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Assessment of cytotoxic and antioxidant properties of copper nanoparticles using green tea and neem formulation- An in-vitro study

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Abstract

Introduction: Copper nanoparticles are of great interest and have applications in various fields due to their antibacterial, antioxidant, cytotoxicity and anticancer properties. Green synthesis of nanoparticle is currently a new approach which is a nontoxic, economical, ecofriendly approach. Our study aim was to assess the antioxidant and cytotoxic effects of green synthesized copper nanoparticles from green tea and neem formulation.

Materials and methodology: 20mM of copper sulphate solution is mixed with 40mL of plant extract and 60 mL of distilled water was added and made it into 100 ml solution. The copper nanoparticles derived using green synthesis was assessed for antibiotic activity by free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Cytotoxicity of copper nanoparticles was evaluated using Brine shrimp assay at 5,10,20,40 and 80µL concentrations.

Results: The change of colour from green to brown and the peak observed in UV-vis- spectrophotometer indicated the synthesis of copper nanoparticles. The antioxidant property was visually observed by the colour change from green to brown indicating that DPPH has undergone a reduction reaction exhibiting scavenging activity. The cytotoxic effects of green synthesised copper nanoparticles from green tea and neem extract showed that there is a concentration dependent increase in the cytotoxic property of copper nanoparticles.

Conclusion: The copper nanoparticles are effectively synthesized using green tea and neem formulation. The results obtained in this study concluded that the copper nanoparticles synthesised from green tea and neem extract showed concentration dependent antioxidant and cytotoxic properties.

Keywords: Nanoparticles, copper, green synthesis, green tea, neem, cytotoxicity

Introduction

Copper is a necessary micro component for human beings, plants and other creatures, which is needed in negligible amounts.[1–3].When comparing with the different other metals nanoparticles available such as, gold, platinum and silver, the copper nanoparticles are more economical[4]. There are many techniques for the synthesis of copper nanoparticles such as, thermal reduction, sonochemical reduction, induced radiation, laser irradiation, capping agent method, vapour deposition method, and microemulsion techniques[5–11]. But these procedures have many disadvantages as they contain various toxic chemicals that are hazardous to human beings as well as to the environment.

Currently green synthesis of nanoparticles has evolved giving a new method for the preparation of nanoparticles. This method is easy, reproducible, economical, also gives a stable product, which does not involve the use of toxic chemicals[12]. The bottoms up method for green synthesis is homogeneous to that of chemical reduction of nanoparticles, the difference being that the chemical reducing agent is substituted with plant, fruit, flower, and algae extracts[13,14].The process of green synthesis is initiated by mixing the natural extracts with a metallic solution. Colour change is observed in the solution when the salt is reduced as a result of biochemical reduction mechanism indicating the nanoparticle synthesis[15].

The antioxidants have a vital role in providing protection against oxidative stress, cardiovascular problems and neurodegenerative diseases[16]. Bacteria, algae, fungi, lichens and plants are known to contain various bioactive compounds like polyphenols, phenolic acids, terpenoids, alkaloids, etc. They show promising antioxidant activity, and are effective in reducing and stabilizing the metallic ions[17]. Antioxidants play a major role in upgrading the wellbeing of life by arresting the emergence of many degenerative diseases. Leaves of the green tea comprises a catechin known as epigallocatechin-3-gallate (EGCG). Catechins in green tea have are natural antioxidants that help in blocking cell damage and supplement many other health benefits. These components can prevent cellular and molecular impairment by reducing the generation of free radicals in the body. Neem (Azadirachta indica) which is a member of the Meliaceae family is rich in natural antioxidants, therefore neem leaves play an important role as health-promoting agent [18].

This study was done to evaluate the antioxidant and cytotoxic effects of green synthesized copper nanoparticles from green tea and neem formulation.

Materials and methodology

2 grams of green tea and 2 grams of neem were measured and added to a conical flask and 100 mL of distilled water was added. Then the extract was heated at 60°C for 7-8 minutes in a heating mantle. Using a Whatman No.1 filter paper the extract was then filtered into another conical flask and the filtrate in the conical flask is the required plant extract.40 ml of plant extract is mixed with 20 millimoles of copper sulphate and 60ml of distilled water in a conical flask. The flask was then placed in the orbital shaker at 65 rpm for 2 days and then in a magnetic stirrer at 450 rpm and the colour change was noted periodically. The UV-Vis absorption spectra of the green synthesized copper nanoparticles were recorded at a different

wavelengths. The copper nanoparticles synthesised from green tea and neem formulation shows the peak of absorption at 340 nm, which indicates the synthesis of nanoparticle.

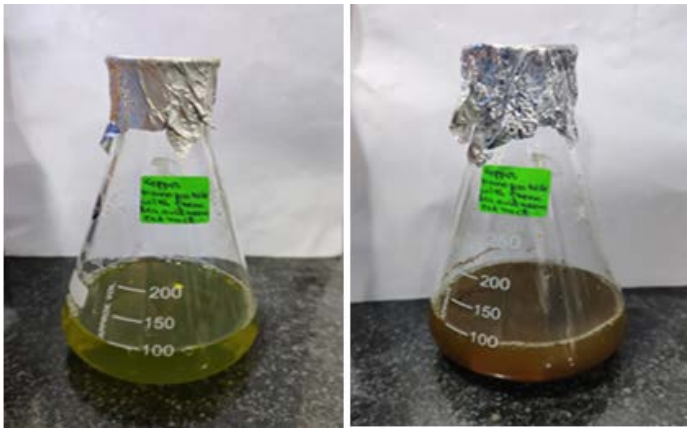

Figure 1: Colour changes from green to brown indicating the synthesis of copper nanoparticles.

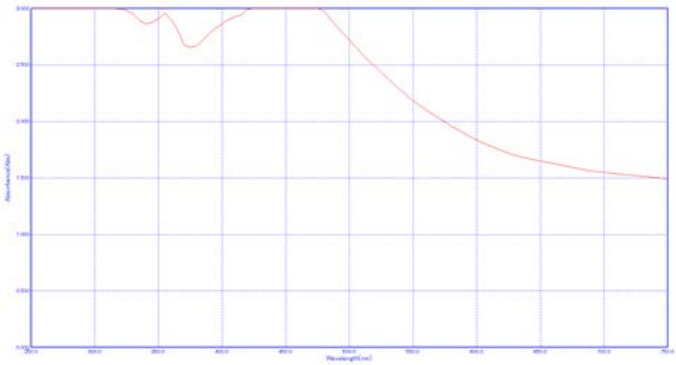

Figure 2: Graphical representation of synthesis of copper nanoparticles.

Antioxidant property of copper nanoparticles

The antioxidant property of copper nanoparticles obtained by green synthesis was assessed by free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Hydrogen donating capacity or scavenging of free radicals by the nanoparticles was assessed by a free radical known as 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Butylated hydroxytoluene (BHT) was used as the positive control. A measured quantity of 1mL of 0.1 mm DPPH in methanol solution was mixed with the copper nanoparticles with a concentration ranging from 5 to 80 µl. Ascorbic acid was kept as the control. The solution was sonicated and incubated for 20 min in light free conditions. After the incubation period the absorbance was determined at a wavelength of 517 nm, which means that the spare electron present in the DPPH free radical is taken up by the antioxidant substance that donates hydrogen. The antioxidant property of the copper nanoparticle was determined by the equation:

$$\% \text{ free radical scavenging effect} = \frac{\text{Absorbance of control - absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Cytotoxic effect of copper nanoparticles

The cytotoxic effect of copper nanoparticles synthesised by neem green tea and neem formulation was evaluated by Brine shrimp assay. A 12 well ELISA plate was taken and 6-8 ml of saltwater was added to 5 wells. Then 10 newly hatched nauplii eggs were added to each of the 5 wells. Copper nanoparticles of different concentrations (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) were added to the 5 wells and then the samples were incubated and kept undisturbed for a period of 24 h. After the incubation period, the numbers of moving and deceased nauplii eggs were estimated and the death (mortality) rate was calculated.

$$\% \text{ mortality} = \frac{\text{Number of deceased nauplii}}{\text{Number of deceased nauplii} - \text{number of moving (live) nauplii}} \times 100$$

Results

The colour change of the solution from green to brown indicates the formation of copper nanoparticles. Various phytochemicals present in plant extract helps to convert copper sulphate to copper nanoparticles which is the reason for the colour change. UV-visible absorption spectra of the copper nanoparticles synthesised using green tea and neem formulation were noted at different wavelengths ranging from 200 nm to 600 nm.

The antioxidant property of copper nanoparticles synthesized using green tea and neem formulation with respect to DPPH free radical was evaluated using

Butylated hydroxytoluene (BHT) as the positive control. The percentage of free radical scavenging effect of copper nanoparticles at various concentrations was evaluated. Violet colored sample turned yellow indicating the reduction of DPPH free radical, thereby exhibiting scavenging activity.

Concentration(µL)	Wavelength(nm)	Abs
10	517	0.460
20	517	0.540
30	517	0.646
40	517	0.826
50	517	0.934

Table 1: Antioxidant property of green synthesised copper nanoparticles from green tea and neem extract.

The cytotoxic effects of green synthesised copper nanoparticles from green tea and neem extract showed a concentration dependent increase. Cytotoxic effect increased with higher concentrations of copper nanoparticles.

Concentration(µL)	Live nauplii (after 24hrs)
0	10
5	9
10	9
20	1
40	0
80	0

Table 2: Cytotoxic effects of green synthesised copper nanoparticles from green tea and neem extract.

Discussion

Copper is the basic and biocompatible element that is well known for its good therapeutic and antibacterial properties since a very long time[19]. Copper nanoparticles are easily synthesized and are economical so it can serve as a substitute for gold and silver nanoparticles. Nowadays, plant materials are used for the synthesis of nanoparticles as it is eco-friendly[20]. Synthesis of nanoparticles from plant extract is arising as an environmentally friendly alternative, as it is economical, nontoxic and eco friendly[21]. Our study evaluated the antioxidant and cytotoxic properties of green synthesised copper nanoparticles from green tea and neem extract. A catechin called epigallocatechin-3-gallate (EGCG) is present in green tea. They are natural antioxidants that help in arresting damage of cells and provide other health benefits. These substances protect cells and molecules from damage by reducing the generation of free radicals in the body. Green tea contains antioxidants such as polyphenols that are capable of neutralizing the free radicals andhelps prevent cell degeneration[22]. Neem (Azadirachta indica) is a good reserve of antioxidants and hence it has got health-promoting effects [18].The study results of Ghimeray et al. summarized that the extracts of leaf and bark of neem have significant antioxidant properties. Our study showed that when the concentration of the copper nanoparticles increased, the cytotoxic and antioxidant properties also increased.

Conclusion

Green synthesis is a nontoxic, ecofriendly and economical method of synthesizing nanoparticles. The copper nanoparticles are effectively synthesized using green tea and neem extract. The results obtained in this study clearly demonstrate that the copper nanoparticles synthesised from green tea and neem formulation have

concentration dependent antioxidant and cytotoxic properties.

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