Effects of differently incubated cupric oxide nanoparticles on the granulosa cells of caprine ovary in vitro

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Abstract
In the nanoscience metal and metal oxide, nanoparticles have a prominent place because of their vast applications. Recent finding shows that in addition to size, there are other critical factors governing the biological response of nanoparticles. These factors include surface chemistry and shape that influences solubility, rate of diffusion, drug delivery, melting temperature, and colour of the nanoparticles. It is thus the present study that was aimed to investigate the effect of temperature on the shape and size of nanoparticles and related cytotoxicity of these particles on ovarian granulosa cells. Cupric oxide nanoparticles (CuONPs) were synthesized using a simple, efficient, and reproducible precipitation method involving the reduction of Cu metal salt with sodium hydroxide and then incubation of the precipitates at 70 °C for 5 h. Subsequently, this prepared sample was divided into 3 subsamples and incubated at 3 different temperatures, i.e. 70 °C, 150 °C, and 350 °C for 5 h to study the effect of temperature on the particles. The products were characterized by XRD, FTIR, HRTEM, and FESEM. Characterization of the particles revealed that all particles were monoclinic crystalline in nature and had a size range from 9 to 60 nm. Particles were of different shapes: spherical, needle, and capsule. The toxicity of each particle was determined on granulosa cells by exposing cells for 24 h at 2 different doses. Toxicological results showed the size and shape-related toxicity of nanoparticles where spherical shapes were significantly more toxic than capsule-shaped particles.

Keywords Cupric oxide nanoparticles · Cytotoxicity · Granulosa cells · XRD · FTIR · HRTEM · FESEM · Temperature

Introduction
Nanomaterials are compounds or substances with a size range from 1 to 100 nm in any one dimension (Buzea et al. 2007). These materials play a vital role in meliorating the quality of human life. By 2024, the worldwide nanotechnology market is estimated to surpass US$ 125 billion (Liu and Xia 2020). Nanoparticles differ from their bulk particles in thermal, mechanical, magnetic, electrical, and optical properties (Buzea et al. 2007). Nanoparticles are employed in array of fields, including chemical, physical, biological, engineering, and material sciences, in addition to electronic sciences (Ingale and Chaudhari 2013). Metal oxides are one of the most intriguing nanotypes by virtue of their numerous applications in nanoelectronics, cosmetics, sensors, water purification, healthcare, sunscreens, coolants, and energy production and storage (Ahamed et al. 2010, 2014; Kandeil et al. 2020). Because of its magnetic properties, ability to traverse the blood–brain barrier (BBB), and huge surface area-to-volume ratio, metal nanoparticles are practiced in biomedical applications such as medication administration, heat ablation of target tumours, and sensing of target molecules (Choi and Wang 2011; Zahin et al. 2020). These nanoparticles are also used in medicines because of their high stability, bio-compatibility, anticorrosive, and photocatalytic properties, while a few also possess the antioxidant defence capacity (Mohammed and Safwat 2020; Dawood et al. 2019a,b). To synthesize these nanoparticles, various approaches have been employed including sol–gel method, precipitation method, vapours method, and ball milling method (Tamilvanan et al. 2014). Because of the variety of applications and synthesis methods, research organizations, government agencies, and even universities are intently
investigating the fate, benefits, toxicity, and direct and indirect effects of metal oxide nanoparticles or nanoparticle-containing products (Tang et al. 2015; Taghavi et al. 2013).

In metal oxides, oxides of transition metals are foremost crucial class of semiconductors and are significantly used in many applications. Unlike other transition metals, copper is a good conductor and does not react with water (Cardeilhac and Whitaker 1988). Copper can act similar to an oxygen carrier and oxidation catalyst (second only to iron); it also exists in many proteins like hemocyanin, ceruloplasmin, monoamine oxidase, galactose oxidase, dopamine β-hydroxylase, superoxide dismutase, and phenolase (Richardson 1997). Copper compounds have a scope of usage in fungicide, bactericide, and algaeicide and also have functional conjugation with photosynthetic enzymes in plants (Lamichhane et al. 2018; Abdel-Latif et al. 2021). Simplest among the family of copper nanoparticles, cupric oxide nanoparticles (CuONPs) are considered as the utmost bio-reactive metal oxide nanoparticles (Karlsson et al. 2008; Lanone et al. 2009; Fu et al. 2014). These particles have a diversity of applications, including veterinary medicine, nano sperm purification, germicides, biocides, food safety, and antibiotic resistance reversal (Hill and Li 2017; Bezza et al. 2020). Furthermore, these nanoparticles have utilization in heat transfer fluids, solar energy, catalysis, conductive coating in lithium-ion batteries, additives in lubricants, coatings for plastics, and gas sensors (Aruoja et al. 2009; Gawande et al. 2016).

The cytotoxic effects of CuO nanoparticles vs bulk particles have been reported in mammalian cells like neuronal cells, lung epithelial cells, cardiac microvascular endothelial cells, and liver cells (Fahmy and Cormier 2009; Ahamed et al. 2010; Sun et al. 2011; Wang et al. 2011; Piret et al. 2012; Siddiqui et al. 2015; Xu et al. 2013). Berntsen et al. (2010) revealed the impaired cell viability and decreased cell contractility in human airway smooth muscle cells on CuONP exposure. A dose-dependent decrease in cell viability of the human pulmonary epithelial cells (A549) by CuONPs also had been disclosed by Ahamed et al. (2010). These studies highlighted the reduction in the size of the particles as the prime cause for NP-induced cytotoxicity. However, emerging studies find that size may not be the sole determining agent, but the shape (Gratton et al. 2008), surface chemistry (Liu et al. 2010a; Studer et al. 2010; Franklin et al. 2007), and particle solubility are all chief determinants in cytotoxicity. As these qualities change, so do these characteristics causing change in particles solubility, melting temperature, optical properties, drug delivery, etc. (Dagher et al. 2014; Misra et al. 2014; Tran and Nguyen 2014; Ahmed et al. 2015).

Ovarian granulosa cells play a pivotal role in the growth and maturation of a follicle (Sharma 2000). Granulosa cell apoptosis, a key biological aspect of programmed cell death that leads to atresia, is cytologically characterised by condensation of cytoplasm and nucleus, pyknotic nuclei, and hazy cytoplasm (Yu et al. 2004; Sharma 2000). In addition to endocrine factors, various xenobiotics and toxic chemicals have also been reported to induce apoptosis in the granulosa cells (Yu et al. 2004; Lima-Verde et al. 2012). Liu et al. (2010b) and Stelzer and Hutz (2009) delineate that nanoparticles like calcium phosphate and gold can induce apoptosis of granulosa cells.

It has been reported that shape of the particles influences the physicochemical properties, bio-distribution, internalization, biological response, and even cytotoxicity of the particles (Huang et al. 2010; Venkataraman et al. 2011). Pal et al. (2007) proved that silver nanoparticles with truncated triangular nanoplates showed a stronger biocidal action than spherical and rod-shaped nanoparticles. Stoehr et al. (2011) also found that wire-shaped silver nanoparticles caused decreased cell viability and increased lactate dehydrogenase (LDH) production in A549 cells, while no such outcomes were observed for spherical silver nanoparticles. Very little is known in regard to shape-related toxicity because most toxicological research focuses on nano vs. bulk particles. As a result, in this study, we have attempted to synthesize four distinct-shaped cupric oxide nanoparticles by adjusting the temperature of the reaction and finding their relative toxicity on the caprine granulosa cells.

**Materials and methods**

The chemicals and accessories were of analytical grade and were collected as per the requirements. Copper (II) acetate monohydrate \([\text{Cu(CH}_3\text{COO)}_2 \cdot \text{H}_2\text{O}]\) (RM 360-250G), glacial acetic acid \((\text{CH}_3\text{COOH})\) (AS001-500ML), polyvinylpyrrolidone \((\text{PVP; MW 40,000})\) (RM854-100G), and culture media \((\text{DMEM})\) (AL 149A-500ML) used were procured from HIMEDIA, while sodium hydroxide pellets \((\text{NaOH})\) (S0290) used were acquired from Rankem. Other chemicals were phosphate buffer saline \((\text{PBS})\) and deionized water \((\text{DI})\). All the chemicals were available in the Reproductive Physiology Lab, Department of Zoology, Kurukshetra University Kurukshetra, India.

**Apparatus and instruments**

Conical flasks, funnel, crucible, spatula, burette and burette stand, culture plates, fine forceps, needle, magnetic stirrer (Popular India), oven (NSW India), furnace (AICIL), CO\(_2\) incubator (BIOSAFE eco), micropipettes (Eppendorf), stereo-microscope (Nikon SMZ1), and fluorescent microscope (Olympus). All the apparatus and instruments were available in Reproductive Physiology Lab, Department of Zoology, Kurukshetra University Kurukshetra, India.
Synthesis of Cupric Oxide NPs

Cupric oxide nanoparticles (CuONPs) were synthesized using the aqueous precipitation method (Ahamed et al. 2014), a type of chemical method which includes the reduction of a metal salt by a reducing agent on the magnetic stirrer. In the method, 0.1 M of copper (II) acetate monohydrate \([\text{Cu(CH}_3\text{COO)}_2\cdot\text{H}_2\text{O}])\) was dissolved in deionized water to form an aqueous solution. This solution was reduced by another aqueous solution of NaOH drop by drop on a magnetic stirrer in a conical flask at the desired molar ratio of 1:10 under the glacial acetic acidic environment. PVP (polyvinylpyrrolidone) was used as a stabilizer to control the growth of CuONPs’ size and morphology. During the addition of the reducing agent, the colour of the solution changed gradually from blue to sea green then from sea green to blackish-brown after which the reaction was stopped (Fig. 1). The blackish-brown solution indicates the synthesis of cupric oxide precipitates in the solution. Obtained precipitates were washed with water and alcohol many times to pull out the impurities present and dried at 70 °C for 5 h (T Group). Particles were divided into 3 groups (E1, E2, and E3) and incubated under different conditions to know the effect of temperature at constant time on the size and structure of nanoparticles (Table 1).

Collection of ovaries and culture of ovarian follicles in vitro

Ovaries of sexually mature, normal cycling Jamnapari breed of goat were brought to the lab in ice-cold 0.9% normal saline at 4 °C from Municipal Slaughter House, Chandigarh (30°70′N, 76°80′S). Follicles (3–8 mm diameter) from ovaries were manually separated using fine forceps and were classified as healthy, pre-atretic, and atretic follicles on the morphometric basis including vascularity, colour, and turbidity of follicular fluid (Sharma and Bhardwaj 2009).

Healthy follicles (pinkish, highly vascularized having amber colour follicular fluid) were cultured for 24 h in 5 groups, i.e. one control and 4 treatment groups (A, B, C, and D). Each treatment group was subdivided into 2 subgroups (1 and 2), where subgroup 1 had 10 µg ml\(^{-1}\) and 2 had 20 µg ml\(^{-1}\) concentration of respective CuONPs in culture media (Dulbecco’s Modified Eagle Medium) supplemented with 200 units of antibiotics (100 IU/ml penicillin and 100 IU/ml streptomycin). These culture media were incubated in a CO\(_2\) incubator (5% CO\(_2\), 95% humidity, and 38 °C temperature). The experimental layout of a follicular culture of goat ovaries is presented in Fig. 2.

Preparation of granulosa cell suspension

Aspiration method was employed to make cell suspension. Treated healthy follicles (3–8 mm) were aspirated with the help of a 20-gauge needle in a 2-ml syringe containing phosphate buffer saline (PBS) at pH 7.4. Cumulus–oocyte complexes (COCs) were removed with the help of micropipettes under stereo-microscope. The remaining cell suspension was washed 3 times by centrifugation method with the help of PBS at 2000 rpm for 5 min each to remove any kind of debris.

Apoptotic assay

The morphometric analysis of the apoptotic granulosa cells was done by Broaddus et al. (1996) method. In this method,
granulosa cells’ apoptosis was evaluated by acridine orange (AO) staining. Each cell suspension prepared from treated follicles was mixed with an equal quantity of AO stain solution. AO stain solution was formulated by dissolving 1 µL of AO in 1 ml PBS. Cells were then observed under the fluorescent microscope. Cells that appeared green were healthy, yellow/orange cells were pre-apoptotic, and red cells were apoptotic. The apoptotic percentage index (API) was deliberated by quantifying healthy, pre-apoptotic, and apoptotic granulosa cells.

**Statistical analysis**

The cytotoxicity data is expressed as mean ± standard error. The cytotoxicity was analysed with the aid of one-way ANOVA with Tukey post hoc test (all treatments were compared to control as well as to one other). SPSS 16.0 was used for the statistical analysis. The p values of less than 0.05 were considered significant.

**Results**

**XRD**

The X-ray diffraction (XRD) confirms the structural characterization of the particles. Samples were scanned in the range of 2θ from 10° to 80° by Panalytical’s X’Pert Pro instrument with Ni filtered using CuKα radiations as an X-ray source (λ = 1.54060Å⁻¹). Peak analysis of the samples showed the reflecting planes of (110), (111), (202), (020), (202), (311), (220), (311) and (004) at 2θ; 57.9°, 61.2°, 65.9°, 67.7°, 72.1°, and 74.9°, respectively (Fig. 3). The reflecting peaks of all the particles were in agreement with the National Bureau of Standards Circular (NBSC)-539 Volume 1 which concluded the presence of monoclinic structure. The absence of an extra peak claims the purity of the substance. When seen in Fig. 3, as we progressed from T to E3 particles, prominent peaks about 35° and 38° were longer and sharper, indicating that the crystallinity order of these particles is E3 > E2 > E1 > T (Kulkarni and Kulkarni 2015; Sedaghat et al. 2006).

The size of the particles using XRD can be evaluated by Debye–Scherrer equation.
where,

\[ T = \frac{k\lambda}{\beta \cos \theta} \]

- \( T \) = Thickness of the crystal
- \( k \) = Dimensionless shape factor, and its value is about 0.9
- \( \lambda \) = Wavelength of the X-rays (1.5405 Å)
- \( \beta \) = Broadening at half the maximum intensity (FWHM) caused by nanoparticle size
- \( \cos \theta \) = Angle between rays in beam and parallel planes (Bragg’s angle)

Debye–Scherrer formula can be used to evaluate the average particle size smaller than 100 nm but larger than 2 nm (Kulkarni and Kulkarni 2015)

The crystal size of particle is given in Fig. 4.

**FTIR**

The Fourier transmission infrared spectroscopic analysis of CuONPs describes the nature of bonds and types of functional groups present (Fig. 4). Different molecules absorb light of different energies in the infrared region of the electromagnetic spectrum, allowing for the identification of different functional groups and compounds (Chopra et al. 2020). Transmittance bands present near the 500 cm\(^{-1}\) and 600 cm\(^{-1}\) wavelength illustrate the presence of Cu–O bonds in samples, particularly Cu–O asymmetric stretching and Cu–O wagging, respectively (Arun et al. 2015).

Figure 5 depicts that with rising in the temperature at constant time duration, CuO bands became sharper and were of considerable depth. The bands present around 1630 cm\(^{-1}\) and 1380 cm\(^{-1}\) were either due to presence of carbonyl group as M–O bond rocking in plane (1383 cm\(^{-1}\)) and M–O bond rocking out of the plane (1634 cm\(^{-1}\)) attached as a bidentate ligand to the CuO (Arun et al. 2015). This presence of the carbonyl group may be due to the carbonyl group (=C=O) present in the pyrrolidone ring (Khan et al. 2020) or due to unreduced carboxyl group copper acetate or glacial acetic acid (Pal et al. 2015).

Whereas the absorbance bands present around 2920 cm\(^{-1}\) wavelength in the T, E1, E2, and E3 nanoparticles represented the asymmetric stretching of -CH\(_2\)- in the sample (Khan et al. 2020). An intense and broad absorbance band present at 3426 cm\(^{-1}\) (T), 3425 cm\(^{-1}\) (E1), 3417 cm\(^{-1}\) (E2), and 3354 cm\(^{-1}\) (E3) wavelength belonged to the stretching mode of hydroxyl group (-OH) of adsorbed water on the surface of CuONPs (Malviya et al. 2015). FTIR peaks have been summarised in Table 2.

**FESEM**

By collating the field emission scanning electron microscopic pictures of different particles, the sequel of different incubation conditions on the morphology of CuONPs were investigated (Fig. 6). The T particles that formed were spherical and clumped together (Fig. 6a). The particles in the E1 were observed to be aggregated and attempting to take needle-like forms (Fig. 6b). In the case of E2 particles (Fig. 6c), however, the situation was flipped, with the
majority of particles being needle-shaped and fewer being aggregated. Finally, E3 particles developed a capsule-like form with distinct borders that allowed them to be well segregated from one another (Fig. 6d).

**HRTEM**

The results of the high-resolution transmission electron micrograph (Fig. 7) of nanoparticles were in agreement with FESEM results, where T particles were spherical (Fig. 7a) and aggregated with an average diameter of 9.42 ± 2.82 nm. E1 particles developed a needle form structure with uneven borders and an average diameter of 56.82 ± 13.61 nm as temperature increased (Fig. 7b). With further increase in temperature, i.e. at 150 °C, the E2 particles showed a diameter of 68.78 ± 18.55 nm and a shape intermediary of needle and capsule with uneven boundaries (Fig. 7c). While E3 particles were capsule...
shape (Fig. 7d) and demonstrated a reverse trend, i.e. size of the particles reduced with an average diameter of 41.62 ± 6.54 nm.

The size distribution pattern of all four types of particles (Fig. 8) demonstrates that the particles are evenly distributed and have a size well below 100 nm. Each nanoparticle’s peak of distribution corresponded to the average particle size.
**Frequency of apoptosis**

The shape and dose-related toxicity of CuONPs observed by the apoptotic percentage index (API) produced by fluorescent labelling of granulosa cells with acridine orange (Fig. 9). The API revealed that in comparison to the control group, the percentage of pre-apoptotic and apoptotic cells increased in all treatment groups, while the percentage of healthy cells dropped (Fig. 10). API also found that higher dose groups had more toxicity than lower dose groups (Fig. 11).

While noticing for significant difference between healthy cells of nanoparticles treated groups and healthy cells of control groups, the difference was reported only in the case of both T nanoparticles doses and higher doses of E1, E2, and E3 nanoparticles compared to control. When we compared pre-apoptotic cells of treated and untreated groups, it was reported that except for E3 nanoparticles at 10 µg ml−1 dose, all types of nanoparticles showed a significant increase in the percentage of pre-apoptotic cells as compared to control (Table 3) (Fig. 11). But when groups were discerned for apoptosis, only those groups treated with higher dose, i.e. 20 µg ml−1, showed significantly higher percentages of apoptotic cells in comparison to the control group.

While comparing one nanoparticle to another nanoparticle, T nanoparticles at higher doses proved significantly more toxic than the rest of the three nanoparticles at 10 µg ml−1 doses when healthy cells were spotted. In case of pre-apoptotic cell evaluation, a significant difference was perceived only between T nanoparticles at 20 µg ml−1 and E3 nanoparticles at 10 µg ml−1. Similarly, while examining all the treatment groups for apoptotic cells, higher doses of T nanoparticles were shown to be significantly different than lower doses of E1, E2, and E3 nanoparticles, while no other...
significant variations between nanoparticles were observed in the case of dose or types of nanoparticles. As per the observations, no significant difference in the number of cells was observed when the lower dose of a nanoparticle was compared to the higher dose of the same nanoparticle, whether it was healthy cell count, pre-apoptotic cell count, or apoptotic cell count. But all types of nanoparticles showed an increase in the percentage of apoptotic cells from lower dose to higher dose at 24 h of cultural duration.

**Discussion**

This study was conducted to examine the effect of the temperature variations on the size and shape of nanoparticles and the impact of different sized and shaped nanoparticles on ovarian granulosa cells. CuONPs were synthesized using the precipitation method approach since this method provides better controllability and reproducibility of the nanoparticles. Ficai and Grumezescu (2017) also found similar peculiarities using the precipitation method. The effects of temperatures on the size and shape of CuONPs were evaluated by techniques like XRD, FTIR, FESEM, and HRTEM. XRD technique was used to confirm that all the particles formed were monoclinic crystals and had a size range from ~12 to ~14 nm. An increase in crystal size with the increase in temperature for T, E2, and E3 nanoparticles can be attributed to the phenomenon of “nuclear aggregation”. Nuclear aggregation is the phenomenon in which rapid formation of crystal nucleus and simultaneous aggregation of these nuclei occurs as the temperature rises. These findings strongly support the earlier reports of Vidyasagar et al. (2012). However, the decrease in E1 crystal size can be attributed to prolonged exposure to 70 °C temperature for 5 h. The nucleation rate for CuONPs remained constant, and thus crystal size did not increase rather it decreased because of the nucleation rate effect as already documented by Liu et al. (2020).
Morphological evaluation done by FESEM revealed that the shape of particles changed from spherical to capsule with the intermediary stages observed in E2 and E3 particles with varied incubation conditions. The FESEM analysis also found that different shapes of nanoparticles produced were actually due to the aggregation between spherical T nanoparticles. HRTEM analysis concluded that while the size of the particles increased up to 150 °C but then decreased. This reduction in the size of E3 nanoparticles compared to E2 and E1 nanoparticles can be attributed to the more evaporation of water and subsequent aggregation of particles at 350 °C as evident from FTIR results where the water absorbance band in the case of E3 nanoparticles is less deep as compared to water absorbance band in T, E1, and E2 particles. Similar results were observed by George et al. (2020) who found that at the maximum calcination temperatures, the size of nanoparticles decreased. The other reason behind this may be the inherent properties of the nanoparticles, in which particles always tend to acquire stable shape and size under particular conditions as reported by Malviya et al. (2015). The reason for the increase in the size of E1 particles compared to the size of T particles in HRTEM analysis which is opposite to XRD analysis can be attributed to the increased growth rate at 70 °C temperature; the similar results have been found in the study of Liu et al. (2020). From FESEM and HRTEM analysis, it can be interpreted that with the increase in temperature at constant incubation duration, the size of CuONPs increases, and particles tend to take a stable shape with smooth boundaries.

Toxicological evaluation of all types of CuONPs (T, E1, E2, and E3) on granulosa cells showed that all these types of nanoparticles exhibited toxicity and triggered an increase in the percentage of pre-apoptotic and apoptotic cells after 24 h of exposure duration. Our results support the earlier findings of Fatahian-Dehkordi et al. (2017). According to El Bialy et al. (2020) and Katsumiti et al. (2018), CuONP cytotoxicity can be attributed to the presence of Cu2+ released by the CuONPs in culture media (2018). Cu2+ on reacting with cells caused increased production of reactive oxygen species (ROS) thus damaged DNA. The significant higher toxicity due to CuONPs at higher doses, when compared to control, can possibly be due to an increase in the concentration of nanoparticles that lead to increased amount of Cu2+ ion release by CuONPs in the medium (Wongrakpanich et al. 2016).

In addition to this, it was also marked that only T nanoparticles at higher doses showed significant differences in the number of apoptotic cells when compared to control and lower doses of E1, E2, and E3 nanoparticles. The heterogeneity in size can be the reason behind significant toxicity of T nanoparticles at higher doses in contrast to lower doses of other nanoparticles. Kim et al. (2012) reported the similar results where small-sized nanoparticles reported more toxicity than large size nanoparticles. However, the significance of higher toxicity that was seen in both doses of T nanoparticles in healthy cells and non-significant toxicity in the case of lower doses of E3 nanoparticles in pre-apoptotic cells when compared to control can be attributed to change in the shape of nanoparticles. The reason for this toxicity may be the rate of release of Cu2+ by different shapes of particles. As reported by Wongrakpanich et al. (2016) and Misra et al. (2014), the rate of release of Cu2+ in the case of spherical nanoparticles was more as compared to other shapes of particles. Misra et al. (2014) specifically reported the reason for this higher release of Cu2+ and concluded that spherical CuONPs have the highest specific surface area (SSA) as compared to the rod and spindle-shaped CuO nanoparticles. Thus, we can also deduce here that capsule-shaped particles seem to have lesser SSA than spherical nanoparticles and needle-shaped nanoparticles, so the release of Cu2+ from capsule-shaped CuONPs is slower, and hence at lower doses (10 µgml−1), the cytotoxicity caused by this type of nanoparticles is also less; hence, it causes less toxicity.

The mechanism behind CuONPs toxicity is yet not confirmed, but an increase in oxidative stress seems to be the main reason as reported by Fahmy and Cormier (2009) and Bernts et al. (2010) where increased oxidation stress was reported in cells cultured with CuONPs. Likewise, increase in levels of lipid peroxidation and ROS production while lowering the levels of antioxidants due to nanoparticles was observed by Ahamed et al. (2010). Cytotoxicity of the CuONPs may be due to DNA damaging capabilities of CuONPs as Akhtar et al. (2016) and Thit et al. (2015) reported that CuONPs can cause genotoxic effects either directly associating with DNA or indirectly by activating phagocytes and macrophages. Time of Cu2+ exposure to the cells might have played some role in the toxicity as Misra et al. (2014) reported that the rate of dissolution of spherical nanoparticles is quicker than the rate of dissolution of other shapes of particles. Thus, cells got exposed to the Cu2+ to a greater extent in the case of spherical particles than other shaped particles.

Conclusion

In the present study, CuO nanoparticles were synthesized at different incubation temperatures (70 °C, 150 °C, and 350 °C). XRD at different angles manifested that all the particles were monoclinic crystal structures. FTIR absorbance revealed that all the particles had CuO stretching. FESEM and HRTEM revealed the significant impact of temperature on the shape of nanoparticles. As with the increase in temperature not just the size of the nanoparticles increased up to a limit but the shape of the particles changed with uneven boundaries to the even boundaries. While there are many
studies providing insight on the toxicity of nanoparticles caused due to their size and surface chemistry, scattered information is available about shape-related nanoparticle toxicity. The present research suggests that shape has a significant impact on the cytotoxicity of the nanoparticles. In this study, spherical particles proved to be more toxic as compared to needle and capsule-shaped particles. Our findings open the door to more investigation into nanoparticle-induced toxicity as a result of shape variations in addition to size and surface chemical differences.

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Author contribution Rajnesh Kumar Sharma: Conceptualization; validation; investigation; resources; proof reading, review and editing; supervision.

Chetan Kumar: Conceptualization; resources; writing, original draft; visualization, funding acquisition.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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