Bioaccumulation and toxicity of titanium dioxide nanoparticles after oral ingestion to male mice

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Abstract

Titanium dioxide nanoparticles (TiO2 NPs) are considered one of the top five nanoparticles used in consumer products. In the present study, male albino mice were used to estimate the effect of (TiO2), using two doses (150, 600) mg/kg. The groups were divided according to the time of exposure (one, fourteen, thirty day), control. The result showed significant increase (p≤0.05) in White Blood Cell (W.B.C); Mean Cell Volume (MCV); Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH) and Platelets (PLT), and a significant decrease in Red Blood Cell (R.B.C.) and Haemoglobin (Hb) after thirty day of exposure. However biochemical parameters include AST peak highest mean value after fourteen day of exposure to the dose (600 mg/kg), while ALT and ALP significantly increased and decreased, respectively. Creatinine and uric acid rose sharply while urea, cholesterol, HDL and LDL decreased. Intra-gastric exposure caused high accumulation in kidney, spleen and liver respectively. In general the doses used caused histological alteration, such as congested dilated portal tract with heavy inflammatory cells infiltration and dilated central venule and glomerular congestion, tubular congestion, atrophy, chronic inflammatory cells infiltration and dilated tubules with flat atrophied lining epithelium of kidney. It is an indication of the different degree of organ injury due to the toxicity of NPs.

Keywords*: *bioaccumulation, titanium dioxide nanoparticles, toxicity, oral.

I. INTRODUCTION

In the last decade, nanotechnology has received a lot of attention from the media and scientific community for its amazing potential properties, ranging from optical properties, flexibility, reactivity, and impressive strength. Nanomaterials (NMs) have been widely used in electronics, cosmetics, drug delivery and antibacterial materials [1]. Correspondingly, titanium dioxide (TiO2) nanoparticles (NPs), which are a main ingredient in sunblock, absorb (UV) light and effectively shield skin from unsafe UV light absorption. In addition, they have amazing anti-microbial and light-weight characteristics. Regardless of amazing commercial benefits, their essence in the nature may cause hazardous biological impacts [2]. On the other hand, the communications of nanoparticles with the delicate surfaces of biological systems like cells play key role in perform their biomedical action and in toxicity [3]. Regarding molecule size and surface features, NPs are enter the cell through different pathways including phagocytosis and pinocytosis. A few pathways rely upon size [4]. Animal exposure to TiO2 NPs can be estimated by measuring level of chemical substances in various body tissues, organs, blood and biochemical test that change according to the type of exposure and dose. These measurements are known as biomarkers. Previous stud-

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ies that assessed organ index, cholesterol, triglyceride, high density lipoprotein cholesterol, low density lipoprotein cholesterol and organic lipid ratio considered them as biomarkers. Thus, change in serum biochemical test can take place after mice were orally administrated with TiO2 NPs in addition to having their liver metabolic functions interrupted [5]. To clarify the interaction of nanoparticles with cell, as well as their entry and transport through the blood stream to other organ, experiments were carried out on red blood cells (RBCs), which lack phagocytic receptors. The outcomes show that TiO2 NP smaller than 200 nm are able to enter red blood cells, while bigger particles were only found attached to the surface of RBC [6]. Nanoparticles that are larger than six nanometer 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 take place [7]. Previous research was focused on the toxicity of nanoparticles by using different in vivo and in vitro test methods to estimate cell uptake and oral absorption of TiO2 NPs [8]. Likewise, the nephrotoxicity and pathology change of kidneys also resulted from exposure to the same type of NPs [9].

II. Materials and Meth-

ODS

a. Animals

Adult male albino mice at age of six to eight weeks and average weight 25±5 g were obtained from Iraqi center for genetics and cancer research. The healthy mice were housed in polypropylene cages under special maintained condition 12±3 hours light / dark cycle and temperature of 25±5°C and 60±10 relative humidity. Food and water were available. The animals were treated and housed in the animal house of Iraqi center for genetics and cancer research / al- Mustansiriyah University.

b. Doses

In the current study, two different concentrations were used. Suspension was prepared by adding a specific amount of nanoparticle (anatase 58.00 nm characterized by scanning probe microscopy) with de-ionized distilled water under sonication for one hour. The first dose was 150 mg/kg while the second dose was 600mg/kg.

c. Treatment

The animals were orally administered every two days with (0.1ml). Groups were subdivided according to exposure time in to three groups: exposure for one, fourteen and thirty day. The two doses in each group were chosen according to a previous paper [10] and pre-experiment study.

d. Procedure

One day after the last treat-

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ment, blood samples were collected from animal by heart puncture and placed into an EDTA tube to determine blood parameters. The blood samples were analysed by using hematology auto analyzer. The other amount of blood was centrifuged at 3000 rpm for ten minute. Serums were collected, frozen and stored until used for assessment under various biochemical test via (Cobas C111 Biochemical Analyzer). Subsequently, mice were anesthetized (by injection with ketamine hydrochloride and xylazine) then dissected. The liver, kidney and spleen were quickly removed for two different purposes. Some organs were placed in Formaldehyde for histopathological examination [11] while others were subjected for study of bioaccumulation of NPs using atomic absorption flame emission spectrophotometer.

III. Statistical analysis

In order to determine the impact oral administration of TiO2 for three times (1 day, 14 day and 30 day) between two doses (150 mg/kg, 600 mg/kg) and control for all parameters. This study utilized analysis of variance, F-test, and t-test. To explain the differences between means, least significant differences (LSD) at $p \leq 0.05$ were utilized and expressed as (mean ± SEM) [12]-[13].

IV. Results and discussion

a. Biochemical tests

In order to evaluate the toxicity of nanoparticles exposure,

the oral route was chosen for experimental animal studies. The groups were divided according to time of exposure to one, fourteen and thirty days. The result was shown in table (I). The mean value of AST enzyme 640.80 ± 12.76 (U/L) was higher in the exposed dose of (600mg/kg) for fourteen day exposure and was lowest 198.30 ± 6.58 (U/L) in exposed dose (150mg/kg) for the same exposure time. The value in control group was 150.00 ± 3.86 (U/L). Because of the small size and difficult clearance via oral administration of TiO2 NPs, previous studies reported that retention half time of TiO2 in vivo was long. In addition to variations in hepatic enzyme ALT, AST and ALP as a result of deposition of NPs, there was also hepatic damage [14]. Furthermore, ALT levels in serum rose after one day of exposure for the two applied doses. The mean values were 211.60 ± 1.50 (U/L) and 137.65 ± 5.05 (U/L). The mean value of control group was 53.10 ± 2.31 (U/L) and decreased in serum after thirty days of exposure to first dose (150mg/kg) which had a recorded mean value of 46.35 ± 0.77(U/L), compared to the mean value of control group at 53.10 ± 2.88 (U/L). The result was considered an indicator of liver disease or destruction in organ [15]. Were ALP declined as compared with control group except after 24 hour of exposure the mean value

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has slightly increased 104.50 ± 2.66 (U/L) , the mean value of control group was 91.10 ± 1.73 (U/L) which record non-significant p ≤0.05 difference as compared with control group. Statistically there were significant differences p≤0.05 among means in different groups. The calculation of uptake, dispersion, and biological effects of ingested NMs is complicated in vivo due to inter-individual differences in the composition, pH, thickness of the mucus layer, gastrointestinal flora, and gastrointestinal passage time [16]. In general, TiO2 NPs is a type of nanoparticles with a low absorption rate when administered orally and few of it were detected in tissue while the remaining were spotted in feces [17]. The finding of this study is in agreement with that of previous studies which indicated that TiO2 NPs make different toxic effects on young and adult rats .These included liver edema, minor injury in the liver and kidney in addition to decreased intestinal permeability [18]. In mice that were orally administered with a single dose of TiO2 NPs (25, 80, and155 nm, 5 g/kg), after 14 days there were changes in serum biochemical parameters (ALT/AST, LDH) and pathology (hydropic degeneration around the central vein and the spotty necrosis of hepatocytes) of liver which indicated that the hepatic injury was induced after exposure to mass different-sized TiO2 particles

[19]. On the other another hand, nephrotoxicity following TiO2 NPs exposure were assessed by measuring creatinine, urea and uric acid level in the serum of experimental animals. The results which show that the highest mean value of creatinine 0.45 ± 0.866 (mg/dl) in two weeks after exposure to TiO2 NPs (600mg/kg) through oral route, with the control group having the lowest mean of creatinine level which was 0.1 ± 0.02 (mg/dl). Urea is a nitrogenous compound containing a carbonyl group attached to two amine groups with osmotic diuretic activity. In vivo, urea is formed in the liver via the urea cycle from ammonia and it is the final end product of protein metabolism. Urea level decreased in all groups after oral exposure compared with control group except after two weeks of exposure to second dose (600mg/kg). The result shows that the mean value was 38.46 ± 0.05 (mg/dl), while control group mean value was 35.50 ± 1.62 (mg/dl). Overall, this increase is not significant ($p \leq 0.05$) compared to control group .This result is due to rapid elimination of NPs from kidney. Other studies show that despite the gradual rise in creatinine level, there were non-significant differences at lower doses. However, there was a gradual reduction in urea, which may be due to toxicity to the rat's kidney [20]. The results in table (I). On another hand, the change in the

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level of lipid between exposed and control groups are shown below in table (I I). Cholesterol had a role in reducing the tissue membrane permeability to neutral solutes [21]. The level of serum cholesterol was varied among groups, with lowest mean value at 84.93 ± 0.72 (mg/dl) and control group mean value at 114.00 ± 1.73 (mg/dl) after fourteen days of exposure to second dose (600mg/kg). According to the result of other studies, the change of total cholesterol in mice after intra-gastric exposure daily during one month was significantly increased with increasing dose [22]. High density lipoproteins was lowest at mean value 66.44 ± 0.13 (mg/dl) following exposure to 600mg/kg after two week of exposure, while mean value of control group was 74.00 ± 1.15 (mg/dl). The results were similar to previous studies- there was a significant elevation in the cholesterol level after 14 days of exposure to TiO2 NPs while HDL level was non-significant in 50 mg/kg group and steep elevation was found in the 100 mg/kg group compared with that of control [14]. Low density lipoproteins had the highest mean value after one day of exposure to the first dose (150mg/kg) and one month of exposure to second dose (600mg/kg). The mean values were 25.20 ± 0.17 (mg/dl) and 22.48 ± 0.09 (mg/dl), while control groups mean values were 15.51 ± 1.73 (mg/

dl) and 16.71 ± 0.63 (mg/dl) respectively. Any elevation of LDL may be worrisome because LDL play a main role in causing and manipulating the progression of atherosclerosis, especially coronary sclerosis [23].

b. Blood parameters

Hematological parameters are important for the assessment of the animal and human physiological status. Hematological indices are closely linked to the animal's response to the environment. Any change of hematological indices is suggestive of possible effects on the hematological characteristics exerted by the location where the animal live [24]. The highest mean value of WBCs recorded in blood sample exposed to first dose (150mg/kg) after four weeks while, lowest mean value was found in blood sample subjected to the same dose after one day of exposure to TiO2 NPs orally as compared to control blood. The platelet number showed significant increase .whereas RBC and Hb decreased in most cases. The decrease in RBCs can be accredited to the decrease in iron within erythrocytes or its content of hemoglobin and this causes the decline of oxygen carrying capacity by blood [25]. In addition, this decrease can be attributed to intoxication [26]. The decrease in Hb may diminish its formation by the decline of pool of succinyl in addition to the pool of glycine [27]. Otherwise the decline in

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Hb resulted from the accumulation of metal inside RBC and stopped the formation of Hb, or may prevent linking iron to globin protein by inhibition of ferrochelatase enzyme [28]. With respect to MCV, which is the indication of the status or size of RBCs, there was an increase during the experiment except after one day of exposure, in mice exposed to (600mg/kg) of TiO2 NPs during 4weeks. MCV value increased significantly ($p \leq 0.05$), which had the highest mean value 46.80 ± 0.81(FL) compared to control and other doses. The results of hematological parameters were showed in table (III).

c. Bioaccumulation

The study of bioaccumulation following TiO2 NPs exposure during 14 and 30 days and subsequent analysis of titanium in tissue samples of organs (kidney, liver and spleen) by Atomic absorption flame emission spectrophotometer (NovAA 350) in illustrated in table (VI). Titanium dioxide nanoparticles can accumulate in organs with different concentration. The highest mean value in liver was 152.78 ± 1.14 (ppm) after thirty days of exposure since oral administration takes longer time post-treatment to reach organs [29]. The accumulation in spleen was higher than liver with the mean value at 200.26 ± 1.02 (ppm) since spleen can mount complex adaptive im-

mune responses, as well as effectively clear pathogens from the blood, remove older erythrocytes and cellular debris [30]. Furthermore, titanium dioxide could accumulate in kidney and has the highest organ accumulation in comparison with liver and spleen with the highest mean value at 205.07 ± 3.27 (ppm). All bioaccumulation in the three organs recorded highest mean value after thirty day of exposure to second dose (600mg/kg). Study of TiO2 nanoparticles in spleen, kidney and lung of female mice after 14 days following exposure to the test substance by gastric gavage showed significant difference with control group [9].

d. Histopathology

The elevation in organs function tests and accumulation of titanium dioxide nanoparticles were coordinated with histopathological change. In this study, the histopathological change of liver and kidney appeared in groups of exposure after 14 and 30 days. The liver is the target organ of toxic substance, thanks to its function in biotransformation and excretion of xenobiotics [31]. The control liver of mice shows normal cords of hepatocytes, as shown in figure (1). The histopathological change of groups that were orally exposed to TiO2 NPs show dilated portal tract with chronic inflammatory cells inflammation. Figure (1) displays the cross sec-

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tion in liver of mice a month after exposure to 600 mg/kg. This result is with agreement of that report which included significant histopathological variations and abnormalities in the liver including inflammatory infiltration, congested hepatic veins, and dilated blood sinusoids. Swollen hepatocyte besides dilated central blood vessels and blood sinusoids showed that the TiO2 NPs may alter the cell permeability in the hepatocyte and blood vessel [32]. Conversely, in the control group of mice, the renal tissue showed normal glomeruli and tubules, as shown in figure (2). The same figure shows the histopathological examination of kidney in which there was glomerular congestion in addition to tubular congestion and atrophy among the group of mice exposed to 600mg/kg of TiO2.

Table- I: mean value ± standard error (SE) of biochemical test of mice exposed to acute doses of titanium dioxide nanoparticles

Dose	1 Day		
	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	170.90 ± 5.77 b	53.10 ± 2.30 c	91.10 ± 1.73 a
150 mg/kg	578.30 ± 1.1a	211.60 ± 1.5 a	104.50 ± 2.66 a
600 mg/kg	579.90 ± 22.86 a	137.65 ± 5.05 b	103.30 ±25.98 a
LSD P ≤ 0.05	47.16	11.50	52.29
Dose	14 Day		
	AST	ALT	ALP
Control	150.00 ± 3.86 c	53.60 ± 2.30 c	98.10 ± 2.30 a
150 mg/kg	198.30 ± 6.58 b	69.60 ± 2.77 b	86.95 ± 2.22 b
600 mg/kg	640.80 ± 12.75 a	101.00 ± 1.15 a	69.90 ± 3.46 c
LSD P ≤ 0.05	29.7	7.56	9.42
Dose	30 Day		
	AST	ALT	ALP
Control	153.90 ± 2.31 c	53.10 ± 2.88 b	104.80 ± 3.23 a
150 mg/kg	201.50 ± 1.44 b	46.35 ± 0.77 c	89.50 ± 2.36 b
600 mg/kg	317.25 ± 6.52 a	60.50 ± 0.28 a	71.20 ± 1.73 c
LSD P ≤ 0.05	14.12	6.00	8.72
Dose	1 Day		
	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	0.10 ± 0.02 b	2.6 ± 0.05 b	37.27 ± 0.69 a
150 mg/kg	0.20 ± 0.003 a	5.95 ± 0.03 a	34.29 ± 0.33 b
600 mg/kg	0.20 ± 0.0003 a	2.85 ± 0.14 a	29.61 ± 0.23 c

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Dose	1 Day		
	AST(U/L)	ALT(U/L)	ALP(U/L)
Control	170.90 ± 5.77 b	53.10 ± 2.30 c	91.10 ± 1.73 a
150 mg/kg	578.30 ± 1.1a	211.60 ± 1.5 a	104.50 ± 2.66 a
600 mg/kg	579.90 ± 22.86 a	137.65 ± 5.05 b	103.30 ±25.98 a
LSD P ≤ 0.05	47.16	11.50	52.29
Dose	14 Day		
	AST	ALT	ALP
Control	150.00 ± 3.86 c	53.60 ± 2.30 c	98.10 ± 2.30 a
150 mg/kg	198.30 ± 6.58 b	69.60 ± 2.77 b	86.95 ± 2.22 b
600 mg/kg	640.80 ± 12.75 a	101.00 ± 1.15 a	69.90 ± 3.46 c
LSD P ≤ 0.05	29.7	7.56	9.42
LSD P ≤ 0.05	0.04	0.31	1.60
Dose	14 Day		
	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	0.10 ± 0.02 c	2.20 ± 0.05 b	35.50 ± 1.62 a
150 mg/kg	0.20 ± 0.000 b	1.50 ± 0.06 c	31.47 ± 1.04 b
600 mg/kg	0.45 ± 0.866 a	3.8 ± 0.00 a	38.46 ± 0.05 a
LSD P ≤ 0.05	0.18	0.15	3.84
Dose	30 Day		
	Creatinine mg/dl	Uric acid mg/dl	Urea mg/dl
Control	0.10 ± 0.02 b	2.6 ± 0.05 c	37.27 ± 1.25 a
150 mg/kg	0.20 ± 0.003 a	3.40 ± 0.06 a	34.38 ± 0.76 ab
600 mg/kg	0.20 ± 0.0026 a	3.02 ± 0.02 b	33.88 ± 0.20 b
LSD P ≤ 0.05	0.04	0.16	2.96

TABLE- II: MEAN VALUE ± STANDARD ERROR (SE) OF LIPID FUNCTION TEST OF MICE EXPOSED TO ACUTE DOSES OF TITANIUM DIOXIDE NANOPARTICLES

Dose	1 Day		
	Cholesterol(U/L)	High density lipoproteins (U/L)	Low Density Lipoproteins (U/L)
Control	113.67 ± 2.30 a	77.05 ± 1.73 ab	15.51 ± 1.73 b
150 mg/kg	115.73 ± 1.01 a	82.22 ± 2.09 a	25.20 ± 0.17 a
600 mg/kg	98.90 ± 6.49 b	68.11 ± 4.47 b	13.68 ± 2.74 b
LSD P ≤ 0.05	13.93	10.46	6.49
Dose	14 Day		
	Cholesterol	High density lipoproteins	Low Density Lipoproteins
Control	114.00 ± 1.73 a	74.00 ± 1.15 a	16.00 ± 0.63 a
150 mg/kg	92.78 ± 3.65 b	76.23 ± 3.29 a	13.25 ± 0.77 b
600 mg/kg	84.93 ± 0.72 b	66.44 ± 0.13 b	9.80 ± 0.05 c
LSD P ≤ 0.05	8.21	6.97	2.00
Dose	30 Day		
	Cholesterol	High density lipoproteins	Low Density Lipoproteins
Control	114.00 ± 1.73 a	71.88 ± 1.15 c	16.71 ± 0.64 c

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Dose	1 Day		
	Cholesterol(U/L)	High density lipoproteins (U/L)	Low Density Lipo-proteins (U/L)
Control	113.67 ± 2.30 a	77.05 ± 1.73 ab	15.51 ± 1.73 b
150 mg/kg	115.73 ± 1.01 a	82.22 ± 2.09 a	25.20 ± 0.17 a
600 mg/kg	98.90 ± 6.49 b	68.11 ± 4.47 b	13.68 ± 2.74 b
LSD P ≤ 0.05	13.93	10.46	6.49
150 mg/kg	101.16 ± 0.782 b	79.83 ± 0.39 b	19.01 ± 0.57 b
600 mg/kg	117.74 ± 0.63 a	92.32 ± 0.05 a	22.48 ± 0.09 a
LSD P ≤ 0.05	4.00	2.44	1.72

Table- III: Mean value ± standard error of hematological parameters in mice exposed to two acute doses of TiO2 NPs.

Parameters	1 Day			LSD P ≤ 0.05	14 Days			LSD P≤0.05	30 Days			LSD P≤0.05
	Control	150 mg/kg	600 mg/kg		Control	150 mg/kg	600 mg/kg		Control	150 mg/kg	600 mg/kg	
WBC (×103/µl)	2.1b ± 0.13	2.16b± 0.44	6.23a± 1.02	2.23	2.5c± 0.12	7.5a± 0.52	4.6b± 0.26	1.19	3.4b± 0.08	8.93a ± 0.19	7.15a± 1.7	3.43
RBC (×106 /µl)	8.52a± 0.01	7.29b± 0.33	8.00ab± 0.27	0.86	8.9a± 0.01	8.04b± 0.01	9.02a± 0.19	0.38	8.7a± 0.01	6.73c± 0.39	7.7b± 0.25	0.93
PLT (×103 /µl)	67b± 5.35	134.6b± 35.74	307.6a± 72.14	161.2	204.3c± 11.13	670b ±47.92	820a± 56.58	149.8	160 b± 7	612.3a± 96.13	360 b ± 106.81	287.43
HGB (g/dl)	13.70a± 0.65	10.80 b± 0.26	11.40b ±0.82	2.15	11.90ab± 0.66	11.30b ±0.17	13.00a± 0.35	1.54	13.80a± 0.64	9.10b± 0.61	10.70b± 0.52	2.05
MCHC (g/ dl)	29.10b± 0.65	31.17a± 0.17	31.60a± 0.47	1.63	29.40b± 0.4	30.35ab± 0.55	31.60a± 0.46	1.64	28.10a± 0.64	31.10b± 0.25	29.75ab± 0.89	2.26
MCV (fL)	48.00a± 0.87	47.67a± 0.63	45.00b± 0.5	2.37	35.00b± 1.11	46.35a± 1.76	45.60a± 0.58	4.32	40.30c± 0.91	43.40b± 0.06	46.80a± 0.81	2.43
MCH (pg)	13.50b± 0.43	14.80a± 0.15	14.17ab± 0.15	0.96	12.50b± 0.47	14.00a ±0.23	14.35a± 0.09	1.07	11.50b± 0.76	13.47a ±0.12	13.80a± 0.17	1.57

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Table- VI: mean value ± standard error (SE) of titanium accumulated in liver, kidney and spleen of mice exposed to acute doses of titanium dioxide nanoparticles after 14 and 30 days

Dose	Liver	Kidney	Spleen	Liver	Kidney	Spleen
	14 Day			30 Day		
Control	2.80 c ± 0.43	0.40 b ± 0.09	1.90 c ± 0.08	2.80 c ± 0.43	0.40 b ± 0.09	1.90 c ± 0.08
150 mg/kg	70.95 a ± 13.05	43.01 a ± 1.18	54.07 b ± 2.08	38.88 b ± 6.55	5.70 b ± 3.29	36.98 b ± 2.05
600 mg/kg	38.85 b ± 2.21	52.91 a ± 11.51	60.00 a ± 1.96	152.78 a ± 1.14	205.07 a ± 3.27	200.26 a ± 1.02
LSD P ≤ 0.05	26.45	23.11	5.72	13.31	9.27	4.58

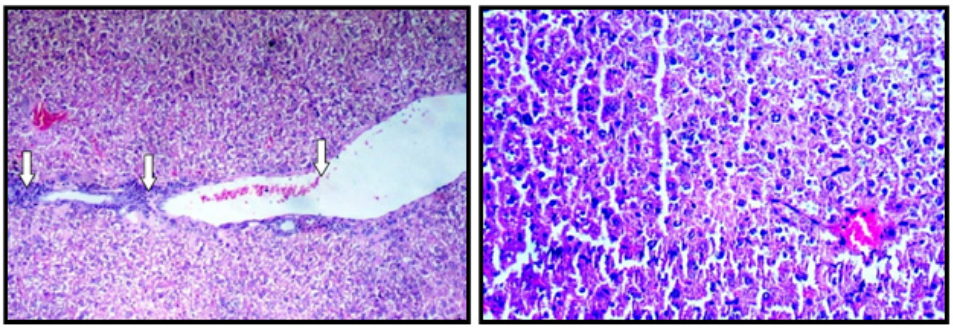

Fig. 1- Histological section representing hepatic tissue show dilated portal tract with chronic inflammatory cells infiltration (white arrows). On the right (control group), cross section in liver of control mice represents hepatic tissue with normal looking cords of hepatocytes, (H&E) (X40).

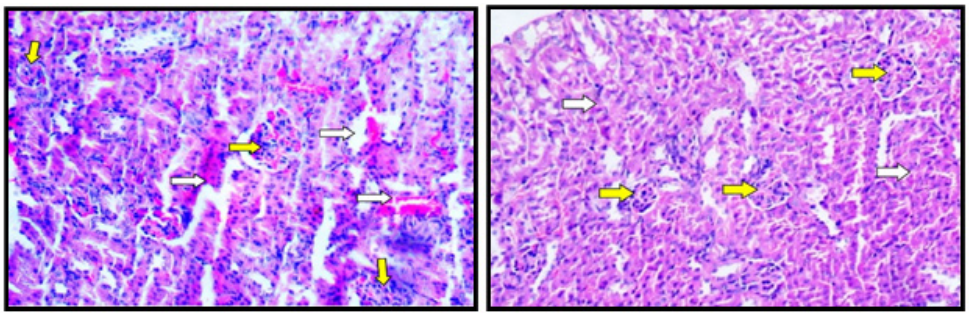

Fig.2- Histological section representing renal issue show glomerular congestion (yellow arrows) & tubular congestion & atrophy (white arrows). On the right (control group), histological section shows normal looking renal tissue (Glomeruli yellow arrow, and Tubules white arrow) (H&E) (X40)

CONCLUSION

On the basis of the result of blood and biochemical tests, titanium dioxide nanoparticles were found to be responsible for induced marked alteration in many blood parameters, liver and kidney function of exposed mice. In most cases, they appear to increase the level of badCholesterol, which may increase the risk of heart diseases. Generally, it might be toxic towards man and the environment, especially at high doses.

REFERENCES

1. Robertson, T. A., W. Y. Sanchez and M. S. Roberts. (2010). Are commercially available nanoparticles safe when applied to the skin? Journal of biomedical nanotechnology. 6 (5). 452-468.

2. Bhatt, I. and B. N. Tripathi. (2011). Interaction of engineered nanoparticles with various components of the environment and

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possible strategies for their risk assessment. Chemosphere. 82 (3). 308-317.

3. Zhao, F., Y. Zhao, Y. Liu, X. Chang, C. Chen and Y. Zhao. (2011). Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. Small. 7 (10). 1322-1337.

4. Kou, L., J. Sun, Y. Zhai and Z. He. (2013). The endocytosis and intracellular fate of nanomedicines: Implication for rational design. Asian Journal of Pharmaceutical Sciences. 8 (1). 1-10.

5. Yang, J., M. Luo, Z. Tan, M. Dai, M. Xie, J. Lin, H. Hua, Q. Ma, J. Zhao and A. Liu. (2017). Oral administration of nano-titanium dioxide particle disrupts hepatic metabolic functions in a mouse model. Environmental toxicology and pharmacology. 49. 112-118.

6. . Rothen-Rutishauser, B. M., S. Schürch, B. Haenni, N. Kapp and P. Gehr. (2006). Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environmental science & technology. 40 (14). 4353-4359.

7. Albanese, A., P. S. Tang and W. C. Chan. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annual review of biomedical engineering. 14. 1-16.

8. Janer, G., E. M. Del Molino, E. Fernández-Rosas, A. Fernández and S. Vázquez-Campos. (2014). Cell uptake and oral absorption of

titanium dioxide nanoparticles. Toxicology letters. 228 (2). 103-110.

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

10. Shrivastava, R., S. Raza, A. Yadav, P. Kushwaha and S. J. Flora. (2014). Effects of subacute exposure to TiO2, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. Drug and chemical toxicology. 37 (3). 336-347.

11. Bancroft, J. D. and M. Gamble, 2008: Theory and practice of histological techniques. Elsevier Health Sciences.

12. Quinn, G. P. and M. J. Keough, 2002: Experimental design and data analysis for biologists. Cambridge University Press.

13. Rosner, B. (2010). Fundamentals of biostatistics, Brooks/Cole, Cengage Learning. Inc., Boston, MA.

14. Vasantharaja, D., V. Ramalingam and G. Aadinaath Reddy. (2015). Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats. Nanomedicine Journal. 2 (1). 46-53.

15. Hassoun, E. and S. Stohs. (1995). Comparative studies on oxidative stress as

ISSN: 2075 - 2954 (Print)

a mechanism for the fetotoxic of TCDD, endrin and lindane in C57BL/6J and DBA/2J mice. Teratology. 51 (3). 186.

16. Fröhlich, E. and E. Roblegg. (2012). Models for oral uptake of nanoparticles in consumer products. Toxicology. 291 (1-3). 10-17.

17. Cho, W.-S., B.-C. Kang, J. K. Lee, J. Jeong, J.-H. Che and S. H. Seok. (2013). Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Particle and fibre toxicology. 10 (1). 9

18. Wang, Y., Z. Chen, T. Ba, J. Pu, T. Chen, Y. Song, Y. Gu, Q. Qian, Y. Xu and K. Xiang. (2013a). Susceptibility of young and adult rats to the oral toxicity of titanium dioxide nanoparticles. Small. 9 (9-10). 1742-1752.

19. Ma, L., J. Zhao, J. Wang, J. Liu, Y. Duan, H. Liu, N. Li, J. Yan, J. Ruan and H. Wang. (2009). The acute liver injury in mice caused by nano-anatase TiO 2. Nanoscale research letters. 4 (11). 1275.

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

21. Yeagle, P. (1991). Modulation of membrane function by cholesterol. Biochimie. 73 (10). 1303-1310.

22. Duan, Y., J. Liu, L. Ma, N. Li, H. Liu, J. Wang, L. Zheng,

C. Liu, X. Wang and X. Zhao. (2010). Toxicological characteristics of nanoparticulate anatase titanium dioxide in mice. Biomaterials. 31 (5). 894-899.

23. Rifai, N., G. R. Warnick, J. R. McNamara, J. D. Belcher, G. F. Grinstead and I. Frantz. (1992). Measurement of low-density-lipoprotein cholesterol in serum: a status report. Clinical chemistry. 38 (1). 150-160.

24. Gabriel, U., G. Ezeri and O. Opabunmi. (2004). Influence of sex, source, health status and acclimation on the haematology of Clarias gariepinus (Burch, 1822). African Journal of Biotechnology. 3 (9).

25. Ololade, I. and O. Oginni. (2010). Toxic stress and hematological effects of nickel on African catfish, Clarias gariepinus, fingerlings. Journal of environmental chemistry and Ecotoxicology. 2 (2). 014-019.

26. Iavicoli, I., G. Carelli, E. Stanek III, N. Castellino and E. Calabrese. (2003). Effects of low doses of dietary lead on red blood cell production in male and female mice. Toxicology letters. 137 (3). 193-199.

27. Tikare, S. N., S. Yendigeri, A. Gupta, S. A. Dhundasi and K. K. Das. (2012). Effect of garlic (Allium sativum) on hematology and erythrocyte antioxidant defense system of albino rats exposed to heavy metals (nickel II & chromium VI). Indian J Physiol Pharmacol. 56 (2). 137-146.

28. Al-Hamdany, A. (2010). The effect of lead acetate on

ISSN: 2075 - 2954 (Print)

histological structure of liver, kidney, spleen and some blood parameters in the white rats Rattus rattus. Msc Degree. Dept. biology, Univ. of Babylon. Iraq.

29. MacNicoll, A., M. Kelly, H. Aksoy, E. Kramer, H. Bouwmeester and Q. Chaudhry. (2015). A study of the uptake and biodistribution of nano-titanium dioxide using in vitro and in vivo models of oral intake. Journal of Nanoparticle Research. 17 (2). 66.

30. Mebius, R. E. and G. Kraal. (2005). Structure and function of the spleen. Nature reviews immunology. 5 (8). 606.

31. Roganovic, Z. and M. Jordanova. (1998). Liver lesions in bleak (Alhurnus alburnus alborella Filippi) collected from some contaminated sites on lake Ohrid. A histopathological evidence. Ekol. Zast. Zivot. Sred. 6. 11-18.

32. Shakeel, M., F. Jabeen, N. A. Qureshi and M. Fakhr-e-Alam. (2016). Toxic effects of titanium dioxide nanoparticles and titanium dioxide bulk salt in the liver and blood of mal