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Toxicological assessment of nano and micron-sized tungsten oxide after 28 days repeated oral administration to Wistar rats

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ABSTRACT

Tungsten oxide (WO_3) nanoparticles (NPs) are being used in various applications. However, the health consequences of WO_3 NPs exposure have not been explored extensively. Hence, the goal of this study was to evaluate the toxicity of WO_3 NPs and their microparticles (MPs) after 28 days repeated oral administration in Wistar rats. The particles were characterised by transmission electron microscopy (TEM), dynamic light scattering (DLS), laser Doppler velocimetry (LDV), Brunner-Emmett-Teller (BET), X- ray diffraction (XRD), and inductively coupled plasma optical emission spectrometer (ICP-OES). Genotoxicity was determined using comet assay in blood and liver and micronucleus test in bone marrow. Biochemical parameters such as aspartate aminotransferase and alanine aminotransferase in serum and reduced glutathione content, catalase and lipid peroxidation in liver tissue were determined. Histopathological changes in tissues were documented. Biodistribution of tungsten (W) in rat's blood, urine, feces and tissues were analysed. The mean size of WO_3 NPs and MPs by TEM was 52 ± 2.97 nm, and 5.73 ± 7.58 µm and morphology were spherical in both the particles. DLS of NPs was 195.6 nm. XRD and BET data of WO_3 NPs and MPs showed a hexagonal and tetragonal crystal structure and surface area of 19.33 and 15.15 (m^2/g), respectively. The results revealed a significant increase in DNA damage and micronuclei, a difference in biochemical levels and histopathological alterations after exposure to 1000 mg/kg dose of WO_3 NPs. W biodistribution was detected in all the tissues in a dose and organ-dependent manner in both the particles. The highest amount of W was found in the liver and lowest in the brain of the treated rats. The tested NPs were found to have little toxicity hazard.

1. Introduction

Significant development and impressive profit have been achieved in the field of nanotechnology. One angle of this commercial enterprise consists of manufacturing nanoparticles (NPs). The properties exhibited by NPs are different from their microparticles (MPs) (> 100 nm) because of their size, structure and high surface area [1]. During the last couple of years, more than 1800 NP containing goods have become available in the market [2,3]. Hence, NPs may gain access to the human body through dermal, inhalation and oral routes during manufacture, use and disposal [4]. Since the NPs are found to be stable, it is anticipated that they may be retained in the human tissues and the environment for a long time. The likely toxicological effects of exposure to NPs are not well explored [5,6]. However, the limited literature indicates potential toxic side effects [7]. Acute and chronic toxicity to humans and environment induced by NPs have recently become a serious concern. Several investigations have evaluated the *in vivo* toxicity of NPs. The results revealed significant genotoxicity and biochemical alterations [8–11]. Further, a few studies have shown that NPs cause oxidative stress [12–15]. The actual mechanism of the carcinogenic effect of metals has not yet been studied. However, there is sufficient evidence to suggest that reactive oxygen species (ROS) play an important role [16]. Thus there is an urgent need to develop rapid, accurate and efficient testing strategies to assess toxic effects of these NPs.

Among several metal oxide NPs, tungsten oxide (WO_3) NPs have attracted an increasing interest due to their large surfaces and high-temperature stability in nanotechnological products [17]. They have

Abbreviations: WO_3 NPs, tungsten oxide nanoparticles; WO_3 MPs, tungsten oxide microparticles; TEM, transmission electron microscopy; DLS, dynamic light scattering; LDV, laser doppler velocimetry; XRD, X-ray diffraction; BET, Brunner–Emmett–Teller technique; ICP-OES, inductively coupled plasma optical emission spectrometer; OECD, Organization for Economic Co-operation and Development; MNT, micronucleus test; % PCE, percentage of polychromatic erythrocytes; MN-PCE, micronucleated polychromatic erythrocytes; MI, mitotic index; bw, body weight; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, platelets; WBC, white blood cells; ANOVA, analysis of variance

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well-known electrical, gasochromic, mechanical, and photo-electrochromic properties that can be used in various applications. WO_3 NPs are very useful as field emission devices, optical devices, light – emitting diodes, chemical sensors, and gas sensors for the determination of ammonia, hydrogen and nitrogen [18–22]. WO_3 NPs have gained considerable importance for discoloration of rhodamine B dye [23]. Further, they have been identified in strategic sectors like aerospace for various engine components, nose cones, jet vanes and kinetic energy projectiles [24]. Besides, WO_3 NPs inhibited the bacterial growth in patients having urinary tract infection with indwelling catheters and gave continuous protection to the site [25].

Possible health impact of WO_3 NPs on humanity and the environment is of great concern due to their increased use. Although nanoscale WO_3 are being used commercially, few studies have demonstrated that exposure to WO_3 NPs may lead to adverse effects. In an *in vitro* study, WO_3 NPs showed a positive mutagenic response in *Salmonella typhimurium* bacterial strains (TA1537 and TA98) using Ames test [26]. Cultured rat primary hepatocytes were used to evaluate the cytotoxicity and DNA oxidation of WO_3 NPs. There was a significant decrease in cell viability and an increase in 8- oxo-2-deoxy guanosine levels [27]. Eleven metal oxide NPs along with WO_3 NPs were examined for cell viability in A549, Caco-2 and 3T3 cell lines. The WO_3 NPs showed a toxic effect (> 100 μg/ml) with neutral red uptake assay [28]. The genotoxic potential of WO_3 NPs was evaluated in bone marrow cells of Sprague-Dawley rats by intraperitoneal (IP) injection daily for 30 days. Significant increase in MN was noticed [29]. Prajapati et al. [30] investigated the pulmonary toxicity of WO_3 NPs in golden syrian hamsters after inhalation for 4 h/day for 4 and 8 days of exposure. The NPs caused significant cytotoxicity, morphological changes in the lung tissue at both the exposure periods. *In vivo* toxicology of WO_3 NPs by oral route has not been reported till date. In view of the above fact, the current study was undertaken to investigate the 28 days repeated oral toxicity study of WO_3 NPs and MPs in albino Wistar male and female rats. The particles were initially characterised by using dynamic light scattering (DLS), laser doppler velocimetry (LDV), transmission electron microscopy (TEM), X- ray diffraction (XRD), Brunauer–Emmett–Teller (BET) and inductively coupled plasma optical emission spectrometer (ICP-OES) before conducting the main study. Genotoxicity was investigated using comet and micronucleus tests. Haematological limits like red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (HCT) and mean corpuscular volume (MCV) were estimated. Biochemical parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reduced glutathione (GSH) content, catalase and lipid peroxidation were determined. Histopathological changes were examined in rat tissues. Furthermore, biodistribution of tungsten (W) in rat's whole blood, urine, feces and tissues were analysed using ICP-OES after 28 days of treatment.

2. Materials and methods

2.1. Chemicals

All the chemicals were purchased from Sigma Chemical Co. Ltd (St Louis, MO, USA). However, Perchloric acid, Triton X-100, DMSO was obtained from Hi-media Laboratories (India) and were of analytical grade. Glassware and Plasticware were purchased locally.

2.2. NPs and MPs

WO_3 NPs ($WO_3 < 100$ nm, 99.8% purity, CAT No. 550086) and WO_3 MPs ($WO_3 < 20$ μm, 99% purity, CAT No. 232785) (according to the manufacture's datasheet) were purchased from Sigma Chemical Co. Ltd (USA).

2.2.1. Characterization of particles

WO_3 NPs and MPs were characterised using BET, DLS, ICP-OES, LDV, XRD and TEM to evaluate the specific surface area, size distribution and zeta potential, purity analysis, state of dispersion, crystal structure and material size.

2.2.2. TEM of WO_3 particles

A TEM was utilised to attain the size and morphology of WO_3 NPs and MPs. The images obtained from the TEM (JEOL, JEM-2100, Japan) at an accelerating voltage of 120 kV. The microscope has a plunge freezer and a cryo transfer holder to fix samples in frozen condition and fitted with a Gatan 2 K × 2 K CCD camera for acquiring high-resolution images. The WO_3 particles were suspended in water (0.01 mg/ml), and one drop of suspension was placed on a carbon-coated copper TEM grid and evaporated at room temperature. The NPs were examined by using advanced microscopy techniques software for the digital TEM camera. This software was calibrated for nanoscale size measurements. For the size determination, 100 particles were calculated from random fields of view and images showing the general morphology of the particles.

2.2.3. DLS and LDV of WO_3 particles in solution

The size of the particles and agglomerates in solution was determined by DLS and LDV with a Zetasizer Nano-ZS from Malvern Instruments (Malvern Instruments, UK). The device has a 4 mW He-Ne 633 nm laser and an electric field generator to measure the samples for LDV. Freshly prepared WO_3 particle suspensions (20 μg/ml in Milli-Q water) were ultrasonicated for 10 min at 90% amplitude, 100 W and 30 kHz using a probe sonicator (UPH 100, Germany). The samples were then transferred to a 1.5 ml cuvette for DLS measurement thereupon 1 ml was transferred to a Zeta Potential cell for LDV. The instrument software was used to evaluate the mean particle diameter from the distribution of particles by the polydispersity index (PdI) which was a criterion for determination of the size ranges of the particles present in the solution. The PdI scale ranged from 0 to 1 where 0 indicates monodispersed and 1 indicates polydispersed state of particles.

2.2.4. BET analysis

The specific surface area (m^2/g) of the particles was determined by N_2 adsorption– desorption measured at 77 K according to the BET protocol using a Quadrusorb-SI V 5.06 analyser (M/S Quanta chrome Instruments Corporation, USA).

2.2.5. XRD of WO_3 particles

The XRD pattern of the WO_3 particles was documented on a Bruker AXS D-8 Advance powder X-ray diffractometer (Shimadzu, Japan). The instrument was operated at a current of 30 mA, voltage of 40 kV and utilizing a CuKα radiation ($\lambda = 1.5406$ Å). The diffractometer was controlled with Datascan software and the scan specifications were set at a scan rate of 1.2° per minute and scan range was $2\theta = 0–80°$.

2.2.6. Purity analysis

The ICP-OES analysis was performed for the purity of the WO_3 NPs according to the methodology described by Yokel et al., 2009. The suspensions (10 mg/10 ml in Milli-Q water) of the particles were ultrasonicated for 10 min with a probe sonicator (UP100H, Hielscher Ultrasonics GmbH, Teltow, Germany) at 90% amplitude (100 W, frequency 30 kHz). The particles were then analysed by ICP-OES.

2.3. Animals

Female and male albino Wistar rats were procured from the National Institute of Nutrition, Hyderabad, India. The rats obtained were 6–8 weeks old and weighed 100–120 g. The animals were acclimatised in our animal house facility in polypropylene cages (5 per group). The animals had access to unlimited standard laboratory pellets for food and tap water for drinking. They were maintained under standard conditions of automated light cycles (12 h light/12 h dark), humidity (55–65%) and temperature (22 ± 3 °C). The study protocols