Renal toxicity of titanium dioxide nanoparticles in male albino rats

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ABSTRACT
Nanoparticles have many characteristics that make them suitable for biological and medical applications. Uptake of these nano particulates into animals and humans bodies through different routes may exhibit potential side effects. Titanium dioxide (TiO2) is a common additive that 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 histopathological ultratraction. 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 K$_\alpha$ radiation, operated at 20 mA and 40 kV) in the 2$\theta$ 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 TiO$_2$ Nps suspension [TiO$_2$Nps were suspended in 1% tween 80 and dispersed by ultrasonic vibration for 15 minutes (Al-Rasheedet al., 2013) and administered via oral gavage in dose of 500 mg kg$^{-1}$ bwt (equal to 1/25 of LD$_{50}$ oral LD$_{50}$ of TiO$_2$ for rats is larger than 12,000 mg/kg body weight (WHO,1969)) 5 days | week throughout all the experiment for 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 coulurimetrically 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 μm thickness 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 deviation]. P<0.05 was in the accepted significance level.

3. Results

3.1. Characterization of nano-TiO2

The results of the X-ray diffraction (XRD) show 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>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Body weight gained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.40±9.47a</td>
</tr>
<tr>
<td>TiO2 NPs</td>
<td>80.60±23.39a</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>The organ to body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.06±.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tio2NPs</td>
<td>3.36±.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

<sup>abc</sup> The means within the same column and bearing different superscripts are significantly different at P<0.05.

![Figure 1: Organ to bodyweight ratio.](image)

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

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

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

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

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Total thiols (nmol/100mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.58 ±1.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tio2NPs</td>
<td>16.30 ±3.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean±SD for 10 rats in each group.
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).
Figure 3: 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 stain showing deposition of hyaline-like and proteinous substances in certain proximal tubules HE 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) and the kidney is particularly susceptible to xenobiotics owing not only to its high blood supply but also its ability to concentrate toxins.

TiO$_2$ 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 TiO$_2$ toxic mechanisms on kidney is an oxidative damage, probably because an imbalance between ROS and their removal damages macromolecules and
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•− and H2O2 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 O2 can 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 sulphhydryl group. Among all the antioxidants that are available in the body. Total thiols 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 et 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 tubules. In contrast, this study revealed 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 antioxidative defense, 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.

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