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Original Article

GREEN AND CHEMICALLY SYNTHESIZED COPPER OXIDE NANOPARTICLES-A PRELIMINARY RESEARCH TOWARDS ITS TOXIC BEHAVIOUR

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ABSTRACT

Objectives: Metal oxide nanoparticles have been widely explored in various applications like biosensors, solar cells, biomedical applications in the recent times. However, biological applications of these nanoparticles needs to be low compatible and this is often found as a limitation. The present study aims to compare CuO nanoparticles prepared by chemical and green route to evaluate its suitability for biological applications.

Methods: CuO nanoparticles prepared by both methods were characterized by using XRD, UV-Visible spectroscopy, FTIR, ZETA for phase structure, size, the functional group presence, thermal stability and surface charge stability respectively. Free radical scavenging potential and phenolic contents, was assessed for biologically prepared CuO nanoparticles.

Results: Green synthesized CuO nanoparticles were found to have higher free radical scavenging potential with good colloidal stability and was also found to have well defined monodisperse nature as compared to chemically synthesized CuO nanoparticles. Evaluation of toxicity in cell line LLC PK1 was found to be slightly toxic.

Conclusion: Green synthesized CuO nanoparticles was found to have good stability when compared to chemically synthesized ones showing that they possess desired attributes to be used for biomedical applications.

Keywords: CuO nanoparticles, Chemical synthesis, *Desmodium gangeticum,* X-ray diffraction, Crystal structure.

INTRODUCTION

Nanotechnology has been focused as an important branch of science in current era of research due to its unique size-related enhanced characteristics, tailoring of desired properties and their reliability in various fields. Metal nanoparticles, due to their outstanding properties especially its small dimensions made them to occupy an important place in diverse field of applications such as conductive inks, sensor devices, catalysis, etc. Many metal nanoparticles are under active research because they possess interesting physical properties differing considerably from that of the bulk phase [1]

Copper is the most important trace element that plays the significant role in biological process. It is essential for proper functioning of human body. Henceforth, considering the importance of copper and its unique properties viz., potent catalytic [2], optical [3] and thermal conduction ability [4], it is widely compared with other metal nanoparticles.

The most common method employed for the synthesis of metal nanoparticles is the reduction of metal ions in suitable media. Though chemical methods have some advantages, it has more limitations such as use of toxic chemicals and production of toxic by-products, often act as the main barrier for its biological application.

Biosynthetic green approach has been reported to be safe and eco-friendly, involving the use of fungi [5], bacteria and plants [6-7], has become a reasonable optional alternative for chemical synthetic method. Phytocompounds in plants such as alkaloid, flavanoids have been reported to possess high reducing potential which makes them ideal for synthesis of nanoparticles with less time consumption, low cost and enhanced efficacy. However, not all phytocompounds are safer as flavonoids are reported to be toxic in earlier studies.

In this study, we synthesized CuO nanoparticle by chemical and green route, using metal reduction method. For the green synthesis, *Desmodium gangeticum* [fabaceae family] was used as reducing agent as it possesses many bioreducing and biostabilizing phytocompounds [8]. It was widely used against inflammation [9], fever, ischemia reperfusion injury [10], myocardial infarction [11], analgesic activities and other neurological imbalances [12]. Synthesized nanoparticles were characterized and compared by evaluating their physico chemical nature, colloidal stability, free radical scavenging efficacy and toxicological effects.

MATERIALS AND METHODS

Materials

Copper sulphate ($CuSO_4$) purchased from Sigma Aldrich, PVP (Polyvinylpyrollidine), NaOH(sodium hydroxide), $NaBH_4$ (sodium borohydride), DPPH, nitro blue tetrazolium, riboflavin, hydrogen peroxide, sodium nitroprusside, Griess reagent, Folin-ciocalteu reagent, sodium carbonate, potassium hexacyano ferrate purchased from Sigma Aldrich; Muller Hinton agar, Agar agar from HI-MEDIA; LLC-PK1 cell line from NCCS Pune, India) were also used.

Preparation of aqueous *Desmodium gangeticum* root extract

One kilogram (1 kg) of fresh secondary roots of DG was sliced and air-dried at room temperature. The sliced, air-dried roots of the plant were milled into fine powder in a commercial blender. The powder was Soxhlet extracted with 2.5 liters of distilled water at 37°C for 24 hours. The combined aqueous extracts were filtered and concentrated to dryness under reduced pressure at 30±1°C. The resulting aqueous extract was freeze-dried, finally giving 18.66 g [i.e., 1.866% yield] of a light-brown, powdery crude aqueous root extract of DG. Aliquot portions of the crude root aqueous extract residue was weighed and dissolved in distilled water (2.5 mg/ml) for use on each day of our experiment. Preliminary qualitative thin layer chromatography analysis of the aqueous extract showed the presence of alkaloids (Dragendorffs reagent), flavones (Shinoda test and Uranium acetate test), Phenols (4 amino anti pyrine test) and steroids (para-toluene sulfonic acid test).

Synthesis of Copper oxide nanoparticles

Chemical synthesis

Copper oxide nanoparticles were prepared in room temperature using $CuSO_4$ (10 mM) as substrate, (20 mM) PVP as capping agent, NaOH (100 mM) as stabilizing agent and $NaBH_4$ (100 mM) as reducing agent. Black colored nanoparticles were formed which was then centrifuged, dried and collected.

Green synthesis

Green synthesis of copper oxide nanoparticles was carried out using CuSO4 (10 mM) as precursor and aqueous DG root extract as bioreducer, mixed in the ratio 1:4 followed by heating @ 80 ^{0}C for 30 minutes. We observed the color change from brown to black which confirms the formation of copper oxide nanoparticles. Thus obtained nanoparticles were dried and stored.

Characterization of Copper oxide nanoparticles

Absorption spectrum of the copper oxide nanoparticles was recorded using UV-Visible spectrophotometer in the wavelength range of 300-800 nm. Crystalline structure of synthesized copper oxide nanoparticles was examined using X-ray diffractometry (Ultima IV, Rigako Make). Diffraction pattern was recorded in a wide range of Bragg angles (θ) using optimum operating parameters (40 kV and 30 mA) with the scanning rate of 2°min^{-1} and Cu Kα target (λ-1.5405 Å). FTIR spectral analysis was used to identify the functional groups present in copper oxide nanoparticles. Transmission spectrum was recorded using the Perkin Elmer FT-IR system (Spectrum GX mode) in the frequency range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. The average hydrodynamic particle size and surface potential was measured by Malvern Zeta Sizer. Measurements were done at 25 °C after the dilution of samples in deionized water (RI 1.330, Viscosity 0.8872) using the electrophoretic light scattering technique.

DPPH, Superoxide, total phenol assay

DPPH (2, 2-diphenyl-2-picryl hydrazyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a color change from deep violet to light yellow which can be measured spectrophotometricly at 518 nm. The scavenging effect of copper oxide nanoparticles was carried out according to Solver-Rivas *et al.* (2000) protocol, with slight modification [13]. The total phenolic content determination was done by using Folin-Ciocalteau reagent in alkaline medium [14]. Superoxide radical scavenging activity was carried out according to Winterbourn *et al.* (1975) [15].

Toxicology

LDH assay was used to measure the cytotoxicity of copper oxide nanoparticles. Cells (LLC-PK1) were cultured in 96 well plates. After cells reached 60% confluent state, copper oxide nanoparticles and its precursor were added and incubated for 24 hours. Later, 50 µl of culture supernatant were collected and treated with the reaction mixture containing NAD^{+}, lactate and phosphate buffer (pH-7.4) with the final volume 200 µl each [16].

Statistical analysis

For the biological assays like DPPH, Superoxide radical scavenging, total phenol content and LDH activity, data signify the mean±S. D. of three samples and are representative of three independent experiments. All statistical analysis was performed using Graph Pad prism 5.

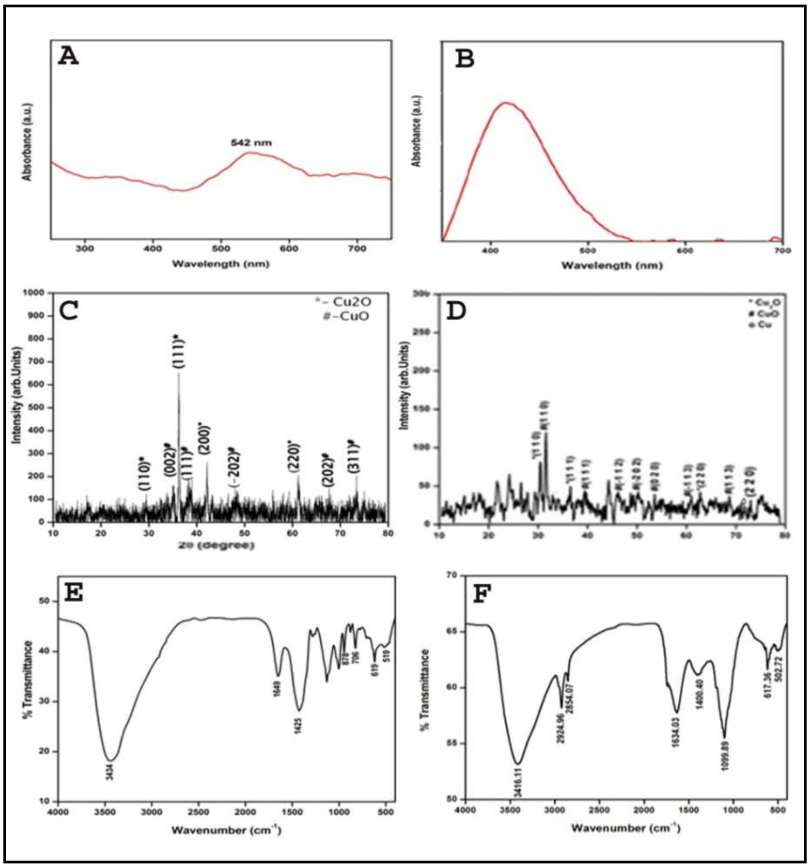

Fig. 1: Characterization of chemical and green synthesized copper oxide nanoparticle by UV-Visible spectrum, X-Ray Diffractometry and Fourier Transform Infrared Spectroscopy

RESULTS AND DISCUSSION

Characterization

CuO nanoparticles were prepared by metal reduction method and confirmed using UV visible spectrophotometer. From Fig.1A & 1B, it was observed that chemically synthesized CuO NPs exhibit broad surface plasmon resonance (SPR) spectral band at 542 nm and green synthesized CuO NPs exhibit sharp SPR band at 415 nm. The oscillation of free electron confined to the surface is responsible for SPR, which is more sensitive towards size of the particle. Hence, lower the lambda max value, smaller the size of the nanoparticles. This result is in accordance with the crystallite size determined from XRD peak broadening.

XRD

Fig. 1C & 1D shows the XRD pattern of CuO nanoparticles. Nanoparticles prepared by both methods were found to exhibit polycrystalline nature. The sharp peaks at 29.54^0, 36.19^0, 42.18^0 and 61.10^0 2θ angles corresponding to (110), (111), (200) and (220) hkl values confirms the Cu_2O crystalline cubic phase (JCPDS file no.05-0667) and peaks at 35.06^0, 38.36^0, 48.17^0, 67.62^0, 73.28^0 corresponding to (002), (111), (-202), (202), (311) hkl reflections representing CuO monoclinic phase (JCPDS file no.05-0661) for chemically synthesized copper oxide nanoparticles. In case of green synthesized nanoparticles presence of two crystalline phase was confirmed viz., monoclinic cupric oxide (CuO) and cubic cuprous oxide (Cu_2O). The peaks observed in the spectrum with 2θ Braggs angles of 30.26^o, 36.45^o and 62.56^o are indexed as (110), (111) and (220) hkl planes that are in good agreement with those of powder Cu_2O from the ICDD card (JCPDS file no. 05-0667). Peaks at 31.6^o, 39.2^o, 46.23^o, 49.7^o, 53.3^o, 60.97^o and 68.3^o are indexed as (110), (111), (-112), (-202), (020), (-113) and (113) hkl planes that are in good agreement with the values of monoclinic cubic phase of CuO (JCPDS file no. 45-0937). Peak with 2θ = 74.18^o matches with the JCPDS pattern of copper oxide nanoparticles (file no. 04-0836) confirming the face centered cubic structure. The peak broadening was used to calculate average crystallite diameter of the CuO nanoparticles. By using Scherrer formula the size was found to be 1.46 nm and 0.76 nm for chemical and green synthesized CuO nanoparticles.

FTIR

In order to understand the nature of capping agent and the presence of different functional groups responsible for the synthesis of monodispersed copper oxide nanoparticles, FTIR measurement was carried out. The interaction between different chemical species and changes in the chemical environment of the sample was studied. The FTIR spectrum of copper oxide nanoparticles is shown in the fig. 1E &1F. The absorption is in the range of 400-4000 cm^{-1}. The spectra showed a main frequency band at 617 cm^{-1} which corresponds to copper oxide in Cu_2O phase [17]. The frequency band at 2928 cm^{-1} in DG IR spectra is shifted to 2924 cm^{-1} in chemical CuO NPs which refers to alkyl C-H stretching. The other frequencies at 3416 cm^{-1}, 2924 cm^{-1}, 2854 cm^{-1}, and 1400 cm^{-1} corresponds to H-O-H stretching, O-H stretching of alcohols/phenols, alkyl C-H stretching and C-O stretching respectively. The spectra of DG extract showed frequency bands 3746 cm^{-1}, 2928 cm^{-1}, 1400 cm^{-1} and 1079 cm^{-1} which is also present in the IR spectra of copper oxide nanoparticles which confirm the presence of DG phyto-constituents in the nanoparticles surface that may act as capping agent. (Data not shown)

In chemically synthesized copper oxide nanoparticles two main peaks were observed 619 cm^{-1}, 519 cm^{-1} which indicates Cu-O stretching [17]. The other peaks 3434 cm^{-1}, 1649 cm^{-1}, 1425 cm^{-1} and peaks found between 706-878 cm^{-1} corresponds to O-H stretching, carbonyl stretching, CH_2 asymmetric bending and M-O-M bending (M=Cu) [18].

Zeta potential

Zeta potential is used to identify the surface charge of the particle which acts as an indicator of their colloidal stability. Nanoparticles with high positive or negative zeta potential show dispersion stability, as a result, do not agglomerate on storage. Zeta potential of chemical synthesized and green synthesized nanoparticles was found to be-5.51 and-6.30 respectively (Fig. 2A & 2B). The hydrodynamic diameter of chemically synthesized CuO nanoparticle was found to be 1757 nm with PDI (poly dispersity index) of 0.972(Fig.2C &2D) This result suggests that the chemically synthesized CuO nanoparticles exhibit intermediate colloidal stability and polydispersed in nature. In case of green synthesis, the hydrodynamic diameter was found to be 1207 with less PDI of about 0.373, which implies the monodispersed nature of the nanoparticles with good colloidal stability.

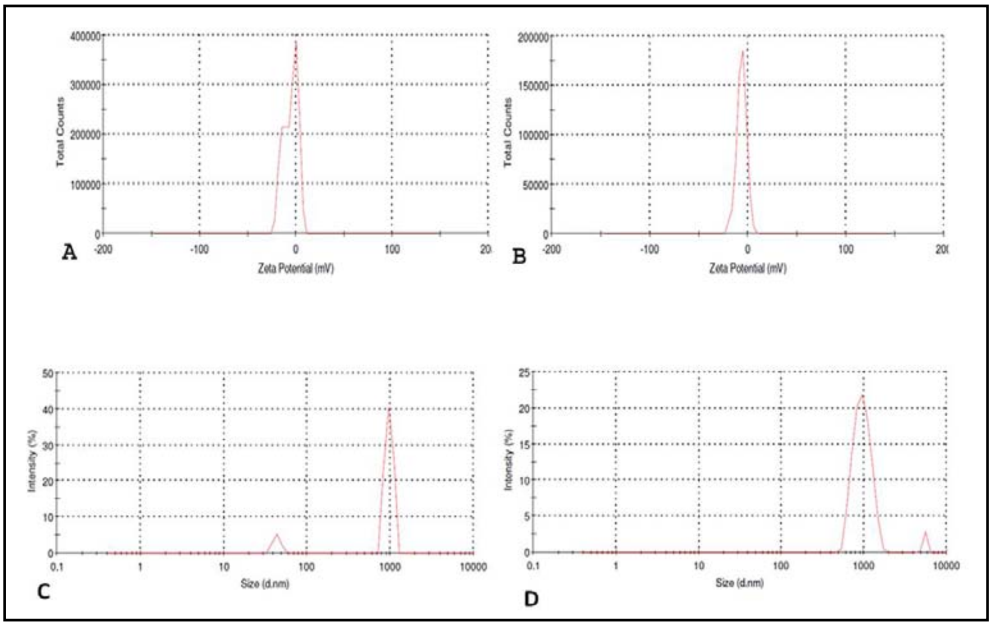

Fig. 2: Zeta potential and size distribution of chemical and green synthesized copper oxide nanoparticle

Antioxidant assay

Both chemical and green synthesized CuO nanoparticles possess dose dependent antioxidant activity against DPPH and O_2^-free radical. Antioxidant potential against DPPH for green synthesized CuO nanoparticle was significant over chemically synthesized CuO nanoparticles. This result was in agreement with fig.3C, where DG synthesized nanoparticles exhibits better superoxide scavenging activity than chemically synthesized one. Higher antioxidant potential of green synthesized CuO nanoparticles may be due to the phytocompounds present in the DG extract which was confirmed by FTIR. Phenolic compounds are commonly found in all sort of plants and are responsible for multiple biological effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage [19]. Furthermore, total phenolic content was measured in both nanoparticles and the results showed maximum phenolic content in DG synthesized nanoparticle which may be responsible for high antioxidant potential.

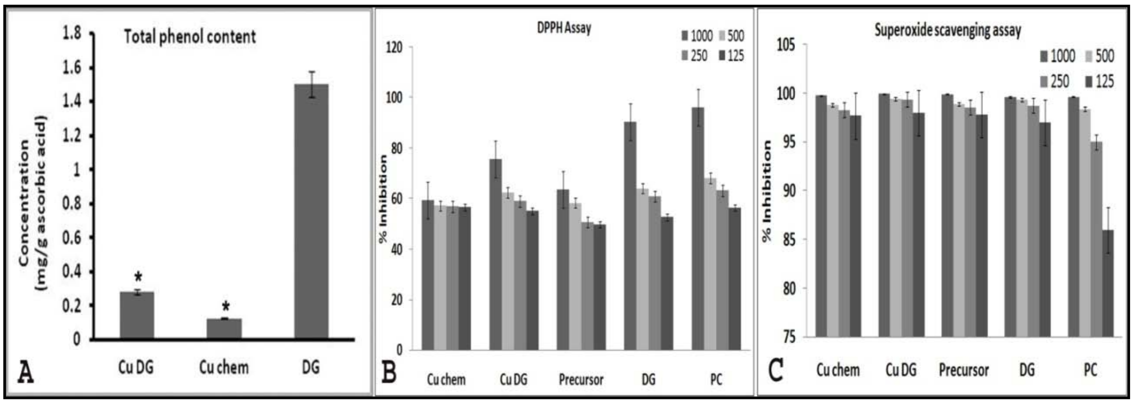

Fig. 3: Antioxidant potential of chemically synthesized and green synthesized copper oxide nanoparticle

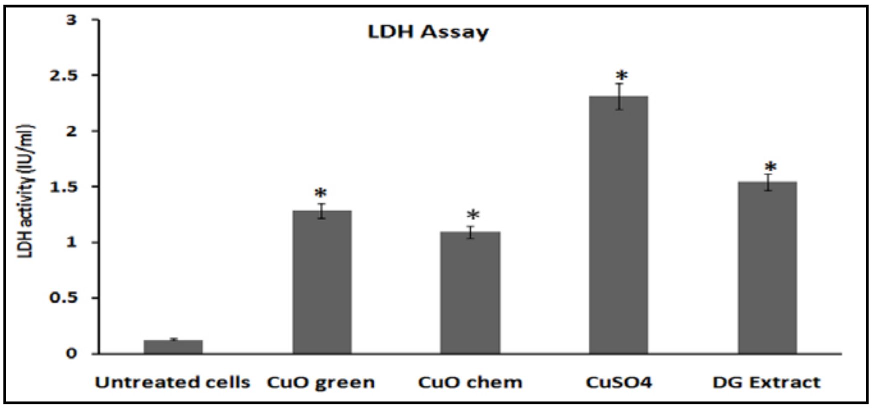

Fig. 4: Toxicological evaluation of copper oxide nanoparticles

In vitro toxicity

Contrary to our assumption, green synthesized nanoparticles showed comparable toxicity as that of chemically synthesized one, as shown in the Fig.4. Toxicity of green synthesized nanoparticle may be attributed to certain phytocompounds of DG such as flavanoids, saponins, that were reported to be cytotoxic [20]. Hence we can ascertain that not all green based technology is eco-friendly. Depending upon its nature, those particles can be used in various streams of applications. Thus green synthesized CuO NPs may find its application in the field of anti-cancer therapy. Further studies have to be done to understand the phytocompounds solely responsible for metal salt reduction as well as enhanced antioxidant potential.

CONCLUSION

We hereby reported the economically feasible biological approach to synthesize polycrystalline copper oxide nanoparticles with high yield when compared to chemical approach. Green synthesized CuO NPs were in well defined monodisperse nature with good colloidal stability and higher free radical scavenging potential as compared to chemically synthesized CuO NPs.

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CONFLICT OF INTERESTS

Declared None

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