Journal of Academia and Industrial Research (JAIR)
Volume 3, Issue 12 May 2015

ISSN: 2278-5213

RESEARCH ARTICLE

Titanium Dioxide Nanoparticles Induced Alteration in Haematological Indices of Adult Male Wistar Rats

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Abstract

Nanoparticles are extensively being used in modern life due to their distinctive properties such as small size and large surface area per mass. These unique features of large surface area may exhibit very high biological reactivity on living organisms. Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in cosmetics, food additives, pharmaceuticals and engineering including electronics. Their wide applications may reveal high exposure to humans, so analysis of its reactivity and toxicity in the body is important. The present study aimed to investigate the effects of rutile and anatase mixed TiO_2 NPs (<100 nm) on the changes of haematological biomarkers in male Wistar rats. Findings showed most prominent changes in red blood cells ($P<0.001$), haemoglobin ($P<0.001$), haematocrit ($P<0.001$), mean corpuscular volume ($P<0.001$), platelets ($P<0.001$) and total platelet crit ($P<0.001$) of TiO_2 NPs intoxicated rats. Our findings indicate that reticulo-endothelial system damage in rat treated with TiO_2 NPs is likely associated with the damage of haemostasis blood system.

Keywords: Nanoparticles, Titanium dioxide, Wistar rats, haematocrit, haematological biomarkers.

Introduction

Nanotechnology is a promising field of science and technology, to deal with the effects of engineered or manufactured nanomaterials and their applications on living organisms. It has been observed that the materials in fine dimensions have high chemical and biological reactivity because of their unique property of large surface area per mass. These properties will dispute to the potential health risk on human population. The total production of nanomaterials was 2000 tons in the year of 2004 and it is likely to increase 58,000 tons in 2020. Such increase is possible to impact the entire ecosystems (Oberdorster *et al.,* 2005). Titanium (Ti) is one among the widely used nanomaterials, which is 9th most abundant metallic element in the earth's crust and it has been frequently enters the food chain to some degree. Oral route is a potential exposure for human population due to TiO_2 NPs used as white pigment on toothpaste and drug products (Ghoropade *et al.,* 1995; Baan *et al.,* 2006; Jovanovic, 2015), in dairy based products, it has been used as a whitener in different types of cheese (Leone, 1973), chocolate, milk powder, soybean products, margarine, processed meat, soda water and sausage casing (JECFA, 2006). Human is estimated to use approximately 300 mg of titanium per day with commercial food items (Dunford *et al.,* 1997). But, India restricts the uses of TiO_2 NPs to chewing gum and bubble gum with a level not to exceed 1% and to powdered concentrate mixes for fruit beverage drinks not to exceed 100 mg/kg (India, 2004a,b). While tremendous positive impacts of TiO_2 NPs are widely publicized, potential risks to environment and human health are just beginning to emerge. Very few studies have employed TiO_2 NPs toxicity in different organisms. Moreover, TiO_2 NPs is an essential man-made product for food additives as well as cosmetics, which could exhibit a variety of toxic responses leading prolonged exposure to high concentration either inhalation or by orally (Chen *et al.,* 2006). The importance of haematological analysis in clinical studies is well established. Haematological markers may be used as suitable indicators of disease or stress in animals (Calabrase *et al.,* 1975). Blood parameters are possibly more rapid and measurable variations under stress condition and are valuable in assessing the health state of human and animals (Hymavathi and Rao, 2000). Particularly, haematological markers are commonly used to resolve systematic relationship and physiological adaptations including the evaluation of general health conditions in human beings (Alkinson and Judd, 1978). Hence, it has long been used as diagnostic tools to examine physiological, pathological and metabolic alterations in living systems (Bansal *et al.,* 1979; Hardikar and Gokhale, 2000). However, TiO_2 NPs toxicity study was poorly documented in different status but not elaborated in haematologic levels. Therefore, the present study is aimed to investigate the oral toxic effects of TiO_2 NPs on haematological biomarkers in adult male Wistar rats.

Materials and methods

Experimental animals: Adult male Wistar rat strain (*Rattus norvegicus*) weighing 240-260 g was used in this study. The animals were maintained as per the Institute Animal Ethical Committee (IAEC) guidelines.

Healthy animals were kept under hygienic conditions, housed in polypropylene cages with soft paddy husk as bedding. Sterilized food and water for rats were available *ad-libitum*. They were acclimated to this environment for 5 d prior to dosing. All experimental protocols were approved by IAEC (138/PHARMA/SCRI, 2013).

TiO_2 NPs dose preparation and treatment: Characterized TiO_2 NPs (mixture of rutile and anatase <100 nm) were purchased from Sigma-Aldrich chemicals Co. (St, Louis, MO, USA). 0.9% saline solution (NaCl) was used as a suspending agent. TiO_2 NPs powder was dispersed onto the surface of saline solution and then, the suspending solutions containing TiO_2 NPs were ultrasonicated for 10 min and mechanically vibrated for 2 or 3 min before the treatment. Animals were randomly selected into three groups: each group contains six rats (n=6), control group treated with normal saline (0.9% NaCl) and two experimental groups (50 and 100 mg/kg BW TiO_2 NPs). TiO_2 NPs suspension (50 and 100 mg/kg BW) were given to rats by intragastric administration every day for 14 d. The symptoms and mortality were observed carefully every day. On the 15^{th} d, all the animals were weighed and blood was collected by the ocular sinus puncture method. Then, the blood samples were stored at room temperature for haematological studies.

Haematological investigation: The blood samples were collected into separate tubes containing ethylene diamine tetra acetic acid (EDTA) anti-coagulant for haematological studies. Haematological parameters including white blood cell count (WBC), numbers of lymphocytes (LYM), percent of lymphocytes (LYM%), numbers monocytes (MONO), percent of monocytes (MONO%), numbers of granulocytes (GRA), percent of granulocytes (GRA%), erythrocytes (RBC), haemoglobin (HGB), haematocrite (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) as well as total platelet crit (PCT) were analyzed using Auto-hematology analyzer (MINDRAY-BC-2800, USA).

Statistical analysis: The data were analyzed using Student's t-test and the data were expressed as mean ± standard error mean (SEM). The value of $p<0.05$ was considered as significant value against the control.

Results and discussion

The knowledge of haematological analysis is a vital tool, which can be used as a sensitive index to observe the internal environment of animals and humans. Considering the major role of haematology, the following haematological markers including WBC, RBC, HGB, HCT, PLT, MCV, MCH, MCHC, RDW, MVP, PDW and PCT as well as total numbers and percent of LYM, MONO and GREA were analyzed. There was no significant changes in WBC, but a noticeable decrease was observed in RBC ($P<0.001$), GRA ($P<0.01$), HGB ($P<0.001$), MCHC ($P<0.01$) and PCT ($P<0.001$) in the TiO_2 NPs treated groups. However, total numbers and percentage of LYM and MON exhibited non-significant changes, while the total number of GRA ($P<0.01$) were decreased in 100 mg/kg BW of TiO_2 NPs treated rats. Conversely, HCT ($P<0.001$), MCV ($P<0.001$), MCH ($P<0.05$), PLT ($P<0.001$), MPV ($P<0.05$) and RDW ($P<0.05$) were significantly increased in the TiO_2 NPs treated groups when compared with control (Table 1). Therefore, the influence of TiO_2 NPs was maximum in 100 mg/kg BW than the 50 mg/kg BW compared to control. In our study, orally administered rats with TiO_2 NPs showed non-significant reduction of WBCs. It is predicted that high concentration of TiO_2 NPs, decreased the number of blood cells due to inhibition of cell activity, stimulation of oxidative stress in cells, reduction of cellular antioxidants and increasing of cells in the immune responses (Nemmar *et al.,* 2008; Bu *et al.,* 2010). Nevertheless, oral administration of high dose of silver NPs for 28 d exposed rats showed liver damage but no genotoxicity in erythrocytes and bone marrow (Kim *et al.,* 2008). However, free radicals induced by NPs can cause destruction of blood cells (Machiedo *et al.,* 1989). The very lesser diameter of the nanoparticles is more reactive to the cells and its molecular effects on intracellular mechanisms will increase. Due to large surface area per mass and more effect on the cell membrane in higher doses, silver nanoparticles leads to effects on WBCs and changes in their mitochondrial enzyme activity of male and female rats (Cheraghi *et al.,* 2013). Jani *et al.* (1994) reported that orally ingested TiO_2 NPs can be absorbed through gastrointestinal tract and pass via the mesentery lymph supply and lymph node to the systemic organs. Therefore, these NPs can interact with systemic circulation and their metabolites such as, coagulation factors, PLT, WBC and RBCs. For this reason, TiO_2 NPs adversely affects the structural and physiological changes of the cells, which can susceptible for RBC and WBCs to be destroyed when they pass recticulo-endothelial systems. We observed significantly increased MCV and decreased MCHC in TiO_2 NPs treated rats, suggesting that TiO_2 NPs may induce a kind of macrocytic and hypocromic anemia in rats. The HGB levels in the TiO_2 NPs treated rat groups attend to decrease and also with according to considerable elevation in MCV, diminishing amount of MCHC in rat groups may be warranted. Significant increases in MCV can be due to interruption in mitotic period and DNA damage is one of the causes can stimulate this process. In this view, it has been reported that silver NPs have possibly to causes DNA damage and chromosomal aberrations are able to stimulate proliferation or arrest in cell lines of zebra fish (Asha Rani *et al.,* 2008).

Table 1. Haematological parameters in rats by oral administration with TiO_2 NPs for 14 consecutive days.

Parameters	Control	50 mg/kg BW TiO_2 NPs treated	100 mg/kg BW TiO_2 NPs treated
WBC (10^9/L)	10.46 ± 0.942	9.18 ± 0.684	9.00 ± 0.470
Lymphocyte (10^9/L)	7.13 ± 0.762	6.66 ± 0.616	6.23 ± 0.331
Monocyte (10^9/L)	0.266 ± 0.033	0.266 ± 0.033	0.216 ± 0.016
Granulocyte (10^9/L)	3.06 ± 0.210	2.50 ± 0.223	2.30 ± 0.093**
Lymphocyte (%)	71.73 ± 1.37	69.25 ± 1.92	67.36 ± 1.75
Monocyte (%)	3.033 ± 0.190	2.83 ± 0.120	2.76 ± 0.192
Granulocyte (%)	29.80 ± 1.66	27.71 ± 1.77	25.5 ± 1.24
RBC (10^{12}/L)	8.43 ± 0.134	8.11 ± 0.236	7.24 ± 0.142***
HGB (g/dL)	14.46 ± 0.327	13.81 ± 0.394	12.08 ± 0.208***
HCT (%)	35.96 ± 0.637	42.38 ± 0.866***	42.7 ± 1.31***
PLT (10^9/L)	202.0 ± 6.28	296.5 ± 13.20***	322.33 ± 24.03***
MCV (fL)	49.73 ± 0.261	50.30 ± 0.446	52.65 ± 0.187***
MCH (pg)	16.63 ± 0.098	16.98 ± 0.188	17.10 ± 0.152*
MCHC (g/dL)	34.08 ± 0.274	33.56 ± 0.128	32.31 ± 0.412**
RDW (%)	10.50 ± 0.143	11.36 ± 0.247*	12.01 ± 0.482*
MPV (fL)	5.80 ± 0.068	6.00 ± 0.057*	6.03 ± 0.042*
PDW	15.05 ± 0.105	15.06 ± 0.075	15.11 ± 0.060
PCT (%)	0.194 ± 0.014	0.177 ± 0.007	0.116 ± 0.004***

Values indicate mean ± SEM from six rats per group (n=6). Statistical significance at *P<0.05, **P<0.01 and ***P<0.001.

This study demonstrated significantly increased PLT and RDW in both 50 and 100 mg/kg BW of TiO_2 NPs exposed rat groups. In contrast, a study indicated that the elevation of MCV, MCH and RDW in mice caused by TiO_2 NPs suggested massive corpuscle anemia and the increases of PLT and MPV showed a possible effect of TiO_2 NPs on blood coagulation, causing a severe damage of PLTs but improving the metabolic function of the bone marrow (Duan *et al.,* 2010). Thus, further the decreased RBC count and HGB concentration caused by high doses of TiO_2 NPs anatase could cause a decrease oxygen content in the blood than might decreased metabolic and immune responses of mice (Duan *et al.,* 2010). Moreover, nanoparticles or nanotubes can affect the endothelial function, leading to sequestration of RBCs and PLTs, which could impair systemic circulation and promote thrombosis (Donaldson *et al.,* 2001; Bihari *et al.,* 2010). Therefore, the toxic effects of nanomaterials and their applications on human is the major concern of health industry. Meanwhile, NPs are able to cross the biological membrane and access to cells, tissues and organs. It can gain access to the blood stream through inhalation or ingestion. This may lead to biochemical toxicity due to accumulation of these particles in systemic circulation as suggested Oberdorster *et al.* (2005).

Conclusion

From the study, it is concluded that exposure of TiO_2 NPs produce an adverse effect in haematological markers of rats. In view of the consequence of WBC in protecting the human body and the vital role of RBC in daily life, any changes made in their structure and number of these cells can cause physiological changes for human body. On the other hand, extensive use of the different composition of TiO_2 NPs in the whole world requires more accurate and complete studies on the effects of these NPs on blood cells. Therefore, such NPs exposure is possible to generate the toxicity in human populations.

Acknowledgements

Authors would like to express their thanks to the Director, Siddha Central Research Institute, (SCRI), Chennai for providing necessary animal house and lab facilities to carry out this study successfully.

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