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Acute and sub-chronic toxicity of titanium dioxide nanoparticles synthesized by microwave-irradiation-assisted hybrid chemical approach

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A lot of research has been focused upon the use of nanoparticles for various consumer applications. Titanium dioxide nanoparticles (TNPs) are now gaining importance to be used in food, environment and drug delivery vehicles. To explore the full potential of TNPs, a complete toxicity analysis is crucial before their commercialization. In the present study, we have evaluated the acute and sub chronic toxicity of TNPs on Wistar rats. In acute toxicity study, the LD_{50} value was found to be more than 2000 mg/kg bw as no mortality was observed. However, in sub-chronic toxicity, there was alteration in liver and kidney enzymes which indicated inflammation in these major organs. There was also accumulation of TNPs in liver and lungs of Wistar rats indicating the inefficient clearance of TNPs from rat bodies. A long term toxicity analysis should be done to evaluate the stability and potential use of TNPs for consumer products.

Keywords: Titanium dioxide nanoparticle, Wistar rats, acute toxicity, sub-chronic toxicity, histopathology.

Introduction

Nanomaterials possess unique properties which are different from their bulk counterparts largely due to their large surface to volume ratio[1]. TNPs have been used in a variety of consumer products ranging from cosmetics, food, paints and in medical devices. This wide range of products makes it of utmost importance to analyse its toxic properties. Since nanoparticle have greater penetration property than their bulk counterparts; the toxicity of these particles are expected to be greater. Interaction of TNPs with biomolecules such as proteins and enzyme gives an insight into how these particles will interact within the body if ingested intentionally or unintentionally[2].

TNPs are reported to be cytotoxic in nature. Several reports have indicated that these nanoparticles can induce oxidative stress and ROS leading to membrane and DNA damage. This structural damage leads to apoptosis or genetic alteration in cells thus affecting the overall health being of the organism[3]. Due to the wide range of products in which TNPs are used, they are either directly ingested or through environmental contamination. They can enter through various routes such as through the gastrointestinal or respiratory system and reach the blood or major organs. Thus, it is necessary to understand the long term effects of TNPs on various biological systems[4].

In this study, we investigated the effects of TNPs on Wistar rats. The acute and sub-chronic toxicity was observed for different concentrations. The effect was observed for liver and kidney enzymes which give a direct correlation hepatic injury and toxicity. The enzymes such as ALP, AST, ALT and GGTP and histopathological changes were observed. It can be noted that the controls and study design is similar to our earlier work on toxicological evaluation of silver nanoparticles[5].

Experimental

Chemicals:

All reagents used were of analytical grade and were used

without further purification. Throughout the procedures, double deionized (DI) water (resistivity ~18.2 MΩ cm^{-1}) was used. The enzymatic kits were procured from M/s Agappe Diagnostics Ltd., India.

TNP synthesis and characterization:

TNPs were synthesized by our previously standardized novel protocol i.e. microwave-irradiation-assisted hybrid chemical approach[6].

Animal model for in vivo study:

Animal experiments were performed after due clearance from the Institutional animal ethics committee (IAEC NO.VIT/IAEC/12/July23/5) and conducted at the institute's animal house. For the present study, female Wistar rats of 6–8 weeks old and 130–150 g in weight were chosen. They were kept in polycarbonate cages and were housed under controlled conditions (22–25°C) on a 12 h light/12 h dark cycle. Animals were maintained on standard pellet feed and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments of Animals (CPCSEA), GoI. The rats were weighed and divided in treatment groups such that the average body weight in each group was similar. All animal procedures were approved by the ethical committee in accordance with our institutional Animal Ethics Committee, 1333/C/12/CPCSEA.

Treatment of animals with TNP:

The animals were first observed for acute toxicity as a preliminary reference for dose selection. According to the OECD test guideline 420, when the toxicity of the test material is not known a limit dose of 2000 mg kg^{-1} body weight should be taken for acute studies (OECD, 2001). TNPs were gavaged (0.2 ml) as an aqueous suspension. The experimental design included four groups with three different doses (500 mg/kg bw, 1000 mg/kg bw and 2000 mg/kg bw) and a control group. The study was carried out for 14 days and 5 animals per group. Clinical observations and mortality checks were conducted once per hour for 6 h after dosing and once daily thereafter for 14 days. Body weights were measured on the day of treatment and on test days 1, 7 and 14.

If no acute toxicity observed-limit dose of 1000 mg/kg bw can be used as higher dose level in sub-chronic studies (OECD, 1998 guidelines). Based on literature survey, the limit dose for sub-chronic studies, 100 mg/kg bw was chosen. TNPs were orally administered as an aqueous suspension. The experimental design included six groups with five different doses (20 mg/kg bw, 40 mg/kg bw, 60 mg/kg bw, 80 mg/kg bw and 100 mg/kg bw) and a control group. The study was carried out for 12 weeks and 5 animals per group. The rats were then analyzed for different parameters as discussed further. During the study period, the clinical signs and mortality of the rats were observed daily and the body weights were recorded weekly[7].

Body weights, feed and water consumption and clinical signs:

Clinical signs like changes in fur, skin, nasal secretions (mucus and bleeding), mucous membranes and eyes, incidence of secretions and excretions, autonomic activity like lacrimation (secretion of tears), piloerection (erection of fur) and unusual respiratory pattern were examined daily. Rats were also monitored for changes in locomotion, gait, posture, response to handling, repetitive circling, or bizarre behavior like self-mutilation, walking backward, etc. Body weights, feed and water intake were measured on the day of treatment and on test days 1, 7 and 14 for acute study. While for sub-chronic study, body weights, feed and water intake were measured once before treatment and then at weekly intervals[8].

Biochemistry panel analysis:

At the end of the study, for both acute as well as sub-chronic, all of the animals were anesthetized with ketamine (22–24 mg/kg bw im) and sacrificed by cervical dislocation. Total blood was collected by cardiac puncture and was analyzed further for various biochemical assays such as total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyl transpeptidase (GGTP). The enzymatic and protein assays were performed as per the instructions provided by M/s Agappe Diagnostics Ltd., India. Various other biochemical parameters were also observed such as albumin (ALB), cholesterol (CHO), creatinine (CRE), glucose (GLU), lactate dehydrogenase (LDH), total protein (TP), inorganic phosphorous (IP), triglyceride (TRG), sodium (Na) and potassium (K). Hematology was analyzed for erythrocyte count (RBC), total leukocyte count (WBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (thrombocytes) count (PLT), mean corpuscular volume (MCV), hematocrits (HCV), lym-

phocytes (LYO), red cell distribution width (RDW), neutrophils (NEU), monocytes (MONO), eosinophils (EOS), basophils (BASO). Hematological parameters were analyzed using an automated hematology analyzer (K-4500, Sysmex Corp., Japan). Differential counts of leukocytes were analyzed with automatic analyzer MICRO HEG-120A (Omron Tateishi Electronics Co. Ltd., Japan).

Histopathology:

At necropsy, all major organs and tissues were cautiously observed macroscopically for any lesions and were surgically removed, washed in isotonic saline and blotted. Major organs such as liver, kidney, pancreas, heart, and lungs were fixed in 10% neutral-buffered formalin and stained with hematoxylin and eosin, and examined microscopically for any histological changes[8].

Determination of TNP concentrations in tissues:

Fractions of sample tissues (liver, kidney, lung, heart and pancreas) were weighed and dried in oven set at 60°C, to constant weight. The samples were placed in test tubes and a volume of 3 ml/100 mg dry weight of nitric acid is added. The tubes are then heated to 120°C. After cooling tubes, minerals were diluted with 10 ml of deionized water. Titanium content was analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The detection limit of titanium was 0.002 mg/L[9].

Statistical analysis:

Data were statistically evaluated using one way ANOVA and expressed as mean ± SEM. $p \leq 0.05$ was considered significant.

Results and discussion

Acute toxicity:

Epidemiology researches have reported that TiO_2 is low toxicity and shows no carcinogenic effect and/or nonmalignant respiratory disease for human[10]. But, recently, Baan's working group of the International Agency for Research on Cancer (IARC) classified pigment-grade titanium dioxide as possibly carcinogenic to human beings (group 2B)[11,12].

Acute study for TNP was carried out for three different doses for 14-day repeated toxicity. No mortalities were observed in both the vehicle control rats and the nanoparticle-administered rats. Thus, the acute oral LD_{50} is estimated to be more than 2000 mg kg^{-1} of body weight. There was also no significant clinical observational change. Moreover, TNPs gavaged rats did not show any significant change in body weight, feed and water intake. At the end of the study, serum was extracted from the sacrificed animals and various biochemical parameters were analyzed. It was observed that except for ALT, all the other enzymes and protein estimation were not significantly altered. As depicted in Table 1, there was significant increase in ALT levels which was dose dependent in nature. Wang *et al.* observed that after oral exposure to 5 g/kg TNP of size 25 and 80 nm, no mortalities were observed. They observed significant alteration in ALT/AST, BUN (blood urea nitrogen), and LDH indicating hepatic and renal toxicity. Also significant lesions of liver and kidneys in female mice were observed[12]. Similarly, Jyotisree *et al*. have reported acute toxicity of 20 nm size TNP when applied topically on Wistar rats. They reported LDH, BUN and creatinine levels indicating hepatic toxicity[13]. Liang *et al.* have reported that an exposure of 50 m^2/g and 210 m^2/g for a period of 7 days does not induce acute toxicity, but induces alteration in hepatic and renal enzymes[14]. However, TNP is reported to have less toxicity at lower doses. Fabian *et al.* have reported that TNP of size 20–30 nm were treated intravenously to Wistar rats and no detectable inflammatory response or organ toxicity was reported[15]. Cho *et al*. also reports that after TNP treatment for 24 h; no toxicity or inflammatory response was reported[16]. This indicates that TNP does not induce toxicity at lower doses and can be safely incorporated in food and pharma industries.

Table 1. Acute toxicity on Wistar rats after treatment with different concentrations of TNPs indicates dose dependent increased levels of ALP

Sr.No.	TiO_2 (mg/ kg bw)	ALP (Unit)
1.	0 (negative control)	120.2±25.2
2.	500	196.8±33.6
3.	1000	215.3±60.1
4.	2000	233.2±29.2

Sub-chronic toxicity:

Body weights, feed and water consumption and clinical signs:

Fine TiO_2 particles of micro size are considered as an inert and nontoxic compound. According to a WHO (1969) report, LD_{50} for rats is >12,000 mg/kg[14]. However, since the

nanoparticles differ from their bulk counterparts both in structural and chemical properties, the toxicity of TNP has to be analyzed for its cautious use in food, pharma and biomedical products. The sub-chronic toxicity study was carried for 12 weeks. During this study, no mortality or treatment-related abnormal clinical symptoms were observed. However, there was a significant reduction in body weight for the higher dose group. As depicted in Fig. 1, the weight of the control rats increased from 141.6±22.7 g to 293.4±24.7 g with the age. However, the higher dose group significantly decreased body weight to 263.8±22.7 and 254.7±10.4 as compared to control for 80 mg/kg bw and 100 mg/kg bw respectively. However, there was not much significant difference for body weight for 20 mg/kg bw to 60 mg/kg bw. Similarly, as depicted in Fig. 2 and Fig. 3, the feed and water intake decreased for the higher doses only but was not significantly reduced.

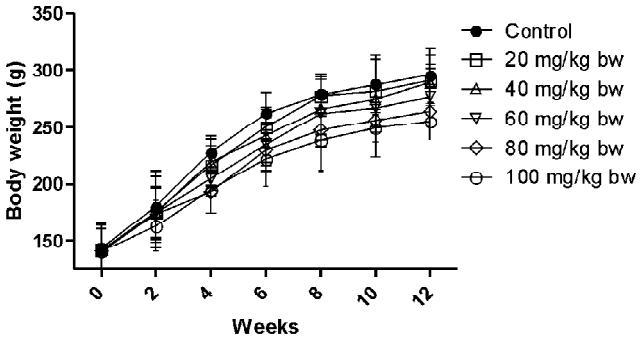

Fig. 1. Body weight of rats after treatment with different concentrations of TNPs. The body weight of control rats increased with age while the body weight of rats decreased after treatment with TNPs. Each data represents the mean ± SD (n = 5).

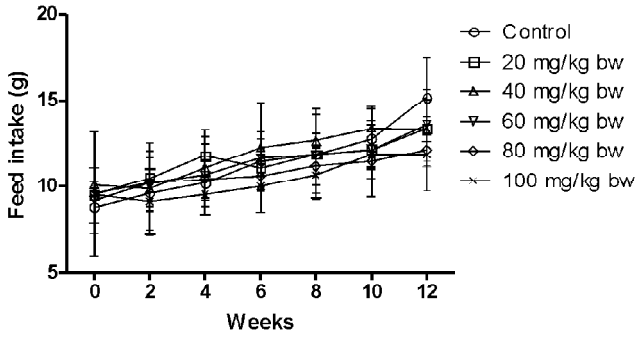

Fig. 2. Feed intake of rats after treatment with different concentrations of TNPs. The feed intake of control rats increased with age while the feed intake of rats decreased after treatment with TNPs. Each data represents the mean ± SD (n = 5).

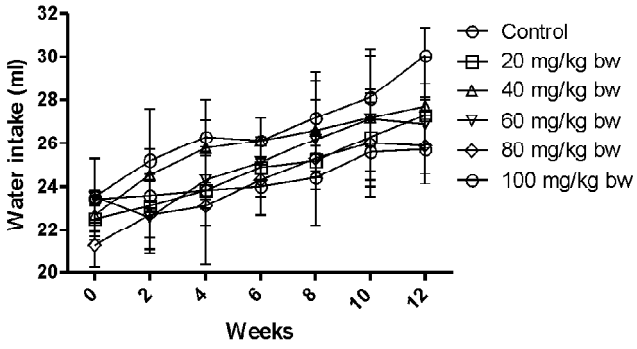

Fig. 3. Water intake of rats after treatment with different concentrations of TNPs. The water intake of control rats increased with age while the water intake of rats decreased after treatment with TNPs. Each data represents the mean ± SD (n = 5).

Three main pathways for absorption of particles across intestinal barriers can occur. First, paracellular transport can take place for small molecules that can pass the tight junctions. Second, transcytosis may appear across enterocytes, but mostly across M-cells located in the Peyer'spatches. And third, particles (nano and microsized) can be transported across degrading enterocytes[17]. Janer *et al*. proposed that transcytosis across enterocytes and transportation across degrading enterocytes could have occurred after exposure to TNPs. Eleonore *et al.* also observed that after administration, TNP enters the systemic circulation by overcoming the epithelial barrier and infiltrate organs[18]. However, it is currently unclear, whether TNPs can permeate through the buccal epithelium and can reach the systemic circulation bypassing the liver. Similarly, Shahare *et al.* indicated that the epithelial cells of gastrointestinal tract get destroyed after exposure to silver nanoparticles and are the reason for the decrease in body weight of mice[19]. Thus, after oral ingestion, TNP interacts with intestinal membrane and disrupts the metabolism of rats which in turn may cause the loss in body weight of rats.

Hematology analysis:

Blood cell count analysis is normally used to detect the hematological toxicity of different chemicals. After 12 weeks of study, hematology parameters were analyzed for various parameters including WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYO, RDW, NEU, MONO, EOS and BASO. Significant changes were observed for WBC, HCT, MCV, MCHC, NEU, EOS and BASO. Table 2 depicts the changes in hematology parameters after TNP treatment. Most of the param-

Table 2. Evaluation of various hematological parameters after exposure to different concentrations of TNP

Hematological	Dose (mg/kg bw)					
Parameters	0 (Control)	20	40	60	80	100
WBC ($10^3/\mu L$)	5.27±0.18	5.32±0.17	5.52±0.52	5.87±0.73	6.03±0.51	6.25±0.79*
RBC ($10^6/\mu L$)	8.31±0.24	8.49±0.14	8.62±0.36	8.81±0.31	8.98±0.28	9.18±0.52
HGB (g/dL)	16.11±0.23	16.07±0.24	16.01±0.14	15.98±0.15	15.97±0.21	15.95±0.25
HCT (%)	34.39±1.36	34.38±0.85	34.35±1.03	34.33±0.71	34.26±1.04	34.17±0.43
MCV (fl)	40.71±1.19	40.78±0.34	40.94±1.05	41.38±0.35	41.53±0.18	41.72±0.81*
MCH (pg)	19.35±0.21	19.38±0.25	19.43±0.61	19.50±0.14	19.54±0.41	19.59±0.27
MCHC (g/dL)	47.21±1.16	47.15±0.75	46.79±1.04	46.32±0.64	46.07±1.12	45.93±1.06*
PLT ($10^3/\mu L$)	770.6±82.7	737.1±57.4	708.1±46.4	692.1±61.3	685.1±43.1	673.1±24.1*
LYO (%)	70.72±3.82	70.54±1.16	69.98±1.23	69.29±2.04	68.74±1.54	68.21±1.69
RDW (%)	19.33±0.83	19.28±1.27	19.21±1.33	19.15±1.74	19.02±2.06	18.96±2.28
NEU (%)	24.19±1.73	24.55±2.07	25.01±2.11	25.81±2.44	26.36±2.09	27.57±1.02*
MONO (%)	3.13±0.95	3.28±0.88	3.41±1.79	3.63±0.61	3.98±0.94	4.53±0.94
EOS (%)	0.41±0.03	0.37±0.03	0.32±0.03	0.28±0.05	0.19±0.06	0.14±0.01*
BASO (%)	0.16±0.02	0.14±0.01	0.11±0.03	0.07±0.04	0.04±0.02	0.01±0.02*

eters were significantly altered for the higher doses, that is for 100 mg/kg bw treatment dose. Younes *et al.* observed that there was no change in RBC or WBC but observed significant change in PLT. This may be due to the fact that Younes *et al*. conducted the study for 14 days, however, our study was for 12 weeks. Platelets are characterized by expert functions in assisting and modulating inflammatory reactions and immune responses[9]. Inflammation was further confirmed in the study by observing the alteration in liver and kidney enzymes. NEU have a major role in innate immunity and during inflammation. In our study, a significant increase in NEU was observed for higher dose which indicates inflammatory response. Goncalves *et al*. have reported that TNP exerts neutrophil agonistic properties and activation of certain cytokines[20]. Hemolysis activity and interaction with HGB have been reported earlier by *in vitro* studies[21,22]. However, we did not observe any significant changes in RBC count or HGB levels. Nemmar *et al*. observed that intratracheal instillation of TNPs experienced a dose-dependent increase in macrophage numbers, influx of neutrophils in their bronchoalveolar lavage fluid and platelet aggregation in whole blood[23]. This indicates that TNP interacts with various biomolecules and activates certain cytokines which in turn generates inflammation.

Serum biochemistry analysis for liver and kidney function:

In our study, we analyzed different parameters for serum biochemistry such as ALB, CHO, CRE, GLU, LDH, TP, GLO, IP, TRG, Na, K, and certain enzymes. As depicted in Table 3, significant changes were observed for the higher doses, that is 80 mg/kg bw and 100 mg/kg bw. A significant change was observed for CHO, GLU, TRG and LDH indicates hepatic toxicity. Shukla *et al*. indicated that TNP induces oxidative stress and triggers DNA damage in mice[24]. Similarly, Shrivastava *et al.* observed altered antioxidant enzymes activities and ROS generation in mice[25]. Thus, it leads to inflammation in liver cells that directly affects the metabolism in rats. Further, liver and kidney enzymes were studied for alteration after exposure to TNP.

As depicted in Fig. 4; total, direct and indirect bilirubin was analyzed. No significant change was observed for the doses 20 mg/kg bw and 40 mg/kg bw. Also, indirect bilirubin was higher at 40 mg/kg bw than any other dose. For direct bilirubin, a significant increase ($p < 0.05$) was observed for 80 and 100 mg/kg bw as compared to control. However, there was also a significant increase ($p < 0.01$) at 60 mg/kg bw which was greater than the higher dose. The total bilirubin was significantly increased ($p < 0.01$) for 60 mg/kg bw, 80 mg/kg bw and 100 mg/kg bw as compared to control. It was also observed for higher doses that the total bilirubin was mostly conjugated (direct) bilirubin. When more than 50% of the total bilirubin is conjugated (direct) bilirubin, hepatocellular injury can be inferred. This suggests that hepatocellular injury may have occurred at higher doses.

Table 3. Evaluation of various biochemical parameters after exposure to different concentrations of TNP

Biochemical	Dose (mg/kg bw)					
Parameters	0 (Control)	20	40	60	80	100
ALB (g/dL)	2.85±0.04	2.81±0.01	2.73±0.06	2.67±0.03	2.55±0.05	2.40±0.03*
CHO (mg/dL)	101.±1.28	113.54±1.85	126.72±1.14	138.62±0.96	143.76±1.06*	161.60±1.59*
CRE (mg/dL)	0.89±0.41	0.93±0.16	0.97±0.14	0.99±0.17	1.03±0.19	1.09±0.18
GLU (mg/dL)	147.62±7.11	151.59±3.56	160.67±4.96	179.47±3.16	191.53±2.70*	206.22±1.69**
LDH (IU/L)	747.33±11.35	726.09±17.96	682.61±11.36	588.47±16.49	527.02±20.01*	484.11±16.56**
TP (g/dL)	6.51±0.19	6.43±0.04	6.27±0.18	6.13±0.15	6.02±0.20	5.92±0.19
IP (mg/dL)	6.79±0.95	6.60±0.71	6.37±0.57	6.08±0.31	5.84±0.75	5.53±0.61
TRG (mg/dL)	40.38±2.48	43.85±2.48	49.47±3.84	52.28±2.57	60.06±1.28*	67.56±3.97*
Na (mmol/L)	151.77±2.47	151.63±0.73	151.96±0.61	152.02±0.61	152.08±0.31	152.08±0.15
K (mmol/L)	4.03±0.51	3.99±0.09	3.94±0.06	3.92±0.07	3.88±0.06	3.82±0.04

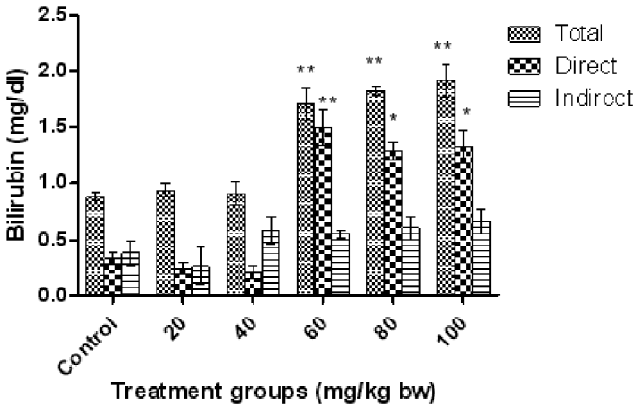

Fig. 4. Effect on total, direct and indirect bilirubin in Wistar rats after treatment with TNPs. Significant increase in total bilirubin was observed for 60, 80 and 100 mg/kg bw. Each data represents the mean ± SD (n = 5).

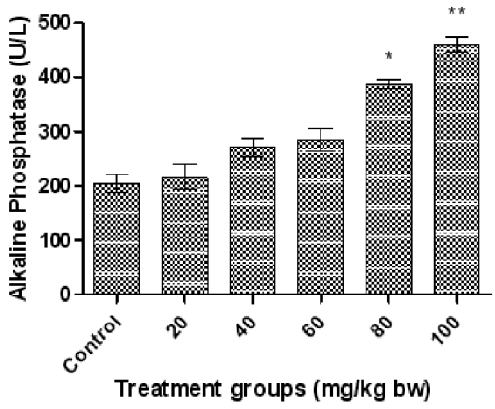

Fig. 5. Effect on ALP in Wistar rats after treatment with TNPs at different concentrations. Significant increase was observed for higher doses. Each data represents the mean ± SD (n = 5).

Alkaline phosphatase activity is a measure of Type II epithelial cell secretory activity and increased ALP activity is considered to be an indicator of Type II cell toxicity[26]. As depicted in Fig. 5, a significant increase was observed for higher doses only. At a dose of 100 mg/kg bw, the increase was more significant ($p < 0.01$) than for 80 mg/kg bw ($p < 0.05$). The level of ALP was more than double for 100 mg/kg bw as compared to control. The levels increased from 205.4± 16.6 U/L of the control group to 388.1±8.3 U/L and 461.7± 13.3 U/L for 80 mg/kg bw and 100 mg/kg respectively. Interestingly, there was no significant increase for 60 mg/kg bw as was observed for total bilirubin.

In the present study, a significant increase in AST levels was observed for the higher and medium dose, that is for 60, 80 and 100 mg/kg bw as depicted in Fig. 6. Interestingly, the

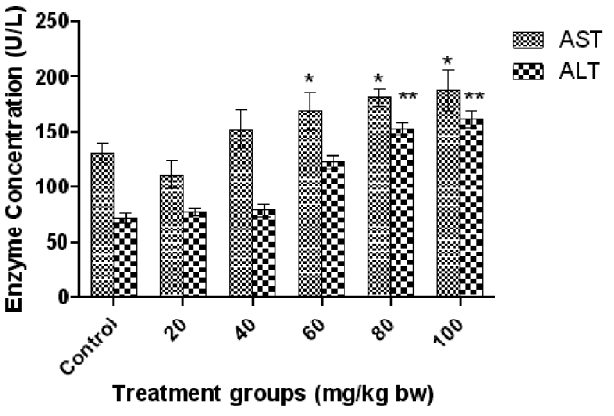

Fig. 6. Effect on AST and ALT in Wistar rats after treatment with TNPs at different concentrations. Each data represents the mean ± SD (n = 5).

levels were significantly higher than the control ($p < 0.05$) but almost similar levels were obtained for 60, 80 and 100 mg/kg bw. The levels of AST increased from 130.4±9.2 U/L of control to 188.1±11.9 U/L at the treatment of 100 mg/kg bw. However, for ALT levels more significant increase was observed ($p < 0.01$) for 80 and 100 mg/kg bw treatment dose than for 60 mg/kg bw ($p < 0.05$) as depicted in Fig. 6. The levels increased from 72.2±4.8 U/L of control to 161.6±7.2 U/L. Younes *et al.* also observed an increase of the AST/ALT enzyme ratio and LDH activity after exposure to TNP intraperitoneally for 14 days. LDH is an important isoenzyme in glycolysis and glyconeogenesis and widely exists in the heart, liver, lung, and many other tissues. When the tissues are subjected to injury, LDH would leak into the serum of blood from organs or cells, which resulted in the increase of LDH activity and its isoenzyme in the corresponding organ[9]. However, some studies have reported no significant change in AST and ALT levels[14,15]. This may be due to lower doses of TNP concentration administered. Fabian *et al.* administered 5 mg/kg body weight of TNP while Liang *et al.* admininstered 50 mg/kg body weight. We observed a significant change at 60 mg/kg body weight for AST but no significant change at 40 mg/kg body weight.

An increase in levels of GGTP was observed after 12 weeks repeated treatment with TNPs. A significant increase ($p < 0.01$) was observed for the higher doses, 15.8±1.4 U/L and 16.2±0.9 U/L for 80 and 100 mg/kg bw respectively as depicted in Fig. 7. Interestingly, a significant increase ($p < 0.05$) was also observed for 40 mg/kg bw whereas no significant change was observed for other enzymes and protein at this treatment dose. Several *in vitro* and *in vivo* studies have indicated the inflammatory action of TNP due to oxidative stress.

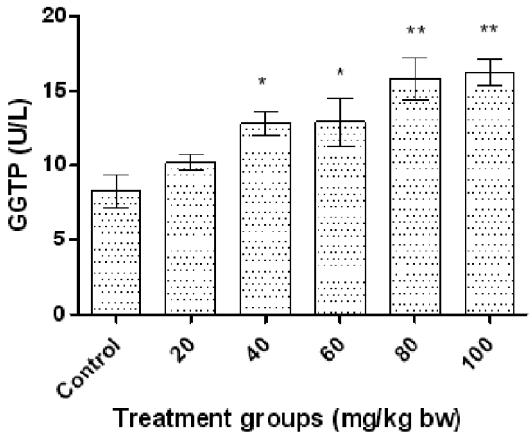

Fig. 7. Effect on GGTP in Wistar rats after treatment with TNPs at different concentrations. Each data represents the mean ± SD (n = 5).

Histopathology:

In order to investigate the toxicity of TNPs and the deposition within the cells, histopathology was performed on tissues from major organs. At the end of the study, rats were sacrificed and the tissues of lungs, liver, kidney, pancreas and heart of the rats of higher treatment dose 100 mg/kg bw were analyzed. Major congestion was observed in the lung tissue as compared to the control as depicted in Fig. 8a. A number of studies have indicated pulmonary toxicity after exposure to TNP. Bermudez *et al.* have reported that an inhalation of 10 mg/m^3 TNP for 13 weeks have induced pulmonary load in rats and mice but not in hamsters. They also reported inflammation response as increased neutrophils and macrophages and fibroproliferative lesions in pulmonary tissue[27]. As indicated in our study, accumulation was more in lungs and liver tissue. MNC (Mononuclear cell) infiltration and congestion was observed for liver tissue as shown in Fig. 8b. Particle deposition was also observed in the liver cells. Federici *et al*. reported that there was minor fatty change and lipidosis in liver, and some hepatocytes showed condensed nuclear bodies (apoptotic bodies) in rainbow trout (*Oncorhynchus mykiss*). They observed that oxidative stress and depletion of glutathione was present in gills, however, no significant accumulation was observed[28].

Tubular degeneration and tubular dilation was observed in kidney tissue. Due to particle deposition, the cells appear to be constricted as indicated in Fig. 8c. Gui *et al.* nephric inflammation, cell necrosis and dysfunction and activation of various inflammatory cytokines indicating nephric injury in mice after TNP exposure[29]. However, no abnormalities were observed for heart (Figs. 8d and 8e) and pancreatic tissue (Figs. 8f and 8g). No severe damage was observed in these tissues and the cellular morphology and membrane integrity was maintained.

Determination of TNP concentrations in tissues:

In the present study, the concentration of TNP was determined by ICP-MS and tabulated in Table 4. The concentration of TNP was maximum in liver tissues than rest of the tissues examined. Previous reports have indicated that TNP induces pulmonary load in rats. Younes *et al*. observed an

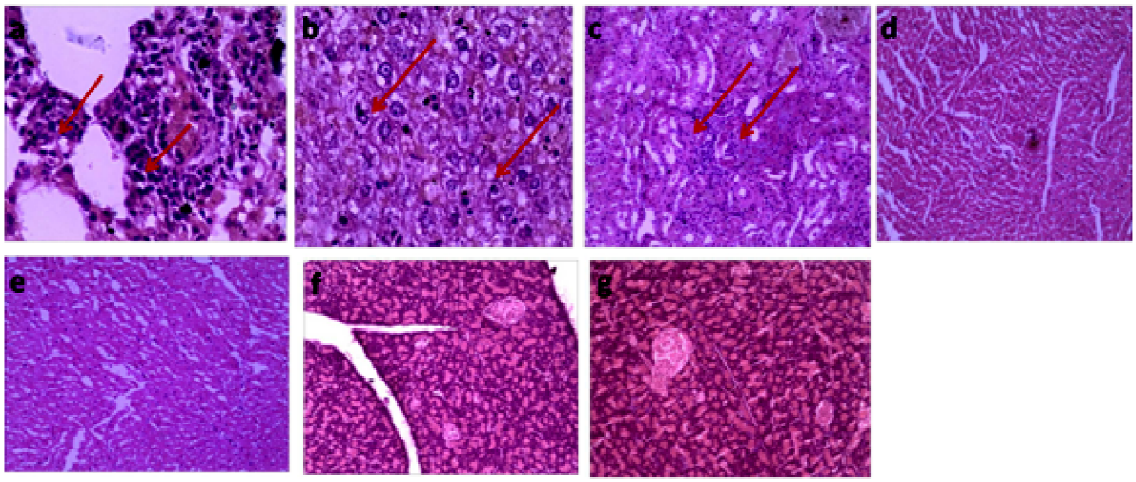

Fig. 8. Histopathology of (a) lung tissue of Wistar rats treated with TNP, arrows indicate congestion in bronchial cells; (b) liver tissue of Wistar rats treated with TNP, arrows indicate MNC (Mononuclear cell) infiltration, particle deposition and congestion in hepatic cells; (c) kidney tissue of Wistar rats treated with TNP, arrows indicate tubular degeneration and tubular dilation in kidney cells; (d) control rats with normal heart cells; (e) heart tissue of Wistar rats treated with TNP, no significant changes were observed after the treatment; (f) control rats with normal pancreatic cells; (g) pancreatic tissue of Wistar rats treated with TNP, no significant changes were observed after the treatment.

Table 4. Concentration of TNP in different major organs as estimated by ICP-AES. Each data represents the mean ± SD (n = 5)

Parameter	Lungs	Liver	Kidney	Heart	Pancreas
TNP (µg of titanium/g of organ)	21±0.3	38.1±0.4	12.3±0.2	0.17±0.01	0.1±0.03

increase in the relative weight of lung tissues after exposure to TNP intraperitoneally. However, they did not observe any significant change in body weight and the relative weight for liver, kidney and brain. This indicates that TNP accumulates more in lung tissue[9].

Conclusions

In this study, a concentration of 40 mg/kg bw was observed to be toxic in nature. It impaired liver and kidney functions as depicted from the enzyme levels. The LD_{50} value was estimated to be more than 2000 mg/kg bw as observed in acute toxicity studies. Particle deposition and congestion was observed in major organs. However, heart and pancreas tissue were not affected even by the higher doses. Thus, TNPs are not completely eliminated from the body and these trapped TNPs in major organs can cause oxidative stress and ROS which leads to chromosomal aberration and DNA damage. On the basis of the data obtained in this study, it is concluded that up to 40 mg/ kg is a safe dose of TNPs and is recommended for use.

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