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RESEARCH ARTICLE

Free Radical Scavenging and Cytotoxic Effect of Copper Nanoparticles Synthesised Using Nutmeg Oleoresin

TRISHA SASIKUMAR[1], ANITHA ROY[2*], S. RAJESHKUMAR[3], LAKSHMI THANGAVELU[4]

[1]*Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai, Tamil Nadu, India, Email: 151901013.sdc@saveetha.com*

[2]*Professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India, Email: anitharoy.sdc@saveetha.com*

[3]*Associate Professor, Nanobiomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India, Email : rajeshkumars.sdc@saveetha.com*

[4]*Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai-77,Tamil Nadu, India, Email: lakshmi@saveetha.com*

**Corresponding Author*

ABSTRACT

Background: Copper nanoparticles that are extremely small and have a high surface to volume ratio can also serve as antifungal/antibacterial agents. They have excellent biocompatibility, are economical, and exhibit low toxicity. Nutmeg or Myristica fragrans is found to have health benefits, including its ability to relieve pain, soothe indigestion, strengthen cognitive function, detoxify the body, boost skin health, alleviate oral conditions, reduce insomnia, increase immune system function, and prevent leukemia, and improve blood circulation.

Aim: The aim of this study was to analyse the free radical scavenging and cytotoxic activities of copper nanoparticles made from nutmeg oleoresin.

Materials and methods: In this study, nutmeg oleoresin was used to synthesise copper nanoparticles. These nanoparticles were subjected to cytotoxic and free radical scavenging assays. The cytotoxicity was studied by brine shrimp lethality assay and the free radical scavenging activity by DPPH assay technique.

Results: The extract shows the highest percentage of live nauplii at 50% for 80µL concentration and antioxidant activity was 84% almost close to the standard at 50µL concentration.

Conclusion: The extract has shown high cytotoxicity and good free radical scavenging with increase in concentration. It also showed good antioxidant activity with DPPH assay. Hence , nutmeg mediated copper nanoparticles may be used in the treatment of cancer and other conditions such as diabetes and cardiovascular disease.

KEYWORDS:
Copper nanoparticles, Nutmeg oleoresin, cytotoxic activity , free radical scavenging activity, green synthesis, eco friendly

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INTRODUCTION

Nanoparticles are one of the most significant wings of biomedical research. Nanoparticles being the same size as human body cells are readily taken in and adopted by the body. They are of the size 1-100nm. By encapsulating medicine in nanoparticles, which are the size of viruses, they are effective in drug delivery systems. (1,2) They can precisely find the diseased cell and carry the medicine to them. They are also used to deliver heat, light, or other substances to specific target cells like cancer cells. (1-3)This technique reduces the damage caused to healthy cells in the body and allows early detection of diseases. It becomes more beneficial when these nanoparticles are synthesized through natural sources. Green synthesis of these nanoparticles provides very little to no side effects on our body, thereby reducing any risk of exposure to

harmful chemicals. With the growing awareness of the benefits of naturally or herbally sourced products, there is an increasing demand for more herbal-based products and services.

Copper nanoparticles that are extremely small and have a high surface to volume ratio and also serve as antifungal/antibacterial agents. The antimicrobial activity is induced by their close interaction with microbial membranes and their metal ions released into solutions. They have excellent biocompatibility, are economical, and exhibit low toxicity. These NPs have superior antibacterial, antimicrobial, and cytotoxic properties.(4),(5) Nutmeg or Myristica fragrans is found to have health benefits, including its ability to relieve pain, soothe indigestion, strengthen cognitive function, detoxify the body, boost skin health, alleviate oral conditions, reduce insomnia, increase immune system function, and prevent leukemia, and improve blood circulation. Nutmeg is also rich in anti-inflammatory compounds called monoterpenes.

Cytotoxicity assays measure loss of some cellular or intracellular structure and/or functions, including lethal cytotoxicity. They thus give an indication of the potential to cause cell and tissue injury and as such have been used by some investigators to predict tissue injury, including eye injury. The cytotoxicity test is one of the biological evaluation and screening tests that use tissue cells in vitro to observe the cell growth, reproduction and morphological effects by medical devices. The type of cytotoxicity test used was brine shrimp lethality assay, the preliminary cytotoxicity assay of plant extract and others based on the ability to kill a laboratory cultured larvae (nauplii). The nauplii were exposed to different concentrations of plant extract for 24 hours. Previously, our team had conducted numerous studies with different oleoresin and plant extracts (6) - (7). This research aims to study the free radical scavenging activity and cytotoxic activity of copper nanoparticles made from nutmeg oleoresin.

MATERIALS AND METHODS

Study Setting

The study was conducted at the Nanobiomedicine lab of Saveetha Dental College after obtaining approval from the Scientific Review Board . Ethical Clearance Number is IHEC/SDC/UG-1913/21/166.

Plant material

Nutmeg oleoresin having product code 4010000484 was obtained from Synthite Industries Pvt.Limited, Kerala. 1mg/ml was used as the stock solution .

Preparation of plant extract

0.2 ml of nutmeg oleoresin was mixed with 100 ml of distilled water and dissolved using a heating mantle with the temperature about 50-60 degree Celsius for 5-10 minutes. The extract was stored in the beaker, covered by using an aluminium foil and was used for biosynthesis of nanoparticles.

Preparation of copper nanoparticles

20mm of anhydrous copper sulphate was prepared by dissolving 0.477 in distilled water. 80ml of this was mixed with 20ml of prepared nutmeg oleoresin solution. Then this mixture was kept in the orbital shaker for 2 days and then colour change was observed initially by visual means and then confirmed by using UV-visible spectroscopy. There was a colour change from dark blue to light blue (Figure 2). The absorbance was noted at regular intervals and a graph was plotted. (Figure 1)The UV spectroscopy revealed a peak at 280 nm .

Cytotoxic Activity

In this test, 6 elisa wells were filled with 2g of non iodised salt dissolved in 200mL of distilled water. In each well, 10 nauplii (freshly brine shrimp larvae) were transferred. Among the 6 wells , 5 wells were loaded with Copper nanoparticles in different concentrations (5µL, 10µL, 20µL, 40µL, 80µL). The last was used as a control without any nanoparticles. Finally the wells were filled with salt water till the brim and observed for changes after 24hrs. (8)(9)(10)(11)(12)(13)(14)

Free radical scavenging activity using DPPH Assay Technique

2ml of nutmeg oleoresin mediated copper nanoparticle was added to five test tubes. 50% of the methanol solution (buffer), 0.1mm of DPPH solution was added to five test tubes. Nutmeg oleoresin mediated copper nanoparticles were added to five test tubes in a different concentration ranging from 10-50 µl. The mixture was then incubated for 30 minutes in a dark place at room temperature. The absorbance was measured using a spectrophotometer at 517 nm. The blank used was methanol solution. Methanol solution mixed with 0.1mM of DPPH solution was used as a control. Ascorbic acid was used as a standard.. Percentage of inhibition was estimated using the equation,(7)

% Inhibition = Absorbance of control - Absorbance of sample x 100 / Absorbance of control.

Colour change was recorded before and after incubation.

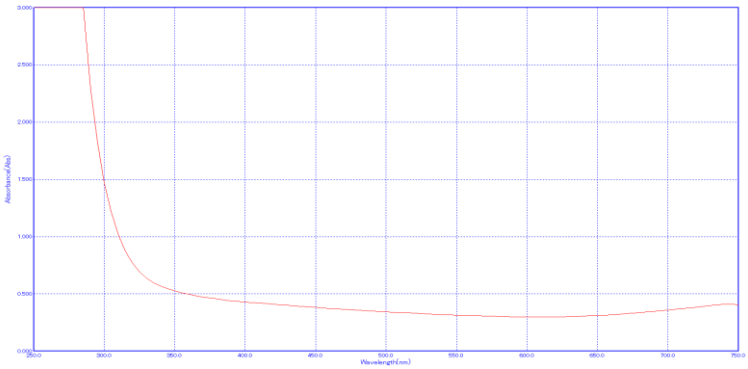

Fig.1: UV -Visible Spectroscopy of nutmeg oleoresin mediated copper nanoparticles .

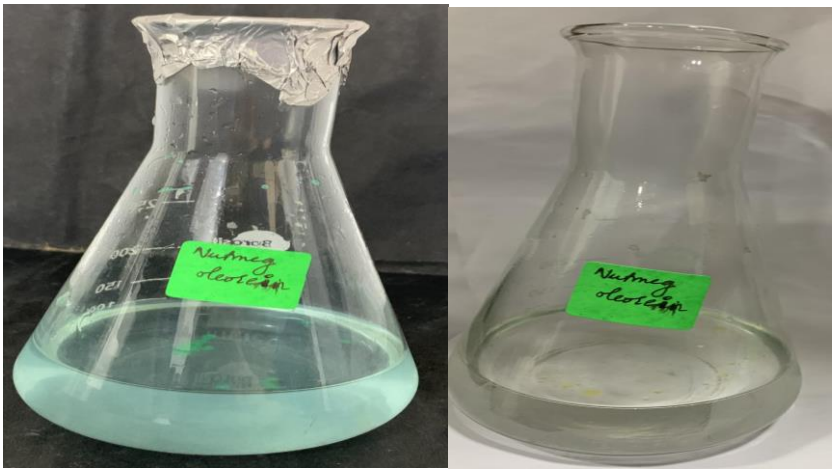

Fig.2: Formation of nutmeg oleoresin mediated copper nanoparticles.

RESULTS

The results have shown that the percentage of live nauplii was 90% at 5µL and was only 50% at highest concentration of 80µL. This proves that the cytotoxicity increased with increase in concentration of the nutmeg oleoresin mediated copper nanoparticles. (Figure 3)The free radical scavenging activity was found to be 18% at 10µL concentration and the highest value was 84% at 50µL. Thereby proving that the free radical scavenging activity increased with increase in concentration. (Figure 3) (15,16)-(17)

Free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In DPPH Assay, violet colour DPPH solution is reduced to a yellow coloured product, diphenyl picryl hydrazine, by the addition of the extract in a concentration dependent manner (Figure 4). The percentage of inhibition increased with increase in concentration of nutmeg oleoresin mediated copper nanoparticles. The percentage of inhibition at 10µL was 64%, at 20µL was 68%, at 30µL was 84%, at 40µL was 86% and 50µLwas 96%. The percentage of inhibition for different concentrations of nutmeg oleoresin mediated copper nanoparticles were: 5µL inhibited 10% of live nauplii, 10µL inhibited 20% live nauplii, 20µL inhibited 20% live nauplii, 40µL inhibited 30% live nauplii and 80muL inhibited 50% live nauplii. (18)-(15)

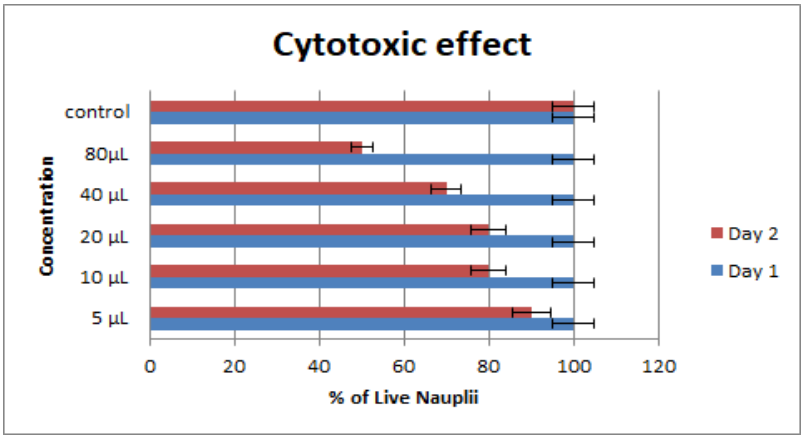

Fig.3: represents the cytotoxic activity by depicting the percentage of live nauplii. X- axis represents the percentage of live nauplii andY- axis represents the quantities of nutmeg oleoresin mediated copper nanoparticles. Blue represents live nauplii on day 1 and red colour represents live nauplii on Day2.

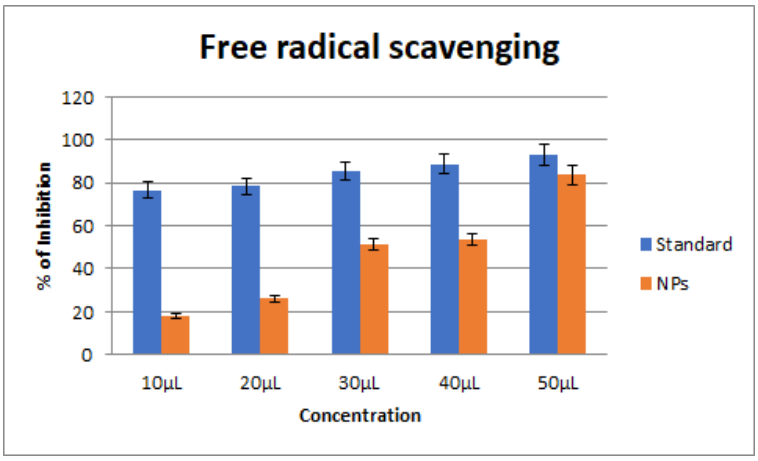

Fig.4: represents the free radical scavenging activity by depicting the percentage of inhibition. X- axis represents different quantities of nutmeg oleoresin mediated copper nanoparticles (orange color) and standard in blue color. Y-axis represents the percentage of inhibition. (n = 3 with mean ± SD)

DISCUSSION

It was concluded that copper nanoparticles provided great clinical significance and further expansion of studies should be conducted for them to be used widely. (19–22) It was concluded that copper nanoparticles show high cytotoxic activity even under low concentrations. They proved that free radical scavenging effects produced by nanoparticles are very significant and are considered a breakthrough in biomedical research. (19–21) A previous study concluded that copper nanoparticles can be produced by green synthesis. (17,23)-(24)

From previous literature, we can conclude that the green synthesis of copper nanoparticles has very little or no side effects in comparison to commercially synthesized medical supplements. The previous author has concluded that copper nanoparticles have high antimicrobial and antifungal activity. (19,20) From previous literature, we know that zinc nanoparticles showcase excellent drug delivery targeted sites without harming other healthy cells of the body. Myristica fragrans is found to have health benefits, including its ability to relieve pain, soothe indigestion, strengthen cognitive function, detoxify the body, boost skin health, alleviate oral conditions, reduce insomnia, increase immune system function, and prevent leukemia, and improve blood circulation. (19) Nutmeg is also rich in anti-inflammatory compounds called monoterpenes. There are previous studies that prove the antimicrobial activity of nutmeg is extremely significant in medical properties. From previous literature, we can conclude that nutmeg has a very high cytotoxic and free radical scavenging effect. (25)

Our team has extensive knowledge and research experience that has translate into high quality publications(26–30) (31) .The limitation of the study was that the study was done only in in-vitro with a single model due to limitation of time .Inorder to take into clinical application level, further studies are needed in animal model and human volunteers.

CONCLUSION

Nutmeg oleoresin mediated copper nanoparticles exhibited excellent cytotoxic activity and very good free radical scavenging activity (32 - 41). Hence nutmeg oleoresin mediated copper oxide may be recommended for its use as an antioxidant and anticancer drug in future after conducting the research in animal models .

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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