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Original Research

Oral toxic exposure of titanium dioxide nanoparticles on serum biochemical changes in adult male Wistar rats

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Abstract

Objective(s): Titanium dioxide (TiO_2) nanoparticles (NPs) are widely used in commercial food additives and cosmetics worldwide. Uptake of these nanoparticulate into humans by different routes and may exhibit potential side effects, lags behind the rapid development of nanotechnology. Thus, the present study designed to evaluate the toxic effect of mixed rutile and anatase TiO_2 NPs on serum biochemical changes in rats.
Materials and Methods: In this study, adult male Wistar rats were randomly allotted into the experimental and control groups (n=6), which were orally administered with 50 and 100 mg/kg body weight of TiO_2 NPs. Toxic effects were assessed by the changes of serum biochemical parameters such as glucose, total protein, albumin, globulin, cholesterol, triglyceride, high density lipoprotein, alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, blood urea nitrogen, uric acid and creatinine. All the serum biochemical markers were experimented in rats, after 14-days of post exposure.
Results: Changes of the serum specific parameters indicated that liver and kidney were significantly affected in both experimental groups. The changes between the levels of total protein, glucose, aspartate transaminase, alanine transaminase and alkaline phosphatase indicate that TiO2 NPs induces liver damage. Significant increase in the blood urea nitrogen and uric acid indicates the renal damage in the TiO2 NPs treated rats.
Conclusion: The data shows that the oral administration of TiO_2 NPs (<100nm) may lead to hepatic and renal toxicity in experimental rats.

Keywords: Nanoparticle, Oral toxicity, Serum biochemical markers, Titanium dioxide, Wistar rat

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Introduction

Nanotechnology is a major innovative and highly hopeful technology in the field of molecular science, which may use in medical, agriculture, industrial, manufacturing and military sectors (1, 2). The basic principle of the nanotechnology has resulted in a reduction of particle sizes, which improves cellular uptake efficiencies and endows novel physical properties that are potentially useful in biomedical research (3, 4).

In recent years, titanium dioxide (TiO_2) nanoparticles (NPs) are consequently used in paints, plastics, papers, ink, cosmetics and skin care products especially in the case of 1-100 nm size (5-9).

However, it has unique characteristics such as small size, large surface per unit mass and high reactivity that NPs can quickly enter the human body and then imposes potential health risk on human welfare (10, 11).

The ultra-sized TiO_2 particles enable them to pass through cell membranes to nuclear membranes; finally they can interrupt on cell ultrastructure and damage the cell membrane (12, 13). TiO_2 is a preferred nanomaterial for experimentalists because it has been used as negative controls in toxicity screening studies as well as suited for low solubility and low toxicity (14-16). The toxic proof of the TiO_2 NPs exposure leads to adverse effects in aquatic organisms also; this was confirmed by Linhua et al (17).

TiO_2 NPs have two major forms of crystal structures, named rutile and anatase. Both are toxic but the anatase NPs may produce much more toxic than rutile NPs and these particles mutually associated with oxidizing mechanisms of an organism, which capable to generating ROS (18) including oxidative stress and DNA damage (19-23).

Humans are highly exposed to TiO_2 NPs because of its extensive area of use. Liver is most vulnerable target organs of those NPs, which act as detoxification of the body. Most of the terrestrial activities, oral uptake of manufactured NPs to human has become so common because those particles are used as color additive for food as well as tooth paste and capsule (24).

In toxicological studies of an acute exposure to NPs, the changes of specific enzyme levels which directly reflects the damage in specific cells and organs (12, 25).

TiO_2 NPs formulated products are easily enter into the human body via different routes with different forms and may interrupt the body metabolism. Until now, most of the toxicological studies of TiO_2 NPs in mammalian models have focused on the hepatotoxicity via inhalation or dermal exposure.

Therefore, the present study is aimed to investigate the toxic effects of mixed rutile and anatase TiO_2 NPs (<100 nm) on *in-vivo* model through repeated oral administration of adult male Wistar rats.

Materials and Methods

Nanomaterials and preparation of treated suspension

Fully characterized mixture of rutile and anatase TiO_2 NPs used in the present study was purchased from Sigma-Aldrich chemicals Co. (St, Louis, MO 63103, USA). Characterization of the TiO_2 NPs as follows: a mixture of rutile and anatase nanopowder, appearance- color-white, powder form with particle size <100 nm and its purity 99.5 % trace metal basis. NPs were suspended in 0.9% saline and that suspension was sonicated for 10 min before the treatment.

Experimental animals and treatment

Adult male Wistar rat strains (*Rattus norvegicus*) weights (240-260g) were used in this study. Animals were housed in polypropylene cages placed in ventilated animal house (Siddha Central Research Institute, Chennai, Tamil Nadu, India). Temperature, humidity and 12 hours light/dark cycle were properly maintained. Distilled water and commercial food pellets for rats were available *ad libitum*.

They were acclimatized for five days prior to the experiment. All procedures were followed as per Institutional Animal Ethical Committee (IAEC) (138/PHARMA/SCRI, 2013).

Animals were randomly dived in to three groups, each group containing six rats (n=6): Control group treated with 0.9 % saline only and the experimental groups treated with TiO_2 NPs suspension (50 and 100 mg/kg of body weight) administered orally through intragastric oral intubation tube for 14 consecutive days. On the 15^{th} day all animals were sacrificed using cervical decapitation. The dose selection based upon the report of World Health Organization in 1969. According to the statement, LD_{50} of TiO_2 for rats is more than 12,000 mg/kg body weight (b.w) after oral administration (26). The dose of 50 mg/kg (b.w) and 100 mg/kg (b.w) of TiO_2 NPs was selected for the present study and exposed to rats every day.

Serum biochemical analysis

Blood samples were collected by the method of ocular vein puncture.

Collected blood samples were allowed to coagulate and the serum part was harvested immediately and utilized for biochemical analysis like, Total protein (TP), Albumin (ALB), Globulin (GLB), Cholesterol (CHOL), Triglycerides (TG) and High density lipoprotein (HDL) were measured by commercially available kit (Siemens Healthcare Diagnostics Ltd.).

The serum levels of alanine amino-transferase (ALT), aspartate aminotrans-ferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBILI) are estimate for liver functional test. Nephrotoxicity was determined by the levels of blood urea nitrogen (BUN), uric acid (UA) and creatinine (CREA) as well as glucose (GLU) levels was also measured in serum using by (Bayer RA-50, Auto-analyzer).

Statistical analysis

Statistical analysis of all data are expressed as mean ± standard error mean (SEM) and also followed by a one-way analysis of variance (ANOVA).

The values of $P<0.05$ was considered as statistical significance. Statistical analysis was performed using SPSS (version 19) software package.

Results

In this study, the GLU values were significantly increased in both the experi-mental groups as compared with that of control. The GLU values were elevated with 50 mg/kg of TiO_2 NPs treated group and more significantly ($P<0.01$) increased with 100 mg/kg treated group (Table 1).

There was no significant difference in ALB, GLB, and TBILI, while the TP was decreased in the serum of 50 mg/kg experimental group, but slightly increased TP was observed from 100 mg/kg TiO_2 NPs treated group.

There was a significantelevation in the CHOL and decrease in TG value noted from both of the experimental groups.

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.

The enzymes AST and ALP levels varied in the serum of both TiO2 NPs treated groups. A remarkable decrease of ALP activity was observed from both groups. AST levels were significantly ($P<0.05$) increased in 100 mg/kg of TiO2 NPs treated group, but not significantly increased in 50 mg/kg group.

There were no changes of ALT levels in both the experimental groups. The renal functional parameters of BUN and UA was increased in both the TiO2 NPs intoxicated groups, while there were no change of CREA in the two experimental TiO2 treated groups.

Table 1. Changes of the serum biochemical parameters in TiO2 NPs treated rats after 14-days oral supervision.

Parameters	Control	TiO_2 NPs treated 50mg/kg (b.w)	TiO_2 NPs treated 100mg/kg (b.w)
GLU(mg/dL)	106.83 ± 4.61	125.16 ± 4.33*	127.0 ± 3.043**
TP (g/dL)	7.83 ± 0.102	7.23 ± 0.200*	8.28 ± 0.153*
ALB (g/dL)	3.33 ± 0.122	3.25 ± 0.102	3.61 ± 0.134
GLB (g/dL)	4.50 ± 0.134	3.98 ± 0.261	4.40 ± 0.269
CHOL (mg/dL)	67.50 ± 4.12	84.33 ± 4.48*	101.16± 8.25**
TG (mg/dL)	191.5 ± 11.46	122.33 ±11.97**	122.83 ± 14.52**
HDL (mg/dL)	38.20 ± 1.056	39.60 ± 0.982	43.20 ± 1.59*
AST (U/L)	130.8 ± 4.48	134.00 ± 4.62	145.20 ± 4.14*
ALT (U/L)	57.20 ± 3.94	49.80 ± 3.95	56.60 ± 3.49
ALP (U/L)	244.0 ± 4.91	219.20 ± 4.70**	216.60 ± 4.92**
TBILI (mg/dL)	0.55 ± 0.093	0.56 ± 0.040	0.50 ± 0.024
BUN (mg/dL)	39.66 ± 1.85	46.50 ± 2.31*	48.16 ± 1.98*
UA (mg/dL)	1.08 ± 0.187	1.40 ± 0.163	1.61 ± 0.118*
CREA (mg/dL)	0.63 ± 0.032	0.61 ± 0.032	0.62 ±0.069

Note: Serum biochemical parameters are expressed as mean ± SEM values, $^{*}p<0.05$, $^{**}p<0.01$ significant difference from the groups (n=6/group).

Discussion

Generally, the impacts of TiO_2 NPs exposure have been experimented in different animals followed by different routs of administration including whole body and dermal exposure as well as gastric lavage and inhalation (27-29). In 1969, WHO reported that the LD_{50} of TiO_2 for rats is more than 12,000 mg/kg (b.w) after oral administration (26). In this study, we selected 50 and 100 mg/kg (b.w) of rutile and anatase mixed TiO_2 NPs exposed rats for 14 consecutive days. Different epidemiological studies have shown those TiO_2 NPs is low toxic and no carcinogenic effects in human (30, 31). But recently, the International Agency for Research on Cancer (IARC) working group was classified the ultrafine TiO_2 particles as possibly carcinogenic to human (32). In our study, mixture of rutile and anatase TiO_2 NPs induced abnormal physiological activities in male rats with emphasis to liver and kidney. Previously, Jani *et al.* (1994) reported that orally ingested rutile TiO_2 NPs can be absorbed via the gastrointestinal tract and pass through the mesentery lymph supply and lymph node to the liver (33). The liver is activated to abolish the side effect induced by these particles. Recently, biodistribution studies

have examined that the ultrafine TiO_2 particles mostly accumulate in the liver followed by kidney and are excreted gradually (34, 35). However, the small size NPs are very difficult to clearance from the liver, resulted in long time retention and induced the liver damage after oral exposure of 5 g/kg TiO_2 NPs in mice (36). Previous studies also reported that the retention halftime of TiO_2 NPs *in vivo* was long because of its small size and very complicated to clearance. Furthermore, intravenously injected TiO_2 NPs on rats with the dose of 250 mg/kg were 69% accumulated in liver at after 5 min and at other hand 80% of particles accumulated in liver at after 15 min (37). In the present investigation oral ingestion of <100 nm TiO_2 NPs may deposit in the liver and leads to hepatic damage conformed by the fluctuations in hepatic marker enzymes ALT, AST and ALP and nephron-markers as while.

Since the liver is the major place for biological changes and it protect the body from foreign substances and xenobiotic chemicals, identifying the causes for hepatic toxicity. The liver excretes the substances into bile; consequently the biliary organism is also exposed to NPs. Previous study have shown that various toxins with diverse mechanisms, including activation of alcohol degeneration, membrane lipid peroxidation, inhibition of protein synthesis, disruption of calcium homeostasis and activation of receptor enzymes, cause damage to liver cells (11). Enzymes such as ALT and AST are the metabolic enzymes in liver, which are dysfunctional enzymes in serum and plasma. The level of these enzymes in the cytoplasm of liver cells is number of times more than extracellular fluid. When the hepatic cells and membrane are damaged or died, the amount of these enzymes raise in the blood stream and this amount of elevation is an indication of the liver damage (38, 39). In the present study, rats were exposed to different doses of TiO_2 NPs; resulted in significant difference between the levels of AST and ALT enzymes within the groups. Although the levels of serum AST were increased in the TiO_2 NPs treated rats when compared to that of controls, this change was not significant in among the experimental groups. Wang et al (36), who reported that the TiO_2 NPs induce acute hepatic damage.

ALP is nothing but a cholestatic liver enzyme. Cholestasis is a state that causes partial or blockage of the bile ducts. Bile duct gives bile from the liver into the gall bladder and intestines. Bile is the fluid secreted from the liver cells helps the body to split the fat, process cholesterol and get free of toxins. If the bile duct is sore or injured, ALP can get backed up and leak out from the liver into the blood stream (40). In this study, reduced ALP and significantly elevated CHOL levels were observed in the serum of TiO_2 NPs treated rats. However, certain liver toxicity was monitored by the raise of CHOL in serum of male rats following the 28-days oral supervision of the low and high dose of silver NPs, and bile duct hyperplasia was observed in the male rats (41). Moreover, our findings records with elevated CHOL levels in the serum of male rats followed by low and high dose of TiO_2 NPs.

Nanoparticles are exposed to reach the systemic circulation after ingestion, inhalation or intravenous injection. They can share out to a number of organs such as liver, spleen, kidney, heart, brain, and ovary (11, 42-45). Meanwhile, kidney has been known to remove the unsafe substances from the blood, thus NPs absorb in to the circulatory system and can be filtered by renal system (46, 47). However, kidney dysfunction was found in rats treated with TiO_2 NPs, because of an increased level of BUN and UA in serum. In contrast, Wang *et al.* (2007) explained that the high levels of BUN and CREA in the serum of mice, which leads to dysfunction and pathological changes of kidneys (36). But the present study indicates there is no significant changes of

CR in the serum of TiO_2 NPs treated rats. On the other hand, the authors accomplished that TiO_2 NPs in higher dose caused severe damage to the liver and kidney and it troubled the equilibrium of blood glucose and lipid in mice (48). Furthermore, Meena and Paulraj(2012) were conformed in the increased levels of BUN in serum is directly associated with the sign of glomerulonephric toxicity, swelling in renal glomerulus, renal tubules crammed with the proteinic fluids because of the hold up of TiO_2 particles in kidneys, observed with 50 mg/kg TiO_2 NPs treated rats (49). Present study confirms, after oral administration of TiO_2 NPs have possible accumulation in the liver, and kidney which induce adverse side effects in the rats ingested with TiO_2NPs.

Conclusion

Present study reflects that TiO_2 NPs has adverse side effects in the physiological system in terms of hepatic and renal toxicity in TiO_2 NPs intoxicated rats. Oral administration of TiO_2 NPs may accumulate in the liver and kidney, which affects in lipid profile too. Hence, the usage of TiO_2 NPs and its formulated food and cosmetic commodity in day today life should be curiously focused in order to ascertain its toxic effects in humans.

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