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.
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 acclimatized 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 15th 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), haematocrit (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 hypochromic anemia in rats. The HGB levels in the \[TiO_2\] NPs treated 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).
This study demonstrated significantly increased PLT and RDW in both 50 and 100 mg/kg BW of TiO₂ NPs exposed rat groups. In contrast, a study indicated that the elevation of MCV, MCH and RDW in mice caused by TiO₂ NPs suggested massive corpuscle anemia and the increases of PLT and MPV showed a possible effect of TiO₂ 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 TiO₂ 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).

Therefore, such NPs exposure is possible to generate the toxicity in human populations.

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References


