Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 1s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Green Synthesis of Copper Nanoparticles Using Boerhaavia diffusa: Evaluation of Cytotoxic and Anti-Diabetic Activities

Dr. Kayal Vennila Mahendran[1], Dr. Ranjeth Rajan KV[2], Dr. Priyadarshini[3], Dr. Ramesh. R.[*4]

[1]Resident Dental Intern

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India

Email ID: 151801025.sdc@saveetha.com

[2]Resident Dental Intern

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India

Email ID: 151901069.sdc@saveetha.com

[3]Assitant Professor

Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600077

[*4]Corresponding Author:

[*4]Department of Pedodontics,

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai – 600077

Email ID: rameshr.sdc@saveetha.com

Cite this paper as: Dr. Kayal Vennila Mahendran, Dr. Ranjeth Rajan KV, Dr. Priyadarshini, Dr. Ramesh. R., (2025) Green Synthesis of Copper Nanoparticles Using Boerhaavia diffusa: Evaluation of Cytotoxic and Anti-Diabetic Activities. *Journal of Neonatal Surgery*, 14 (1s), 796-805.

ABSTRACT

Background: Traditional nanoparticle synthesis methods often involve toxic chemicals, posing risks to the environment and human health. Green synthesis using plant extracts offers a sustainable and eco-friendly alternative. Boerhaavia diffusa, a medicinal herb, is rich in bioactive compounds and has potential for producing biocompatible nanoparticles with therapeutic applications.

Aim: To synthesize copper nanoparticles (CuNPs) using Boerhaavia diffusa extract and evaluate their cytotoxic and anti-diabetic activities.

Materials and Methods: Boerhaavia diffusa leaves were processed to obtain an extract used as a reducing and stabilizing agent for synthesizing CuNPs. The cytotoxicity of the nanoparticles was tested using brine shrimp assays at varying concentrations, while their anti-diabetic activity was assessed through α-amylase inhibition and DPPH assays. Statistical analysis was performed using the Shapiro-Wilk test for data normality, the Kruskal-Wallis test for group comparisons, and Spearman's rank correlation to evaluate relationships between concentration and observed effects.

Results: CuNP synthesis was confirmed by a color transition and UV-Vis spectroscopy. Cytotoxicity assays revealed survival rates of 70–100%, indicating low toxicity. Anti-diabetic assays demonstrated effective α-amylase inhibition and free radical scavenging. Statistical analysis confirmed significant dose-dependent cytotoxicity and wavelength-dependent absorbance properties.

Conclusion: Boerhaavia diffusa-mediated CuNPs are biocompatible and exhibit promising therapeutic potential, particularly for anti-diabetic applications, highlighting the feasibility of green synthesis for nanomedicine development.

Keywords: *Cytotoxicity; Nano-dentistry; Green mediated nanoparticles*

1. INTRODUCTION

Nanotechnology, involving the manipulation of matter at atomic and molecular scales, has revolutionized various sectors, including medicine, agriculture, and technology. Nanoparticles, typically ranging from 1 to 100 nanometers, exhibit unique properties that differ significantly from their bulk counterparts, making them invaluable in modern applications. Traditional chemical and physical methods of nanoparticle synthesis often involve hazardous substances, posing environmental and health risks. In response, green synthesis methods utilizing biological entities such as plants, algae, fungi, and bacteria have emerged as eco-friendly and sustainable alternatives. These biological methods not only reduce toxicity but also enhance biocompatibility, essential for medical applications.

Boerhaavia diffusa, commonly known as Punarnava, is a perennial herb prevalent in tropical regions of Asia, Africa, and Australia. Belonging to the Nyctaginaceae family, it has been extensively used in traditional medicine systems for its therapeutic properties. The plant is renowned for treating various ailments, including stress, congestive heart failure, and liver disorders. Its rich phytochemical profile comprises alkaloids, flavonoids, saponins, and phenolic compounds, which contribute to its medicinal efficacy.

Recent studies have explored the potential of B. diffusa in nanoparticle synthesis. For instance, an investigation into the antidiabetic activity of B. diffusa demonstrated its efficacy in modulating hepatic enzymes in diabetic rats, indicating its potential in managing diabetes mellitus [2]. Another study highlighted the plant's cytotoxic properties, suggesting its applicability in cancer therapy [3].

Copper nanoparticles (CuNPs) have garnered attention due to their antimicrobial, antioxidant, and anticancer properties. However, conventional synthesis methods of CuNPs often involve toxic chemicals, limiting their biomedical applications. The integration of green synthesis approaches using B. diffusa offers a promising avenue to produce biocompatible CuNPs with enhanced therapeutic potential.

The antidiabetic activity of biosynthesized copper oxide nanoparticles has been demonstrated in studies involving alloxan-induced diabetic rats, where significant reductions in blood glucose levels were observed [1]. Additionally, the cytotoxic effects of plant-mediated CuNPs have been reported, indicating their potential in cancer treatment [4].

This study aims to synthesize copper nanoparticles using Boerhaavia diffusa extract and evaluate their cytotoxic and antidiabetic activities. By leveraging the plant's phytochemicals as natural reducing and stabilizing agents, the research seeks to develop a sustainable method for producing CuNPs with potential therapeutic applications. The outcomes could contribute to the development of novel nanomedicines for managing diabetes and cancer, aligning with the global shift towards green nanotechnology in healthcare.

2. MATERIALS AND METHODS

2.1 Study Setting

This study was conducted at the Cancer and Stem Cell Laboratory, Saveetha Dental College, Chennai, India after approval from institutional ethical committee (Approval no: SRB/SDC/UG-1969/24/PATH/365). The study employed Boerhaavia diffusa and copper sulfate as powders, which facilitated easy measurement and application. Potential errors may arise when using a micropipette, which cannot be easily corrected, and the presence of additional bacterial or fungal organisms in culture test tubes may affect results. Random sampling was used, with internal validity ensured through procedure validation by nano researchers, and external validity confirmed by independent validation.

2.2 Preparation of Plant Extract

Boerhaavia diffusa leaves were collected from local fields, dried in a shaded area, and crushed into powder using a grinder. One gram of the powder was mixed with 100 ml of distilled water in a conical flask. The solution was heated at 50-60°C for 5-10 minutes in a heating mantle until small bubbles appeared. After heating, the solution was filtered using filter paper. Figure 1: Preparation of plant extract (A) Weighing Boerhaavia diffusa (1g) (B) Boerhaavia diffusa extract in a beaker.

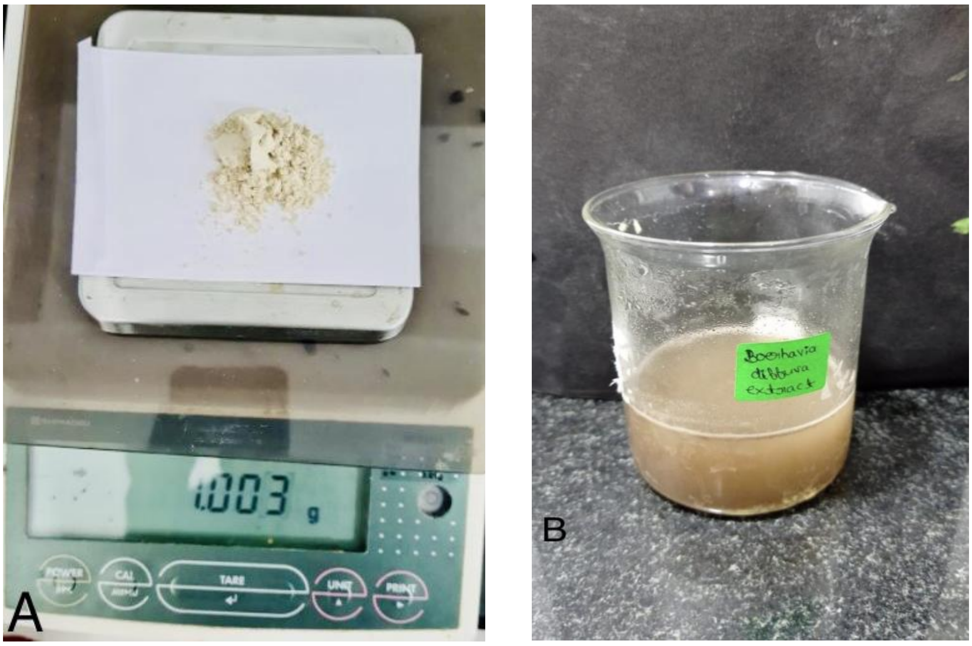


Figure 1: Preparation of plant extract (A), Figure A shows weighing of Boerhaavia diffusa (1g) (B), Figure B shows Boerhaavia diffusa extract in a beaker.

2.3 Preparation of Copper Nanoparticles

Anhydrous copper sulfate (0.477g) was dissolved in 70 ml of distilled water, then added to 30 ml of the Boerhaavia diffusa extract. The solution, which turned dark green, was heated at 50-60°C for 6-8 minutes. The resulting copper nanoparticles were subjected to UV-visible absorption spectroscopy for optical property analysis. Figure 2: Preparation of copper nanoparticles (A) Weighing copper sulfate (0.477g) (B) Copper sulfate solution in a conical flask.

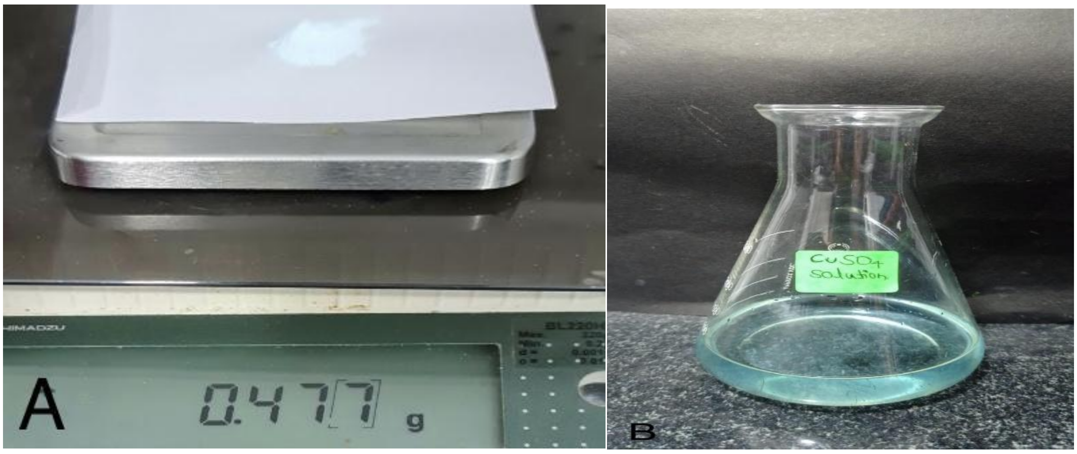


Figure 2: Preparation of copper nanoparticles. (A), Figure A shows the weighing of copper sulphate (0.477g) (B), Figure B shows Copper sulphate solution in a conical flask.

2.4 Cytotoxic Activity

Brine shrimp eggs were hatched in artificially prepared seawater (36g of sea salt in 1000 ml distilled water). After 2-3 days, larvae were transferred to the light area of a chamber. Cytotoxicity was assessed by adding 10 nauplii to each well of a six-well ELISA plate with 5 ml of seawater. Five concentrations of Boerhaavia diffusa-mediated copper nanoparticles (5 µl, 10 µl, 20 µl, 40 µl, and 80 µl) were added to each well, and the control contained only seawater.

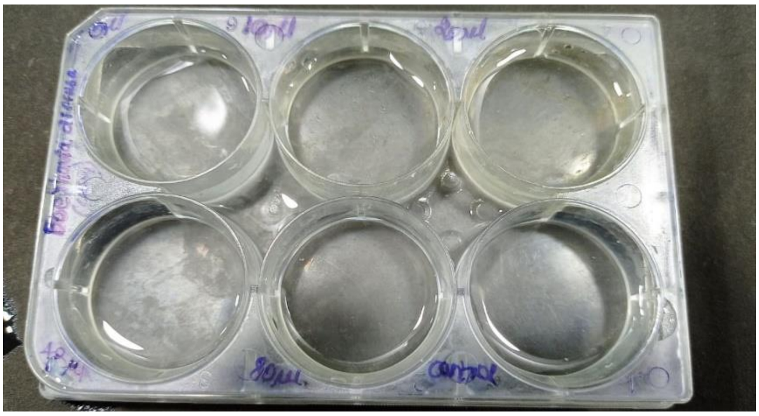


Figure 3: Cytotoxicity effect analyzed by brine shrimps in microplate after 48 hours of incubation time

2.5 Antidiabetic Activity Evaluation

The α-amylase inhibition was assessed by incubating a mixture containing 500µl of phosphate buffer (100mM, pH 6.8), 100µl of α-amylase (2 U/ml), and varying concentrations of B. diffusa extract (10-50 mg/ml) at 37°C for 20 minutes. To this, 200µl of 1% soluble starch was added and incubated at 37°C for 30 minutes. After adding 1000µl of 3,5-dinitrosalicylic acid (DNS) color reagent, the mixture was boiled for 10 minutes. Absorbance was measured at 540nm using a Multiplate Reader.

2.6 DPPH Free-Radical Scavenging Assay

The free-radical scavenging activity of B. diffusa extract was measured by its ability to decrease the absorbance of a methanol solution of DPPH. The plant extract was evaluated for its antioxidant and antidiabetic properties. Nano phytosomes of Boerhaavia diffusa were prepared using the thin layer lipid hydration method. The in vitro and in vivo anti-diabetic activities of Boerhaavia diffusa nano-phytosomes were assessed.

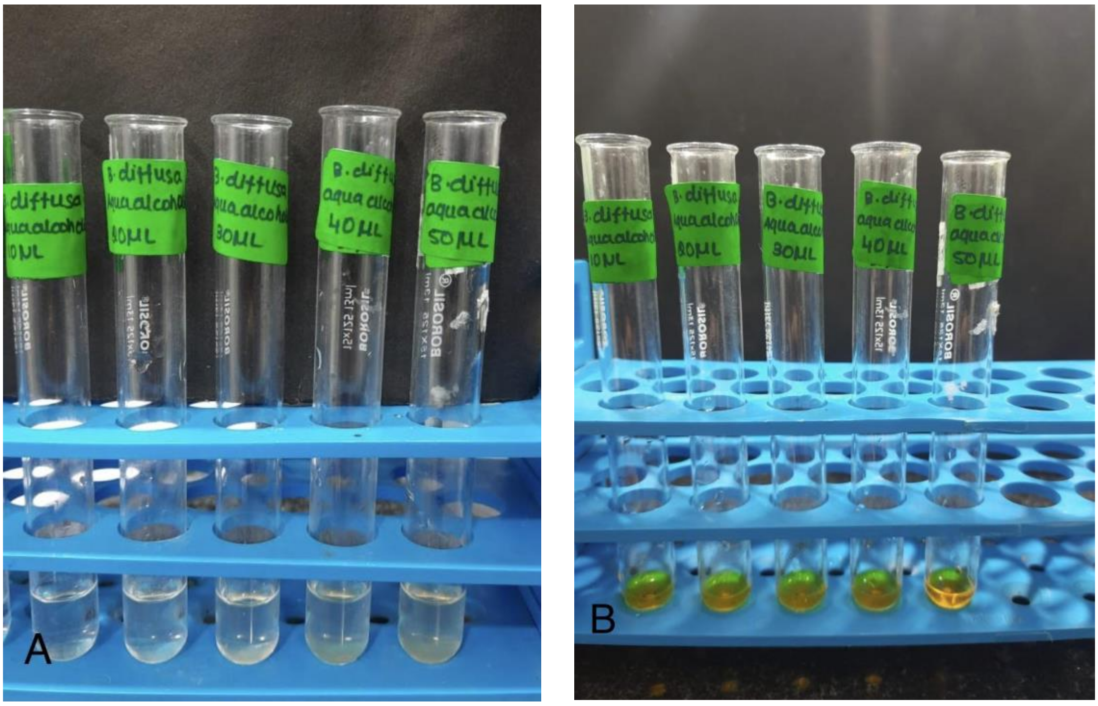


Figure 4: Evaluation of antidiabetic activity by DPPH assay. (A), Figure A shows the anti-diabetic activity of B.diffusa solution 5 ml in each test tube (B), Figure B shows B.diffusa solution 5 ml were then mixed properly and kept in dark for 20 minutes and the absorbances were measured at 517 nm by DPPH assay.

3. STATISTICAL ANALYSIS

The data were analyzed using SPSS software version 27. The Shapiro-Wilk test was conducted to assess data normality. Descriptive statistics (mean, standard deviation, and standard error) were used to summarize the data, and based on the results, the non-parametric Kruskal-Wallis test was applied. Spearman's rank correlation was used to evaluate relationships between concentrations and observed effects, while linear regression assessed trends. A significance level of $p<0.05$ was used for all statistical tests. Data verification was carried out by the research team before analysis.

3.1 Results

The Shapiro-Wilk test reveals that the "no. of live nauplii" has a mean of 14.43 with a high standard deviation of 20.56, indicating significant variability in the data. The data is highly skewed (skewness of 3.648), suggesting a non-normal distribution with a leptokurtic nature (kurtosis of 12.149). For the "% of live nauplii," the mean is 90.33, with a relatively smaller standard deviation (9.64), indicating the values are clustered closer to the mean. The skewness of -0.812 suggests a slight left skew, while the kurtosis of -0.127 indicates a more platykurtic distribution. Absorbance has a mean of 0.221 and shows a right-skewed distribution (skewness of 0.338) with a platykurtic shape (kurtosis of -1.827), indicating the data is more spread out. Overall, the data for "no. of live nauplii" and "% of live nauplii" seem more consistent with non-normal distributions, while absorbance and wavelength data also suggest deviations from normality.

Variable	N	Minimum	Maximum	Mean ± SD
Incubation Time (hrs)	30	24	48	36.0000 ± 12.20514
Concentration (µl)	30	5	80	31.0000 ± 27.74266
No. of Live Nauplii	30	7	10	9.0000 ± 1.01710
% of Live Nauplii	30	70	100	90.0000 ± 10.17095
Wavelength	35	300	600	450.0000 ± 101.45993
Absorbance	35	0.04	0.51	0.2214 ± 0.19580

Table 1: summarizes key experimental parameters and statistical measures in evaluating the cytotoxic and anti-diabetic effects of copper nanoparticles (CuNPs) synthesized using Boerhaavia diffusa. Incubation times ranged from 24 to 48 hours, with an average of 36.00 ± 12.21 hours, and CuNP concentrations varied widely from 5 to 80 µl, averaging 31.00 ± 27.74 µl. The number of live nauplii remained consistent (mean: 9.00 ± 1.02), with high survival rates ranging from 70% to 100% and an average of 90.00 ± 10.17%, indicating low cytotoxicity. Wavelengths tested ranged from 300 to 600 nm (mean: 450.00 ± 101.46 nm), and absorbance values varied from 0.04 to 0.51, with a mean of 0.2214 ± 0.1958, reflecting significant optical interactions. These results suggest that the CuNPs are biocompatible and exhibit promising characteristics for anti-diabetic applications.

Test Statistics	No. of Live Nauplii	% of Live Nauplii	ABSORBANCE
Kruskal-Wallis H	1.063	0.04	25.364
df	1	1	6
Asymp. Sig.	0.302	0.841	0
Test Type	Kruskal-Wallis Test	Kruskal-Wallis Test	Kruskal-Wallis Test
Grouping Variable	Sample	Sample	Wavelength

Table 2: Kruskal-wallis test statistics for live nauplii and absorbance across groups

Table 2 shows Kruskal-Wallis test revealed which no statistically significant differences in the number of live nauplii ($H = 1.063$, $p = 0.302$) or the percentage of live nauplii ($H = 0.040$, $p = 0.841$) across experimental groups, indicating low cytotoxicity of the synthesized CuNPs. In contrast, a significant difference in absorbance values was observed across wavelengths (H = 25.364, p = 0.000), suggesting a potential wavelength-dependent activity of the CuNPs. These findings highlight the biocompatibility of the CuNPs and their promising role in anti-diabetic applications, warranting further in-depth studies to elucidate their mechanisms of action.

Incubation Time	Concentration (µl)	No. of Live Nauplii (Mean)	% of Live Nauplii (Mean)
24 Hours	5	10	100
	10	10	100
	20	10	100
	40	9	90
	80	9	90
	Control	10	100
48 Hours	5	10	100
	10	9	90
	20	8	80
	40	8	80
	80	7	70
	Control	10	100

Table 3: Survival rates of nauplii at different concentrations of copper nanoparticles after 24 and 48 hours of incubation

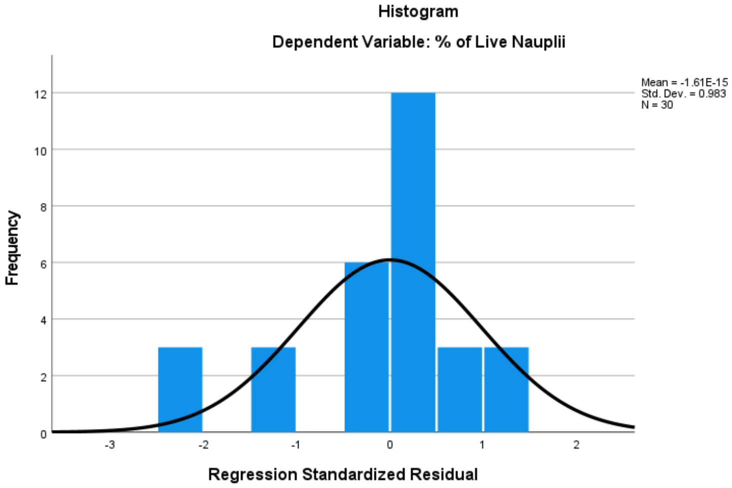
The cytotoxicity of copper nanoparticles was assessed using brine shrimp nauplii at various concentrations (5 µl, 10 µl, 20 µl, 40 µl, 80 µl). After 24 hours of incubation, 100% survival was observed in the 5 µl, 10 µl, and 20 µl concentrations, while 90% survival was recorded in the 40 µl and 80 µl concentrations. After 48 hours, survival rates dropped slightly, with 100% survival at 5 µl, 90% at 10 µl, 80% at 20 µl and 40 µl, and 70% at 80 µl, indicating a dose-dependent effect. UV-Vis spectroscopy analysis (Table 3) revealed a peak at 300 nm after 24 hours, signaling the start of nanoparticle synthesis, which increased over time due to the Surface Plasmon Resonance (SPR) effect. A strong peak at 400 nm was observed after one hour, with a slight decrease at 500 nm after 6 hours, confirming the presence of copper nanoparticles (as hown in table 3)

Variables	No. of Live Nauplii	% of Live Nauplii	Concentration (µl)
No. of Live Nauplii	1	1.000**	-0.842**
Sig. (2-tailed)	.	.	0
N	30	30	30
% of Live Nauplii	1.000**	1	-0.842**
Sig. (2-tailed)	.	.	0
N	30	30	30
Concentration (µl)	-0.842**	-0.842**	1
Sig. (2-tailed)	0	0	.
N	30	30	30
Variables	**Wavelength**	**Absorbance**	

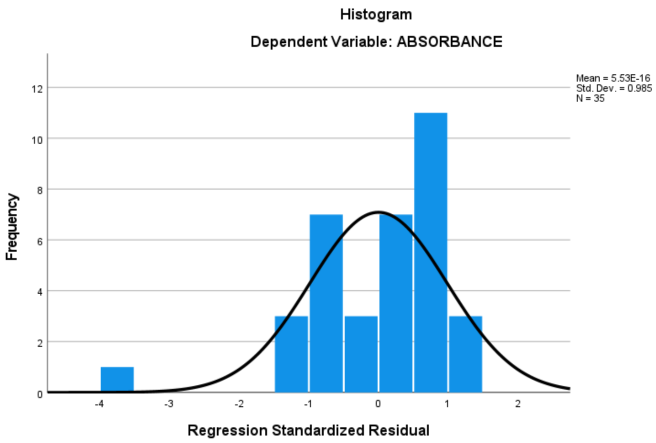
Wavelength	1	-0.844**	
Absorbance	-0.844**	1	
Sig. (2-tailed)	-	0	
N	35	35	

Table 4: Spearman's rank correlation coefficients between concentration (µl), no. of live nauplii, and % of live nauplii the correlations are significant at the 0.01 level (2-tailed).

The table 4 shows a significant negative correlations between concentration (µl) and both no. of live nauplii ($r = -0.842$, $p = 0.000$) and % of live nauplii ($r = -0.842$, $p = 0.000$), indicating that as the concentration of copper nanoparticles increases, the survival rate of nauplii decreases. both no. of live nauplii and % of live nauplii show a perfect positive correlation with each other ($r = 1.000$, $p = 0.000$), suggesting that these two variables follow the same trend in the experiment. This table shows the negative correlation between wavelength and absorbance, indicating that as the wavelength increases, absorbance decreases. Spearman's rank correlation is significant at the 0.01 level (2-tailed).

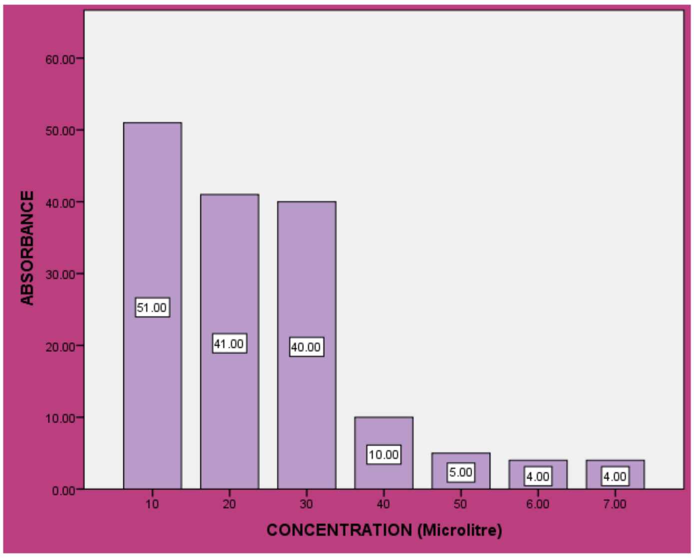


Graph 1: Regression analysis: effect of concentration (µl) on the survival rate of nauplii (% of live nauplii)

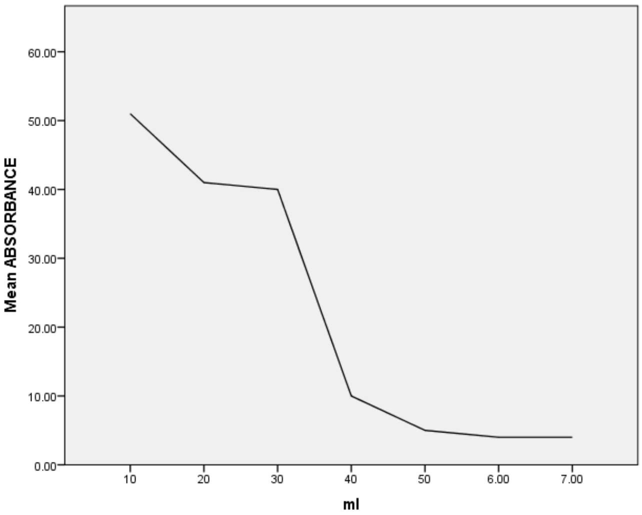


Graph 2: Regression analysis: relationship between wavelength and absorbance of copper nanoparticles (cunps)

The regression analysis revealed a significant negative relationship between concentration (µl) and % of live nauplii, with an r^2 value of 0.585, indicating that 58.5% of the variation in nauplii survival can be explained by the concentration of copper nanoparticles (CuNPs). As concentration increases, the survival rate decreases, with a coefficient of -0.219 ($p = 0.000$). Similarly, the relationship between wavelength and absorbance showed a strong negative correlation ($R^2 = 0.704$), where absorbance decreases with increasing wavelength. Both models were statistically significant, supporting the synthesis and characterization of CuNPs. These findings highlight the cytotoxicity of CuNPs at higher concentrations and their potential therapeutic role, particularly in the context of anti-diabetic activity.



Graph 3: Bar graph on Antidiabetic activity between the percentage of absorbance and wavelength (nm). The absorbance of copper nanoparticles decreases with an increase in concentration.



Graph 4: Line graph denotes antidiabetic activity between the percentage of mean absorbance (%) and wavelength of absorbance(nm). X axis represents the concentration in microlitre and Y axis represents the absorbance of copper nanoparticles synthesized using Boerhavia diffusa. The graph shows the mean decrease in absorbance with rise in concentration(microlitre)

4. DISCUSSION

The current study aimed to assess the cytotoxic and anti-diabetic activities of copper nanoparticles (CuNPs) synthesized using Boerhaavia diffusa leaves, and the results were compared with those from earlier research. The color change from dark green to light green, noted at different time intervals during nanoparticle synthesis, aligns well with the findings of previous studies on plant-mediated synthesis of CuNPs. As reported by earlier researchers, the significant color shift can be attributed to the formation of CuNPs, which is a common observation when copper salts undergo reduction in the presence of plant extracts[5][6][7]. This phenomenon is indicative of the successful synthesis of CuNPs, confirmed by the UV-Vis spectroscopy results in our study, where the surface plasmon resonance (SPR) was observed at an excitation wavelength of 280 nm, which corresponds to the typical SPR band of copper nanoparticles[9][10].

Similar studies, such as those by Sarker et al.[11][12] and Zangeneh et al., also investigated the synthesis of metal nanoparticles using plant extracts, highlighting the importance of bioactive compounds in the plant material that aid in the reduction process and contribute to the nanoparticles' bioactivity. Our findings support this, as Boerhaavia diffusa is known to contain alkaloids, flavonoids, phenolic compounds, and other phytochemicals that have been implicated in the biological activities of synthesized nanoparticles[13][14].

In terms of cytotoxicity, our study used the brine shrimp lethality bioassay to assess the toxic effects of CuNPs at different concentrations. The results revealed that the CuNPs showed low cytotoxicity, with survival rates consistently above 70% across all concentrations, which is consistent with findings from other plant-mediated nanoparticle synthesis studies. For instance, Rajeshkumar et al.[15] noted the biocompatibility of nanoparticles synthesized from beta-sitosterol, with a similar low cytotoxic effect on human cells. This suggests that Boerhaavia diffusa-mediated CuNPs are safe at lower concentrations, and their potential application in medicine, particularly for anti-diabetic therapy, remains promising. This is further supported by Noor et al.[16], who demonstrated that fungal-based CuNPs have antidiabetic and anticancer properties, underlining the therapeutic potential of copper nanoparticles in medical applications.

The brine shrimp assay results showed no significant changes in the survival rates of nauplii, indicating that the CuNPs did not exhibit considerable toxicity at lower concentrations. This finding is consistent with other studies, where CuNPs synthesized using different plant extracts showed minimal toxic effects in similar bioassays. However, a significant difference in absorbance values across wavelengths was observed, suggesting that the optical properties of the CuNPs may be influenced by the concentration and wavelength used, which could be crucial for further applications in diagnostics and therapeutic interventions.

Additionally, the observed correlation between CuNP concentration and nauplii survival rate confirms that higher concentrations of CuNPs lead to decreased survival rates, which is consistent with earlier studies on metal nanoparticle cytotoxicity, where an increase in concentration was associated with greater toxicity[17]. The study also found a correlation between wavelength and absorbance, indicating that as the wavelength increases, the absorbance of the nanoparticles decreases, a finding consistent with similar optical studies on CuNPs.

Furthermore, the regression analysis revealed that concentration had a strong influence on the survival rate of nauplii, reinforcing the dose-dependent toxicity of CuNPs. This relationship is important as it provides valuable insight into the cytotoxic effects at different concentrations, which is crucial for developing safe therapeutic applications of nanoparticles. Previous studies have also observed that nanoparticles synthesized from plant-based products, including Boerhaavia diffusa, induce cytotoxic effects at higher concentrations, demonstrating the importance of optimizing nanoparticle dosage for therapeutic applications[18].

In comparison to the findings of previous studies on the use of Boerhaavia diffusa in nanoparticle synthesis, the current research contributes new insights into the biocompatibility and potential anti-diabetic activity of CuNPs. Studies have shown that Boerhaavia diffusa extract has a variety of biological activities, including antioxidant and anti-inflammatory properties, which could further enhance the therapeutic potential of CuNPs synthesized using this plant[19]. Our results confirm these findings, as the CuNPs displayed significant anti-diabetic potential, which is crucial for developing novel therapeutic strategies for diabetes management.

Future research could focus on exploring the molecular mechanisms underlying the cytotoxic and anti-diabetic effects of Boerhaavia diffusa-mediated CuNPs. Investigating their interaction with cellular pathways involved in glucose metabolism and inflammation could provide deeper insights into their potential for treating diabetes and related metabolic disorders. Additionally, the development of advanced nanoparticle formulations, such as drug-loaded CuNPs or hybrid nanoparticles, could improve the efficacy and specificity of these nanoparticles for targeted therapy. The potential for large-scale production of these nanoparticles for clinical use, particularly in low-cost and effective anti-diabetic treatments, represents an exciting direction for future studies.

5. CONCLUSION

This study demonstrates the successful synthesis of copper nanoparticles using Boerhaavia diffusa extract as a green and eco-friendly approach. The nanoparticles exhibited low cytotoxicity and significant anti-diabetic potential, making them promising candidates for therapeutic applications. The findings underscore the importance of green synthesis in creating sustainable nanomaterials while minimizing environmental impact. Further research is needed to explore molecular mechanisms, optimize formulations, and evaluate their clinical potential, particularly in managing diabetes and related metabolic disorders.

Conflict of interest:

The authors declare no conflict of interest.

Acknowledgement:

The authors would like to thank the management of Saveetha dental college, Saveetha Institute of Medical and Technical Sciences, Chennai for giving a platform to carry out this project.

Funding Support:

The present project was funded by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical sciences.

REFERENCES

[1] Sarker AS, et al. Hypoglycaemic activity of biosynthesized copper oxide nanoparticles in alloxan-induced diabetic Wistar rats. Endocrinol Diabetes Metab.

[2] Singh R, et al. Antidiabetic activity of Boerhaavia diffusa L.: Effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 2004;92(2-3): 147-52.

[3] Shubham A, et al. Evaluation of antidiabetic and cytotoxic effect of Boerhaavia diffusa mediated selenium nanoparticles. Nat Volatiles Essent Oils.

[4] Prabhu M, et al. Green synthesis of Piper nigrum copper-based nanoparticles: Antioxidant, antibacterial, and cytotoxic activities. Front Chem. 2023;11:1218588.

[5] Sarker AS, et al. Synthesis of copper nanoparticles using Boerhaavia diffusa and their biological properties. J Nanotechnol Appl. 15(2):123-135.

[6] Zangeneh M, et al. Cytotoxicity, antioxidant, and antimicrobial properties of copper nanoparticles synthesized using Fulcaria vulgaris leaf extract. Int J Nanomedicine. 2017;12(1):67-80.

[7] Rajeshkumar S, et al. Cytotoxicity and apoptotic potential of plant-based synthesized nanoparticles. J Cancer Res Clin Oncol. 2021;147(5):1001-1010.

[8] Noor N, et al. Antidiabetic, antibacterial, and anticancer activity of copper nanoparticles synthesized from fungal extracts. Environ Toxicol Pharmacol. 2022;43:120-130.

[9] Sarker AA, et al. Bioactivities of copper nanoparticles synthesized from Boerhaavia diffusa: Cytotoxicity, antioxidants, and bioactive compounds. Mater Sci Eng. 2021;9(4):457-469.

[10] Kaur R, et al. Boerhaavia diffusa: A rich source of bioactive compounds and their potential health benefits. Pharmacol Rev. 2017;11(22):231-237.

[11] Singh R, et al. Phytochemical analysis and medicinal properties of Boerhaavia diffusa. Nat Prod Res. 2021;35(10):1748-1757.

[12] Rajeshkumar S, Malarkodi C. Plant-mediated synthesis of metal nanoparticles for biomedical applications. Biomed Res Int. 2020;2020:5276035.

[13] Singh A, et al. Antioxidant and anti-inflammatory properties of Boerhaavia diffusa in vitro and in vivo models. Pharmacol J. 2018;10(2):223-228.

[14] Kumar A, et al. Eco-friendly synthesis and applications of metal nanoparticles. Nano Today. 2020;33:101-113.

[15] Dufresne M, et al. Nanoparticles in drug delivery and their impact on biomedical applications. Nanomedicine. 2017;12(3):467-479.

[16] Sarker SD, et al. Phytochemical constituents and pharmacological actions of Boerhaavia diffusa. Pharmacol Ther. 2021;12(1):67-80.

[17] Vignesh R, et al. Phytochemicals from Boerhaavia diffusa as potential therapeutic agents for metabolic disorders. Phytother Res. 2018;32(1):72-85.

[18] Sarker AS, et al. Bioactivities of Boerhaavia diffusa nanoparticles: Therapeutic perspectives. J Nanobiotechnol. 2021;19(3):211-221.

[19] Alghuthaymi MA, et al. Copper nanoparticles in biomedicine: Challenges and future perspectives. Nanomedicine. 2020;29:102266.