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Copper oxide nanoparticles induces oxidative stress and liver toxicity in rats following oral exposure

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ABSTRACT

The present study aimed to evaluate the effect of Copper Oxide (CuO) Nanoparticles (NP) on liver following oral exposure in rats. A total of 18 male wister rats were used in the experiments including a control group (6 rats). NP were given to the rats with a doses (5, 50 mg/kg b.w./day) via oral gavage and a control group (received only 200 µl PBS). The treatment was continued for 14 days. The supernatants of rat Liver tissue homogenates were used to analyze for glutathione levels (GSH), Catalase, superoxide dismutase (SOD), the extent of lipid peroxidation products (Malondialdehyde, MDA). Oral administration of NP to rats caused a significant ($P < 0.05$) dose dependent alterations in antioxidant enzyme activities. Data results clearly showed the significant decrease ($p < 0.05$) in GSH, Catalase (CAT) and SOD activity, whereas the lipid peroxidation product (MDA) levels were increased ($p < 0.05$). In conclusion, oral exposure of CuO nanoparticles to rats causes significant toxicity to the liver and it might be due to oxidative stress.

1. Introduction

Nanoparticles (NP) with < 100 nm size, are widely used nanotechnology [1,2]. Metal-oxide NP contain heavy metal ions and other organic and inorganic groups which make them unique in their functional groups, electronic properties, shape, surface structure and aggregation behavior. Because of these particular characteristics make the nanoparticles suitable for various applications in modern industries [3]. However, widespread use of nanomaterials may lead to environmental contamination and human exposure by inhalation, dermal and oral routes, raising concerns about their potential toxicity [4]. The extreme usage of these NP brings challenges to the environment and to humans. With sizes smaller than cellular organelles, nanoparticles can easily penetrate through basic biological structures [5]. Administration routes (oral, inhalation, subcutaneous, injection etc.) of NPs are also known to have significant effects on their toxicities in mammals [6].

Copper oxide nanoparticles (CuO NPs) have attracted attention and used in industrial, commercial fields like medicine, engineering for their photovoltaic and photoconductive properties [7]. Copper oxide is a semiconductor metal with unique optical, electrical and magnetic properties and it has been used for various applications, such as the development of supercapacitors, near-infrared filters, in magnetic storage media, sensors, catalysis, semiconductors, etc. [7]. Particularly, CuO NPs useful in the pharmaceutical industry especially in the production of anti-microbial fabric treatments or prevention of infections caused by E.coli and methicillin-resistant S.aureus. [8]. In this study, we aimed at investigating the effects of oral exposure of CuO NP on the antioxidant enzyme activities in the liver in Wistar rats.

2. Materials and methods

2.1. Nanoparticles

CuO NPs (size < 50 nm, surface area 29 m^2/g, crystalline shape, diameter < 50 nm, length < 50 nm) purchased from Sigma Aldrich, USA. Suspension of CuO NPs was prepared using Phosphate Buffer Saline (PBS) and subsequently sonicated and mixed vigorously with a sonicator (20 min).

2.2. Animals and treatment

Male wistar albino rats (Mahaveer Enterprises, Hyderabad, India) of 8 weeks old at study start (180–200 g) were selected. A total of 18 rats were used in the experiments as each experimental group consisted of 6 rats, including a control group (6 rats). NPs were given to the rats with a doses (5, 50 mg/kg b.w./day) via oral gavage and a control group

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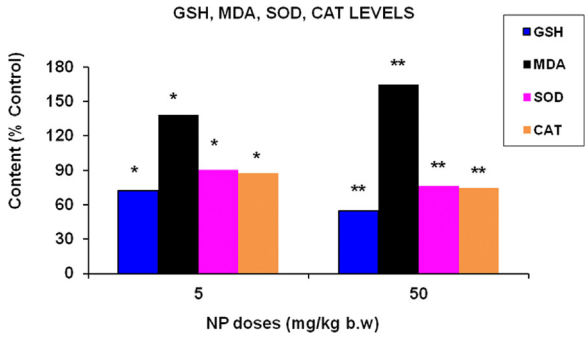

Fig. 1. GSH, MDA, SOD and CAT levels in liver homogenate of rats exposed to CuO NP.
$P < 0.05$, $p < 0.01$ vs control.

(received only 200 µl PBS). The treatment was continued for 14 days. Rats were killed with high doses of anesthesia after 14 days and dissected carefully using sterile equipment. Liver tissues were collected from all the group of rats and stored at −80 °C until further use. Homogenization of the liver was done in homogenization buffer (1:10, w/v) containing 100 mM potassium buffer (pH 7.4), 100 mM KCl and 1 mM EDTA for 90 s and then homogenates were centrifuged at 10,000 *g* for 30 min (+4 °C) to obtain supernatants. The supernatants of liver tissues of rats were used to analyze for glutathione levels [9], Catalase [10], superoxide dismutase (SOD) [11], the extent of lipid peroxidation (Malondialdehyde, MDA) by measuring the thiobarbituric acid reactive substances (TBARS) [12]. One-way analysis of variance (ANOVA) and Dennett test were used for statistical evaluation of results and $p < 0.05$ was considered as statistically significant.

3. Results

No mortality was found with oral administration of CuO nanoparticles for 14 days. The antioxidant enzyme levels in rat liver following exposure of NPs are presented in Fig. 1. Oral administration of NPs (with 5, 50 mg/kg b.w) to rats caused significant ($P < 0.05$) alterations in antioxidant enzyme activities. It was found the significant dose dependent decrease ($p < 0.05$) in GSH, Catalase (CAT) and SOD activity, whereas the lipid peroxidation product (MDA) levels were increased ($p < 0.05$). Statistically significant reduction in reduced glutathione, catalase and SOD activity represents the reduction of antioxidant enzyme levels following exposure of CuO nanoparticles and significant increase in lipid peroxidation levels indicate the tissue damage and oxidative stress. These above results confirm the liver toxicity and induction of oxidative stress by CuO nanoparticles in rat livers following their exposure for 14 days.

4. Discussion

Present study results clearly showed that oral exposure of CuO NP to rats for 14 days causes a significant decrease in liver antioxidant enzyme activity i.e. decrease in glutathione, SOD and Catalase levels, whereas the marker for oxidative stress i.e MDA levels (Fig. 1) were significantly increased indicating the induction of oxidative stress, which causes toxicity to the liver. These results were supported by our previous reports [13–15]. Moreover, our results are consistent with previous results suggesting that toxicity of CuO NPs mediated through ROS generation and oxidative stress [16–18]. It was reported that CuO nanoparticles induced dose dependent toxicity and oxidative stress in rats by generation of Reactive Oxygen Species (ROS) by lowering the antioxidants SOD and Catalase levels. Thus, it was confirmed that CuO nanoparticles induces toxicity and oxidative stress both in vivo as well as in vitro. Recently, Assadian et al., [8] also reported the in vitro cytotoxicity of CuO-NP, which was associated with significant increase at intracellular ROS level with effective induction of oxidative stress. The capability of NP to produce free radicals is one of the primary mechanisms of NPs toxicity [19–21]. It may result in oxidative stress, inflammation, and consequent damage to proteins, membranes, and DNA [22,23]. In conclusion, oral exposure of CuO nanoparticles to rats causes significant toxicity to the liver and it might be due to oxidative stress. More extensive studies would be needed to verify the safety issues related to increased usage of CuO NPs by consumers.

Conflict of interest

No conflicts of interest.

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