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GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING ALOE BARBADENSIS (ALOE VERA) LEAF EXTRACT: OPTICAL CHARACTERIZATION AND IN VITRO CYTOTOXICITY EVALUATION IN 3T3-L1 MOUSE FIBROBLASTS

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ABSTRACT

Background: Copper oxide nanoparticles (CuO NPs) are increasingly explored for biomedical applications owing to their antimicrobial, antioxidant, and wound-healing-promoting properties. Conventional physical and chemical synthesis routes involve toxic reducing and capping agents that limit biocompatibility. Green synthesis using plant extracts offers an eco-friendly, low-cost alternative, but the biosafety of the resulting nanoparticles must be established before biomedical use.

Objective: To green-synthesize CuO NPs using Aloe barbadensis (Aloe vera) leaf extract as a reducing and capping agent, characterize the nanoparticles by UV–visible spectroscopy, and evaluate their dose-dependent cytotoxic potential on 3T3-L1 mouse fibroblast cells as a preliminary safety assessment prior to downstream biomedical formulation.

Methods: Dried Aloe barbadensis powder was extracted in boiling distilled water and filtered. The extract was combined with 30 mM copper sulfate solution and stirred continuously for 48 hours at 600 rpm to yield CuO NPs, which were characterized by UV–visible spectroscopy (250–650 nm) and recovered by centrifugation. Cytotoxicity was assessed by MTT assay on 3T3-L1 fibroblasts exposed to CuO NPs (25–250 µL/mL) for 24 hours (n = 3 per group), and morphological changes were assessed by phase-contrast microscopy.

Results: A concentration-dependent decline in cell viability was observed, from 97.38 ± 3.69% at 25 µL/mL to 47.35 ± 9.62% at 250 µL/mL, relative to untreated control (100%). Viability reduction reached statistical significance from 200 µL/mL onward ($p < 0.05$). The half-maximal inhibitory concentration (IC_{50}) was estimated at approximately 243 µL/mL. Phase-contrast microscopy revealed progressive cell rounding, shrinkage, and reduced confluency at 100 and 200 µL/mL compared with the smooth, spindle-shaped morphology of control fibroblasts.

Conclusion: Aloe barbadensis-mediated green synthesis yielded CuO NPs that were well tolerated by 3T3-L1 fibroblasts at concentrations up to approximately 150 µL/mL, with cytotoxicity emerging only at higher doses. These findings support a favourable biosafety margin for low-to-moderate concentrations of green-synthesized CuO NPs and provide a rational dose ceiling for their incorporation into biomedical formulations such as nanoparticle-augmented platelet-rich plasma for chronic wound healing.

KEYWORDS: Green synthesis; Copper oxide nanoparticles; Aloe barbadensis; MTT assay; Cytotoxicity; 3T3-L1 fibroblasts; Nanotoxicology; Wound healing

1.INTRODUCTION

Nanotechnology has emerged as a transformative discipline in biomedicine, enabling the engineering of particles with dimensions in the 1–100 nm range that display physicochemical properties distinct from their bulk counterparts. Among metal oxide nanomaterials, copper oxide nanoparticles (CuO NPs) have attracted considerable research interest because of their favourable electrical, catalytic, optical, and antimicrobial characteristics, together with their comparatively low production cost relative to noble-metal nanoparticles such as silver and gold.

Conventional physical and chemical methods of nanoparticle synthesis, including precipitation, sol-gel processing, and thermal decomposition, typically require hazardous reducing agents, high energy input, and generate toxic by-products that restrict biomedical translation. Green synthesis using plant extracts has therefore gained prominence as a sustainable alternative, in which phytochemicals such as flavonoids, polyphenols, and polysaccharides act simultaneously as reducing and capping agents, yielding nanoparticles with improved biocompatibility.

Aloe barbadensis Miller (Aloe vera) is a succulent plant widely used in traditional medicine for its wound-healing, anti-inflammatory, and antioxidant properties, attributable to bioactive constituents including anthraquinones, polysaccharides,

and glycoproteins. These same constituents have been shown to mediate the bioreduction of copper salts into copper oxide nanoparticles, and Aloe barbadensis-derived extracts have previously been used to synthesize monodisperse CuO nanoparticles with defined optical properties, as well as related metal oxide nanoparticles such as indium oxide.

While the antimicrobial and antioxidant potential of green-synthesized CuO NPs has been explored extensively, comparatively fewer studies have systematically characterized the dose-dependent effect of Aloe barbadensis-synthesized CuO NPs on mammalian fibroblast viability, which is a necessary preliminary step before such nanoparticles can be considered for incorporation into wound-care or regenerative formulations. Fibroblasts are the principal effector cells of dermal wound repair, and any candidate nanomaterial intended for topical or intra-lesional biomedical use must first demonstrate an acceptable safety margin in this cell type.

CuO NPs have been reported to induce dose-dependent cytotoxicity in a range of mammalian cell lines, an effect that is largely attributed to the generation of reactive oxygen species (ROS), depletion of intracellular glutathione, and consequent oxidative damage to mitochondrial membranes, ultimately triggering apoptotic cell death. Establishing the concentration range over which green-synthesized CuO NPs remain biocompatible with fibroblasts is therefore essential to defining a safe working dose for subsequent biomedical application.

The present study was undertaken to (i) green-synthesize CuO NPs using an aqueous Aloe barbadensis leaf extract as a reducing and stabilizing agent, (ii) characterize the resulting nanoparticles by UV–visible spectroscopy, and (iii) evaluate their dose-dependent cytotoxic and morphological effects on 3T3-L1 mouse fibroblast cells using the MTT assay and phase-contrast microscopy, in order to define a biocompatible concentration range for future incorporation into wound-healing formulations.

2. MATERIALS AND METHODS

2.1 Chemicals and plant material

Dried powdered Aloe barbadensis (Aloe vera) was procured from a local Ayurvedic store in Poonamallee, Chennai, Tamil Nadu. Copper sulfate ($CuSO_4$), used as the precursor salt, and all other chemicals were of analytical grade. Distilled water was used throughout for extract preparation and nanoparticle synthesis.

2.2 Preparation of the herbal extract

One gram of dried Aloe barbadensis powder was weighed and added to 100 mL of distilled water. The mixture was boiled at 50–60 °C for 15–20 minutes, after which the extract was filtered through muslin cloth to remove particulate debris. The clarified filtrate was retained as the herbal reducing agent for nanoparticle synthesis.

2.3 Green synthesis of copper oxide nanoparticles

A 30 mM copper sulfate precursor solution was prepared by dissolving copper sulfate in 50 mL of distilled water. This solution was combined with 50 mL of the Aloe barbadensis extract and stirred continuously for 48 hours at 600 rpm using a magnetic stirrer at room temperature. A visible colour change in the reaction mixture, consistent with nanoparticle formation, was noted over the course of stirring. Following synthesis, the nanoparticle suspension was centrifuged at 8000 rpm for 10 minutes; the resulting pellet was separated, washed, and redistributed into sterile containers for further characterization and biological testing.

2.4 UV–visible spectroscopic characterization

The formation of CuO NPs was monitored by UV–visible spectroscopy across a wavelength range of 250–650 nm, which is consistent with the characteristic surface plasmon resonance absorption reported for green-synthesized copper oxide nanostructures in the literature.

2.5 Cell line and culture conditions

The 3T3-L1 mouse fibroblast cell line was used as the in vitro model for cytotoxicity evaluation. Cells were cultured under standard conditions (37 °C, humidified atmosphere with 5% CO_2) in complete growth medium supplemented with 10% fetal bovine serum and antibiotics, and were sub-cultured upon reaching sub-confluence.

2.6 MTT cytotoxicity assay

Cell viability was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay, a standard method for evaluating cellular proliferation and cytotoxicity. 3T3-L1 cells were seeded in culture plates and exposed to CuO NPs at concentrations of 25, 50, 100, 150, 200, and 250 µL/mL for 24 hours, alongside an untreated control group, with each concentration tested in triplicate (n = 3). Following treatment, MTT reagent was added and formazan absorbance was recorded as optical density (OD). Percentage cell viability at each concentration was calculated relative to the mean OD of the untreated control, which was set at 100%.

24h							
	CuO Np's (µl/ml)						
	Control	25	50	100	150	200	250
O.D	0.826	0.824	0.812	0.809	0.625	0.475	0.353
O.D	0.798	0.792	0.779	0.765	0.732	0.512	0.326
O.D	0.625	0.582	0.579	0.556	0.549	0.492	0.365
%	100	99.758	98.30508475	97.94188862	75.66585956	57.50605327	42.78450363
	100	99.2481203	97.619	95.865	91.72932331	64.160401	40.85213033
	100	93.12	92.64	88.96	87.84	78.72	58.4
Mean	100	97.37532985	96.18804412	94.25551676	85.07839429	66.79548476	47.34554465
SE	0	3.694026949	3.091783393	4.70219005	8.38024859	10.84968358	9.622071187
p-Value		0.161262269	0.05890007	0.061562673	0.059530384	0.028964494	0.022166727

2.7 Phase-contrast morphological evaluation

Morphological changes in 3T3-L1 cells following 24-hour exposure to CuO NPs at 100 and 200 µL/mL, alongside untreated controls, were examined using an inverted phase-contrast microscope to qualitatively assess alterations in cell shape, confluency, and attachment.

2.8 Statistical analysis

Data are expressed as mean ± standard error (SE) of three independent replicates per concentration. Statistical significance between control and treatment groups was determined, with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1 UV–visible spectral characterization

Progressive colour change of the reaction mixture over the 48-hour stirring period provided the first visual evidence of copper oxide nanoparticle formation. UV–visible spectroscopic scanning across the 250–650 nm range confirmed the characteristic absorption profile expected for CuO nanostructures synthesized via the Aloe barbadensis-mediated bioreduction route, consistent with prior reports of Aloe-mediated green synthesis of monodisperse copper oxide nanoparticles.

3.2 Dose-dependent cytotoxicity of CuO NPs on 3T3-L1 fibroblasts

Exposure of 3T3-L1 fibroblasts to increasing concentrations of CuO NPs for 24 hours produced a clear concentration-dependent decline in cell viability, as determined by the MTT assay (Table 1, Figure 1). Mean cell viability remained largely preserved at lower concentrations, measuring 97.38 ± 3.69% at 25 µL/mL and 94.26 ± 4.70% at 100 µL/mL relative to control. A more pronounced decline was evident from 150 µL/mL onward (85.08 ± 8.38%), with viability falling to 66.80 ± 10.85% at 200 µL/mL and 47.35 ± 9.62% at 250 µL/mL. The reduction in viability reached statistical significance at 200 µL/mL ($p = 0.029$) and 250 µL/mL ($p = 0.022$) compared with untreated control. Based on linear interpolation of the dose–response curve, the half-maximal inhibitory concentration (IC_{50}) was estimated at approximately 243 µL/mL.

Table 1. Effect of increasing concentrations of green-synthesized CuO NPs on 3T3-L1 fibroblast viability at 24 hours (MTT assay, n = 3). *p < 0.05 vs. control.

Concentration (µL/mL)	Mean OD ± SD (n = 3)	Cell viability (% of control, Mean ± SE)	p-value (vs. control)	Significance (p < 0.05)
0 (Control)	0.750 ± 0.109	100.00 ± 0.00	—	No
25	0.733 ± 0.131	97.38 ± 3.69	0.161	No
50	0.723 ± 0.126	96.19 ± 3.09	0.059	No
100	0.710 ± 0.135	94.26 ± 4.70	0.062	No
150	0.635 ± 0.092	85.08 ± 8.38	0.060	No
200	0.493 ± 0.019	66.80 ± 10.85	0.029*	Yes

Concentration (µL/mL)	Mean OD ± SD (n = 3)	Cell viability (% of control, Mean ± SE)	p-value (vs. control)	Significance (p < 0.05)
250	0.348 ± 0.020	47.35 ± 9.62	0.022*	Yes

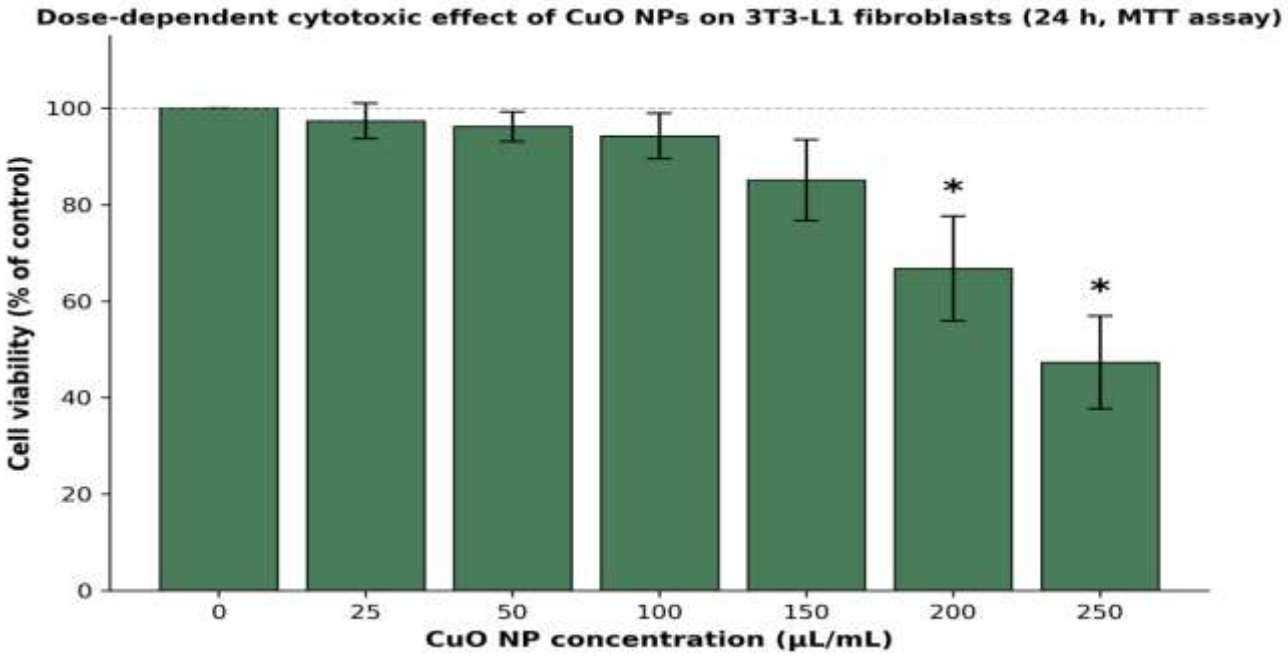

Figure 1. Dose-dependent effect of CuO NPs on 3T3-L1 fibroblast viability at 24 hours. Data are expressed as mean percentage viability ± SE (n = 3) relative to untreated control. * indicates statistical significance (p < 0.05) compared with control.

3.3 Morphological alterations induced by CuO NPs

Phase-contrast microscopic examination corroborated the viability findings. Control 3T3-L1 fibroblasts displayed a characteristic elongated, spindle-shaped morphology with uniform monolayer confluency. Cells exposed to 100 µL/mL CuO NPs showed a modestly reduced confluency with early morphological alteration, while cells treated with 200 µL/mL CuO NPs exhibited more pronounced changes, including cell rounding, shrinkage, loss of the characteristic spindle shape, and reduced overall cell density, consistent with the substantial decline in viability observed at this concentration.

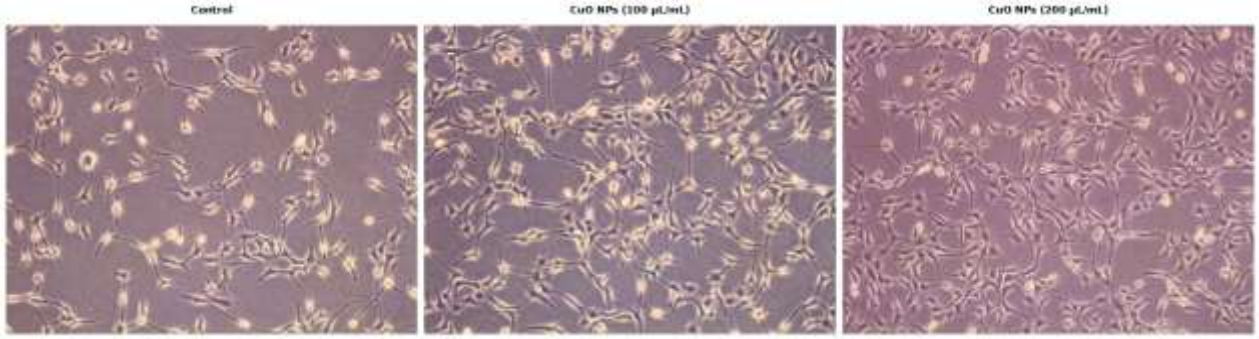

Figure 2. Phase-contrast micrographs of 3T3-L1 fibroblasts after 24-hour exposure to CuO NPs: (a) untreated control showing normal spindle-shaped morphology; (b) 100 µL/mL CuO NPs showing early morphological change; (c) 200 µL/mL CuO NPs showing marked cell rounding, shrinkage, and reduced confluency.

4. DISCUSSION

This study demonstrates that Aloe barbadensis leaf extract can effectively mediate the green synthesis of copper oxide nanoparticles, consistent with earlier reports describing Aloe-mediated bioreduction of copper salts into monodisperse CuO nanostructures with defined optical properties. The phytochemical constituents of Aloe barbadensis, including polysaccharides and phenolic compounds, are thought to serve a dual role as reducing and capping agents, a mechanism analogous to that described for Aloe vera-mediated synthesis of other metal oxide nanoparticles such as indium oxide.

The dose-dependent cytotoxicity observed in the present study is consistent with a broad body of literature describing concentration-dependent reductions in mammalian cell viability following CuO NP exposure. Comparable dose-dependent MTT-assay findings have been reported in mouse embryonic BALB 3T3 fibroblasts exposed to CuO nanoparticles, as well as in human hepatocarcinoma and

other cell lines, where cytotoxicity has been attributed principally to nanoparticle-induced generation of reactive oxygen species, depletion of cellular antioxidant defences, and consequent oxidative damage to mitochondrial membranes culminating in apoptotic cell death. The relatively preserved viability observed at low-to-moderate concentrations (up to approximately 150 µL/mL) in the present study, followed by a steep decline at 200–250 µL/mL, mirrors this general pattern of a biocompatible threshold beyond which oxidative injury becomes dominant.

The corresponding morphological changes observed by phase-contrast microscopy, namely progressive cell rounding, shrinkage, and reduced confluency at higher concentrations, are typical early morphological correlates of oxidative stress-induced cytotoxicity and are consistent with descriptions of nanoparticle-induced cytoskeletal disruption and membrane destabilization reported for copper oxide nanoparticles in fibroblast and epithelial cell models.

From a translational perspective, these findings carry particular relevance for the development of copper nanoparticle-based biomedical formulations intended for chronic wound management, in which controlled, low-dose copper delivery is thought to promote angiogenesis, collagen synthesis, and antimicrobial protection without inducing cytotoxic injury to resident fibroblasts. The present data suggest that concentrations up to approximately 150 µL/mL of Aloe barbadensis-synthesized CuO NPs may be reasonably well tolerated by fibroblasts, providing a rational upper limit for dose selection in such formulations, while concentrations approaching or exceeding the estimated IC_{50} of 243 µL/mL should be avoided in biomedical applications requiring fibroblast viability.

This study has certain limitations. Structural and elemental characterization of the synthesized nanoparticles by complementary techniques such as X-ray diffraction, Fourier-transform infrared spectroscopy, and electron microscopy was outside the scope of the present preliminary evaluation, and would strengthen future confirmation of nanoparticle size, crystallinity, and surface chemistry. Additionally, the cytotoxicity assessment was limited to a single time point (24 hours) and a single cell line; extension to additional exposure durations, primary human dermal fibroblasts, and mechanistic assays of oxidative stress and apoptosis would further substantiate the biosafety profile of these nanoparticles prior to clinical translation.

5. CONCLUSION

Aloe barbadensis leaf extract successfully mediated the green synthesis of copper oxide nanoparticles, offering an eco-friendly alternative to conventional chemical synthesis routes. Cytotoxicity evaluation in 3T3-L1 mouse fibroblasts demonstrated that these nanoparticles are well tolerated at concentrations up to approximately 150 µL/mL, with significant dose-dependent cytotoxicity emerging only at 200 µL/mL and above, and an estimated IC_{50} of approximately 243 µL/mL. These findings define a preliminary biocompatible concentration range that can inform the rational design of CuO nanoparticle-based biomedical formulations, including nanoparticle-augmented platelet-rich plasma preparations for chronic wound healing, and support further preclinical characterization of this green-synthesized nanomaterial.

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Conflict of Interest

The authors declare no conflict of interest.

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Nil.

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