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Acute oral toxicity study of magnesium oxide nanoparticles and microparticles in female albino Wistar rats

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ABSTRACT

Advancements in nanotechnology have led to the development of the nanomedicine, which involves nanodevices for diagnostic and therapeutic purposes. A key requirement for the successful use of the nanoparticles (NPs) in biomedical applications is their good dispensability, colloidal stability in biological media, internalization efficiency, and low toxicity. Therefore, toxicological profiling is necessary to understand the mechanism of NPs and microparticles (MPs). MgO NPs have attracted wide scientific interest due to ease of synthesis, chemical stability and unique properties. However, their toxic effects on humans should also be of concern with the increased applications of nano MgO. The present study was aimed to assess the toxicological potential of MgO NPs in comparison to their micron counterparts in female Wistar rats. Toxicity was evaluated using genotoxicity, histological, biochemical, antioxidant and biodistribution parameters post administration of MgO particles to rats through oral route. The results obtained from the investigation revealed that the acute exposure to the high doses of MgO NPs produced significant ($p < 0.01$) DNA damage and biochemical alterations. Antioxidant assays revealed prominent oxidative stress at the high dose level for both the particles. Toxicokinetic analysis showed significant levels of Mg accumulation in the liver and kidney tissues apart from urine and feces. Further, mechanistic investigational reports are warranted to document safe exposure levels and health implications post exposure to high levels of NPs.

1. Introduction

Nanomaterials (NMs) are been used in biomedical applications as cosmetics, food products, antibiotic products and therapeutic preparations (Sahoo et al., 2007; Zhang et al., 2012). Among these NMs, nanoparticles (NPs) have good dispensability, colloidal stability in biological media, high internalization efficiency, and produce lower toxicity (Zhang et al., 2008) than those of corresponding microparticles (MPs). The unique physicochemical properties of NPs are responsible for their increased cell permeability and increased quantum effects as a function of their size (Hochella et al., 2008; Ivask et al., 2015). The utilization of NPs in various fields has increased throughout the world exponentially. However, the concerns that deliberate or accidental human and environmental exposure to NPs may lead to significant adverse effects (Oberdörster et al., 2007) which are inevitable. To overcome debates and concerns, nanotoxicology has emerged to address toxicological impacts of engineering NPs and safe use for mankind and environment (Drobne, 2007). The fact that NPs have been eliciting biological responses resulting in cellular toxicity and genotoxicity than MPs was demonstrated in several studies with different NPs (Arnold, 2013; Chen et al., 2008; Singh et al., 2013b; Wang et al., 2007; Zhang et al., 2010). Therefore, toxicological profiling is necessary to predict the potential hazards associated with the exposure of these particles. Consequently, NPs are able to get entry into the body via inhalation, dermal and oral routes. Moreover, Most of the health hazards occur through the entry of the NPs into GI tract could be via accidental ingestion through varied sources such as at manufacturing industries, contact with nano-structured surfaces, or along with the drinking water or eating food contaminated with NPs (Ahamed et al., 2011).

Among the known metal oxides magnesium oxide (MgO) NPs have attracted wide scientific interest due to ease of synthesis, chemical stability, unique properties and extensive applications in various fields. The main applications of nano MgO in chemical industry as catalyst, synthesis of petro-chemical products, corrosion

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Abbreviations

MgO NPs	Magnesium oxide nanoparticles
MgO MPs	Magnesium oxide microparticles
TEM	Transmission electron microscopy
DLS	Dynamic light scattering
LDV	Laser doppler velocimetry
XRD	X-ray diffraction
BET	Brunner–Emmett–Teller technique
OECD	Organization for economic co-operation and development
MNT	Micronucleus test
% PCE	Percentage of polychromatic erythrocytes
MN-PCE	Micronucleated polychromatic erythrocytes
ROS	Reactive oxygen species
ICP-OES	Inductively coupled plasma optical emission spectrometer
ANOVA	Analysis of variance

inhibitors, surface coatings, fuel additives, refractory fiber board and metallic ceramics (Krishnamoorthy et al., 2012; Moorthy et al., 2015). In the field of medicine, MgO NPs are being used in antacid preparations, detoxifying agents, bio molecular detection and diagnostics (Bertinetti et al., 2009; Martínez-Boubeta et al., 2010). Similarly, despite the wide applications of MgO nanoparticles, there is serious lack of knowledge related to their impact on human health and environment.

Very few investigations conducted with MgO NPs using *in vitro* and *in vivo* models revealed interesting and conflicting results. MgO NPs were tested for cytotoxicity against human umbilical vein endothelial cells (HUVECs) revealed that the treatment of these particles significantly enhanced the NO release and total antioxidation competence of the HUVECs. (Ge et al., 2011). However, these particles were least effective in inducing cytotoxic effects against human astrocytoma U87 cells (Lai et al., 2008) and human cardiac microvascular endothelial cells (HCMECs) (Sun et al., 2011). Another study reported on human liver epithelial cancer cell line revealed no significant genotoxic effects at the investigated concentrations (Kumaran et al., 2015). Ghobadian et al. (2015) studied on zebrafish embryos reported that these MgO NPs induced cellular apoptosis and intracellular ROS. An *in vivo* study conducted with MgO NPs on rats produced dose-dependent pulmonary toxicity after acute intratracheal instillation (Gelli et al., 2015). Another study reported reduction in total antioxidant capacity in serum of rats after acute intratracheal instillation of MgO NPs (Kiranmai and Reddy, 2013).

We are aware that the acute oral toxicity of MgO NPs and MPs in albino Wistar rats has not been previously investigated. *In vivo* studies for the toxicological evaluation of NPs are important because animal systems are extremely complicated and their interactions with biological systems could lead to novel immune response, metabolism patterns, biodistribution and clearance which provide useful information on likely health hazards assessment in mankind (Fischer and Chan, 2007). Three different doses (test groups) were used in the current investigation which ranged from least or no toxicity to highly toxic. The low dose of 100 mg/kg body weight (bw), was chosen to replicate probable human exposure as the workers unintentionally get exposed to the NPs through hands and to the mouth during manufacturing processes in work space (Singh et al., 2013b). Further, the highest dose of 1000 mg/kg bw, which showed symptoms of toxicity was chosen to see the effect when large quantities of NPs are released accidentally into the environment, and reach the human body (Kumari et al., 2014a).

The acute oral toxicity study was conducted as per Organization for Economic Co-Operation and Development (OECD) test guideline known as the “acute oral toxicity-fixed dose method” (OECD 420, 2001). The procedure provides information on the hazardous properties and allows the substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of chemicals. As per the test guideline, the observation period is 14 days. It consisted of a sighting study and a main study. In the sighting study, single female rat was administered with 5, 50, 300 and 2000 mg/kg bw dose sequentially. The main study was conducted with the single acute dose of 2000 mg/kg (limit test). Histopathological studies were also carried in animals dosed with 3, 50, 300 and 2000 mg/kg bw. The above mentioned “acute toxicity study” was used to select the doses for further experiments consisting of genotoxicity, biochemical and biodistribution studies and were carried out with 100, 500 and 1000 mg/kg bw doses. The characterization of NPs is required before predicting the toxicity to biological components (Murdock et al., 2008). Hence, in current study, the NPs and MPs were characterized using transmission electron microscopy (TEM), dynamic light scattering (DLS), laser Doppler velocimetry (LDV), X-ray diffraction (XRD) and Brunner–Emmett–Teller (BET) analysis were measured.

Genotoxicity studies are intended to evaluate the effects of test chemicals on DNA/chromosome which may lead to oncogene activation or functional loss of tumor suppressor gene results in mutations and cancer inductions. The *in vivo* genotoxicity was studied using comet (Tice et al., 2000; OECD 489, 2014), micronucleus (MNT) (Schmid, 1975) and chromosomal aberration (CA) assays (Adler, 1984). The CA assay involves analysis of mitotically arrested metaphase cells for the presence of structural chromosomal alterations induced by the test compounds. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzyme analysis has become a standard measure of hepatotoxicity. The presence of these markers in the blood in significant quantities suggests tissue damage (Ophardt, 2003). Therefore, these enzyme biomarkers were estimated to determine the functional status of liver and kidney upon oral exposure to MgO NPs and MPs. The present study also aimed to explore the oxidative stress induction ability of MgO particles. Hence, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) and Lactate dehydrogenase (LDH) levels were evaluated in serum, liver and kidney homogenates in the treated rats in comparison to controls. Further, hematology and serum markers were also estimated.

Histopathological examination is necessary to determine the morphological changes owing to NPs exposure and also for assessing the toxicological effects on liver, kidney, heart, spleen and brain (Reddy et al., 2017). NPs have different rates of absorption, persistence, distribution, accumulation, and elimination patterns when compared to MPs resulting in a potent interaction with animal models (Dumala et al., 2017). Metal content analysis is required to estimate the amount of Magnesium (Mg) that reached the target tissue or site after oral administration of NPs and MPs. Hence, the amount of metal content in rat's whole blood, liver, kidney, heart, brain, spleen, lungs, urine and feces was also analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) to estimate the uptake and retention of MgO particles and also for determining the anatomic fate, clearance and biological effect of these substances.