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Original Research Article

Renal toxicity of titanium dioxide nanoparticlesin male albino rats

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ABSTRACT

Nanoparticles have many characteristics that make them suitable for biological and medical applications. Uptake of thesenano particulates into animals and humans bodiesthrough different routes may exhibit potential side effects. Titanium dioxide (TiO2) is a common additivethat is increasingly used in consumer products, food,pharmaceutical dosage forms and cosmetic articles. In this study, the effects of oral administration of TiO2 nanoparticles (500 mg| kg .bw) for 60 days were investigated on kidney function and histopathological changes. The body weight gain and kidney/body weight ratio showed no significant changes in comparison with control group. There was a significant decrease in total thiol levels in kidney homogenate, the biochemical changes was supported by histopathologicalultration. In conclusion the data shows that the oral administration of TiO2 NPs may lead to renal toxicity in experimental rats.

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1. Introduction

hours light / dark cycle were properly maintained. Distilled water and commercial food pellets for rats were available ad libitum. The rats were acclimated to the environment for 5 days

2.3. Characterization of nanoparticles anatase Tio_2 by using XRD method:-

XRD obtained using a Philips APD-3720 diffract meter (Cu Kα radiation, operated at 20 mA and 40 kV) in the 2θ range of 5–70 at a scanning speed of 5°/min.

2.4. Animal grouping and experimental design:

The male albino rats were equally divided into 2 groups as following:

Group 1: received 1% tween 80 and served as (-ve) control

Group 2: received Tio2 Nps suspension [Tio_2Nps were suspensed in 1% tween 80 and dispersed by ultrasonic vibration for 15 minutes**(Al-Rasheedet al., 2013)**and administrated via oral gavage in adose of500 mg| kg .bwt (equal to 1/25 of LD50 (oralLD50 of TiO2 for rats is larger than 12,000 mg/kg body weight **(WHO,1969)**5 days | week throughout all the experimentfor 60 days.

Twenty four hours after the last dose, rats were weighed, anesthetized with amixture of alcohol, chloroform and ether (1:2:3) respectively. kidneys was quickly excised, weighed and homogenized in a saline solution (0.9 % NaCl) (10% w/v) using Teflon homogenizer (Glas-Col, Terre Haute, USA), The homogenates were centrifuged at 3000 r.p.m. for 15 minutes and the supernatants were kept at -20 C° for the assay of biochemical parameters (oxidative status). The other part of kidneys was put in formalin for histopathological examination.

2.5.Organ to body weight ratio "coefficient of kidney":

After weighing the body and kidney, coefficients of kidney mass to bwt were calculated as the ratio of kidney :"wet weight ,mg" to bwt (g)

2.6. Biochemical analysis:

Determination of total thiols content:-

The method of **Kosteret al. (1986**) was adopted for estimation of tissue total thiols content.

Principle:-

The method is based upon the reduction of Ellman's reagent (5,5`-dithiobis-(2-nitrobenzoic acid) by thiols containing compounds to form 5-thio-2-nitrobenzoic acid which can be measured colourimetrically at 412 nm.

Reagents:-

1. Phosphate buffer (0.1 M, pH 7.4): prepared by dissolving 0.087g potassium dihydrogen phosphate and 0.38g disodium hydrogen phosphate in 50 ml distilled water.

2. Ellman's reagent (2mM): prepared by dissolving 80 mg of 5.5`-dithiobis-(2-nitrobenzoic acid) in 100 ml of 1% sodium citrate.

Procedure:-

50 µl of kidney homogenates was mixed with 0.75 ml phosphate buffer and 0.2 ml Ellman's reagent. Mixing was followed by incubation for 5 min. at 37 C. Distilled water instead of the sample was used for blank preparation. Absorbance was measured at 412 nm against blank.

2.7. Histopathology

Collected samples from kidneys were fixed in 10% buffered formalin at room temperature. Samples were trimmed for a size of one cubic centimeter. Sections underwent

routine histological procedures of dehydration, clearing and paraffin embedding. Sections of 4-6 µmthickness were stained with Hematoxylin and Eosin **(Howard et al. 2004).**

2.8. Statistical analysis

The primary data were analyzed for descriptive statistics using one-way ANOVA analysis and Duncan's multiple range tests. The statistical analyses were calculated, using Statistical Package for Social Sciences (SPSS version 20.0,2011). The data values were expressed as [mean concentration ± SD standard devision]. P<0.05 was in the accepted significance level.

3. Results

3.1. Characterization of nano-TiO2

The results ofthe X-ray diffraction (XRD) shows the nano-Tio2 which was used in this study, was anatase phase with the size of 63.8 nm.

3.2. Effect of nano-Tio2 on body weight gain

Data showing the effect of nano-Tio2 on body weight gain of administered rats were represented in table (1) and illustrated in figure (1).

The Tio2 Nps -administrated rats showed no significant decrease (P< 0.05) in body weight as compared to the normal ones.

Table 1:Effect of Tio2 Nps on body weight gain (mean **± SD**).

Experimental conditions	**Body weight gained**
Control	85.40±9.47[a]
Tio2NPs	80.60±23.39[a]

Values are the mean±SD for 10 rats in each group.

[abc] The means within the same column and bearing different superscripts are significantly different at P<0.05.

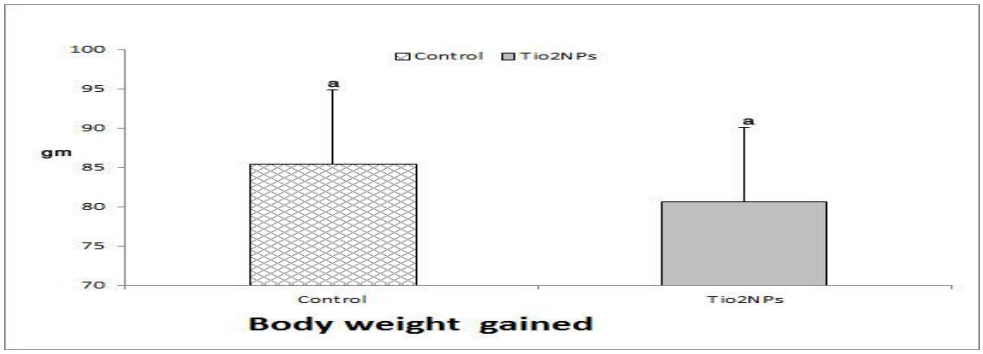

Figure 1**:** Effect of Tio2 Nps on body weight gain**.**

3.3. Body weight and coefficient of the kidney

There were no significant differences in food and water intake in the nano-TiO2-treated animals compared to control groups. The organ to body weight ratios of the kidney of animals treated with nano-TiO2 did not show any significant differences from control group Figure (2)& table (2)

Table 2: The organ to body weight ratio

Experimental conditions	The organ to body weight ratio
Control	3.06±.29[a]
Tio2NPs	3.36±.35[a]

Values are the mean±SD for 10 rats in each group.

[abc] The means within the same column and bearing different superscripts are significantly different at P<0.05.

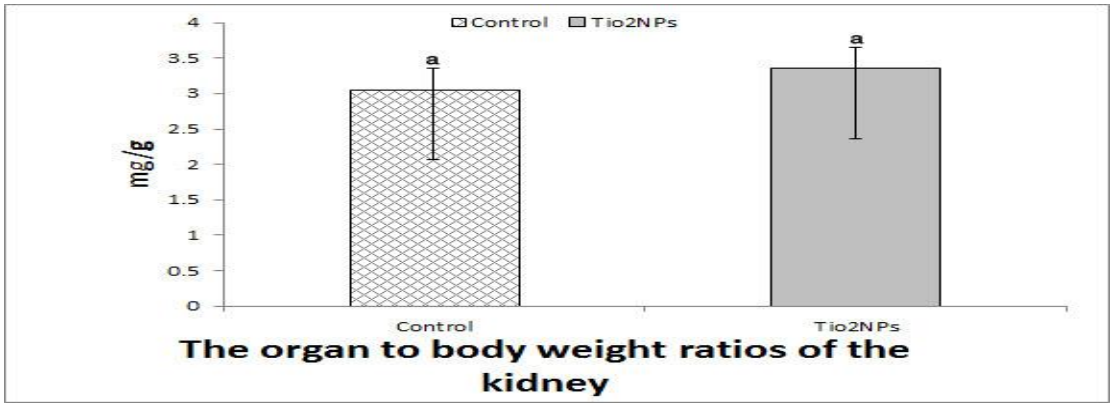

Figure 1: Organ to bodyweight ratio.

3.4. Effect of Tio2 Nps on total thiols content in rats.

Data showing the effect of Tio2 Npson total thiols content were represented in table (3) and illustrated in figures (3).

Tio2 Nps -administration caused marked depletion (P < 0.05) of total thiolsas compared to control group.

Table 3: The effect of Tio2 Nps on total thiols content

Experimental conditions	Total thiols (nmol/100mg tissue)
Control	23.58 ±1.98[b]
Tio2NPs	16.30 ±3.54[a]

Values are the mean±SD for 10 rats in each group.

[abc] The means within the same column and bearing different superscripts are significantly different at P<0.05

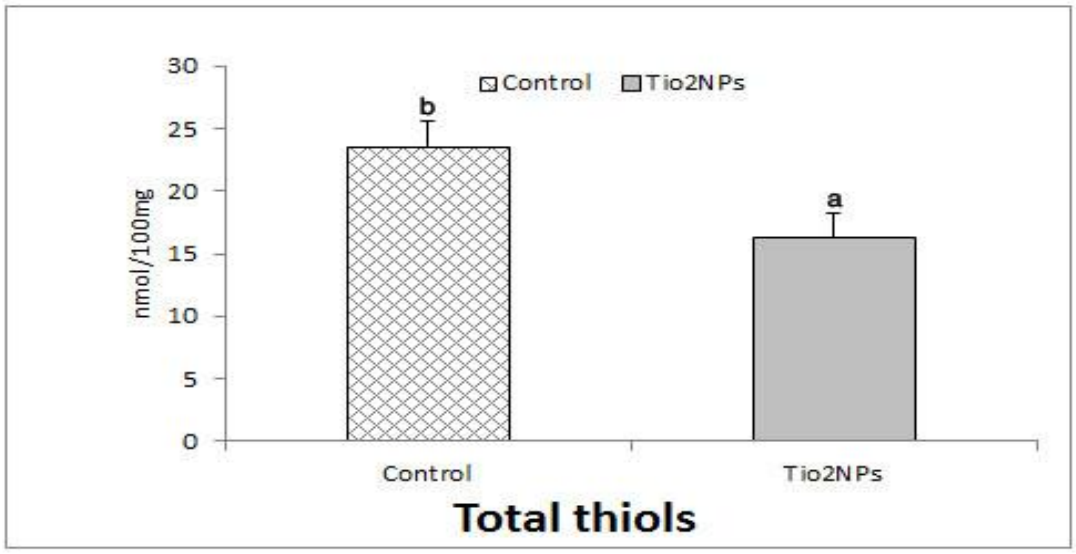

Figure 2:The effect of Tio2 Nps on total thiols content

3.5. Histopathology

Histopathological examination showed normal histological structure of the kidneys including renal tubules and glomerular tuft in control group (Fig. 4). While in Tio2 Nps group mild changes could be detected in the form of deposition of hyaline-like and proteinous substances in certain proximal tubules, dilatation of Bowman's capsule of some glomeruli and very mild degeneration changes in epithelium of the proximal tubules (Fig.5). Additionally, congestion of intertubular blood capillaries and glomerular tuft was detected (Fig.6)

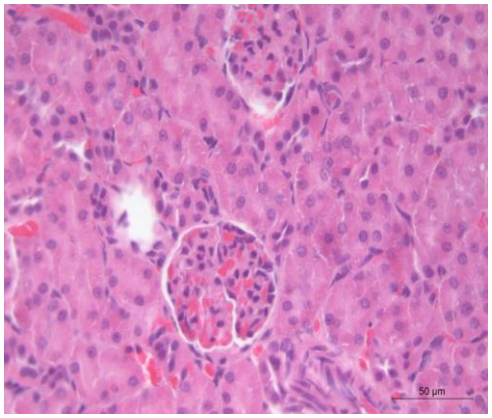

Figure3:Section of rat's renal cortex routinely stained with Hematoxylin and Eosin stain Showed normal histological structure of the kidneys. HE x400.

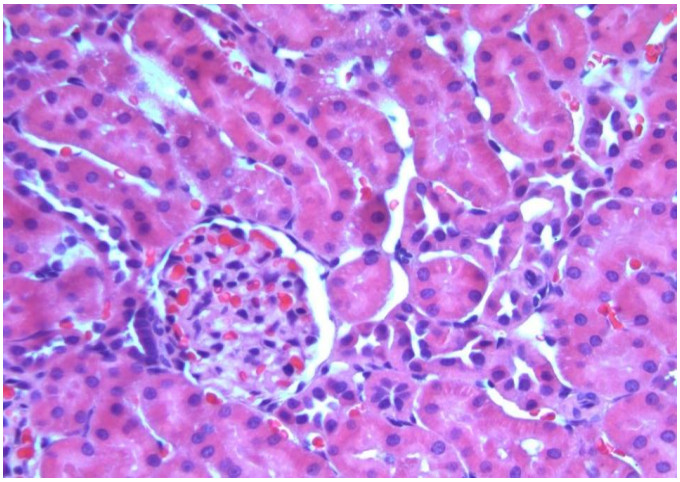

Figure 4: Section routinely stained with Hematoxylin and Eosin stainshowing deposition of hyaline-like and proteinous substances in certain proximal tubulesHE x400.

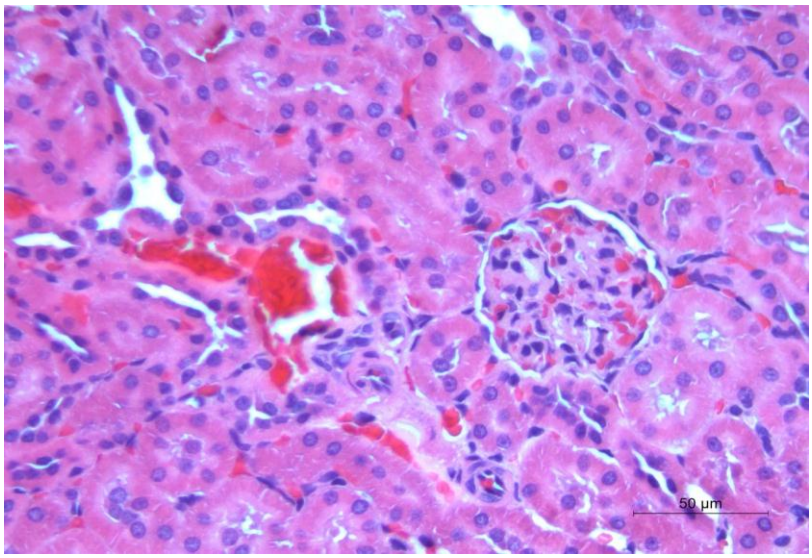

Figure 5:Congestion of intertubular blood capillaries.HE x400.

4. Discussion:

Broad application of TiO_2 NPs confers substantial potential for human exposure and environmental release, which inevitably allows for a potential health risk to humans, livestock, and the eco-system **(Longet al. 2007)**andthe kidney is particularly susceptible to xenobiotics owing not only to its high blood supply but also its ability to concentrate toxins.

TiO2 nanoparticles can damage DNA and cause cell death by induced oxidative stress. In addition, these nanoparticles can produce reactive oxygen species (ROS) and reduce cell antioxidants such as glutathione and vitamin E **(Johnstonet al. 2009).**

One of the nano-anatase TiO2 toxic mechanisms on kidney is an oxidative damage, probably because an imbalance between ROS and their removal damages macromoleculesand

membranes. It had been demonstrated that nano-TiO2 particles (25nm) stimulate ROS in BV2 microglia **(Long et al. 2007).**

TiO2 NPs-induced accumulation of ROS such as $O2^{\bullet-}$ and H_2O_2 in mouse kidneys resulted in oxidative damage of biological macromolecules, including lipid, protein, and DNA peroxidation. This oxidative damage happened before TiO2 NP-induced renal inflammation or apoptosis became evident **(Valko et al. 2006).**

ROS is the physiological products generated during aerobic metabolism in mammalian mitochondria. The intracellular ROS level is balanced through balancing the metabolism (by antioxidant enzymes and scavengers). A number of possible signaling pathways can describe the ROS association with apoptosis including death pathways involving cell-surface receptors (external) and mitochondrial pathways (internal) **(Jin et al., 2005).**

The abundance of long chain polyunsaturated fatty acids in the composition of renal lipids makes the kidney vulnerable to damage caused by ROS**(Ozbek, 2012).**

oxidative stress leads to lipid peroxidation, which is the result of an Interaction between H2O2 and O2can create OH and, 1O2 which are far more destructive and peroxidate the unsaturated lipid of the cell membrane(Fridovich,1978).Oxidative stress has been implicated as a factor that contributes to various forms of cell death, including as a specific inducer of apoptosis(Liu et al. ,2010).

Thiols are the organic compounds that contain a sulphydryl group. Among all the antioxidants that are available in the body.Totalthiols composed of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins.Thiol (-SH) groups present on protein are considered as major plasma antioxidants in vivo. They play a significant role in defense against reactive oxygen species**(Himmelfarbet al., 2000; 2001)** . Apart from their role in defense against free radicals, thiols share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level **(Prakash et al., 2009).**

Oxidative stress and thiol status, oxidation of cysteine (reduced thiols) residues can lead to the reversible formation of mixed disulfides between protein thiol groups and low–molecular-mass thiols (S-thiolation), particularly with GSH (S-glutathionylation). Protein S-glutathionylation can directly alter or regulate protein function (redox regulation) and may also have a role in protection from irreversible (terminal) oxidation. Sglutathiolation of protein cysteine residues protects against higher oxidation states of the protein thiol, thereby preserving the reversibility of this type of modification**(Prakash et al., 2009).**This study demonstrated that protein thiols are sacrificed to quench ROS which are produced excessively in Tio2 Nps - administrated rats.

Pathologically, the adverse effect of nanoparticles of titanium dioxide in the kidney was studied by **Wang et al.(2007)** who showed that TiO2 nanoparticles had affect kidneys and caused pathological changes and nephron-like toxicity characterized by inflammatory changes in the glomeruli of the kidney as well as renal tubles. In contrast, this study revelaed the presence of mild degenerative changes either on the glomeruli or renal tubules .

5. Conclusion:

This study showed that there were no significant changes in body weight gain or in kidney /body weight ratio in rats that administered Nano-TiO2 in comparison with control. Oral

administration of Nano-TiO2 may accumulate in kidney resulted in kidney toxicity and antioxidative responses. Nano-TiO2 could cause obvious ROS accumulation, which was attributed to the decrease of antioxidativedefense, resulting in serious nephritis. Hence, the usage of Nano-TiO2 and its formulated food and cosmetic commodity in day today life should be curiously focused in order to ascertain its toxic effects in exposed animals and humans.

References

Al-Rasheed NM1, Faddah LM, Mohamed AM, Abdel Baky NA, Al-Rasheed NM, Mohammad RA. (2013). "potential impact of Quercetin and idebenone against immuno-inflammatory and oxidative renal damage induced in rats by Tio2". J .Oleo sci.62(11)961-971

Brunet, L., D.Y. Lyon, E.M. Hotze, P.J. Alvarez, and M.R. Wiesner. (2009). Comparativephotoactivity and antibacterial properties of C-60 fullerenes and titanium dioxide nanoparticles.Environmental Science & Technology 43: 4355–60.

Fartkhooni FM, NooriA, MohammadiA(2016). Effects of Titanium Dioxide Nanoparticles Toxicity on the Kidney of Male Rats. International Journal of Life Sciences 10 (1); 65 - 69

Fridovich, I. (1995). Superoxide radical and superoxide dismutases.Annu Rev Biochem., 64: 97–112

Hedenborg M., (1988). Titanium dioxide induced chemiluminescence of human polymorphonuclearleukocytes, Int. Arch. Occup. Environ. Health 61, pp. 1–6.

Himmelfarb J, McMongale E, McManaminE (2000). Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. Kidney Int.;58:2571–8.

Himmelfarb J, McMongale E (2001). Albumin is the major plasma protein thiol target of oxidant stress in uremia. Kidney Int.60:358–63.

Howard, D.W., Lewis, E.J., Keller, B.J., SMITH Smith, C.S., (2004). Histological techniques for marine bivalve mollusks and crustaceans. Oxford: NOAA Technical Memorandum NOS NCCO

Jin Z, El-DeiryW (2005). Overview of cell death signaling pathways.CancerBiolTher.; 4(2): 139-163

Johnston HJ, Hutchison GR, Christensen FM, et al (2009). Identification of the mechanisms that drive the toxicity of TiO2 particulates: The contribution of physicochemical characteristics. Part FibreToxicol. 6: 33.

Kang SJ, Kim BM, Lee YJ, Hong SH and Chung HW (2009). Titanium Dioxide Nanoparticles Induce Apoptosis Through the JNK/p38-Caspase-8-Bid Pathway in Phytohemagglutinin-Stimulated Human Lymphocytes.*BiochemBiophysi Res communi386 : 682-687.*

Kang SJ, Kim BM, Lee YJ, Hong SH and Chung HW (2009). Titanium Dioxide Nanoparticles Induce Apoptosis Through the JNK/p38-Caspase-8-Bid Pathway in Phytohemagglutinin-Stimulated Human Lymphocytes. BiochemBiophysi Res communi386 : 682-687.

Koster, J.F.; Biemond, P. and Swaak, A.J. (1986). Intracellular and extracellular sulphydryl levels in rheumatoid arthritis. Ann. Rheum. Dis., 45(1):44-46.

Liu, J., N. Li, J. Wang, Y. Duan, J. Yan, H. Liu, H. Wang, and F. Hong (2010). Oxidative stress inthe brain of mice caused by translocatesnanoparticulate TiO2 delivered to the abdominal cavity.Biomaterials31: 99–105.

Liu R, Yin LH, Pu YP, Li YH, Zhang XQ, Liang GY, Li XB, Zhang J, Li YF, Zhang XY (2010). The immune toxicity of titanium

dioxide on primary pulmonary alveolar macrophages relies on their surface area and crystal structure. J Nanosci Nanotechnol.;10:8491–8499. doi: 10.1166/jnn.2685

Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, Hester S, Lowry GV, Veronesi B (2007). Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons in vitro. Environ Health Perspect 115: 1631–1637. 10.1289/ehp.10216

Mital GS and Manoj T (2011). A Review of TioNanoparticle.*ChineseSci Bull 56 : 1639-21657*

OberdoG (2005).¨ rster, E. Oberdo¨ rster, and J. Oberdo¨ rster, Nanotoxicology: An emerging disciplineevolving from studies of ultrafine particles, Environ. Health Persp. 113, pp. 823–839.

OzbekE (2012). Induction of oxidative stress in kidney.Int J Nephrol, 2012: 465-897

Prakash M, Shetty MS, Tilak P, Anwar N (2009). Total Thiols: Biomedical Importance And Their Alteration In Various Disorders. Online J Health Allied Scs.8(2):2

Valko, M.; Rhodes, C. J.; Moncol, J.; Izakovic, M.; Mazur, M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160,1−40

Warheit D.B., Hoke R.A., Finlay C., Donner E.M.,.Reed K.L, and Sayes C.M., (2007). Development ofa base of toxicity tests using ultrafine TiO2 particles as a component of nanoparticle riskmanagement, Toxicol.Lett. 171, pp. 99–110.

World Health Organization (WHO). FAO Nutrition Meetings Report Series No. 46A: 1969. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anti-caking agents and certain other substances. WHO/ FOOD ADD/70.36

Wang J, Zhou G, Chen C,Yu H and et al (2007). Acue Toxicity and Biodistribution of Different Sized Titanium Dioxide Particles in Mice after Oral Administration. Eur J ToxicolHyg Environ 168 : 176-185

Zhao J , Li N , Wang S , Zhao X, Wang J, Yan J , Ruan J , Wang H & Hong F (2010). The mechanism of oxidative damage in the nephrotoxicity of mice caused by nano-anatase TiO2 , Journal of Experimental Nanoscience, 5:5, 447-462, DOI: 10.1080/17458081003628931