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Antioxidant, Anti-Inflammatory and Cytotoxic Effects of Vernonia Amygdalina Mediated Copper Nanoparticles

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ABSTRACT

Background: Vernonia amygdalina is generally known as sour leaf or bitter leaf which is one among the medically significant plants in African and Mediterranean regions. Hence, this study aids the biosynthesis of copper nanoparticles using V.amygdalina for the first time and further it aims to investigate the cytotoxicity, antioxidant and anti-inflammatory properties of the V.amygdalina mediated copper nanoparticles.

Methods: Cytotoxic effect of Copper sulphate nanoparticles reinforced with Vernonia amygdalina extract were assessed using Brine Shrimp Assay at 5 µL, 10 µL, 20 µL, 40µL and 80µL, anti-inflammatory activity using Bovine Serum Albumin (BSA) and antioxidant activity using DPPH Assay at 5 µL, 10 µL, 20 µL, 30 µL, 50 µL.

Results: As the concentration increased, the cytotoxicity of the nanoparticles increased. It was found that the values for anti-inflammatory properties of nanoparticles was higher than the standard values at 30µL, 40µL, 50 µL concentrations. Percentage of inhibition was highest at 40 µL (85.7%) and 50 µL (92.6%). The values for antioxidant properties of nanoparticles were found to be higher than the standard values at 10 µL and 20 µL and almost equal in 30 µL concentrations.

Conclusion: Vernonia amygdalina mediated copper nanoparticles have very less cytotoxic effects. It also has a potential to serve as a good anti-inflammatory and antioxidant agent.

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INTRODUCTION

Metallic nanoparticles are multidimensional in nature, and they have been tremendously used in a variety of sectors of industries and medicine including drug delivery, wastewater treatment, and DNA analysis, as antibacterial agents and biosensors and in solar power generation and catalysis. Many researchers have turned their centre in favour of metallic nanoparticles like copper, gold , silver due to their magnificent physicochemical(1,2) electronic, chemical(3), catalytic, optical, antifungal applications mechanical, electrical, and thermal conduction properties. . In recent years, copper nanoparticles (Cu NPs) have received much attention from researchers due to its applications in industries and medicine. Many methods are available to synthesize copper nanoparticles which includes physical, chemical, biological, thermal reduction, chemical reduction, sonochemical reduction, microwave heating etc(4,5). Above mentioned methods like physical and chemical

methods utilize toxic chemicals that are expensive and difficult to scale up in large quantities (6–8). Biosynthesis of nanoparticles using crude extracts of leaves, seed's and animal's chitin has been adapted to overcome the toxic effects of the above mentioned methods. This method of synthesis is free of toxic, biocompatible, cost-effective, and eco-friendly(9) (10) (5,11).

Biosynthesis implies the utilization of green technology used for the reduction process of metal ion into bulk metallic nanoparticles formulation in the redox reaction to obtain nano-sized particle which includes crude extracts from leaves of plants that are enriched in metabolites such as flavonoid, alkaloid, tannin, saponin and phenolic acid (12) (13)(14). The study on antibacterial effects of copper nanoparticles using E. coli and Bacillus subtilis revealed the fact that the Cu NPs exhibited superior antibacterial activity compared to the silver nanoparticles. (15). But no research has been conducted as far as green synthesis of Cu NPs is concerned using extracts of medicinal plants like Vernonia amygdalina.

Vernonia amygdalina is generally known as sour leaf or bitter leaf due to its bitter flavour, which usually grows in tropical Africa as a small bush with 22 cm to 5 m long and belongs to the family of Asteraceae kin(16). V. amygdalina is one among the medically significant plants used against malaria, helminth infections, gastrointestinal disorders, and fever(17). In traditional medicine , V.amygdalina has been used to treat malaria, fever and inflammatory diseases. V. amygdalina produces a variety of flavonoids and bitter sesquiterpene lactones which contribute to the bioactivities of this plant(18). It can be speculated that the antioxidant properties of Vernonia amygdalina can be attributed to the presence of these flavonoids.

This study aids the biosynthesis of copper nanoparticles using V.amygdalina for the first time and further it aims to investigate the cytotoxicity, antioxidant and anti-inflammatory properties of the V.amygdalina mediated copper nanoparticles. Our team has extensive knowledge and research experience that has translate into high quality publications (19–28)

Aim of the study

To investigate the cytotoxicity, antioxidant and anti-inflammatory properties of the V.amygdalina mediated copper nanoparticles.

Objectives

To investigate the cytotoxicity of Vernonia amygdalina mediated copper nanoparticles.

To investigate antioxidant properties of Vernonia amygdalina mediated copper nanoparticles

To investigate anti-inflammatory properties of Vernonia amygdalina mediated copper nanoparticles

Null hypothesis

There is no difference in the cytotoxicity, antioxidant and anti-inflammatory properties of the V.amygdalina mediated copper nanoparticles.

Alternate hypothesis

There is a difference in the cytotoxicity, antioxidant and anti-inflammatory properties of the V.amygdalina mediated copper nanoparticles.

MATERIALS AND METHODS

Preparation of aqueous leaf extract

Vernonia amygdalina was brought from the Nigerian market. The collected leaves of V.amygdalina were washed 3–4 times using distilled water and dried for 7-14 days. The well dried leaves were made into powder. The collected powder was stored in air-tight containers. About 1 g of V.amygdalina powder was dissolved in distilled water and boiled for 5–10 min at 60–70°C. The solution was filtered by using Whatman no. 1 filter paper. The filtered extract was collected and stored in 4°C for further use.

Synthesis of NPs

The synthesis of copper nanoparticles, 20 ml of V.amygdalina leaf extract was added with 80 ml of CuSO4 kept under constant stirring using a magnetic stirrer at 45–50 °C for 6–7 h. At the end of the step green colour was obtained after the centrifugation process the product was washed twice with deionized water and dried in a hot air oven at 100 °C for 3 h. Finally, the dried powder was stored and properly labeled and used for further analysis (Figure 1).

Preparation of NPs

CuSO4 NPs solution is centrifuged using Lark refrigerated centrifuge at 800 rpm for 10 min and the pellet is collected and washed with distilled water twice. The final purified pellet is collected and dried at 100–150°C for CuSO4 NPs for 24 h. Finally, the NPs powder is collected and stored in an air-tight Eppendorf tube.

Characterization of copper nanoparticles

The synthesis of NPs solution preliminary characterized using UV-visible spectroscopy. About 3 ml of the solution is taken in curettes and scanned in double-beam UV-visible spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for the graphical analysis. The aqueous copper nanoparticles and the optical properties were characterized by UV-spectrophotometer (UV-2450, Shimadzu). The synthesized copper nanoparticles were identified by X-ray diffraction; the Fourier transform infrared spectroscopy

analyzed the functional and chemical group (range of 4000–400 cm) and transmission electron microscopy (JEOL JEM3100F).

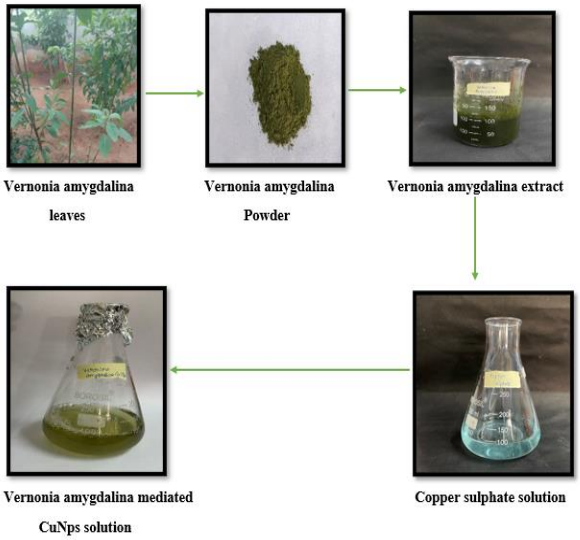


Figure 1: The scheme of synthesis of Vernonia amygdalina mediated Cu NPs.

Cytotoxic Effect

Setup preparation: The artemia tank was filled with 6 litres of distilled water. To that 50 grams of iodine free salt was added and mixed well using a spatula. 2 capsules containing 15 grams of Brine Shrimp eggs were added to the tank and left undisturbed for 5 minutes for proper soaking in salt water. Microscopic picture of nauplii is shown in figure 2.

After that, airline tip was placed inside the artemia tank and the aeration level was increased to maximum level. After 24 hours of incubation, the nauplii hatch out from the brine shrimp eggs, and observed using a stereomicroscope.

The cytotoxicity CuSO4 nanoparticles reinforced with Vernonia amygdalina extract was assessed using Brine shrimp assay. 12 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. CuSO4 nanoparticles reinforced with Vernonia amygdalina were added to each well at different concentrations (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} - \text{number of live nauplii}} \times 100$$

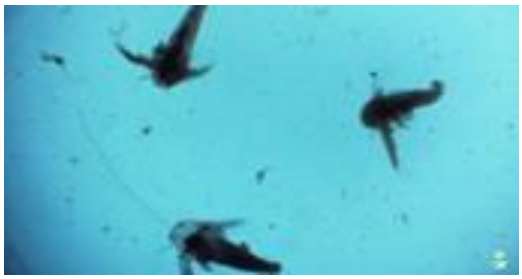


Figure 2: Microscopic picture of nauplii

Anti-Inflammatory Activity (Protein Denaturation Assay)

Bovine serum albumin (BSA) was used as a reagent for the assay. BSA makes up approximately 60% of all proteins in animal serum. It's commonly used in culture, particularly when protein supplementation is necessary and the other components of serum are unwanted. BSA undergoes denaturation on heating and starts expressing antigens associated with Type III hypersensitivity reaction which are related to a disease such as rheumatoid arthritis, glomerulonephritis, serum sickness, and systemic lupus erythematosus.2 ml of 1% bovine albumin fraction was mixed with 400 µl of plant crude extract in different concentration (500–100 µg/mL), and the pH of reaction mixture was adjusted to 6.8 using 1N HCl. The reaction mixture was incubated at room temperature for 20 min and then heated at 55°C for 20 min in a water bath. The mixture was cooled to room temperature, and the absorbance value was recorded at 660 nm. An equal amount of CuSO4 nanoparticles reinforced with Vernonia amygdalina was replaced with dimethyl sulfoxide for control. Diclofenac sodium in different concentrations was used as standards. The experiment was performed in triplicate.

Test Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the CuSO4 nanoparticles reinforced with Vernonia amygdalina was taken in 5 test tubes respectively. To each test tube 2 ml of 1% Bovine Serum Albumin (BSA) was added. 390 µL, 380 µL, 370 µL, 360 µL and 350 µL of distilled water was added to the test tube containing 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of CuSO4 nanoparticles respectively.

Control Group

2 mL of Dimethyl Sulphoxide (DMSO) was added to 2 mL of BSA solution.

Standard Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of Diclofenac Sodium was taken in 5 test tubes respectively. To each test tube 2 mL of 1% Bovine Serum Albumin (BSA) was added. The test tubes were incubated at room temperature for 10 minutes. Then they were incubated in a water bath at 55 oC for around 10 minutes. Absorbance was measured at 660 nm in UV Spectrophotometer

% Inhibition was calculated using the following formula:

% of inhibition =(Control OD − Sample OD % Control OD) × 100

Antioxidant Activity

DPPH radical scavenging assay was performed to monitor the antioxidant potential of plant crude extract. DPPH (oxidized form) is a stable lipophilic free radical, nitrogen-centered with purple color. The antioxidant can donate an electron to DPPH radical and the change in absorbance at 517nm will follow. Color will change to pale yellow gradually.

Test Group

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the CuSO4 nanoparticles reinforced with Vernonia amygdalina was taken in 5 test tubes respectively. To each test tube 1 ml of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was added. 1990 µL, 1980 µL, 1970 µL, 1960 µL and 1950 µL of 50% methanol solution was added to the test tube containing 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of nanoparticles respectively.

Control Group

1 mL of DPPH was added to 2 mL of methanol solution.

Standard Group

Ascorbic acid was used as standard The test tubes were incubated in a dark cupboard for around 20 minutes. Absorbance was measured at 517 nm in the UV Spectrophotometer.

% Inhibition was calculated using the following formula:

% of inhibition = (Control Absorbance − Sample Absorbance% Control Absorbance)× 100

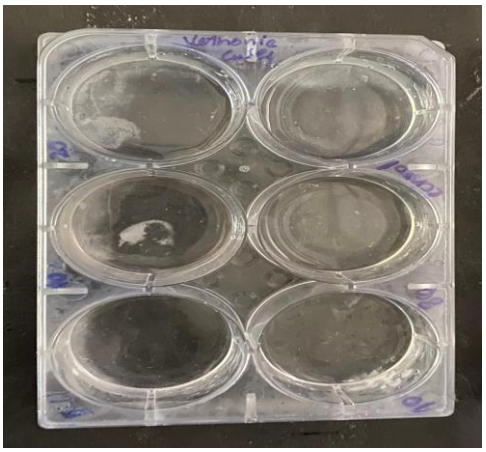
RESULTS

Table 1 depicts the cytotoxicity of CuSo4 nanoparticles reinforced with Vernonia amygdalina extract. At 5 µL and 10µL concentration no death of nauplii found, 20 µL there was a death of 10% of nauplii, at 40 µL there was a death of 10% of nauplii and at 80 µL there was a death of 30% of nauplii. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased.

Table 1: Cytotoxicity of Vernonia amygdalina mediated CuSO4 Nanoparticles

Conc in µl	Viable nauplii	Percentage death of nauplii
	Aqueous extract	Aqueous extract
5 µl	10	0%
10 µl	10	0%
20 µl	9	10%
40 µl	9	10%
80 µl	7	30%

3A



3B

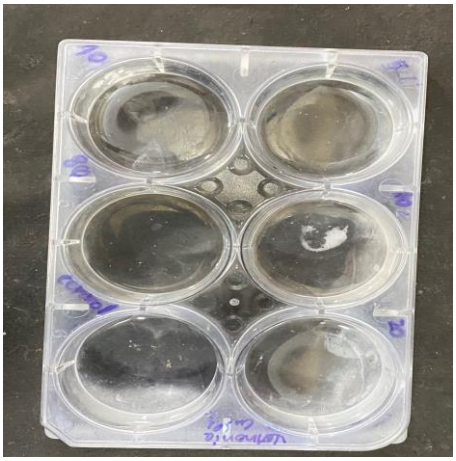


Figure 3A: depicts the pre-set up of cytotoxicity test and figure 3B depicts the post setup after 24 hours of cytotoxicity test.

4A 4B

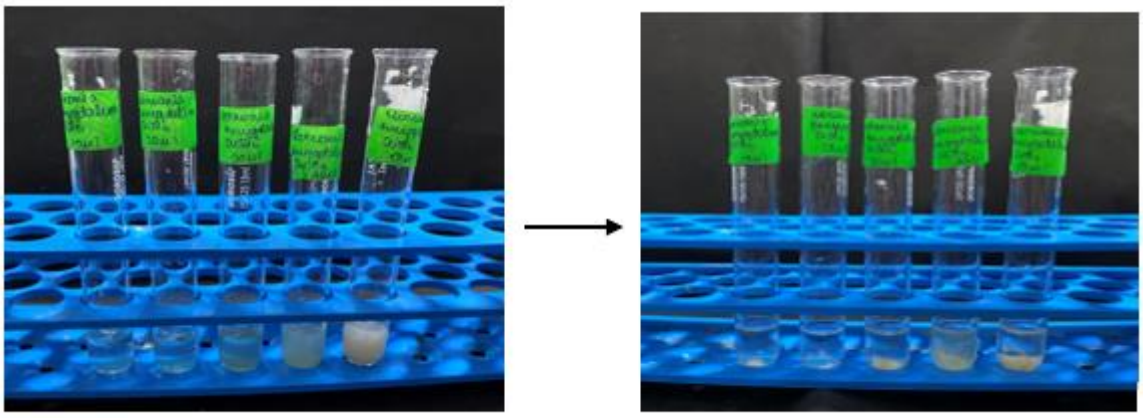


Figure 4A depicts the anti- inflammatory property of CuSO4 nanoparticles reinforced with Vernonia amygdalina extract pre-incubation. Figure 4 B depicts the antiinflammatory property of CuSO4 nanoparticles reinforced with Vernonia amygdalina extract post-incubation (Colour change).

4C

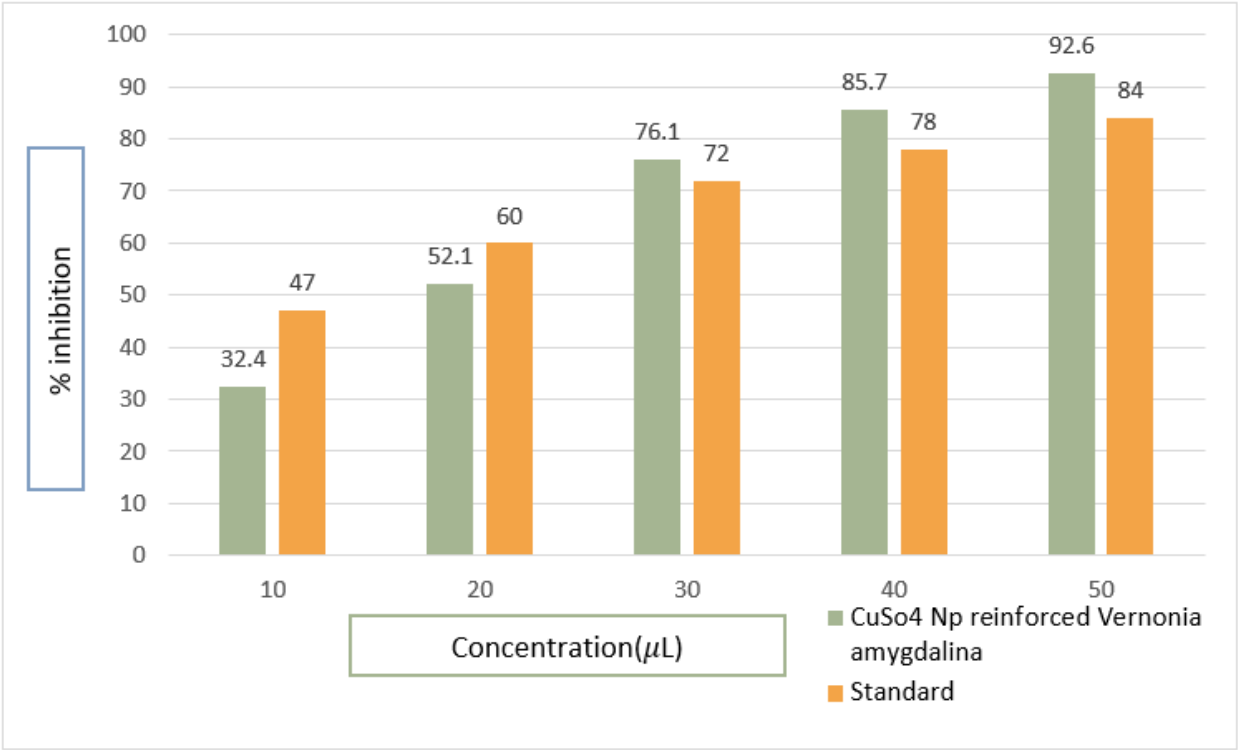


Figure 4C depicts the anti- inflammatory property of CuSO4 nanoparticles reinforced with Vernonia amygdalina at various concentrations compared with the standard values.

It was found that the values for anti-inflammatory property of nanoparticles was higher than the standard values at 30µL, 40µL, 50 µL concentrations.

5A 5B

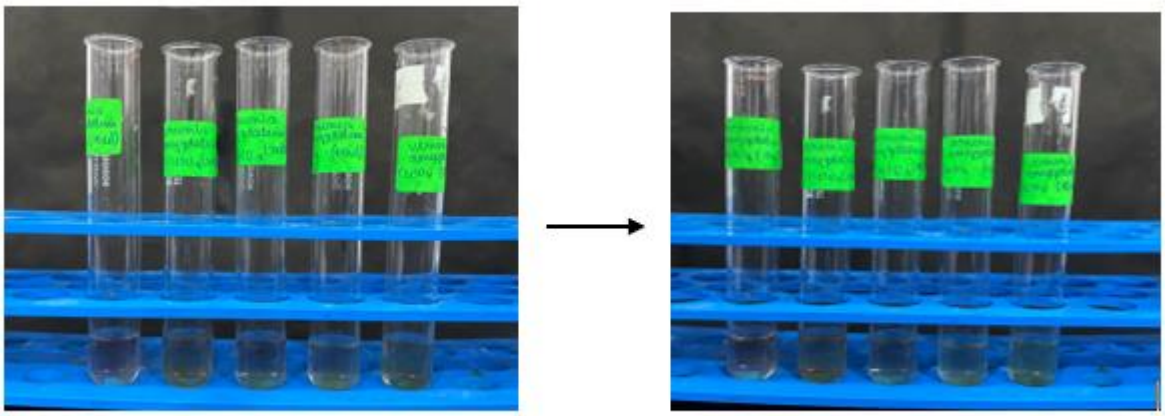


Figure 5A depicts the antioxidant property of CuSO4 nanoparticles reinforced with Vernonia amygdalina extract preincubation. Figure5B depicts the antioxidant property of CuSO4 nanoparticles reinforced with Vernonia amygdalina extract post-incubation.

5C

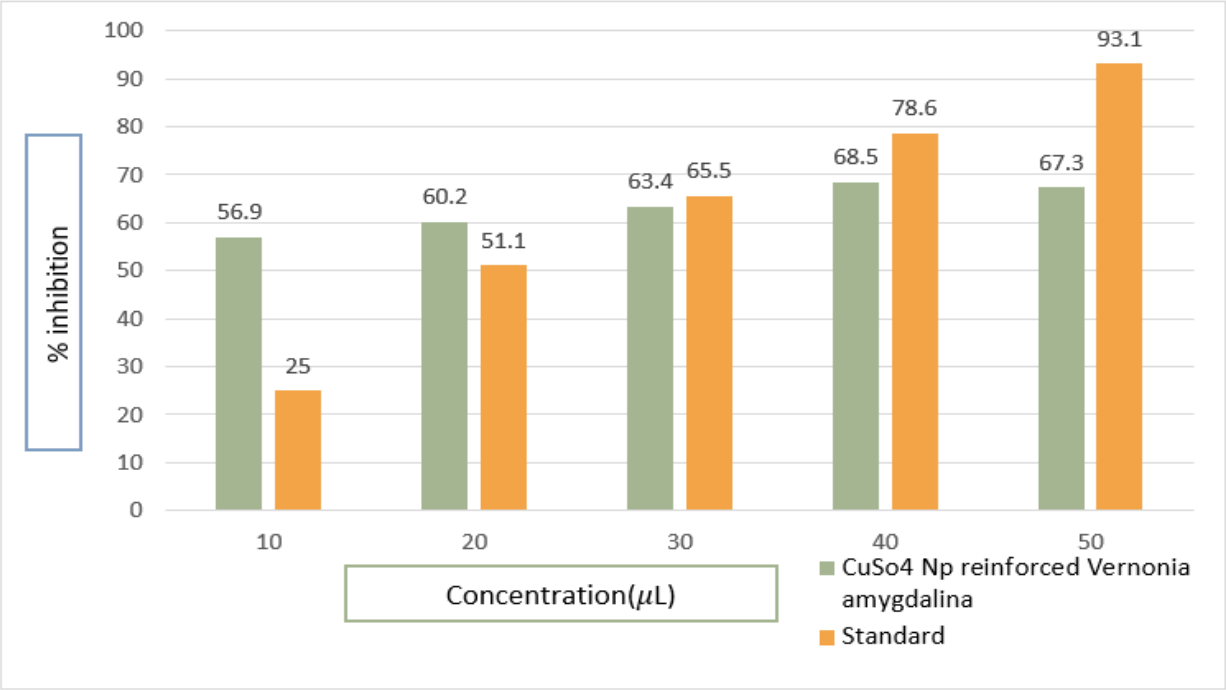


Figure 5C depicts the antioxidant property of CuSO4 nanoparticles reinforced with Vernonia amygdalina extract at various concentrations compared with the standard values.

The values for antioxidant properties of nanoparticles were found to be higher than the standard values at 10 µL and 20 µL and almost equal in 30 µL concentrations.

DISCUSSION

There was an expeditious advancement in nanoparticle synthesis. Compared with the earlier physio-chemical methods which require toxic chemicals for stability, Green nanotechnology using plants becomes an emerging eco-friendly alternative and plant extract mediated nanoparticles synthesis is also cost effective(29). In this study, Vernonia amygdalina was incorporated in Copper nanoparticles using green synthesis and evaluated antioxidant, cytotoxicity and anti-inflammatory.

In the present study, at 5 µL and 10µL concentration no death

of nauplii found, 20 µL there was a death of 10% of nauplii, at 40 µL there was a death of 10% of nauplii and at 80 µL there was a death of 30% of nauplii. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased. Percentage of inhibition of protein denaturation (antiinflammatory activity) was 32.4% at 10 µL concentration, 52.1% at 20 µL, 76.1% at 30 µL ,85.7% at 40 µL and highest at 50 µL (92.6%) (Figure 4C). Percentage of inhibition of DPPH free radicals (antioxidant activity) was 56.9% at 10 µL concentration, 60.2% at 20 µL, 63.4% at 30 µL, 68.5% at 40 µL and 67.3% at 50 µL(Figure 5C).

From the above results of Vernonia amygdalina mediated copper nanoparticles, when concentration of the nanoparticles increased antioxidant, cytotoxicity and anti-inflammatory activity also increased. The highest percentage of death of nauplii was at 50 µL concentration of Copper nanoparticles reinforced with Vernonia amygdalina extract. For both antioxidant and anti-inflammatory activity, the activity was highest at 50 µL concentration. It was found that the values for anti-inflammatory property of nanoparticles was higher than the standard values at 30µL, 40µL, 50 µL concentrations. The values for antioxidant properties of nanoparticles were found to be higher than the standard values at 10 µL and 20 µL and almost equal in 30 µL concentrations.

Studies have been conducted in Vernonia amygdalina extract but in Zinc nanoparticles(30,31). The mechanisms of cytotoxicity from Copper sulphate was not totally understood, but the generation of sulphate from the copper are believed to be major constituents(32).

Many studies revealed that Vernonia amygdalina have antioxidant and anti-inflammatory properties(33–35). The copper nanoparticles are continuously used for the advanced biomedical applications(36). The free radical scavenging activity of copper nanoparticles is very close to the standard ascorbic acid. It can be speculated that the antioxidant properties of Vernonia amygdalina can be attributed to the presence of these flavonoids(37).

Based on the findings of the study we can say that reinforcing copper nanoparticles with Vernonia amygdalina has a synergistic effect and can be used as an alternative to commercially available anti-inflammatory and antioxidant agents.

Limitations

The study was conducted in vitro, so it cannot be assumed that the results of cytotoxicity, anti-inflammatory activity and antioxidant activity could be translated into clinical effectiveness.

Recommendations

- This product can be given to the patients in the form of a mouthwash.

- In further studies, in vivo studies are recommended with people' acceptance values as well.

CONCLUSION

From the above study findings, Vernonia amygdalina mediated copper nanoparticles have very less cytotoxic effects. It also has a potential to serve as a good anti-inflammatory and antioxidant agent. Future studies to be conducted after incorporating Vernonia amygdalina into dentifrices or mouthwashes.

REFERENCES

1. Syed A, Saraswati S, Kundu GC, Ahmad A. Biological synthesis of silver nanoparticles using the fungus Humicola sp. and evaluation of their cytoxicity using normal and cancer cell lines [Internet]. Vol. 114, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2013. p. 144–7. Available from: http://dx.doi.org/10.1016/j.saa.2013.05.030
2. Ramanathan R, O'Mullane AP, Parikh RY, Smooker PM, Bhargava SK, Bansal V. Bacterial Kinetics-Controlled Shape-Directed Biosynthesis of Silver Nanoplates UsingMorganella psychrotolerans [Internet]. Vol. 27, Langmuir. 2011. p. 714–9. Available from: http://dx.doi.org/10.1021/la1036162
3. Huang J, Lin L, Li Q, Sun D, Wang Y, Lu Y, et al. Continuous-Flow Biosynthesis of Silver Nanoparticles by Lixivium of SundriedCinnamomum camphoraLeaf in Tubular Microreactors [Internet]. Vol. 47, Industrial & Engineering Chemistry Research. 2008. p. 6081–90. Available from: http://dx.doi.org/10.1021/ie701698e
4. Dastjerdi R, Montazer M. A review on the application of inorganic nano-structured materials in the modification of textiles: Focus on anti-microbial properties [Internet]. Vol. 79, Colloids and Surfaces B: Biointerfaces. 2010. p. 5–18. Available from: http://dx.doi.org/10.1016/j.colsurfb.2010.03.029
5. Santhoshkumar J, Agarwal H, Menon S, Rajeshkumar S, Venkat Kumar S. A biological synthesis of copper nanoparticles and its potential applications [Internet]. Green Synthesis, Characterization and Applications of Nanoparticles. 2019. p. 199–221. Available from: http://dx.doi.org/10.1016/b978-0-08-102579-6.00009-5
6. Rasheed T, Bilal M, Iqbal HMN, Li C. Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications [Internet]. Vol. 158, Colloids and Surfaces B: Biointerfaces. 2017. p. 408–15. Available from: http://dx.doi.org/10.1016/j.colsurfb.2017.07.020
7. Ravichandran V, Shalini S, Kumar KS, Rajak H, Agrawal RK. Design, Synthesis and Evaluation of Thiourea Derivatives as Antimicrobial and Antiviral Agents [Internet]. Vol. 16, Letters in Drug Design & Discovery. 2019. p. 618–24. Available from: http://dx.doi.org/10.2174/1570180815666180801120440
8. Ravichandran V, Vasanthi S, Shalini S, Shah SAA, Harish R. Green synthesis of silver nanoparticles using Atrocarpus altilis leaf extract and the study of their antimicrobial and antioxidant activity [Internet]. Vol. 180, Materials Letters. 2016. p. 264–7. Available from: http://dx.doi.org/10.1016/j.matlet.2016.05.172
9. Rajeshkumar S, Malarkodi C, Gnanajobitha G, Paulkumar K, Vanaja M, Kannan C, et al. Seaweed-mediated synthesis of gold nanoparticles using Turbinaria conoides and its characterization. Journal of Nanostructure in Chemistry. 2013 Jun 7;3(1):44.
10. Rajeshkumar S, Roy A, Santhoshkumar J, Lakshmi T, Gurunathan D, Others. Antibacterial Activity of Silver Nanoparticles Mediated Aloe vera with Neem Against Dental Pathogens. Indian Journal of Public Health Research & Development [Internet].

2019;10(11). Available from: http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09760245&AN=141274474&h=lnDe035w62%2FBuN9KSaqtrcASu6TS50jTmBHIxQiuM%2BjoOVytPAIoQaVUxASS%2BWHunXrabSTfqkuectle6HKFfA%3D%3D&crl=c

11. Karthiga P, Rajeshkumar S, Annadurai G. Mechanism of Larvicidal Activity of Antimicrobial Silver Nanoparticles Synthesized Using Garcinia mangostana Bark Extract. J Cluster Sci. 2018 Nov 1;29(6):1233–41.
12. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018 Oct;117:91–5.
13. Das M, Smita SS. Biosynthesis of silver nanoparticles using bark extracts of Butea monosperma (Lam.) Taub. and study of their antimicrobial activity [Internet]. Vol. 8, Applied Nanoscience. 2018. p. 1059–67. Available from: http://dx.doi.org/10.1007/s13204-018-0721-0
14. Azmath P, Baker S, Rakshith D, Satish S. Corrigendum to "Mycosynthesis of silver nanoparticles bearing antibacterial activity" [Saudi Pharm. J. 24 (2016) 140–146] [Internet]. Vol. 25, Saudi Pharmaceutical Journal. 2017. p. 148. Available from: http://dx.doi.org/10.1016/j.jsps.2016.03.001
15. Murthy HCA, Desalegn T, Kassa M, Abebe B, Assefa T. Synthesis of Green Copper Nanoparticles Using Medicinal Plant Hagenia abyssinica (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties. J Nanomater [Internet]. 2020 Jun 1 [cited 2021 Jan 21];2020. Available from: https://www.hindawi.com/journals/jnm/2020/3924081/
16. Ejike CE, Ndukwu MC. Pre-harvest and Post-harvest Factors Affecting Bioactive Compounds From Vernonia amygdalina (Del.) [Internet]. Vol. 11, Research Journal of Medicinal Plants. 2017. p. 32–40. Available from: http://dx.doi.org/10.3923/rjmp.2017.32.40
17. Magadula JJ, Erasto P. ChemInform Abstract: Bioactive Natural Products Derived from the East African Flora [Internet]. Vol. 41, ChemInform. 2010. Available from: http://dx.doi.org/10.1002/chin.201015227
18. Nangendo G, Stein A, Gelens M, de Gier A, Albricht R. Quantifying differences in biodiversity between a tropical forest area and a grassland area subject to traditional burning [Internet]. Vol. 164, Forest Ecology and Management. 2002. p. 109–20. Available from: http://dx.doi.org/10.1016/s0378-1127(01)00603-x
19. Neelakantan P, Grotra D, Sharma S. Retreatability of 2 mineral trioxide aggregate-based root canal sealers: a cone-beam computed tomography analysis. J Endod. 2013 Jul;39(7):893–6.
20. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. Risk Manag Healthc Policy. 2021 Jul 7;14:2851–61.
21. Sheriff KAH, Ahmed Hilal Sheriff K, Santhanam A. Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College [Internet]. Vol. 11, Research Journal of Pharmacy and Technology. 2018. p. 543. Available from: http://dx.doi.org/10.5958/0974-360x.2018.00101.4
22. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther. 2021 Mar 18;12(1):192.
23. Jayaraj G, Ramani P, Herald J. Sherlin, Premkumar P, Anuja N. Inter-observer agreement in grading oral epithelial dysplasia – A systematic review [Internet]. Vol. 27, Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2015. p. 112–6. Available from: http://dx.doi.org/10.1016/j.ajoms.2014.01.006
24. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.
25. Li Z, Veeraraghavan VP, Mohan SK, Bolla SR, Lakshmanan H, Kumaran S, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway [Internet]. Vol. 203, Journal of Photochemistry and Photobiology B: Biology. 2020. p. 111773. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111773
26. Gan H, Zhang Y, Zhou Q, Zheng L, Xie X, Veeraraghavan VP, et al. Zingerone induced caspase-dependent apoptosis in MCF-7 cells and prevents 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in experimental rats. J Biochem Mol Toxicol. 2019 Oct;33(10):e22387.
27. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res. 2019 Sep;80(6):714–30.
28. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev. 2014 Mar 17;8(1):244.
29. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract [Internet]. Vol. 22, Biotechnology Progress. 2006. p. 577–83. Available from: http://dx.doi.org/10.1021/bp0501423
30. Liu H, Kang P, Liu Y, An Y, Hu Y, Jin X, et al. Zinc oxide nanoparticles synthesised from the Vernonia amygdalina shows the anti-inflammatory and antinociceptive activities in the mice model [Internet]. Vol. 48, Artificial Cells, Nanomedicine, and Biotechnology. 2020. p. 1068–78. Available from: http://dx.doi.org/10.1080/21691401.2020.1809440
31. Onasanwo S, Oyebanjo O, Ajayi A, Olubori M. Mechanisms of action of the anti-nociceptive and anti-inflammatory effects of leaf extract of Vernonia amygdalina [Internet]. Vol. 6, Journal of Intercultural Ethnopharmacology. 2017. p. 1. Available from: http://dx.doi.org/10.5455/jice.20170330010610
32. Arumugam DG, Sivaji S, Dhandapani KV, Nookala S, Ranganathan B. Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp [Internet]. Vol. 19, Biocatalysis and Agricultural Biotechnology. 2019. p. 101132. Available from: http://dx.doi.org/10.1016/j.bcab.2019.101132
33. Habtamu A, Melaku Y. Antibacterial and Antioxidant Compounds from the Flower Extracts of Vernonia amygdalina [Internet]. Vol. 2018, Advances in Pharmacological Sciences. 2018. p. 1–6. Available from: http://dx.doi.org/10.1155/2018/4083736
34. Phytochemical screening, antioxidant and antibacterial investigation of methanolic extract of the leaf of Vernonia amygdalina (girawa) [Internet]. Vol. 10, International Journal of Green and Herbal Chemistry. 2020. Available from: http://dx.doi.org/10.24214/ijghc/hc/9/4/52331
35. Oriakhi K, Oikeh EI, Ezeugwu N, Anoliefo O, Aguebor O, Omoregie ES. Comparative Antioxidant Activities of Extracts of Vernonia amygdalina and Ocimum gratissimum Leaves [Internet]. Vol. 6, Journal of Agricultural Science. 2013. Available from: http://dx.doi.org/10.5539/jas.v6n1p13
36. Alzahrani E, Chemistry Department, Faculty of Science, Taif University, 888-Taif, Kingdom of Saudi Arabia. Synthesis of Copper Nanoparticles with Various Sizes and Shapes: Application as a Superior Non-Enzymatic Sensor and Antibacterial Agent [Internet]. International Journal of Electrochemical Science. 2016. p. 4712–23. Available from: http://dx.doi.org/10.20964/2016.06.83
37. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int J Environ Res Public Health. 2011 Jun;8(6):2533–55.