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Toxicological Evaluation of Copper Oxide Nanoparticles using *Allium cepa*

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Abstract
Development of nanoparticles is increasing due to their wide applications in various fields including sensing technologies, sieving properties, electronics and biomedical applications. Copper oxide (CuO) nanoparticles are being used in different fields of research. Hence their exposure to animals including humans is increasing. Thus there is a dire need to evaluate the toxicity of CuO nanoparticles using different plant and animal models. We procured copper oxide nanoparticles synthesized at pH 7 and 10 of the precursor solution. Different concentrations (0.1 and 0.01 g/100ml) were made and used for exposure treatments using Allium cepa. CuO nanoparticles showed a dose dependent toxicity in Allium cepa shoot length analysis test. A reduction in the shoot lengths was observed in the treated onion bulbs as compared to the controls. CuO nanoparticles synthesized at pH 10 were found to be more toxic as revealed by shorter shoot lengths as compared to pH7 treated samples.

Keywords: Copper oxide nanoparticles; Metal oxide; Shoot length; Allium cepa; Toxicity

Introduction

Numerous innovative nanomaterials have drawn a lot of interest recently because of their enormous potential for use in industries including agriculture, biomedicine, food safety, and food packaging (Singh, 2016). Nanoparticle development is accelerating as a result of nanoparticles' expanding array of uses in industries including biomedicine, electronics, sieving, and sensing (Jeong, et al., 2016; Park, et al., 2016; Shanmugam et al., 2024; Sivakumar et al., 2023; Goyal et al., 2023; Rani et al., 2024; Mahajan, 2023; Ghosh et al., 2024; Bhatt et al., 2024). The number of nanoparticles being mixed into the environment is rising as quickly as their rate of production. These nanoparticles go through a lot of physical and chemical changes in various environments, which might alter their toxicity and composition. The hazardous characteristics of numerous kinds of nanoparticles are reported by several investigations (Singh and Singh, 2017; Singh, 2017). It is crucial to consider how physical and chemical alterations to nanoparticles will affect their biological activity. Nanoparticles are more hazardous than their bigger counterparts due to their huge surface area. There are several ways that nanoparticles might enter the human body, including the skin, the mouth, or by inhalation (Assadian, et al., 2017). Nanoparticle toxicity might result through protein misfolding, DNA damage, mitochondrial damage, membrane damage, or the generation of reactive oxygen species as a result of nanoparticle entrance into the human body.

The simplest copper compound, CuO, has potential physical features that might be used in gas sensing, photovoltaics, high temperature superconductivity, and electrodes for batteries. CuO nanoparticles have a number of crucial roles in everyday life, notably in the disciplines of technology, engineering, and medicine. Due to its bactericidal and fungicidal properties, copper nanoparticles have been used in nano-biotechnological applications to promote agriculture (Nagaonkar et al., 2015). Nanocrystalline CuO is an extremely effective catalyst for oxidizing carbon monoxide. Recent research on CuO nanoparticles has been conducted in several scientific domains (Khoshnamvand et al., 2018; Lai et al., 2018; Senobari and Nezamzadeh-Ejhieh, 2018). When copper ions surpass the physiological tolerance range in living creatures, which can happen when they are in ionic form, they can cause toxicity. Copper is one of the essential metals for sustaining homeostasis in many different types of animals. Due to the development of nanotechnology, it is crucial for the scientific community to investigate the potential toxicological and health impacts of CuO nanoparticles.

These nanoparticles have been used as sensors, gaseous and solid ceramic pigments, catalysts, semiconductors, semiconductor devices, and magnet rotatable devices. CuO nanoparticles have also been used in the pharmaceutical sector, particularly in the development of anti-microbial fabric treatments and the treatment of illnesses brought on by methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* (Assadian et al., 2017). Copper oxide nanoparticles have been the subject of several research utilizing a variety of models, such as *Oryza sativa* (Liu et al., 2018), *Pisum sativum* (Ochoa et al., 2018), mice (Lai et al., 2018; Libalova et al., 2018) and *Mytilus edulis* (Chatel et al., 2018). Loss of mitochondrial membrane potential, lysosomal membrane leakiness, and a large rise in intracellular ROS were observed to be linked with CuO nanoparticle cytotoxicity (Assadian et al., 2017). Here, we used *Allium cepa* shoot analysis to examine the toxicity of CuO produced at two precursor solution pH levels (pH 7 and 10). This preliminary investigation will undoubtedly advance our understanding of the toxicological features of CuO nanoparticles.

Materials and methods

Procurement of copper oxide nanoparticles

CuO nanoparticles were procured in the powder form which were synthesized from cupric nitrate ($Cu(NO_3)_2.3H_2O$) and citric acid monohydrate ($C_6H_8O_7.H_2O$) at pH 7 and 10 of the precursor solution. The CuO powder samples were designated as NP1 and NP2 corresponding to samples synthesized at pH value 7 and 10 respectively.

Characterization of nanoparticles

FESEM and HRTEM analysis were used to characterize the nanoparticle samples. The surface topography of CuO powder samples was studied by scanning electron micrographs taken using JEOL JSM-6700F with a beam voltage of 30 KV. TEM images were taken using the transmission electron microscope system (HRTEM, model FEI Technai 30) operated at 300 kV.

Treatment solutions

Two concentration groups per CuO powder samples, NP1 and NP2 were made and named as per the table 1.

Table 1. Formulation of different treatment concentrations of CuO nanoparticles solutions.

Sample	Concentration (g/100ml)	Sample annotation
Control	-	A and B
CuO pH7 (NP1)	0.01	C
	0.1	D
CuO pH10 (NP2)	0.01	E
	0.1	F

Allium cepa shoot test

Test Material

Onion bulbs (*Allium cepa* L.) of average size (15-20 mm diameter) were purchased from the local market. The onion bulbs were sun-dried for 5 weeks. The roots of the dried bulbs were shaved off from the base with a sharp blade. This exposed the fresh meristematic tissues and the bulbs were placed in distilled water to protect the primordials from drying up.

Treatment of test material

After removing excess water with a blotting paper, the bases of the onion bulbs were dipped in solutions of all the test solutions as described in table 1. Two onion bulbs were used for each sample concentration and control (tap water). The experiment was run for 14 days. After the exposure time was over, onions were subjected to further analysis.

Shoot length measurement

After the exposure period, onion bulbs were taken for the shoot length measurement. The shoot length (cm) of all onion bulbs was measured on the 14th day using a calibrated ruler. After taking the shoot lengths, the mean was calculated for each concentration treatment. The mean shoot length of the control samples was also calculated.

Results and Discussion

FESEM and HRTEM analysis

The FESEM and HRTEM images of both CuO samples are shown in Figure 1. NP1 sample showed a greater extent of agglomeration as compared to NP2. The decrease in agglomeration in case of NP2, as observed in FESEM images (Figure 1B) of samples prepared with high pH value, may be attributed to the large quantity of the gas evolved. Figure 1D also shows a sharp particle distribution and low agglomeration.

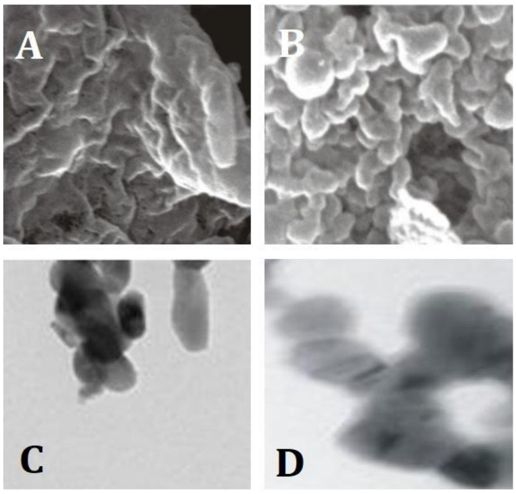

Figure 1. FESEM and HRTEM images of CuO nanoparticle samples **A:** FESEM image of CuO NPs synthesized at pH7 of precursor solution; **B:** FESEM image of CuO NPs synthesized at pH10 of precursor solution; **C:** HRTEM image of CuO NPs synthesized at pH7 of precursor solution, and **D:** HRTEM image of CuO NPs synthesized at pH10 of precursor solution

Shoot length measurement

Onion bulbs were taken for the shoot length measurement. The shoot length was measured on the 14th day using a calibrated ruler. Table 2 and Figure 2 show the mean shoot lengths among different concentration groups of CuO nanoparticles and controls.

Table 2. Mean shoot lengths and length reduction from control among different concentration groups of CuO nanoparticles synthesized at pH7 (NP1) and pH10 (NP2).

Concentration group	Shoot length (cm)	Length reduction from control
A	22.2	-
B	19.4	-
C	17.6	3.2
D	9.5	11.3
E	12.2	8.6
F	0.5	20.75

Mean shoot length = 20.8 cm

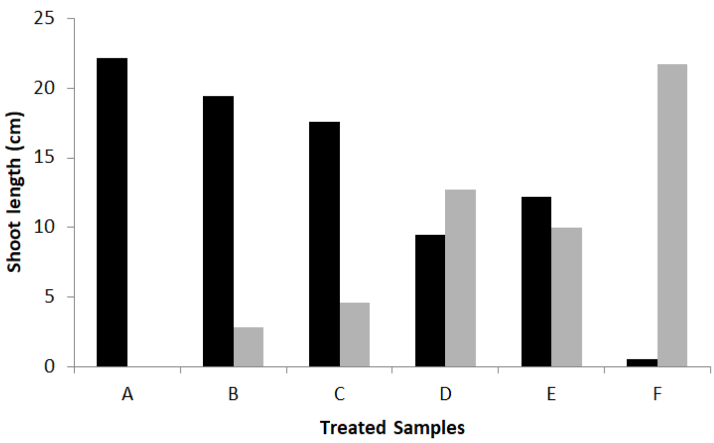

Figure 2. Comparative bar graphs showing mean shoot lengths (cm) among different groups of exposed samples. Black bars represent shoot length and grey bars represent shoot length reduction.

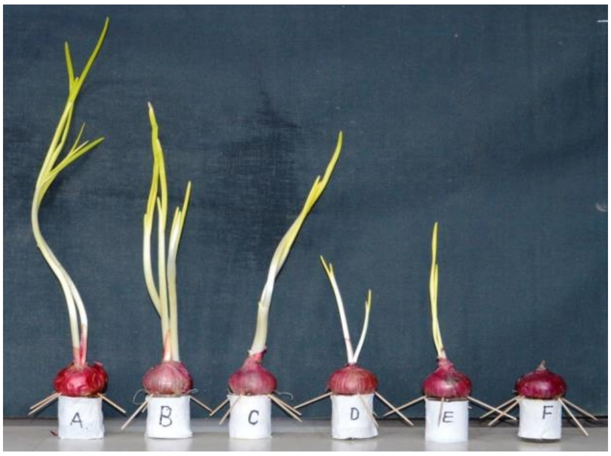

Figure 3. shows the comparative shoot lengths among controls (A and B) and two groups of CuO nanoparticles synthesized at pH7 (C and D) and pH10 (E and F) of the precursor solution

It was seen that as the treatment concentration increased, the mean shoot length decreased. On the 14th day, a maximum mean shoot length was seen in the control group A and B (20.8 cm). A mean shoot length of 17.6 cm was seen in the group C of NP1 with a shoot length reduction of 3.2cm. Similarly, group E showed a mean shoot length of 12.2 with a reduction of 8.6cm. The high concentration groups (D and F) showed higher shoot reductions. Group D and F showed a shoot length of 9.5 and 0.5cm respectively.

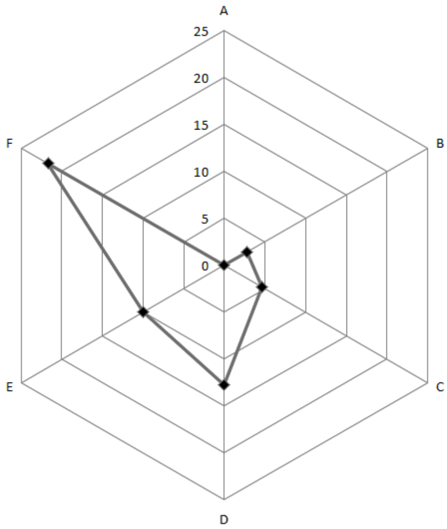

Fig. 4. Reduction in the mean shoot length after exposure to different concentrations of CuO nanoparticles synthesized at pH7 (NP1) and pH10 (NP2).

Figure 4 shows the comparative mean shoot length reduction among control groups and CuO nanoparticle groups synthesized at pH7 and 10 of the precursor solution. It is seen that as the treatment concentration increased per group, the mean shoot length decreased. At 14th day, maximum mean shoot length reduction was seen in group F (20.75cm) as compared to the control group. It is clearly demonstrated that on the 14th day, the reduction in the mean shoot lengths at different concentrations was higher in NP2 groups (E and F) as compared to NP1 (C and D). Thus, higher toxicity of NP2 group nanoparticles was recorded which were synthesized at pH10 as compared to NP1 group. This higher reduction may be attributed to decreased agglomeration as well as smaller particle size of NP2 group nanoparticles.

Conclusion

Copper oxide (CuO) nanoparticles are being used in different research fields and hence their exposure to animals including humans is increasing. Here the toxicity of CuO nanoparticles synthesized at pH7 and pH10 was evaluated using *Allium cepa* shoot analysis. The nanoparticles were characterized using SEM and TEM. Nanoparticles synthesized at pH10 were found to have smaller particle sizes as compared to particles synthesized at pH7. Two concentrations (0.1 and 0.01 g/100ml) were used to treat *Allium cepa*. CuO nanoparticles showed a dose dependent toxicity at

14th day of the experiment with a reduction in the mean shoot length in the treated onion bulbs as compared to the controls (0.01 g/100ml: CuO pH7, 3.2 and CuO pH10, 8.6 Vs Control, 20.8 cm; 0.1 g/100ml: CuO pH7, 11.3 and CuO pH10, 20.75 Vs Control, 20.8 cm). CuO nanoparticles synthesized at pH 10 were found to be more toxic as compared to pH7 samples resulting in higher shoot length reduction. pH plays a vital role in determining the particle size of the synthesized nanoparticles, which in-turn decides the toxicity. Conclusively, mixing of toxic CuO nano-powders to the environment should be checked so as to minimize the nanoparticle related health hazards.

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ZS and NK conceived the concept, wrote and approved the manuscript.

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Ethics approval
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