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Research Article

Effects of Titanium Dioxide Nanoparticles Toxicity on the Kidney of Male Rats

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ABSTRACT

Nanoparticles have many characteristics that make them suitable for biological and medical applications. These special features cause some adverse effects. Despite the widespread use of titanium dioxide nanoparticles, few studies were conducted on the toxicity of these nanoparticles in biological systems. In this study, the effects of intraperitoneal injections of TiO2 nanoparticles (30,50,70 mg/kg) were investigated on kidney function and histology. The creatinine levels did not change but the amount of urea and uric acid showed the significant changes in comparison with control group. But, by passing the time, the uric acid changes back to normal. In kidney tissue, the changes such as deposition of hyaline-like materials, the swelling, dilatation of Bowman's capsule and degenerations were seen. It seems by passing time, The temporary disorders have been removed and the renal function has returned toward normal. However, further investigations are needed to measure the oxidative stress.

INTRODUCTION

Nanotechnology involves the study, control, and composition of materials at the nanometer scales (Biazar et al. 2010). Reducing the size of the nanoparticles leads to the increase in surface area and provides the absorbtion possibility for further chemical molecules on the surface and this increases the reactivity of the particles, leads to increase in the effects of toxicity in these materials (Donaldson et al. 2004). However, some properties of nanoparticles are toxic but some of them may be useful and enable them to fight against diseases at the cellular level so that they can be used in treating the disease (Buzea et al. 2007). However; it is necessary to discover the characteristics of nanoparticles for a successful development of Nano-drugs (Biazar et al. 2010). Among the metal oxide nanoparticles, titanium dioxide nanoparticles due to optical, electrical and high catalytic properties have important usages in various industries; including in industrial pigments. It is also used as photocatalist in environmental purging, in sunscreen creams, in purifing the water, filteration of gases, especially in air polution, destruction of cancer cells, producing the cosmetic and health equipments, providing the protective coating against ultra violet (UVL) (Mital and Manoj 2011).

Recent studies show that nanoparticles such as TiO2 after entry into cells, by initiating inflammatory mechanisms, apoptosis and to generate the oxygen free radicals destruct the nucleus and DNA, and also alter the functions of cell (Kang et al. 2009).

In general, given the numerous applications of nanomaterials in various industries and existance of many hypotheses on the destructive effects of nanoparticles on living organisms, any research is very important in this field. Due to the nanoparticles (between 5-20 nm) can have high usage in drug and gene delivery and other applications in medicine and biology (Bonnemain 1998), so in this study, the effect of titanium dioxide nanoparticles on the kidney of male- wistar rats were studied.

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MATERIALS AND METHODS

TiO2 nanoparticles

Titanium dioxide nanoparticles manufactured by Spain Neutrino Company (Tehran, Iran) was purchased. The results of X-ray diffraction (XRD) shows the nano-TiO2 which was used in this study, was mainly anatase and crystal phase with the size of approximately 10-15nm (Figure 1). And also the purity of nanoparticles was calculated by using the test ICP-MS (99.986%). Table 1 shows the characteristics of the TiO2 nanoparticles.

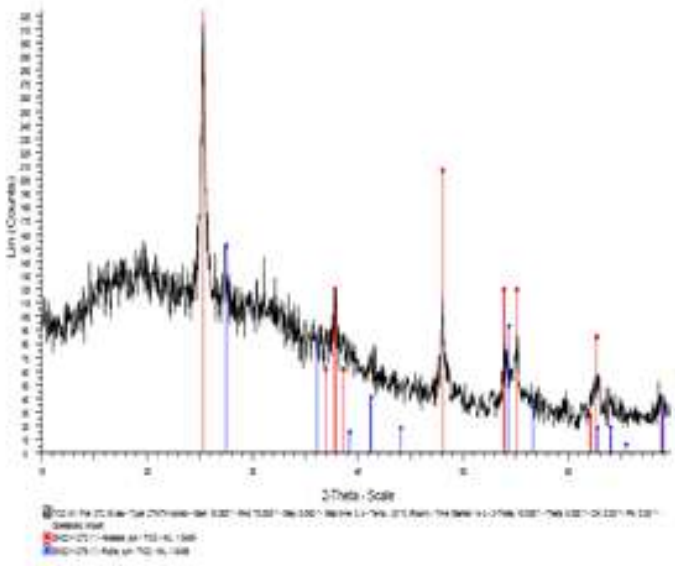

Figure 1. The XRD pattern of titanium dioxide nano-particles

Table 1: Physical parameters of nano TiO2 used in present study. These features is more important in chemical and biological properties of TiO2 nano-particles

Color	With
Morphology	Spherical
Crystallinephase	78.8 % Anatasa,21.2 % Rutilo
Specificsurfacearea	100-150 m^2/g
Density	3.84 g/cc
Size	10-15 nm
Purity	99.986 %

The breeding, treating and classification of experiments samples

In this study, 64 Wistar- adult- male rats were purchased from School of Pharmacy at Isfahan university of medical sciences with the average weight of 250±15g. These animals were kept under identical conditions in order to conform and adaptation with laboratory two weeks before experiments. The samples were under 20c-25c with natural light and randomly divided into 8 groups; the control group(without injection in order to achieve the baseline of renal parameters), the placebo group (received normal saline) and 6 treated groups. The treated ones are follows: the treated groups in the first stage included: 30-1-treated group, 50-1-treated group and 70-1-treated group: intraperitoneal titanium dioxide nanoparticle suspension (30, 50 and 70 mg/kg) was injected respectively for 21 days an alternate day (11 times). Then the samples were taken blood one day after the last injection.

The treated groups in the second stage included: 30-2-treated group, 50-2-treated group and 70-2-treated group: intraperitoneal titanium dioxide nanoparticle suspension (30, 50 and 70 mg/kg) was injected respectively for 21 days an alternate day (11 times). Then the samples were taken blood 21 days after the last injection.

In order to provide the desired dose, titanium dioxide nanoparticle powder was placed in saline under ultrasound for 20 minutes in the ultrasonic device (Parsonic made in Iran) and before each injection the suspension was physically shaken.

Blood analysis

Before taking blood, mice were weighed and then taking blood was conducted directly from the heart of rats which had been anesthetized by chloroform. Each blood samples was slowly poured into a test tube. Then, they were separated by a centifuge device of blood serum and the renal factors such as urea, uric acid and creatinine were measured by the kits of German Hitachi Company (Hitachi Automatic Analyzer, Roche).

Histological studies

After anesthetizing the rats, the kidneys were placed in 10% formalin and after preparation of the tissue sections, they were stained with hematoxylin-eosin and were studied by an optical microscope equipped with a camera.

Statistical analysis

In this study, the comparison of data was performed by using one-way analysis of variance (ANOVA) and Duncan's multiple range test to ensure over 95%. The mean of body weight of rats was compared with paired–t– test. To analyze the date, SPSS- 18- statistical software was used.

RESULTS

The results of changes in rats' body weight in different groups

According to Table 2 one way-variance analysis (ANOVA) showed that the mean of rats' body weight in different groups at the beginning of the experiment (weight 1) was

not significant. The average weight of rats in different groups after treatment (weight 2) showed no significant difference (P =0.96, F=0.255). Paired t-test showed that body weight of rats in all groups before and after treatment did not show any significant difference (P=0.521, F= 0.604). This is due to the natural growth of rats.

The changes of kidney factors

Table 3 shows the concentration mean of serum uric acid in the first stage of treated groups (30-1, 50-1, 70-1) or one day after the last injection had significant increase. This increase depended on the concentration (P<0.05; F= 2.741). Then the increased value of uric acids returned to its normal value by passing 21 days after the last injection in the second stage of treated groups (30-2, 50-2, 70-2).

Table 2: The mean of change in rats' body weight in different groups

Groups	Weight before injection (g)	Weight before blooding (g)
Control group	251.37±125.17	213.50±27.86
Placebo group	224.12 ±49.91	228.25±29.38
30-1	226.87 ±18.69	226.75±15.69
50-1	236.87 ± 28.14	232.25±20.13
70-1	224.75±53.11	229.12±37.19
30-2	226.75±30.36	222.75±29.86
50-2	228.50±27.86	210.75±29.86
70-2	217.87±43.42	239.75±49.7

The 30-1, 50-1, 70-1 groups are doses of 30, 50, 70 mg/kg 24 hours after the last injection.
The 30-2, 50-2, 70-2 groups are doses of 30, 50, 70 mg/kg 21 days after the last injection.
The results of paired t-test showed that injection of titanium dioxide nanoparticles has no effect on the weight of rats in different groups.

Table 3: Effect of titanium dioxidenanoparticles (TiO2 NPs) on kidney parameters in different groups (One-Way ANOVA)

Groups	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Control	20.37±1.18	1.41±0.27	0.57±0.04
Placebo	19.82±1.69	1.56±0.54	0.55±0.05
30-1	20.62±1.59	$^{\alpha}$2.20±0.94	0.57±0.07
50-1	18.87±2.90	$^{\alpha}$2.28±0.88	0.55±0.05
70-1	18.12±3.04	$^{\alpha}$2.68±1.15	0.51±0.06
30-2	*26.62±2.92	1.15±0.96	0.52±0.04
50-2	*26.12±4.64	1.61±1.05	0.55±0.07
70-2	*23.50±2.39	1.76±0.85	0.52±0.08
	P<0.001, F=11.248	P<0.05 F=2.741	P=0.4 F=1.044

The 30-1, 50-1, 70-1 groups are doses of 30, 50, 70 mg/kg 24 hours after the last injection.
The 30-2, 50-2, 70-2 groups are doses of 30, 50, 70 mg/kg 21 days after the last injection.
*Significant increase in the mean of value of urea in 3 treated groups in the second stage
αSignificant increase in the mean of value of uric acid in the 3 treated groups in the first stage.
Each number is the mean of 8 blood samples in each group.

The mean of concentration of urea in the second stage of treated groups (30-2, 50-2, 70-2) has significantly increased (P<0.001; F=11.248). Of course, this increase does not depend on concentration so that in the concentration of 30 mg/kg; the 30-2-treated group with the highest value (26.62±2.92mg/dl) and in the concentration of 70mg/kg; the70-2-treated group with lowest value have been measured (23.50±2.39mg/dl). Mean of creatinine concentration of different treated groups showed no significant difference (P =0.4; F = 1.044).

The Changes of kidney tissue

The results of the histopathological examination revealed that in the different -treated groups, one day and 21 days after the last injection, changes including deposition of hyaline-like materials in some proximal tubules, swelling and dilatation of Bowman's capsule and degeneration changes in epithelium of the proximal tubules were observed. These changes have been presented in Figures 2. However, in the morphological characteristics of kidney were seen no difference.

DISCUSSION

Hypotheses which have been proposed about probable damages resulted from nanotechnology are threatening the development of nanotechnology. Unless the correct information about what the risks are and how to avoid them to be published (Zhang et al. 2010). Nanotitanium dioxide is one of the nanoparticles which is frequently used with different capabilities and wide ranges in sciences including; pharmaceuticals, cosmetics, medicine, engineering, and on such things as water treatment, purification and filtration of gases, especially air, decolorization, neutralizing and destroying the cancer cells (Mital and Manoj 2011). On the other hand, the survival of TiO2 nanoparticles in environment and in food chain causes the continued poisoning resulted from it (Ahamed et al. 2010). Thus, up to now, despite numerous studies about the use of titanium dioxide in various sciences, few studies on the toxicity of titanium dioxide nanoparticles have been performed in biological systems (Mital and Manoj 2011).

As the measure of plasma concentrations of creatinine, urea and uric acid is usually a marker of kidney function and its other conditions (Ahamed et al. 2010), so in the present study, in order to state any comment about nephrotoxicity, we investigated the above factors and also renal tissue structure. Because kidney is one of the organs to collect and disposal of waste materials.

Eradication of creatinine in the blood stream is done by the kidneys , so to measure creatinine in the blood can indicate the function of kidneys (Palm and Lundbland 2005) and if there is a failure in renal function, the

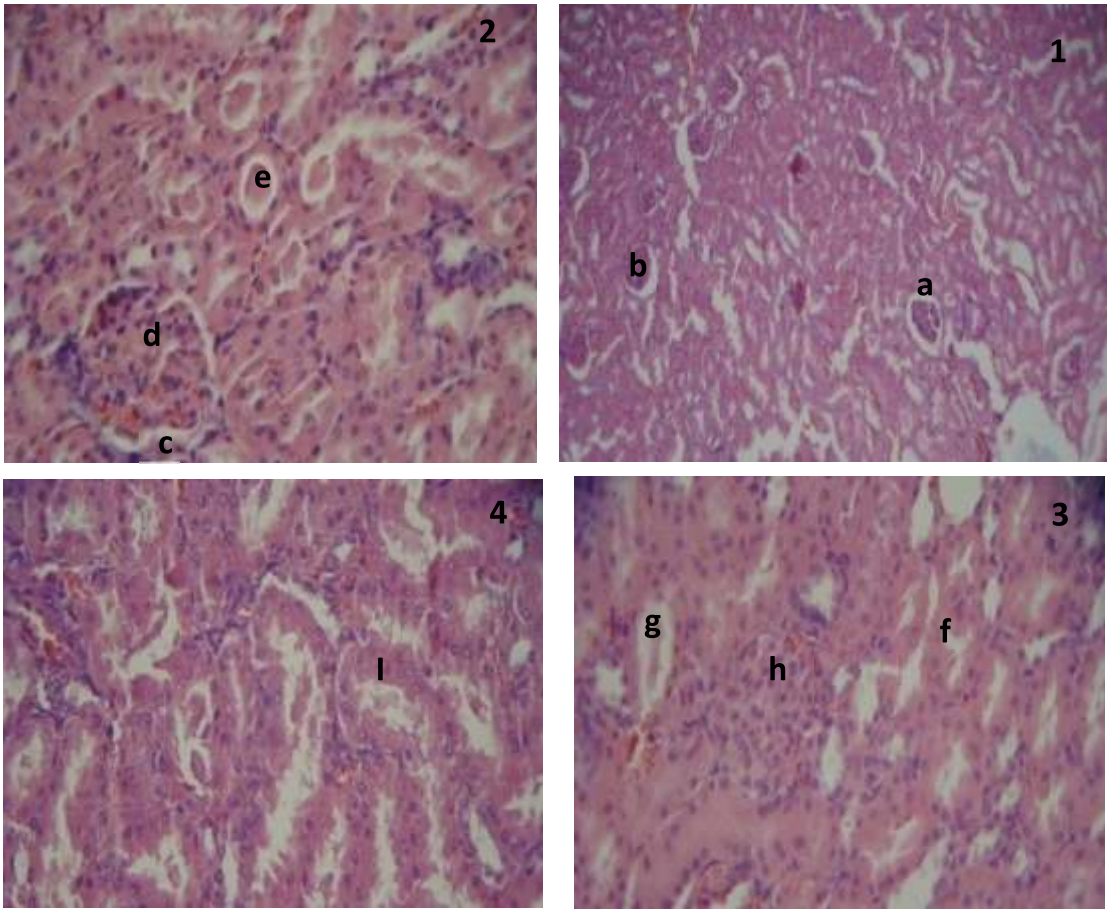

Figure 2. Sections of rat's kidney were prepared with staining of hematoxylin-eosine and enlarged 400×. Fig. 1. shows control groups and placebo which includes (a): Bowman's capsule (b): Glomeruli, Fig. 2 showing treated group receiving 30mg /kg in both stages;including (c): swelling and dilatation of Bowman's capsule, (d): Inflamed glomeruli, (e): deposition of hyaline-like materials, Fig. 3 indicates treated group receiving 50mg/kg in both stages, including (f): degeneration changes, (g): deposition of pink materials,(h): swelling of Bowman's capsule. Fig. 4 showing treated group receiving 70 mg/kg in both stages and includes(I): degeneration changes.

creatinine level of serum rises (Abdelhalim and Jarrar 2011). Thus, as shown in Table 3, serum creatinine level did not change in the two-stage study and showing the normal renal function and lack of effect of TiO2 nanoparticles on creatinine excretion by kidneys.

Regulation of urea by kidneys is a vital part of the rats body metabolism. In addition, to the role of urea as a carrier of waste nitrogen, it plays some interactions in the system of nephrons (Zhang et al. 2012). Serum urea level in the first stage of treatment groups in each 3 doses of 30, 50 and 70 mg/kg one day after the last injection, had no changes in comparison with the control and placebo groups. But, 21 days after the last injection, serum urea level in the second stage of the treatment groups in each 3 doses 30, 50, 70 mg/kg showed the significant increase ($p<0.001$).While serum uric acid levels in the first stage showed the significant-dose-dependent increase ($p<0.05$) but in the second stage after 21 days, uric acid level return to normal. However, the factors such as kidney disease, renal failure, cancer and gout can cause the increase in uric acid (Lewinski et al. 2008). Thus, the increase of uric acid one day after the last injection may cause the renal failure, kidney disease and the toxic effects of titanium dioxide nanoparticles on kidney. In this regard, it can be noted to the studies that nanoparticles of TiO2 have increased the levels of urea (Tang et al. 2010, Guo et al. 2009), creatinine (Tang et al. 2010, Zhao et al. 2010) and uric acid (Zhao et al. 2010, Liu et al. 2009). On the other hand, there are some studies that nanoparticles of TiO2 have reduced the level of urea (Liu et al. 2009, Zhao et al. 2010) and creatinine (Wang et al. 2009). The difference in the results obtained from animals treated with nanoparticles may be due to the type of animal, different physical and chemical properties of nanoparticles, the use of nanoparticles in oral, respiratory, dermal and number of injections (Shubayer et al. 2009). Histopathologic examination of kidney, as in Figure 2 was showed some deposition of hyaline-like materials, degeneration in the number of proximal tubules and swelling of a few glomeruli were observed.

Regarding the effect of nanoparticles of titanium dioxide in the kidney, Wang et al. believe that nanoparticles of TiO2 have been stored in the cells of kidney and caused the pathological changes and nephron-like toxicity in the form of inflammation of the glomeruli of the kidney. And also particles of 25 nm TiO2 can significantly increase the urea level of serum compared with the control group (Wang et al. 2007). However, the investigations show that

gold nanoparticles increase the amount of urea, but urea level return to normal after some time. This is due to the initial shock of kidney that gradually overcome and the renal function returned to normal (Zhang et al. 2012). In the present study, the uric acid level returns to normal gradually and possibly changes of kidney tissue are compensated. It might be due to the gradual disposal of nanoparticles which had been accumulated in the body.

One of the reasons confirms, it is that the titanium dioxide nanoparticles get hunk in the abdominal cavity in both phases of the research. In other words, disappearing the properties and features of nanoparticles after entering the body, is one of the factors that justifies the effects of slight toxic or non-toxic nanoparticles. In animals, many of nanoparticles due to binding to body organic molecules are changed into hunks. So, many special properties such as size, shape, surface charge, and the ability to penetrate into most organs and tissues disappear (Garcia et al. 2005). In relation to the lack of toxicity of nanoparticles can mention to studies that suggest by passing time and after one month that the density of nanoparticles is reduced in the liver, spleen, kidneys and lungs, also, the distinctive changes have not been reported in the enzymes level which have been measured in blood serum and no toxicity in the organs which confirms the nanoparticles of titanium dioxide can be safety used in low doses (Fabion et al. 2008). In another study, the researchers injected intra-peritoneally nano-titanium dioxide (20 mg/kg) with different sizes to the mice. After one week, they did not find any clear toxicity, mortality and distinctive changes in liver and kidney (Han et al. 2009). According to Han et al. 2009, titanium dioxide nanomaterials, without any toxicity, are excreted through the digestive system and urinary. As Borm et al. stated in his study; when nanoparticles enter the blood, they may be removed by different mechanisms depending on the path of absorption and character of their surface. The most common way to remove the nanoparticles is through the kidneys. This process involves filtration of blood in the glomeruli of the kidney nephron (Borm et al. 2006).

CONCLUSION

Finally, with regard to the use of nanoparticles of 5-20 nm in the medicine and biologic fields (Bonnemain 1998) and the results of the present study suggested that the lack of change in rats' body weight during the experimental period, no change in creatinine level and returning the increased uric acid into the level of control group within 21 days after the last injection, and no change in the appearance of kidneys in different groups and reports from other researchers, can conclude that the in vivo use of titanium dioxide nanoparticles in small amounts in the medical fields, probably does not cause any toxicity and certain disturbances in the body. Though, more researches are necessary on the effect of these nanoparticles on the organs and other blood factors. Also, additional studies should be treated in different periods and number of injections by examining oxidative stress.

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