncture and placed into EDTA tubes to determine blood parameters that included RBCs count, WBCs count, Hb, MCV, MCH, and MCHC. The blood samples (1ml) were analysed using Mindray hematology auto analyser. The other amount of blood (1ml) was centrifuged at 3000 rpm for 15 minutes and serum was collected and stored frozen until used for assessment of various biochemical tests using Cobas C111 Biochemical Analyzer. Subsequently, mice were anesthetized by the injection of 0.1ml of a mixture containing ketamine hydrochloride (0.4ml) and xylazine 0.6ml. Then, liver, kidney and spleen were removed quickly and used for two different purposes; parts of the organs were placed in formaldehyde for histopathological examination [11] whereas the other parts were used for studying the bioaccumulation of NPs using atomic absorption flame emission spectrophotometer [12]. The organ weights were recorded. According to the ratio of organ weight to body weight in order to determine the coefficients of liver, spleen and kidneys as calculated in the following equation:

Organ Index = organ wet weight (mg)/body weight (g) × 100%.

Statistical analysis

Analysis of variance (ANOVA), F-test, t-test were used to statistically analyse the data. To explain the differences between the results (expressed as mean ± SEM), least significant differences (LSD) was used at $p \leq 0.05$ [13, 14].

Results and discussion

Haematological parameters are closely linked to the animal's response to the environment, which means that any change of haematological parameters is suggestive of possible effects on the haematological characteristics exerted by the location where the animal lives [15]. Our results of blood tests are shown in Table-1. The number of WBCs in the present study varied between $6.9 \pm 1.56 \times 10^3/\mu\text{l}$ in animals treated with the first dose 150mg/kg after one day of exposure to $10 \pm 0.8 \times 10^3/\mu\text{l}$ for the same dose after 30 days of exposure, while the values in the control group were $2.9 \pm 0.23 \times 10^3/\mu\text{l}$ and $3.4 \pm 0.09 \times 10^3/\mu\text{l}$, respectively. There was a significant difference ($p \leq 0.05$) between the exposed and control groups. This result proved that the highest mean of exposure to TiO_2 NPs from one day to thirty days is accomplished with the dose of 150 mg/kg, which has the highest absorption rate after intraperitoneal injection in comparison with the higher concentration. In addition, these differences reflect alterations in body defence mechanisms, as the fluctuations in WBCs count could occur as a result of nonspecific responses of the immune system against tension situations. This state usually occurs in inflammations or bacterial or parasitic infections [16]. In contrast, the red blood cells showed a decline in numbers from the first day to thirty days of exposure. In the case of haemoglobin, the control mice showed a mean value of 10.20 ± 0.17 g/dl, which was higher in the treated mice after one day and peaked at 13.10 ± 0.17 g/dl after fourteen days. However, after thirty days, Hb level showed a significant decrease using both doses compared with the control group (LSD = 2.79). The decline in RBCs number and haemoglobin level can be due to the immune responses and the lower metabolic activities in mice. Regarding MCHC, MCV and MCH, the results showed significant reduction for the two doses and all periods of exposure, except for the value of MCHC after one day of exposure which showed a non-significant difference with the control group. A previous study reported that no significant haematological toxicity could be detected after two weeks of intraperitoneal exposure to TiO_2 NPs, Nonetheless, they noted a significant increase of platelet count after one day of treatment [17].

Table 1-Haematological parameters in mice exposed to two acute doses of TiO₂ NPs (Mean value \pm standard error).

Parameters	Dose	1 day				14 day				30 day				
		Control	150 mg/kg	600 mg/kg	LSD P \leq 0.05	Control	150 mg/kg	600 mg/kg	LSD P \leq 0.05	Control	150 mg/kg	600 mg/kg	LSD P \leq 0.05	
WBC ($\times 10^3/\mu\text{l}$)		2.9 b	6.9 a	1.56 b	3.18	3.5 b	4.3a	1.08 c	0.31	3.4 c	10	8.2 b	1.71	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		\pm
		0.23	1.56	0.21		0.1	0.11	0.03		0.09	0.8	0.08		
RBC ($\times 10^6/\mu\text{l}$)		8.13 a	7.52 b	7.78 ab	0.47	9.77 a	9.08 b	4.89 c	0.29	8.7 a	7.9 ab	7.14 b	0.96	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		
		0.1	0.04	0.21		0.13	0.06	0.01		0	0.38	0.29		
HGB (g/dl)		10.20 b	10.87 ab	11.63 a	1.09	10.30 b	13.10 a	7.00 c	0.68	13.80 a	10.50 b	10.10 c	2.79	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		
		0.17	0.27	0.44		0.29	0.17	0.06		0.82	0.65	0.92		
MCHC (g/dl)		30.60 a	31.33 a	31.03 a	1.01	31.20 b	32.85 ab	35.40 a	2.89	28.10 c	30.43 b	32.50 a	1.73	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		
		0.23	0.32	0.32		1.44	0.03	0.06		0.7	0.5	0.06		
MCV (fL)		41.40 b	46.23 a	48.27 a	4.72	33.70 c	43.85 a	40.45 b	3.18	40.30 b	43.10 a	43.35 a	1.5	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		
		1.56	1.22	1.28		1.56	0.32	0.03		0.65	0.06	0.38		
MCH (pg)		12.50 b	14.40 a	14.93 a	1.82	10.50 b	14.35 a	14.25 a	3	11.50 b	14.35 a	14.25 a	3	
		\pm	\pm	\pm		\pm	\pm	\pm		\pm	\pm	\pm		
		0.81	0.26	0.33		1.5	0.09	0.03		1.5	0.09	0.03		

Small letters indicate comparisons in the rows. Similar letters reflect non-significantly differences between means at $p \leq 0.05$ using LSD test.

Liver functions were evaluated by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) for treated mice, the results of which are shown in Table-2. There is a significant difference for AST and ALT among two different doses of treated groups compared to control group. The results showed that highest mean value of AST was 935.20 ± 5.02 U/L recorded in the sample treated with 600mg/kg for thirty days, while the control sample had a mean of 253.90 ± 2.31 U/L. Statistical analysis indicated that all doses of exposure showed a marked significant elevation in AST level, reflecting liver damage or dysfunction as a result of toxicant effects. Such abnormalities are detected by the occurrence and activity of AST and ALT, which are regularly known as aminotransferases that, under normal conditions, reside in the hepatic cells while they leak into the blood when these cells are injured [18]. Regarding our results of ALT (Table-2), the values varied from minimum of 39.60 ± 1.21 U/L in the sample exposed to 600mg/kg for 14 days to a maximum of 397.05 ± 1.59 U/L in the sample exposed to 600mg/kg for thirty days, whereas the values for the control groups were 53.60 ± 2.60 U/L and 53.10 ± 2.89 U/L, respectively. The level of these enzymes increased as a result of damaged membranes of hepatic cell or their death and, thus, this elevation is an indication of liver damage [19]. In addition, a decline in ALP level was recorded after fourteen and thirty days, reaching a lowest mean value of 52.95 ± 2.34 U/L in the dose of 150mg/kg after thirty days, whereas the value in the control group was 178.10 ± 1.73 U/L. On the other hand, nephrotoxicity following TiO₂ NPs exposure was assessed by measuring creatinine, urea and uric acid levels in the serum of experimental animals. After 30 days of intraperitoneal injection of TiO₂ NPs, creatinine mean level showed a highest mean value of 0.25 ± 0.028 mg/dl using the higher dose 600mg/kg as compared with the control group which recorded a mean value of 0.1 ± 0.02 mg/dl. These results indicated a slight significant difference ($p \leq 0.05$) in creatinine level between the exposure and control groups. As related to uric acid, the results demonstrated that the highest mean value was 5.65 ± 0.03 mg/dl in the group of mice exposed to 150mg/kg of TiO₂ NPs after 14 days of exposure,

whereas the mean value for the control group was 2.20 ± 0.05 mg/dl. This increase of creatinine in serum refers to a renal dysfunction as a result of diminished glomerular filtration rate of creatinine [20]. In addition, urea mean levels varied among the different groups of different doses, reaching a highest mean value 39.31 ± 1.2 mg/dl in 600mg/kg after 14 days of exposure, the mean value of control group of 35.5 ± 1.62 mg/dl, as observed in Table-2. This elevation is due to increased protein catabolism or increased levels of the enzyme responsible for urea production [21].

Table 2-Biochemical test results in mice exposed to two acute doses of TiO₂ NPs (Mean value \pm standard error).

Parameters Dose	Liver function			Kidney function		
	1 Day					
	AST U/L	ALT U/L	ALP U/L	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	135.33 \pm 1.73 c	40.17 \pm 1.44 c	66.46 \pm 1.44 b	0.10 \pm 0.02 b	2.6 \pm 0.05 a	29.27 \pm 0.75 a
150 mg/kg	408.25 \pm 0.26 a	89.60 \pm 0.06 a	144.00 \pm 4.27 a	0.20 \pm 0.003 a	2.7 \pm 0.00 a	30.31 \pm 0.05 a
600 mg/kg	228.90 \pm 7.85 b	51.60 \pm 0.40 b	140.55 \pm 3.95 a	0.2 \pm 0.003 a	2.2 \pm 0.06 b	30.73 \pm 0.82 a
LSD P \leq 0.05	16.07	3	11.98	0.04	0.16	2.22
14 day						
Parameters Dose	AST U/L	ALT U/L	ALP U/L	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	250.00 \pm 1.73 b	53.60 \pm 2.59 b	178.10 \pm 2.30a	0.10 \pm 0.02 c	2.20 \pm 0.05 c	35.5 \pm 1.62 c
150 mg/kg	773.70 \pm 4.73 a	96.35 \pm 0.72 a	76.00 \pm 4.73c	0.25 \pm 0.029 a	5.65 \pm 0.03 a	30.13 \pm 0.06 a
600 mg/kg	253.90 \pm 5.60 b	39.60 \pm 1.21 c	91.10 \pm 3.23b	0.20 \pm 0.000 a	3.20 \pm 0.06 b	39.31 \pm 1.2 b
LSD P \leq 0.05	15.05	5.4	12.34	0.07	0.16	4.03
30 day						
Parameters Dose	AST U/L	ALT U/L	ALP U/L	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	253.90 \pm 2.30 b	53.10 \pm 2.88 b	178.10 \pm 1.73 a	0.1 \pm 0.02 b	2.60 \pm 0.05 b	37.27 \pm 1.25 a
150 mg/kg	195.60 \pm 0.40 c	46.75 \pm 0.14 b	52.95 \pm 2.33 c	0.15 \pm 0.029 b	1.85 \pm 0.03 c	31.09 \pm 0.03 b
600 mg/kg	935.20 \pm 5.02 a	397.05 \pm 1.5 a	93.50 \pm 0.28 b	0.25 \pm 0.028 a	3.95 \pm 0.03 a	33.37 \pm 0.06 b
LSD P \leq 0.05	11.07	6.58	5.84	0.09	0.13	2.51

Small letters indicate comparisons in columns. Similar letters reflect non-significantly differences between means at $p \leq 0.05$, using LSD test.

Cholesterol is a versatile lipid that has essential roles as a constituent of cell membrane, building blocks of vitamin D, and steroid hormones such adrenal gland hormone, aldosterone and cortisol, in addition to sex hormones and their derivatives [22]. The results in Table-3 for intraperitoneal injection of TiO₂ NPs demonstrate an induction of a slightly significant decrease ($p \leq 0.05$) in cholesterol level of the group exposed to 600mg/kg after one day and thirteen days of exposure. The mean values were 96.28 ± 4.24 mg/dl and 91.03 ± 0.43 mg/dl, respectively, while those of the control group were 113.67 ± 2.31 mg/dl and 105.60 ± 1.73 mg/dl, respectively. The decrease in lipid concentration observed in the present study can be attributed to its utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in cytoplasm [23].

Monitoring of HDL-cholesterol in the serum or plasma is of clinical significance as its concentration is important in the assessment of atherosclerotic risk. Elevated HDL-cholesterol concentrations protect against coronary heart disease (CHD), whereas reduced HDL-cholesterol concentrations, particularly in combination with elevated triglycerides, increase cardiovascular disease risk [24]. The results of high density lipoproteins can be summarized by a decreased concentration in all groups, with the lowest mean value being 62.70 ± 0.23 mg/dl, while that for the control group was 70.98 ± 1.15 mg/dl after thirty days of exposure to 600mg/kg. One exception was in the group of fourteen days exposure which showed an increased HDL mean value of 98.39 ± 2.55 mg/dl for 600mg/kg dose, whereas the mean value of the control group was 74.00 ± 1.15 mg/dl. The decline of

HDL, in addition to playing a role in lipid transport, may have protective functions. Moreover, as part of the immune response, cytokine-induced increases in serum lipid levels may play a role in host defence by decreasing the toxicities of biological and chemical agents [25].

On the other hand, the results demonstrated a slight increase in the level of low density lipoproteins in all exposed groups, with the highest mean value being 28.28 ± 0.15 mg/dl after fourteen days of exposure, while the control group value was 16.00 ± 0.63 mg/dl. Elevated LDL concentrations in the blood and the increase in their residence time, coupled with an increase in the biological modification rate, result in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as by smooth muscle cells in vessel walls [26].

Table 3-Results of lipid function parameters of mice exposed to acute doses of titanium dioxide nanoparticles through intraperitoneal route.

Lipid profiles			
1day			
Parameters mg/dl Dose	Cholesterol	High density lipoproteins	Low Density Lipoproteins
Control	113.67 ± 2.31 b	77.05 ± 1.73 a	15.51 ± 1.73 b
150 mg/kg	131.28 ± 0.57 a	69.00 ± 0.26 b	28.20 ± 0.60 a
600 mg/kg	96.28 ± 4.14 c	73.46 ± 2.46 ab	17.52 ± 0.75 b
LSD $P \leq 0.05$	9.56	6.04	3.97
14 day			
Parameters mg/dl Dose	Cholesterol	High density lipoproteins	Low Density Lipoproteins
Control	114.00 ± 1.73 b	74.00 ± 1.15 b	16.00 ± 0.63 c
150 mg/kg	114.89 ± 0.35 b	67.91 ± 0.08 c	28.28 ± 0.15 a
600 mg/kg	121.84 ± 2.35 a	98.39 ± 2.55 a	21.06 ± 1.01 b
LSD $P \leq 0.05$	5.89	5.60	2.41
30 day			
Parameters mg/dl Dose	Cholesterol	High density lipoproteins	Low Density Lipoproteins
Control	105.60 ± 1.73 a	70.98 ± 1.15 a	16.51 ± 0.28 b
150 mg/kg	101.57 ± 0.54 b	67.64 ± 0.70 b	17.65 ± 0.17 a
600 mg/kg	91.03 ± 0.43 c	62.70 ± 0.23 c	16.80 ± 0.23 b
LSD $P \leq 0.05$	3.73	2.75	0.82

Small letters indicate comparisons in column, while similar letters are non-significantly differences between means at $p \leq 0.05$ using LSD test

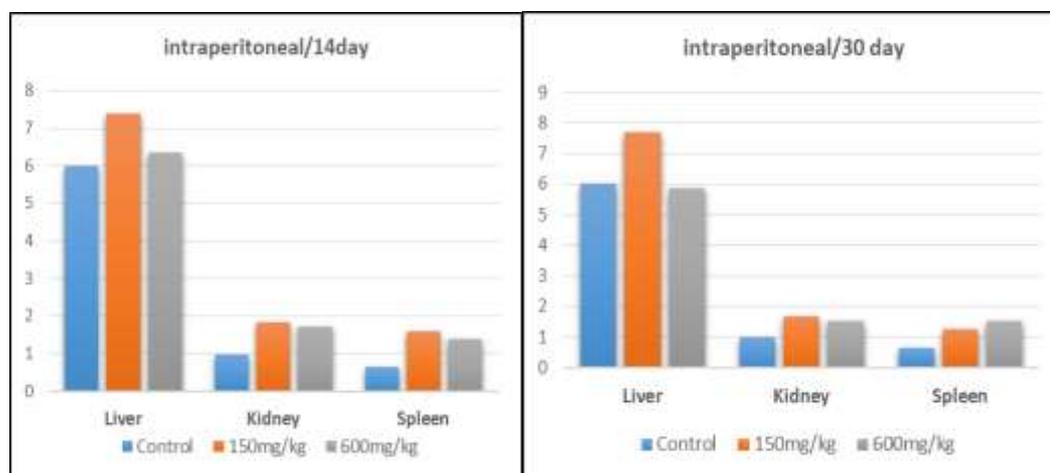
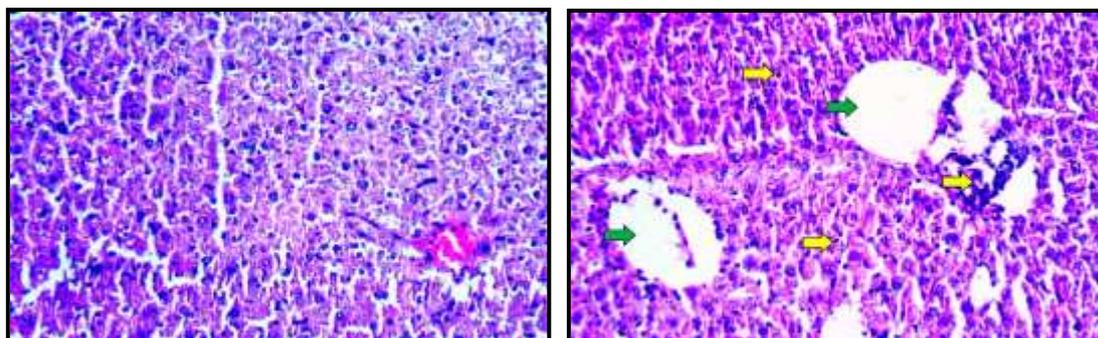
In the current study, we determined the distribution of titanium dioxide NPs following intraperitoneal injection (Table-4) to gain an insight into the kinetics of this material and its accumulation in three different organs, namely the liver, kidneys and spleen. Following injection of TiO_2 NPs for two weeks and after one day of the last injection, subsequent analysis of titanium in tissue samples of organs was performed via atomic absorption flame emission spectrophotometer. The mean value in organ samples, calculated from the average of three duplicate values, was highest after two weeks of exposure to the higher dose 600mg/kg. This can be due to many ways for elimination of nanoparticles, including via urine from kidneys and bile from liver [27]. In our study, the spleen was the major site for NPs distribution, followed by liver and kidney. This result showed that accumulation of TiO_2 NPs in these organs is also related to organ to body weight ratio [28]. In the present study, the results of organ index showed that the higher dose 600mg/kg caused variable differences in all organs. However, the results in general refer to a slight increase of organ index, which might be attributed to accumulation of nanoparticles and dilation of blood vessels as a result of high absorption rate of the dose 150mg/kg in comparison with the other concentration. The results of organ index are shown in Figure-2.

Table 4-Results of titanium accumulation in liver, kidney and spleen of mice exposed to acute doses of titanium dioxide nanoparticles after 14 and 30 days through intraperitoneal route.

Dose	bioaccumulation					
	14 Day			30 day		
	Liver	Kidney	Spleen	Liver	Kidney	Spleen
150 mg/kg	52.66 b ± 1.55	69.08 b ± 1.47	76.03 b ± 4.11	19.47 a ± 0.34	1.34 ab ± 0.32	11.17 b ± 2.45
600 mg/kg	149.69 a ± 5.30	111.49 a ± 4.23	178.89 a ± 1.83	23.76 a ± 3.29	3.23 a ± 1.03	66.79 a ± 2.03
LSD P ≤ 0.05	11.06	8.94	8.99	6.67	2.17	6.37

Small letters indicate to comparison in column, similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test)

This result is due to the retention of TiO_2 nanoparticles in the liver and kidney, as a result of size and difficulty of clearance of TiO_2 [29]. These effects possibly resulted in liver injury, as demonstrated in Figure-3 which represents histological changes in liver of mice injected intraperitoneally with 600 mg/kg of TiO_2 NPs. These alterations were reflected by dilated central venule along with chronic inflammatory cells infiltration of hepatic parenchyma and the central venule. On another hand, the appearance of inflammatory cells in liver tissue suggests that the TiO_2 NPs can cooperate with enzymes and other proteins in the liver interstitial tissue, affecting the antioxidant defence mechanisms and leading to reactive oxygen species creation that may result in an inflammatory response [30]. In our study, this action resulted in an increased organ index of liver as a result of accumulation of blood as great than normal volume that was approved by dilated central venule in the histopathology sections. The sections of kidneys taken from mice treated with the dose of 600mg of TiO_2 NPs (Figure-4) showed congestion of tubular tissue, tubular atrophy, and dilated tubules with flat atrophied lining epithelium. Generally, the organ to body ratio in our study was increased after two weeks and one month in the group treated with the lower dose 150 mg/kg , as a result of high absorption rate of that concentration in comparison with the higher one.

**Figure 2-** Organ index (mg/g) of groups exposed to TiO_2 NPs through intraperitoneal injection.**Figure 3-** Cross section in liver of mice showing dilated central venule (Green arrows) and chronic inflammatory cells infiltration the hepatic parenchyma & central venule (yellow arrows), as compared

to liver of control mice (on the left) which represents hepatic tissue with normal looking cords of hepatocytes, (H&E) (40X).

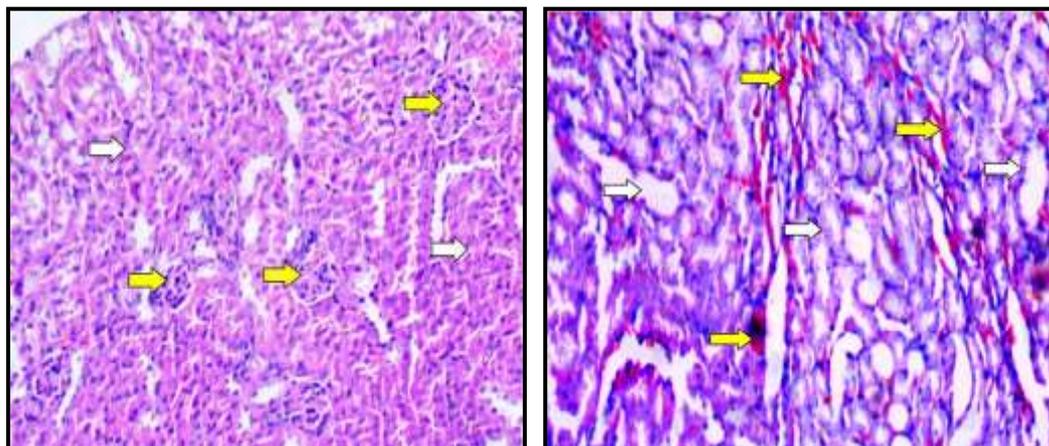


Figure 4- Histological section of renal tissue showing congestion of tubular tissue (yellow arrows), tubular atrophy (white arrows), and dilated tubules with flat atrophied lining epithelium, as compared to kidney of control mice (on the left) showing normal looking renal tissue (Glomeruli: yellow arrow; Tubules: white arrow), (H&E) (40X).

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