Environmental Science and Pollution Research (2025) 32:21703–21721
https://doi.org/10.1007/s11356-025-36935-x

RESEARCH ARTICLE

Sub-chronic oral exposure to titanium dioxide nanoparticles induces neurotoxicity in Wistar rats: evidence from mitochondrial, Micro-CT, and behavioral analyses

Sara Bouzenzana[1] · Rachid Rouabhi[2,3] · Abdellali Bouzenzana[4] · Besma Benaziz[5,6] · Khadidja Bekhouche[5] · Kamel Gaci[7] · Tahar Goudjil[2]

Received: 26 June 2025 / Accepted: 28 August 2025 / Published online: 12 September 2025

Abstract

Given their widespread use in food, cosmetics, pharmaceuticals, agriculture, and industry, titanium dioxide nanoparticles (TiO_2-NPs) pose a potential risk due to frequent and prolonged exposure, raising concerns about their impact on both human and environmental health. A few numbers of studies were interested to the neurotoxicity of TiO_2 NPs through the oral pathway. This study aimed to evaluate the neurotoxic effects of sub-chronic oral exposure to TiO_2-NPs in Wistar rats and investigate their toxic mechanisms using an integrated approach that links structural, cellular, behavioral, and biochemical changes, contributing to a better understanding of their toxicity and helping to mitigate potential risks to human health and the environment. In this study, animals received daily oral gavage of TiO_2-NPs (31–15 nm) at doses of 215 or 500 mg/kg for 90 days. Oxidative stress markers were assessed using brain tissue homogenates, including antioxidant enzymes (CAT, SOD, GPx, GSH, GST) and lipid peroxidation levels. Biochemical parameters Such as carbohydrate, lipid, and protein content were also analyzed. Mitochondrial function was evaluated using an oxygen electrode system, along with assessments of mitochondrial permeability and swelling. Brain structural changes were examined using the Micro-CT Skyscan 1276 system and histological analysis. Neurobehavioral functions were assessed through standardized behavioral tests evaluating memory, learning, locomotor activity, and anxiety. Results showed that high-dose exposure led to significant oxidative stress, evidenced by decreased levels of antioxidant enzyme defenses (CAT, SOD, GPx, GSH, GST) that caused a buildup of ROS and oxidative stress, which increased MDA levels and caused membrane damage and morphological brain alterations, as confirmed by the micro-CT and the histopathological changes. Micro-CT revealed structural disorganization and reduced contrast between brain regions, while histopathological analysis confirmed neuronal degeneration, cerebral edema, and inflammatory infiltration, indicating pronounced neurotoxicity and extensive cellular damage within the brain parenchyma induced by TiO_2 nanoparticles. Mitochondrial dysfunction was also observed, including impaired respiration, elevated permeability, and swelling, as assessed. Biochemical analyses revealed disruptions in brain carbohydrate, lipid, and protein content. Neurobehavioral assessments demonstrated deficits in memory, learning, locomotor activity, and increased anxiety. These findings highlight the dose-dependent neurotoxic potential of sub-chronic TiO_2-NP exposure and emphasize the need for further investigation and regulatory oversight.

Keywords Titanium dioxide nanoparticles · Neurotoxicity · Oxidative stress · Mitochondrial dysfunction · Micro-CT · Behavioral deficits · Wistar rats · Nanotechnology

Introduction

In recent years, nanotechnology has experienced rapid growth globally, with nanoparticles (NPs), defined as materials smaller than 100 nm, being widely applied across various industries (Ferreira et al. 2013). These nanoparticles are extensively incorporated in sunscreens, the food industry, cosmetics, paints, paper, plastics, wastewater treatment, and

Responsible Editor: Lotfi Aleya

Extended author information available on the last page of the article