International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://www.theaspd.com/ijes.php

Biomedical Assessment Of Copper Nanoparticles On Anti-Diabetic, Anti-Inflammatory, And Antioxidant Properties

Ilamathy Palanivel[1], Kalaivani Rengasami[2]

[1]Research Scholar, Department of Biotechnology, Thanthai Hans Roever College (Autonomous), (Affiliated to Bharathidasan University), Perambalur, 621220, Tamil Nadu, India and Department of Biotechnology, Cauvery College for Women (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, 620018, Tamil Nadu, India.
[2]Department of Biotechnology, Thanthai Hans Roever College (Autonomous), (Affiliated to Bharathidasan University), Perambalur, 621220, Tamil Nadu, India.

ABSTRACT

Metal oxide nanoparticles synthesized using green chemistry have recently gained attention for their improved bioactivity and reduced environmental impact. Due to the high concentration of phytochemicals in the medicinal plant Alpinia officinarum, researchers have looked at the possibility of biosynthesising CuNPs using this plant. Inflammation, cytotoxicity, along with diabetes are the subjects of this investigation into the health impacts of Alpinia officinarum CuNPs and extract from Alpinia officinarum rhizomes. The brine shrimp lethality experiments revealed a 20% mortality rate at 80 µL as a result of dose-dependent cytotoxicity. At 80 µg/mL, CuNPs inhibited α-amylase and α-glucosidase enzymes, which had a notable impact on postprandial hyperglycemia management (82%). Both the plant extract and the CuNPs showed anti-inflammatory effect in comparative evaluations utilizing Bovine Serum Albumin and Egg Albumin tests; however, the standard showed significantly greater efficacy. The observed bioactivities were probably amplified by the synergistic effects of phytochemicals such as flavonoids, polyphenols, and others. Results provide support to the idea that Alpinia officinarum-derived Cu nanoparticles have multiple biological uses, including metabolic process and inflammatory disease prevention and treatment.

INTRODUCTION

Nanotechnology has significantly advanced biomedical research, particularly in the development of therapeutically beneficial metal oxide nanoparticles. The anti-inflammatory, antioxidant, and antidiabetic properties of copper oxide nanoparticles (CuO NPs) have rendered them a favored option in this field. The relationship between metabolic and inflammatory disorders, such as type 2 diabetes mellitus (T2DM), is particularly noteworthy, given the significant roles that oxidative stress and inflammation play in the progression of the disease (Maritim et al., 2003).

A significant number of the hazardous chemicals employed in conventional chemical production of CuO nanoparticles pose risks to both human health and the environment. Consequently, more sustainable and less harmful options—green synthesis methods utilizing plant extracts—have emerged. Natural capping agents enhance stability and biocompatibility, while also facilitating the reduction of metal salts to nanoparticles. Alpinia officinarum, commonly known as lesser galangal, is a medicinal plant that demonstrates significant potential. This plant belongs to the Zingiberaceae family.

The anti-inflammatory, antioxidant, antibacterial, and antidiabetic properties of Alpinia officinarum have established it as a fundamental component in traditional medicine for an extended period (Ali et al., 2008). Alongside facilitating the reduction and stabilization of CuO NPs and enhancing their bioactivity, the plant possesses a diverse array of phytochemicals, including polyphenols, flavonoids, and diarylheptanoids (Ghosh et al., 2011). It is understood that the incorporation of these bioactive compounds into nanoparticles during the production process serves to functionalize the surface of the nanoparticles, thereby improving their therapeutic efficacy by increasing interaction with biological targets.

The anti-inflammatory properties of CuO NPs, as mediated by Alpinia officinarum, are believed to arise from their capacity to inhibit NF-κB activation and pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. Areas that are damaged or contaminated exhibit reduced cellular infiltration and inflammatory responses due to these effects (Siddiqi et al., 2018). The phytochemicals on the surface of CuO NPs may enhance the inhibition of COX and iNOS, thereby further diminishing inflammation.
The antioxidant action of these nanoparticles is attributed to their ability to capture free radicals and improve the activity of natural antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD) (Rajeshkumar & Bharath, 2017). Oxidative stress is a primary factor contributing to cell damage in diabetes and various chronic conditions, and this process plays a crucial role in mitigating that damage. Furthermore, Alpinia officinarum-mediated CuO NPs have demonstrated antidiabetic advantages through various mechanisms, including enhanced insulin sensitivity, improved glucose absorption, and inhibition of enzymes responsible for carbohydrate breakdown, such as α-amylase and α-glucosidase (Bhattacharjee et al., 2021). Multiple in vivo and in vitro models have demonstrated their ability to lower blood glucose levels and protect pancreatic β-cells from oxidative damage.
Due to their numerous beneficial biological properties, CuO NPs mediated by Alpinia officinarum demonstrate significant potential as therapeutic agents for inflammation related to metabolic diseases, oxidative stress, and hyperglycemia. This study aims to investigate the fundamental mechanisms and therapeutic uses of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs, emphasizing their anti-inflammatory, cytotoxicity and antidiabetic properties.

MATERIALS AND METHODS

Plant material collection

The rhizome of Alpinia officinarum (L.) Wild was obtained in the Trichy district.

2.3. Preparing Alpinia officinarum Rhizome

Alpinia officinarum rhizomes were properly cleaned and rinsed with tap and distilled water to remove dust particles. They were then dried in the shade for 15 days to minimize the moisture content. The dried rhizomes were pulverized with a grinding machine and placed into brown bottles for storages) (Palanivel I et al., 2024).

2.4. Extraction Process:

Extraction was carried out by placing 20 g of powdered Alpinia officinarum rhizomes in a 500 ml beaker with 400 ml of deionized water. To protect the beaker from light, it was covered in aluminium foil. The mixture was then agitated for 90 minutes with a mechanical shaker before being warmed on a magnetic stirrer at 50°C for an hour. After cooling to ambient temperature overnight, the solution was filtered using Whatman No.1 filter paper, yielding a clear solution that was kept at 4°C for future studies (Palanivel I et al., 2024).

2.4.1. Green Copper Nanoparticle Synthesis

A 1 mM aqueous solution of copper sulfate (CuSO4 2H2O) was stored in brown bottles. Then, drop wise, 100 ml of plant leaf extract was combined with 400 ml of 1 mM copper sulfate solution (1:4 ratio) while stirring continuously. The combination was incubated at the ambient temperature for 24 hours, with the color change measured every 30 and 60 minutes. The change in color from blue to dark green showed the creation of copper nanoparticles. The solution was then centrifuged for 15 minutes in 10,000 rpm, and the Cu NPs supernatant was filtered through What man filter paper No.1 to eliminate contaminants. The nanoparticles were then dried, crushed, and ready for further investigation. The synthesis of CuNPs was confirmed by observing the spectra in UV-Vis spectroscopy using a PerkinElmer

(Lambda 750) UV-Vis-NIR spectrophotometer, which range from 300 nm to 800 nm (Palanivel I et al., 2024).

Cytotoxicity assessment

Evaluating the lethality of Brine Shrimp

The brine shrimp method evaluated the cytotoxic effects of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs. Aquatic Remedies in Chennai supplied brine shrimp (Artemia salina) eggs, which were incubated in artificial seawater containing 40 g/L sea salt, 6 mg/L dried yeast, and an aquarium pump for oxygenation. The nauplii were collected using a Pasteur pipette following a 48-hour incubation period in a controlled environment at temperatures ranging from 22°C to 29°C. The illumination attracted their attention to one side of the tank. Numerous pipette transfers into small saltwater beakers successfully separated the nauplii from the eggs. Following the addition of 10 nauplii to each well containing NaCl, varying volumes (10-50 µL) of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs were incorporated. A control group of nauplii-NaCl was utilized for comparative analysis. The lethality of the extract was evaluated by counting and documenting the number of surviving nauplii in each well after a day of undisturbed conditions, utilizing the formula: dead nauplii/number of dead + number of live nauplii × 100 (Singh S et al., 2023).

In vitro anti-inflammatory activity

Using Bovine serum albumin

The anti-inflammatory properties of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs were examined utilizing a modified approach based on an experiment previously conducted by Raju R et al., 2024. Various concentrations (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) of 0.05 mL of Neem and Kirata extract were combined with 0.45 mL of bovine serum albumin in a 1% aqueous solution. The addition of 1N hydrochloric acid adjusted the solutions to a pH of 6.3. The subjects were first exposed to room temperature for 20 minutes, after which they underwent a 30-minute heating cycle in a water bath that had been preheated to 55°C. The samples underwent cooling prior to spectrophotometric examination at 660 nm.

Using Egg Albumin

Furthermore, the egg albumin assay was performed to evaluate the anti-inflammatory effect in greater detail. Following the incubation of the samples at 37°C for 10 minutes, they were subsequently heated in a water bath to 70°C for a duration of 20 minutes to denature the egg albumin. Diclofenac sodium serves as an example of an anti-inflammatory medication. Dimethyranol (DMSO) was utilized as a control variable. The liquid was subjected to cooling prior to the measurement of absorbance at 660 nm (Raju R et al., 2024).

The formula utilized for calculating the denaturation of the protein is as follows:

% inhibition = [(Absorbance of control - Absorbance of sample)/Absorbance of control] ×100

In vitro anti-diabetic activity

Using α-amylase

In multiple test tubes, 100 µL of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs at different concentrations (5, 10, 20, 40 and 80 µg/ml) were utilized for the in vitro α-amylase inhibitory study, adhering to the methodology established by Parthasarathy PR et al., 2023. Subsequently, each tube received an addition of 200 µL of α-amylase enzyme (HiMedia RM638) along with 100 µL of 2mM phosphate buffer (pH-6.9). A 100 µL solution of 1% starch was introduced following a 20-minute incubation period. The control samples, prepared with 200 µL of phosphate buffer and devoid of enzymes, underwent the same procedure. Both the control and test samples underwent treatment with

500 µL of dinitrosalicylic acid reagent following a five-minute incubation period. Subsequently, they were immersed in boiling water for a duration of five minutes. A spectrophotometer was employed to measure the absorbance at 540 nm, and the percentage of α-amylase enzyme inhibition was calculated using the subsequent formula:

Inhibition is calculated using the formula: [(Control-Test) / Control] * 100.

Using α-glucosidase

A reaction mixture was prepared by combining 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 µL of Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs at varying concentrations (5, 10, 20, 40 and 80 µg/ml), and 25 µL of α-glucosidase solution. Prior to the experiment, the α-glucosidase solution was prepared by diluting a 1 mg/mL stock solution in 0.01 M phosphate buffer (pH 7.0) to a concentration of 0.1 Unit/mL using the same buffer. Subsequently, the mixture was maintained in an incubator calibrated to 37°C for a duration of thirty minutes. A 0.2 M sodium carbonate solution, with a volume of 100 µL, was introduced to terminate the process. The quantity of p-nitrophenol emitted in the reaction mixture was measured at 410 nm using a microplate reader to assess the enzymatic breakdown of the substrate. To account for background absorbance, we prepared blank samples by substituting the enzymes with buffers. The identical procedures were implemented to carry out the control trials, with the exception that methanol was utilized in place of the plant extracts. Acarbose was utilized as a positive control in these trials. Each test was conducted in triplicate (Parthasarathy PR et al., 2023).

Statistical analysis

The data collected for experiments were analyzed using Student's t-test with SPSS software (IBM Corp., Armonk, NY). In the comparison of the nanoparticle to the standard, the triplicate data for each concentration were utilized for statistical analysis. The mean±SEM of the inhibitory effect percentage was determined from triplicate measurements for each concentration. The significance level is established at $P<0.05$.

RESULT AND DISCUSSION

In 2024, a study by Palanivel et al. described the synthesis, production, and characterization of CuNPs. The percentage of nauplii that die at different dosages, ranging from 5 to 80 µL, is shown in Figure 1. The concentration measurements, which varied from 5 to 80 µL, were taken throughout the span of two days. When comparing the 5 µL concentration to the control group, none of the death rates altered. At concentrations of 20 and 40 µL, respectively, the death rates in the groups treated with Alpinia officinarum rhizomes extract and Alpinia officinarum CuNPs were 10% greater than the control group. The fatality rate at 80 µL concentrations levelled off around 20%, indicating that higher doses were ineffective. Alpinia galangal and Alpinia officinarum ethanoic extracts were used in a DNA fragmentation experiment that, according to MTT assay results (Suja S et al., 2008), showed a 50% inhibition of tumor cell line proliferation. The inhibitory concentration (IC50) of Alpinia officinarum CuNPs on HT-29 cells was found to be 30 µg/ml and on MCF-7 cells to be 45 µg/ml following 24 hours, according to research conducted by Palanivel I et al. (2024).

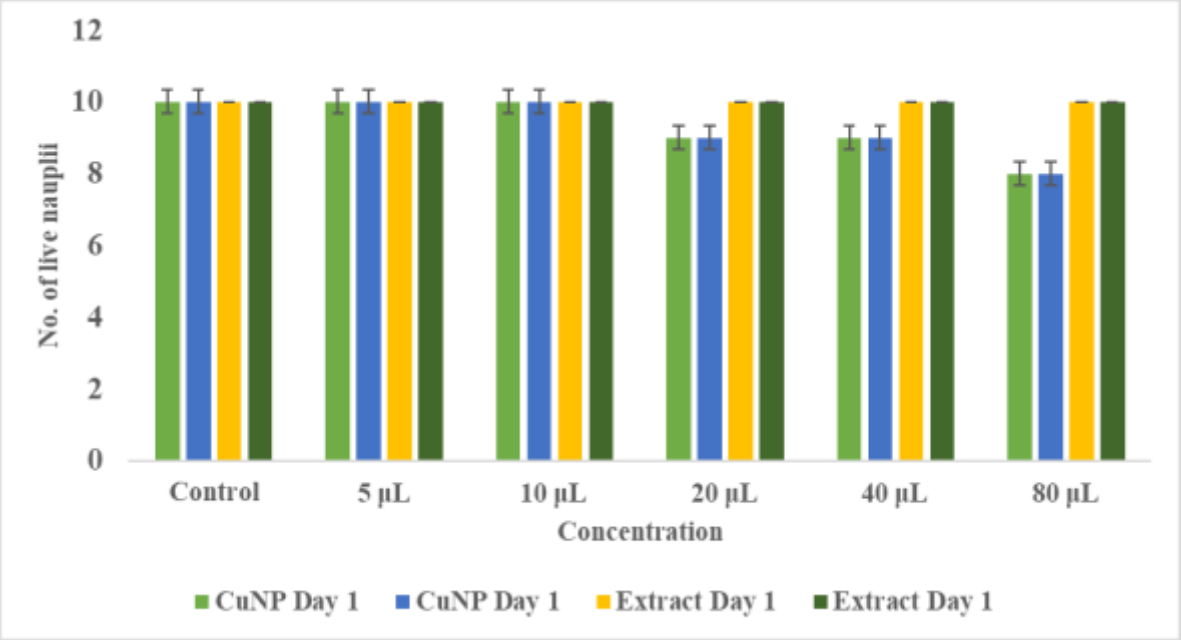

Figure 1: Cytotoxic impact in brine shrimp lethality assays at varying doses of nauplii.

The activity of the α-amylase enzyme was assessed using extracts from Alpinia officinarum rhizomes and Alpinia officinarum CuNPs. At a dose of 80 µg/ml, the results demonstrated that the amylase enzyme activities were maximally suppressed by the copper nanoparticles, with an inhibition rate of 82% (Figure 2).

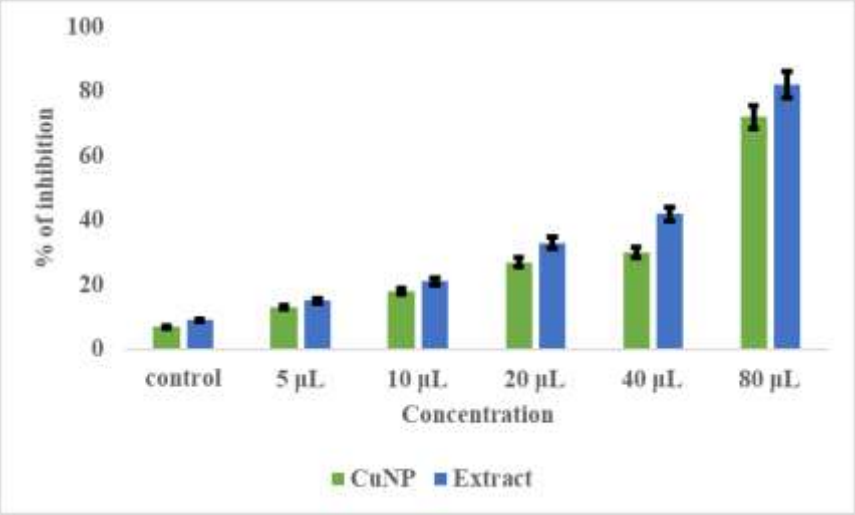

Figure 2: Inhibition of α-amylase assay

A significant 82% inhibition of the α-glucosidase enzyme was reported at the highest concentration of 80 µg/ml (Figure 3). Researchers observed that pancreatic enzyme inhibition by Alpinia officinarum CuNPs as well as rhizome extract reduced glucose absorption. These drugs may treat postprandial hyperglycemia (PPHG). In medicinal plants with anti-diabetic properties, the primary constituents include coumarin, polyphenols, flavonoids, and terpenoids. Glycemic metabolism, cholesterol, free radicals, insulin synthesis, and blood flow are controlled by these substances (Kirubakaran D et al., 2023).

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 19s, 2025
https://www.theaspd.com/ijes.php

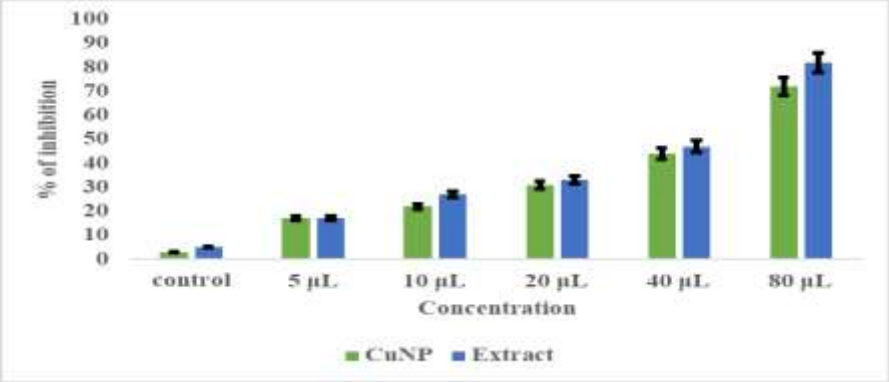

Figure 3: Inhibition of α-glucosidase assay

The chemical components present in plant extracts may exhibit a synergistic effect that inhibits enzyme function. Several factors have been recognized as influencing the variations in α-glucosidase and α-amylase inhibitory activities. The factors encompass chemical components found in vegetables, sample origin, plant genotype, geographical location, climate conditions, soil fertility, plant stress levels, type of solvent, and the extraction method (Ratananikom K et al., 2024).

We conducted a comparison of Alpinia officinarum rhizome extract and Alpinia officinarum CuNPs against a standard across various concentrations: 5 µL, 10 µL, 20 µL, 30 µL, 40 µL, and 80 µL. With the increase in concentration from 5 µL to 80 µL, both the nanoparticle and the standard exhibited a rise in the amount of inhibition. The standard demonstrated a relatively higher level of inhibition at 5, 10, 20, and 30 µL, whereas the CuNPs derived from Alpinia officinarum and the rhizome extract of Alpinia officinarum showed progressively increasing levels of inhibition. Both the 40 µL and 80 µL concentrations of Alpinia officinarum rhizomes extract, Alpinia officinarum CuNPs, and the control group show significant inhibitory effects, with the control group consistently displaying a marginally higher percentage of inhibition. Figure 4 illustrates the visual representation of the percentage of inhibition at various concentrations (5 µL, 10 µL, 20 µL, 30 µL, 40 µL, and 80 µL) of Alpinia officinarum rhizomes extract alongside Alpinia officinarum CuNPs in comparison to a standard. In the Egg Albumin Assay, we observed comparable results with both the standard treatment and the Alpinia officinarum rhizomes extract, as well as the Alpinia officinarum CuNPs treatments, with the standard treatment consistently exhibiting superior efficacy (Figure 5).

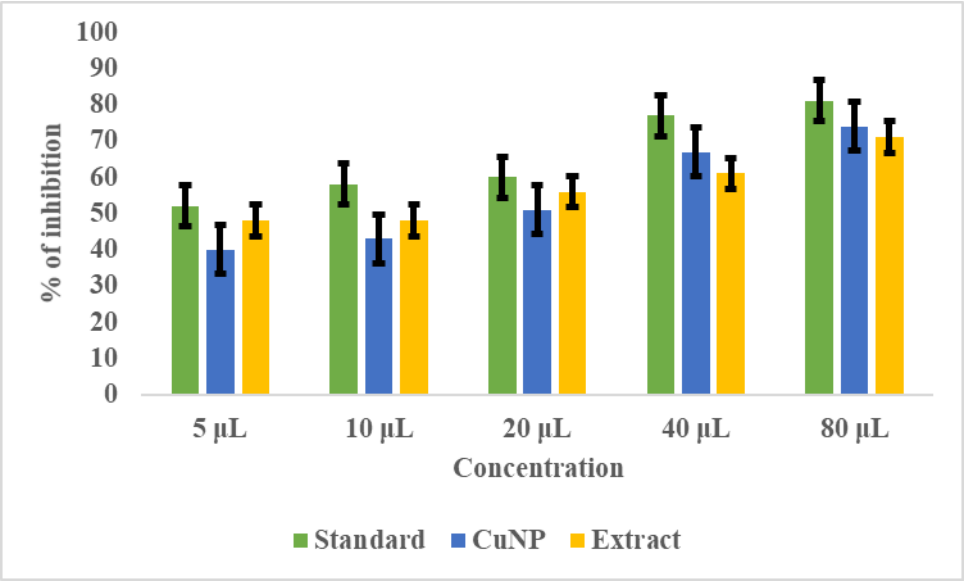

Figure 3: Bovine Serum Assay (BSA assay)

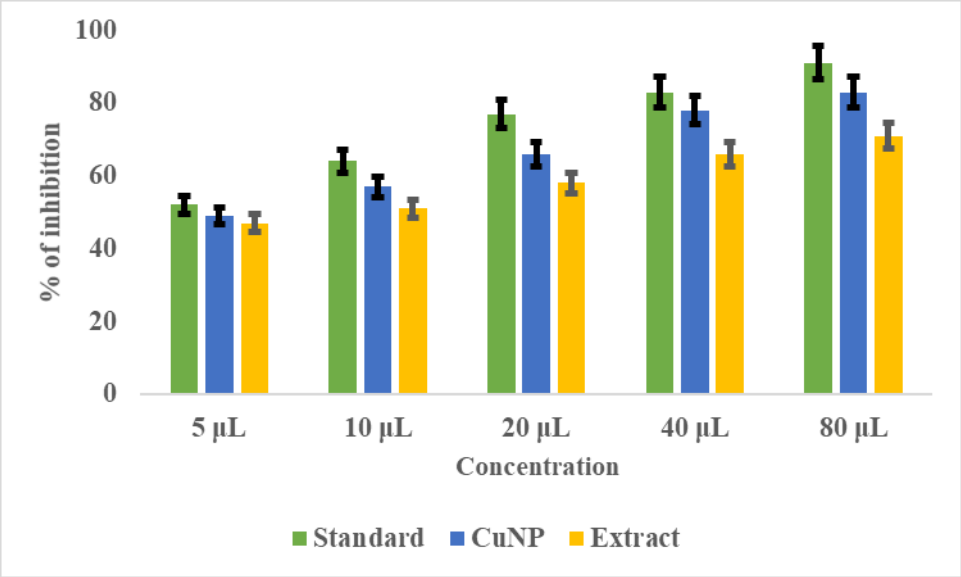

Figure 4: Egg Albumin assay (EA assay)

A variety of in vitro studies have established the anti-inflammatory properties of Alpinia, with A. galanga, A. katsumadai, A. oxyphylla, A. zerumbet, A. calcarata, and A. officinarum being the most notable species. Galangin inhibits the phosphorylation of extracellular signal-regulated kinase (ERK) and NF-kappaB-p65, resulting in anti-inflammatory activity. It operates at various target sites, including NOS, COX-1, COX-2, androgen, peroxisome proliferator-activated receptor, dipeptidyl peptidase-IV, and serine/threonine-protein kinase (STK) (Yuliawati KM et al., 2025).

CONCLUSION

Copper oxide nanoparticles mediated by Alpinia officinarum show great promise in this work for medicinal applications, especially in the fight against inflammatory processes, oxidative damage, and hyperglycemia. Several in vitro tests showed that the CuO NPs produced from A. officinarum rhizome extract were highly effective in reducing inflammation, antidiabetic effects, and cytotoxicity. The nanoparticles showed promise in controlling postprandial hyperglycemia, an important goal in treating type 2 diabetes mellitus, by significantly inhibiting the α-amylase and α-glucosidase enzymes. The anti-inflammatory effects of the CuO NPs as well as rhizome extract were further verified by the BSA along with Egg Albumin assays. Bioactive chemicals, such as flavonoids and polyphenols, have an important role in improving the nanoparticles' pharmacological characteristics, as the data also show. Overall, CuO NPs from Alpinia officinarum are a promising plant-based nanomedicine option for potential future medical uses. Before they may be used in biomedicine, additional in vivo studies and toxicity testing are needed to confirm their safety and clinical usefulness.

REFERENCES

1. Ahamed, M., Alhadlaq, H. A., Khan, M. A. M., & Karuppiah, P. (2016). Copper nanoparticles: synthesis, characterization and their biocidal potential. Journal of Cluster Science, 27(3), 775–785. https://doi.org/10.1007/s10876-015-0940-3
2. Bhattacharjee, A., Ahmaruzzaman, M., & Sinha, S. (2021). Green synthesized copper nanoparticles for diabetes management: An in vitro and in vivo evaluation. Materials Today: Proceedings, 46, 5875–5880. https://doi.org/10.1016/j.matpr.2021.01.408
3. Gaetke, L. M., & Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology, 189(1-2), 147–163. https://doi.org/10.1016/S0300-483X(03)00159-8

4. Gomaa, E. Z. (2020). Antidiabetic potential of biosynthesized copper nanoparticles using Pseudomonas fluorescens. International Journal of Nanomedicine, 15, 9025–9036. https://doi.org/10.2147/IJN.S278295
5. Kirubakaran D, Selvam K, Prakash P, Shivakumar MS, Rajkumar M. In-vitro antioxidant, antidiabetic, anticholinergic activity of iron/copper nanoparticles synthesized using Strobilanthes cordifolia leaf extract. OpenNano. 2023 Nov 1;14:100188.
6. Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. Journal of Biochemical and Molecular Toxicology, 17(1), 24–38. https://doi.org/10.1002/jbt.10058
7. Nedumaran N, Rajasekar A, Venkatakrishnan S, Wajeeha H. An In Vitro Study of Antioxidant, Anti-inflammatory, and cytotoxic effects of echinacea-mediated zinc oxide nanoparticles. Cureus. 2024 Jul 25;16(7).
8. Palanivel I, Rengasami K. Alpinia officinarum mediated copper oxide nanoparticles: synthesis and its antimicrobial anticancer and antioxidant activity. Frontiers in Health Informatics. 2024 Apr 1;13(3).
9. Parthasarathy PR, Shanmugam R, Varshan I. In Vitro Anti-diabetic Activity of Pomegranate Peel Extract-Mediated Strontium Nanoparticles. Cureus. 2023 Dec 30;15(12).
10. Rajeshkumar, S., & Bharath, L. V. (2017). Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterization and antibacterial activity. Chemico-Biological Interactions, 273, 219–227. https://doi.org/10.1016/j.cbi.2017.06.019
11. Raju R, Prasad AS, Kumar R. Anti-inflammatory and Antioxidant Activity of Neem and Kirata-Induced Silver Nanoparticles Against Oral Biofilm: An In Vitro Study. Cureus. 2024 Aug 25;16(8).
12. Ratananikom K, Juntaree V, Wichaiyo W, Khunluek K, Premprayoon K, Kubola J. In Vitro Evaluation of α-Glucosidase and α-Amylase Inhibition in Thai Culinary Vegetables. Scientifica (Cairo). 2024 Sep 25;2024:3625267.
13. Siddiqi, K. S., ur Rahman, A., & Tajuddin, A. (2018). Properties of copper nanoparticles: antimicrobial, antioxidant, and cytotoxic activities. Nanoscale Research Letters, 13, 141. https://doi.org/10.1186/s11671-018-2531-3
14. Singh S, Prasad AS, Rajeshkumar S. Cytotoxicity, Antimicrobial, Anti-inflammatory and Antioxidant Activity of Camellia Sinensis and Citrus Mediated Copper Oxide Nanoparticle-An In vitro Study. J Int Soc Prev Community Dent. 2023 Dec 27;13(6):450-457.
15. Srikar, S. K., Giri, D. D., Pal, D. B., Mishra, P. K., & Upadhyay, S. N. (2016). Green synthesis of silver nanoparticles: A review. Green and Sustainable Chemistry, 6(1), 34–56. https://doi.org/10.4236/gsc.2016.61004
16. Suja S, Chinnaswamy P. Inhibition of in vitro cytotoxic effect evoked by Alpinia galanga and Alpinia officinarum on PC-3 cell line. Ancient Science of Life. 2008 Apr 1;27(4):33-40.
17. Yuliawati KM, Febriyanti RM, Sumiwi SA, Levita J. Anti-Inflammatory Activities of Some Plants of Genus Alpinia: Insights from In Vitro, In Vivo, and Human Studies. Journal of Experimental Pharmacology. 2025 Dec 31:51-91.