RESEARCH PAPER

Evaluation of Glycyrrhiza glabra-Mediated Graphene Oxide and Copper Oxide Nanoparticles for Embryonic Cytotoxicity: An In Vitro Comparative Study

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Abstract

Background

Nanotechnology has significantly advanced biomedical and dental research through the development of nanoparticles with enhanced antimicrobial and regenerative properties. Graphene oxide (GO) and copper oxide (CuO) nanoparticles are widely investigated because of their unique physicochemical and biological characteristics. However, concerns regarding nanoparticle-induced toxicity necessitate detailed biocompatibility evaluation before clinical application.

Aim

To evaluate and compare the embryonic cytotoxic effects of graphene oxide (GO) and Glycyrrhiza glabra-mediated copper oxide (CuO) nanoparticles using zebrafish embryos as an experimental model.

Materials and Methods

Wild-type zebrafish embryos were exposed to different concentrations (5, 10, 20, 40, and 80 µg/mL) of green-synthesized GO and CuO nanoparticles. Nanoparticles were synthesized using Glycyrrhiza glabra extract as a reducing and stabilizing agent. Embryos were monitored for hatching rate, viability, mortality, and developmental abnormalities over the exposure period. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

Results

CuO nanoparticles demonstrated a significant concentration-dependent reduction in embryo hatching rates, with the lowest hatching observed at 80 µg/mL. GO nanoparticles maintained relatively stable hatching rates at lower concentrations but showed significant reduction at higher concentrations. Embryo viability decreased progressively with increasing GO concentration, whereas CuO nanoparticles demonstrated comparatively lesser effects on viability except at the highest concentration. Statistical analysis revealed highly significant intergroup differences for both hatching and viability rates ($p < 0.001$).

Conclusion

Both GO and CuO nanoparticles demonstrated dose-dependent embryotoxicity in zebrafish embryos. Green-synthesized GO nanoparticles exhibited comparatively lower toxicity than CuO nanoparticles, particularly at lower concentrations, suggesting improved biocompatibility and safer potential for biomedical applications. Nevertheless, higher concentrations of both nanoparticles produced significant developmental toxicity, highlighting the need for further molecular and long-term in vivo safety evaluations.

Keywords: Graphene oxide; copper oxide nanoparticles; zebrafish embryos; embryotoxicity; nanotoxicology; green synthesis

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INTRODUCTION

Nanotechnology has revolutionized the field of dentistry by introducing advanced nanomaterials, clinical tools, and devices, offering unprecedented potential for comprehensive oral healthcare (1).The term "nano" originates from the Greek word "nanos," meaning "small," and represents one-billionth of a unit (2). Nanomaterials are ultrafine solid particles ranging from 1 to 100 nanometers in size. Their distinctive physicochemical properties, including small particle size, high surface area-to-mass ratio, and enhanced reactivity, make them highly promising for antibacterial applications (3,4). Nanoparticles within this field can be categorized based on their shape, size, and chemical characteristics, into carbon-based NPs, metal NPs, ceramic NPs, lipid NPs, semiconductor NPs, and polymeric NPs (5). Nanoparticles (NPs), synthesized from various metals and metal oxides, provide a versatile platform for a wide range of innovative applications (6). Graphene, an advanced

carbon-based nanomaterial with outstanding physical, chemical, and biological characteristics. It possesses a wide variety of oxygen-containing functional groups, including carboxyl, hydroxyl, and keto groups on its surface. Its biomedical applications include drug and gene delivery, biosensing, tissue engineering, bioimaging, and photothermal therapy (7).

Copper and copper-based nanoparticles, known for their strong antibacterial effects, are effective against a broad range of bacterial species (8,9). Both metallic copper and copper oxide nanoparticles demonstrate a broad spectrum of antibacterial toxicity, even against multidrug-resistant bacteria such as MRSA, often referred to as the "superbug" (10,11). In the present study, copper oxide nanoparticles were green synthesized using *Glycyrrhiza glabra* extract as a biological reducing agent. Green synthesis involves eco-friendly approaches for the production of materials, chemicals, and nanoparticles. This method employs biological agents such as plant leaf extracts and microorganisms including fungi, bacteria, and algae to minimize toxicity (12). The rapidly expanding interest in the biomedical applications of nanoparticles necessitates a thorough evaluation of their potential toxicity. Despite the numerous benefits they offer, there remain concerns regarding their possible adverse effects on living organisms (13). Toxicology, which investigates the harmful effects of various elements on living systems and the environment, has gained increased relevance with the rise of nanotechnology. The increased exposure of nanoparticles in both the environment and among humans has become a significant concern regarding their impact on human health(14). The intersection of nanotechnology and toxicology has thus given rise to the field of 'nanotoxicology' (15).

The primary objective of medicine and public health is to prevent harm, reflecting the Hippocratic principle of "primum non nocere," meaning "first, do no harm." (16). This study is designed to assess the cytotoxic effects of graphene oxide (GO) nanoparticles and *Glycyrrhiza glabra*-mediated copper oxide (CuO) nanoparticles on zebrafish embryos. Different nanoparticle concentrations were assessed for their effects on embryo viability, hatching rate, mortality, and developmental abnormalities to compare their toxicological profiles and biocompatibility.

MATERIALS AND METHODS

Fish Maintenance and Cu Oxide and GO Nanoparticle Exposure

Wild-type zebrafish (*Danio rerio*), obtained from regional vendors in India, were kept in separate tanks under well-maintained conditions. The water temperature was set at 28 ± 2°C, with a 14-hour light and 10-hour dark cycle, and the pH was maintained between 6.8 and 8.5. The fish were fed twice a day with commercially available dried bloodworms or other suitable food options. For embryo collection, one female zebrafish was paired with three males in breeding tanks. The resulting fertilized eggs were carefully gathered and rinsed three times with freshly prepared E3 medium, excluding methylene blue. The cleaned eggs were then transferred into culture plates of different well sizes (6, 12, or 24 wells), with a density of 20 embryos per 2 mL of solution in each well. Both experimental and control setups were conducted in triplicate to ensure reliability

Nanoparticle preparation

Synthesis of Graphene Oxide (GO) Nanoparticles

Graphene oxide (GO) nanoparticles were prepared using a modified Hummers' technique . Briefly, 1 g of graphite powder was added to 25 mL of concentrated sulfuric acid under ice-cold conditions. Subsequently, 0.5 g of sodium nitrate and 3 g of potassium permanganate were incorporated gradually while maintaining the temperature below 5°C to avoid excessive oxidation. The mixture was then stirred continuously at room temperature for approximately 2 hours.

Following oxidation, 50 mL of distilled water was added slowly, and the reaction mixture was heated to nearly 98°C for 15 minutes. The reaction was terminated by adding 100 mL of distilled water along with 5 mL of hydrogen peroxide, resulting in a visible yellow coloration. The obtained product was repeatedly washed with distilled water and centrifuged at 10,000 rpm until neutral pH was achieved. The purified GO nanoparticles were dried at 60°C and stored in airtight containers. For experimental use, 0.100 g of GO powder was dispersed in 5 mL of ethanol

Green Synthesis of Copper Oxide (CuO) Nanoparticles

Copper oxide nanoparticles were synthesized using a green synthesis method with *Glycyrrhiza glabra* root extract . Initially, 1 g of dried root powder was boiled in 100 mL distilled water at 50°C for 20 minutes, followed by filtration to obtain the plant extract. Separately, a 30 mM copper sulfate solution was prepared in distilled water and mixed with the extract in equal proportions.

The reaction mixture was continuously stirred at 450 rpm for 48 hours. Formation of CuO nanoparticles was confirmed by colour change and ultraviolet-visible spectroscopy analysis between 250–650 nm. After synthesis, the nanoparticles were collected by centrifugation at 8000 rpm for 10 minutes, thoroughly washed, and refrigerated for subsequent use

Healthy, fertilized embryos were then exposed to different concentrations of GO and CuO nanoparticles (5, 10, 20, 40, and 80 µg/mL) for durations ranging from 24 to 72 hours post-fertilization. Control groups

were kept in E3 medium, while dead embryos in the nanoparticle-treated groups were removed every 12 hours. To protect the embryos from light, all experimental plates were covered in foil and kept at a constant temperature of 28°C .

Assessment of Zebrafish Embryos

During the nanoparticle exposure period post-fertilization, the development of zebrafish embryos was carefully observed using a stereo microscope. The embryos were exposed to varying concentrations of GO and CuO nanoparticles (5, 10, 20, 40, and 80 µg/mL) for periods ranging from 24 to 72 hours, with the development process closely monitored at regular intervals. Mortality and hatching rates were recorded every 24 hours to track the effects of the nanoparticle treatments. The primary endpoints of the study focused on assessing embryo and hatchling mortality, the hatching rate, and identifying any developmental malformations in both the treated and control groups. Photographs of any malformed embryos were taken using a COSLAB Model HL-10A light microscope, and the proportion of abnormal embryos was documented at 24-hour intervals throughout the exposure period.

Statistical Analysis

All experimental data were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software version 23.0 (IBM Corp., Armonk, NY, USA). Intergroup comparisons were carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple group comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1. Hatching Rate (%) – CuO Exposure

Concentration	Mean ± SD (%)	One-way ANOVA (F)	p-value
Control	99.3 ± 1.15	201.6	< 0.001
5 µg/mL	99.3 ± 1.15		
10 µg/mL	80.0 ± 2.00		
20 µg/mL	78.7 ± 3.06		
40 µg/mL	60.0 ± 2.00		
80 µg/mL	58.7 ± 3.06		

Table 2. Hatching Rate (%) – GO Exposure

Concentration	Mean ± SD (%)	One-way ANOVA (F)	p-value
Control	99.3 ± 1.15	69.2	< 0.001
5 µg/mL	99.3 ± 1.15		
10 µg/mL	99.3 ± 1.15		
20 µg/mL	98.0 ± 2.00		
40 µg/mL	80.0 ± 2.00		
80 µg/mL	78.0 ± 2.00		

Table 3. Viability Rate (%) – GO Exposure

Concentration	Mean ± SD (%)	One-way ANOVA (F)	p-value
Control	99.3 ± 1.15	173.7	< 0.001
5 µg/mL	99.3 ± 1.15		
10 µg/mL	98.0 ± 2.00		
20 µg/mL	80.0 ± 2.00		
40 µg/mL	78.0 ± 2.00		
80 µg/mL	60.0 ± 2.00		

Table 4. Viability Rate (%) – CuO Exposure

Concentration	Mean ± SD (%)	One-way ANOVA (F)	p-value
Control	99.3 ± 1.15	34.5	< 0.001
5 µg/mL	99.3 ± 1.15		
10 µg/mL	99.3 ± 1.15		
20 µg/mL	98.7 ± 1.15		
40 µg/mL	98.0 ± 2.00		
80 µg/mL	80.0 ± 2.00		

Table 5. Tukey's Post Hoc Comparison for Hatching Rate (%) Following GO and CuO Exposure

Comparison	CuO (p-value)	GO (p-value)
10 µg/mL vs 20 µg/mL	0.973	0.909
10 µg/mL vs 40 µg/mL	< 0.001	< 0.001
10 µg/mL vs 5 µg/mL	< 0.001	1.000
10 µg/mL vs 80 µg/mL	< 0.001	< 0.001
10 µg/mL vs Control	< 0.001	1.000
20 µg/mL vs 40 µg/mL	< 0.001	< 0.001
20 µg/mL vs 5 µg/mL	< 0.001	0.909
20 µg/mL vs 80 µg/mL	< 0.001	< 0.001
20 µg/mL vs Control	< 0.001	0.909
40 µg/mL vs 5 µg/mL	< 0.001	< 0.001
40 µg/mL vs 80 µg/mL	0.973	0.671
40 µg/mL vs Control	< 0.001	< 0.001
5 µg/mL vs 80 µg/mL	< 0.001	< 0.001
5 µg/mL vs Control	1.000	1.000
80 µg/mL vs Control	< 0.001	< 0.001

Table 6. Tukey's Post Hoc Comparison for Viability Rate (%) Following GO and CuO Exposure

Comparison	GO (p-value)	CuO (p-value)
10 µg/mL vs 20 µg/mL	< 0.001	0.993
10 µg/mL vs 40 µg/mL	< 0.001	0.874
10 µg/mL vs 5 µg/mL	0.932	1.000
10 µg/mL vs 80 µg/mL	< 0.001	< 0.001
10 µg/mL vs Control	0.932	1.000
20 µg/mL vs 40 µg/mL	0.733	0.993
20 µg/mL vs 5 µg/mL	< 0.001	0.993
20 µg/mL vs 80 µg/mL	< 0.001	< 0.001
20 µg/mL vs Control	< 0.001	0.993
40 µg/mL vs 5 µg/mL	< 0.001	0.874
40 µg/mL vs 80 µg/mL	< 0.001	< 0.001
40 µg/mL vs Control	< 0.001	0.874
5 µg/mL vs 80 µg/mL	< 0.001	< 0.001
5 µg/mL vs Control	1.000	1.000
80 µg/mL vs Control	< 0.001	< 0.001

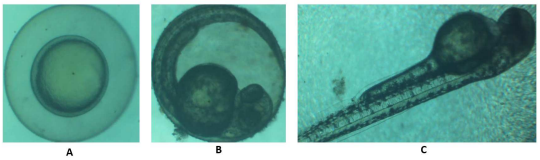


Figure 1. Zebrafish embryos exposed to GO nanoparticles at **(A)** 24 hours post-fertilization, **(B)** 48 hours post-fertilization, and **(C)** 72 hours post-fertilization.

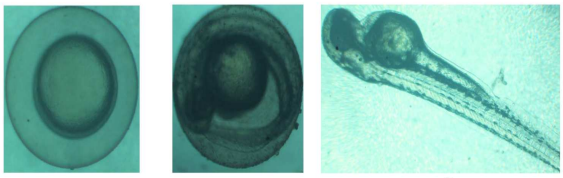


Figure 2. Zebrafish embryos exposed to CuO nanoparticles at (A) 24 hours post-fertilization, (B) 48 hours post-fertilization, and (C) 72 hours post-fertilization.

The hatching rate analysis following CuO nanoparticle exposure demonstrated a concentration-dependent reduction in embryonic development (Table 1). The control and 5 µg/mL groups exhibited similar hatching rates of 99.3 ± 1.15%, whereas significant reduction was observed from 10 µg/mL onwards. The hatching rate decreased to 80.0 ± 2.00% at 10 µg/mL and 78.7 ± 3.06% at 20 µg/mL, followed by a marked decline to 60.0 ± 2.00% and 58.7 ± 3.06% at 40 µg/mL and 80 µg/mL, respectively. One-way ANOVA revealed statistically highly significant intergroup differences ($F = 201.6$, $p < 0.001$). Tukey's post hoc analysis further demonstrated significant differences between higher concentrations (40 and 80 µg/mL) and lower concentration/control groups (Table 5).

GO nanoparticle exposure demonstrated relatively stable hatching rates at lower concentrations (Table 2). The control, 5 µg/mL, and 10 µg/mL groups exhibited identical hatching rates of 99.3 ± 1.15%, while a slight reduction was observed at 20 µg/mL (98.0 ± 2.00%). However, higher concentrations demonstrated notable reduction in hatching, with rates decreasing to 80.0 ± 2.00% and 78.0 ± 2.00% at 40 µg/mL and 80 µg/mL, respectively. One-way ANOVA demonstrated statistically significant differences among the groups ($F = 69.2$, $p < 0.001$). Tukey's post hoc analysis confirmed significant differences primarily between higher concentrations (40 and 80 µg/mL) and lower concentration/control groups (Table 5).

Embryo viability following GO nanoparticle exposure demonstrated a progressive concentration-dependent decline (Table 3). The control and 5 µg/mL groups exhibited viability rates of 99.3 ± 1.15%, whereas a slight reduction was observed at 10 µg/mL (98.0 ± 2.00%). Significant reduction in viability was observed from 20 µg/mL onwards, with viability decreasing to 80.0 ± 2.00% at 20 µg/mL and 78.0 ± 2.00% at 40 µg/mL. The lowest viability rate was observed at 80 µg/mL (60.0 ± 2.00%). One-way ANOVA revealed highly significant intergroup differences ($F = 173.7$, $p < 0.001$). Tukey's post hoc analysis demonstrated significant differences between lower concentration groups and concentrations ≥20 µg/mL (Table 6).

In contrast, CuO nanoparticle exposure demonstrated relatively stable viability rates at lower concentrations (Table 4). The control, 5 µg/mL, and 10 µg/mL groups showed similar viability rates of 99.3 ± 1.15%, with only minimal reductions observed at 20 µg/mL (98.7 ± 1.15%) and 40 µg/mL (98.0 ± 2.00%). A marked reduction in viability was observed only at the highest concentration of 80 µg/mL, where the viability rate decreased to 80.0 ± 2.00%. One-way ANOVA demonstrated statistically significant differences among the groups ($F = 34.5$, $p < 0.001$). Tukey's post hoc analysis confirmed statistically significant differences primarily involving the 80 µg/mL group when compared with lower concentration and control groups (Table 6). Overall, GO nanoparticles demonstrated greater reduction in embryo viability, whereas CuO nanoparticles exerted greater effects on embryo hatching at higher concentrations.

Zebrafish embryos exposed to GO nanoparticles demonstrated progressive developmental changes over time. At 24 hours post-fertilization, normal embryonic morphology was observed. Mild developmental alterations were evident at 48 hours post-fertilization, while at 72 hours post-fertilization, elongated body morphology and structural abnormalities were observed, indicating time-dependent embryotoxic effects of GO nanoparticles. (Fig 1)

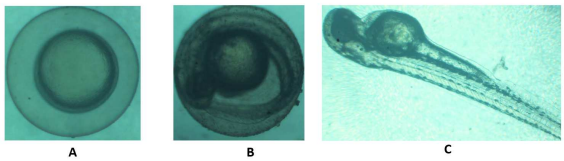


Figure 2. Zebrafish embryos are exposed to CuO nanoparticles at (A) 24 hours post-fertilization, (B) 48 hours post-fertilization, and (C) 72 hours post-fertilization.

As shown in Figure 2, zebrafish embryos exposed to CuO nanoparticles demonstrated progressive developmental abnormalities over time. At 24 hours post-fertilization, normal embryonic morphology was observed. Developmental alterations became evident at 48 hours post-fertilization, while at 72 hours post-fertilization, pronounced structural abnormalities and altered body morphology were observed, indicating the embryotoxic effects of CuO nanoparticles.

DISCUSSION

The rapid advancement of nanotechnology has significantly influenced biomedical science and dentistry through the development of engineered nanomaterials with unique physicochemical and biological properties. Despite these promising

applications, concerns regarding nanoparticle-associated toxicity remain a major obstacle for their safe clinical translation (17). Among these nanomaterials, graphene oxide (GO) and copper oxide (CuO) nanoparticles have attracted substantial attention because of their antimicrobial activity, regenerative potential, drug delivery applications, and biomaterial enhancement properties (18). The findings of the present study revealed that CuO nanoparticles produced greater reduction in hatching rate, whereas GO nanoparticles exerted comparatively greater effects on embryo viability at increasing concentrations. Nanoparticles possess high surface area-to-volume ratio, increased surface reactivity, nanoscale dimensions, and enhanced biological interaction capabilities that contribute to their therapeutic efficacy (5). However, these same properties may also induce oxidative stress, membrane disruption, inflammatory responses, and developmental toxicity (19). Nanotoxicology has therefore emerged as an important discipline aimed at understanding nanoparticle interactions with living systems and identifying potential biological hazards associated with nanoparticle exposure (20). Nanoparticle toxicity is influenced by concentration, particle size, morphology, aggregation state, surface chemistry, and exposure duration (21) .

Zebrafish embryos are increasingly recognized as an ideal vertebrate model for nanotoxicological investigations because of their optical transparency, rapid embryonic development, genetic similarity to humans, and suitability for high-throughput toxicity screening (22). Furthermore, the transparent chorion allows direct visualization of developmental abnormalities, hatching behavior, and embryonic mortality following nanoparticle exposure (23). Zebrafish embryos also respond sensitively to nanoparticle-induced oxidative stress and developmental disruption, making them reliable models for evaluating embryotoxicity (24). In the present study, zebrafish embryos exposed to GO and CuO nanoparticles exhibited concentration-dependent impairment in both hatching and survival, thereby validating the sensitivity of this model for developmental toxicity assessment.

One of the major findings of the present investigation was the significant reduction in hatching rates following CuO nanoparticle exposure. The control and 5 µg/mL groups maintained normal hatching rates, whereas significant decline was observed from 10 µg/mL onwards, with severe suppression at 40 and 80 µg/mL concentrations. Delayed hatching and increased embryonic mortality were observed following CuO nanoparticle exposure (25). Likewise, CuO nanoparticles significantly impair embryonic development through oxidative stress-mediated toxicity and membrane damage (26). The release of Cu^{2+} ion induces mitochondrial injury, inflammatory signaling, cellular membrane damage, and apoptosis .Copper oxide metal ion release significantly enhances oxidative stress and cytotoxicity compared with many other nanoparticle systems (27). In contrast to CuO nanoparticles, GO nanoparticles demonstrated relatively stable hatching rates at lower concentrations, with significant reductions occurring mainly at 40 and 80 µg/mL. These findings indicate that GO nanoparticles exhibit comparatively lower embryotoxicity than CuO nanoparticles. Similar observations were reported in by Chen et al., where lower GO concentrations caused minimal developmental toxicity (28). The comparatively safer profile of GO may be attributed to its carbon-based structure, reduced metal ion release, and enhanced hydrophilicity resulting from oxygen-containing functional groups such as hydroxyl, epoxy, and carboxyl groups(19,3). Concentration-dependent GO toxicity has been reported associated with oxidative stress generation, mitochondrial dysfunction, apoptosis, delayed hatching, and developmental abnormalities (29). Similarly, the present study demonstrated that higher GO concentrations reduced embryo viability. GO nanosheets may adhere to embryonic membranes and interfere with nutrient transport, gas exchange, and cellular signaling pathways (30). The present study demonstrated that embryo viability following GO exposure declined from 20 µg/mL onwards, reaching the lowest survival rate at 80 µg/mL.Collectively, GO toxicity is highly concentration-dependent and becomes biologically significant beyond threshold exposure levels. Interestingly, the present investigation revealed distinct toxicological patterns between GO and CuO nanoparticles. CuO nanoparticles exerted greater effects on hatching rate, whereas GO nanoparticles demonstrated comparatively greater reduction in embryo viability. This differential behavior may be explained by variations in nanoparticle composition, ion release characteristics, aggregation behavior, surface chemistry, and cellular uptake mechanisms (3).

The microscopic observations presented in Figures 1 and 2 demonstrated progressive developmental alterations in zebrafish embryos following exposure to GO and CuO nanoparticles. Morphological abnormalities such as altered body structure, developmental delay, and abnormal embryonic elongation suggest interference with normal embryogenesis and cellular organization. Comparatively greater structural alterations observed in the CuO nanoparticle group indicate increased embryotoxic potential, which may be associated with copper ion release and oxidative stress-mediated

cellular damage. Elsaesser et al. reported that nanoparticle exposure can induce reactive oxygen species generation, mitochondrial dysfunction, and membrane disruption during embryonic development (15).The comparatively milder alterations observed in the GO group suggest relatively better biocompatibility at lower concentrations, which is consistent with previous studies reporting lower toxicity profiles of graphene-based nanomaterials at controlled concentrations (18,30). Another noteworthy aspect of the present study is the use of Glycyrrhiza glabra-mediated green synthesis for nanoparticle reduction. Green synthesis approaches using plant extracts have gained considerable interest because they eliminate hazardous chemical reducing agents and may improve nanoparticle biocompatibility (31). Glycyrrhiza glabra contains flavonoids, glycyrrhizin, polyphenols, and saponins with antioxidant and reducing properties capable of facilitating nanoparticle stabilization and reduction (32). Phytochemical-mediated synthesis may reduce residual chemical toxicity and improve nanoparticle stability compared with conventional chemical synthesis methods (33). The findings of the present study have important implications for biomedical and dental nanotechnology. GO nanoparticles are increasingly explored for regenerative scaffolds, biosensors, implant coatings, antimicrobial formulations, and drug delivery systems because of their favorable mechanical and antibacterial properties (34). Similarly, CuO nanoparticles are widely investigated for their potent antimicrobial activity against oral pathogens and multidrug-resistant bacteria (35). However, the embryotoxic effects observed in the present study emphasize the importance of balancing therapeutic efficacy with biological safety. The comparatively lower toxicity profile of GO observed in the current investigation suggests that GO-based nanomaterials may possess greater potential for safer biomedical applications when used at controlled concentrations. In contrast, the higher toxicity associated with CuO nanoparticles indicates that their concentration and exposure duration must be carefully regulated before clinical application (35,3).

Overall, the present study demonstrated that both GO and CuO nanoparticles exert concentration-dependent embryotoxic effects in zebrafish embryos. CuO nanoparticles produced greater reduction in hatching rates, whereas GO nanoparticles affected embryo viability at higher concentrations. These findings highlight the importance of comprehensive nanotoxicological evaluation prior to biomedical application and contribute valuable evidence regarding the relative safety profiles of GO and CuO nanoparticles synthesized using Glycyrrhiza glabra-mediated green reduction methods. The primary innovation of this study lies in the direct comparative analysis of the toxic effects of GO and CuO nanoparticles on embryonic development, particularly focusing on both hatching and viability rates. Previous studies have individually assessed the toxicity of these nanoparticles, but our research provides a side-by-side comparison under identical experimental conditions. This comparative approach offers a clearer understanding of their relative toxicities, aiding in the safe application of these materials in various fields.

Limitations

The present study was limited to a zebrafish embryo model and may not completely represent nanoparticle behavior in mammalian or human biological systems. Molecular mechanisms underlying toxicity, including oxidative stress, apoptosis, and inflammatory pathways, were not evaluated. In addition, detailed physicochemical characterization of nanoparticles such as particle size analysis, zeta potential, SEM, TEM, and FTIR evaluation was not performed. The study also assessed only short-term embryonic exposure without investigating long-term developmental or genetic effects.

Future scope

Future studies should focus on evaluating the molecular mechanisms underlying GO and CuO nanoparticle toxicity using oxidative stress assays, gene expression analysis, and apoptotic biomarkers. Long-term in vivo studies using mammalian models are necessary to confirm the safety and biocompatibility of these nanoparticles. Further research should also investigate optimization of green synthesis protocols to improve nanoparticle stability while minimizing toxicity. In addition, incorporation of graphene-based green-synthesized nanoparticles into dental biomaterials, tissue engineering scaffolds, antimicrobial coatings, and regenerative applications may provide promising clinical benefits with improved biosafety.

CONCLUSION

Both GO and CuO nanoparticles showed dose-dependent embryo toxicity in zebrafish embryos. GO nanoparticles demonstrated comparatively lower toxicity than CuO nanoparticles at lower concentrations, indicating better biocompatibility. Further studies are required to confirm their safety for biomedical applications.

CONFLICT OF INTEREST

No conflict of interest

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