Research Article

Anti-inflammatory and cytotoxic potential of *Asparagus racemosus* mediated copper nanoparticles

C. Prathiba Reichal, Lakshminarayanan Arivarasu*, S. Rajeshkumar

ABSTRACT

Introduction: Nanoparticles hold properties such as stability, reactivity, and physicochemical properties and measured in the range of 1–100 nm. They have a high surface area-to-volume ratio. And hence, it is a growing field in the modern era of sciences. Copper nanoparticles have certain special features functioning as less toxic, heat transfer properties, low cost, high friction of atoms, and as high surface area-to-volume ratio due to their size, shape, morphology, crystalline phase, and composition. The plant *Asparagus racemosus* is referred as the "Queen of Herbs" in ayurvedic health system as it treats various diseases. The current study evaluates the anti-inflammatory properties of *A. racemosus* extract, the green synthesis, characterization of CuNPs, and screening of their cytotoxic activity. **Aim:** The aim of the present study is to assess the anti-inflammatory and cytotoxic activity of *A. racemosus* mediated copper nanoparticles and considering the efficacy of *A. racemosus* mediated copper nanoparticles. **Materials and Methods:** The methodology includes the green synthesis of *A. racemosus* mediated copper nanoparticles followed by the tests for anti-inflammatory and cytotoxicity activity. **Results:** The anti-inflammatory action and cytotoxicity activity are highly significant and its efficiency increases with increase in the concentration. **Conclusion:** *A. racemosus* mediated copper nanoparticles showed expressive anti-inflammatory and cytotoxic activity and hence they can be used as a potent anti-inflammatory and cytotoxic agents.

KEY WORDS: Anti-inflammatory, *Asparagus racemosus*, Copper nanoparticles, Cytotoxicity

INTRODUCTION

Nanoparticles are particles that possess stability, reactivity, and physicochemical properties and measured in the range of 1–100 nm. Plant extracts as a promising approach for the synthesis of nanoparticles avoid the errors of chemical methods.[1-4] Green synthesis procedures are simple, non-toxic and these constituents are used as reducing agents to reduce the metal ions to their analog nanoparticles and act as protecting agents by capping. The advantages of using plant extract is, to maintain the microbial cell culture and avoid complex purification steps, rapid, economical, and eco-friendly.[5-7] Nanoparticles not only circulate in the body but also enter into cells and as the ability to bind with specific cells. Numerous uses are afforded by nanotechnology for instance cancer therapy, bioimaging, antibacterial, cytotoxic potential, and so on. Copper nanoparticles have certain special features functioning as less toxic, heat transfer properties, low cost, high friction of atoms, and as high surface area-to-volume ratio.[8-10]

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The plant *Asparagus racemosus* is a cherished as a medicinal plant that belongs to the family of Asparagaceae. It is referred as the "Queen of Herbs" in ayurvedic health system as it treats various diseases.[11] It is acknowledged as a diuretic, antispasmodic, refrigerant, galactagogue, aphrodisiac, rejuvenating, antiseptic, and carminative. It is proved to be highly effective in problems that are related with female reproductive system by increasing the production of milk through drug therapy and not by physiological effect. It is attested to have antitussive and antiprotozoal effect in sulfur dioxide-induced cough in mice and inhibitory effect in *Entamoeba histolytica in vitro*.[12] Immunoadjuvant potential is evaluated by its impact on systemic Th1/Th2 immunity and thus it is used against tetanus and diphtheria.[13] Methanolic extracts are evaluated to have anti-amnesic activity as they significantly reverse scopolamine and sodium nitrite levels.[14] *A. racemosus* Wild. is described

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for the prevention and treatment of Type 2 diabetes mellitus along with no side effects due to its inhibitory effects on α-amylase and α-glucosidase.[15] The herb is used to treat nervous disorders, tumors, neuropathy, hepatopathy, hyperdipsia, immunostimulant, dysentery, inflammation, and dyspepsia. Shatavarins (steroidal saponins), sarsapogenins are natural compounds present in *A. racemosus* known to possess anti-inflammatory and antioxidant property.[16,17] The dried root extracts of this plant consist of trace minerals such as cobalt (22.00 mg/g), zinc (53.15 mg/g), manganese (19.98 mg/g), calcium, potassium zinc, selenium, magnesium, and some miscellaneous essential fatty acids, gamma linolenic acids, diosgenin, Vitamin A, B1, B2, C, and E, Mg, P, Ca, Fe, and folic acid.[18]

A. racemosus extract exhibits immunopharmacological activity in cancer chemotherapy. Reports have stated that shatavarin IV rich fraction (AR-2B) unveils anticancer effects in both *in vitro* and *in vivo* methods. Rats affected by mammary carcinogenesis showed inhibition of 7,12-dimethylbenzanthracene from the extracts of this plant.[14] Other properties comprise antiulcer, immunomodulatory, antidiarrheal, antihepatotoxic, antioxidant, phytoestrogenic, and adaptogenic properties.[19] Several studies have reported the macrophagic activity and delaying of tumor development in experimental animals by wild-type *A. racemosus*. It helps to restore the lymphocyte and neutrophils count and hence named as immunomodulator. The herb helps to stimulate immune cell proliferation by influencing interleukin-12 and inhibits the production of IL-6. Many studies have testified that IL-6 prevents differentiation of Th1 and stimulates the production of (CD4) T cells and thereby increases the differentiation of Th2 cells.[13] The current study evaluates the anti-inflammatory properties of *A. racemosus* extract, the green synthesis, characterization of CuNPs, and screening of their cytotoxic activity.

MATERIALS AND METHODS

Preparation of Plant Extract

A. racemosus extract is purchased commercially. The extract is diluted with 100 ml of distilled water and boiled for 20 min. The extract is then filtered using Whatman filter paper and allowed to stand for 40 min undisturbed. A 60 ml of filtered extract is obtained and used for green synthesis.

Synthesis of CuNPs

A 4 nM (0.636 g) of copper (II) sulfate is weighed and mixed with distilled water of 100 ml and mixed with the filtered extract. The extract is permitted to stand in stirrer for a duration of 1 h and kept in the shaker for intermixing of the particles to obtain green synthesis. The reduction of copper (II) sulfate to CuNPs was periodically monitored by ultraviolent–visible (UV) spectrometer. UV–visible spectral analysis was done for an interval of every 2 h. After 3 days of synthesis, the extract is collected and centrifuged for 10 min. Nanoparticles are found to be settled down in the centrifuge tube. Filler and scrapers are used to remove the nanoparticles from the tube and stored at optimum temperature. The color change is represented in Figure 1.

Anti-inflammatory Assay

A 2 ml of 1% bovine albumin fraction was mixed with 400 µl of *A. racemosus* mediated copper nanoparticles in different concentrations (150–25 µg/mL) and the pH of 6.8 is adjusted using 1 N HCl. Incubation at room temperature is done for 20 min and then the mixture is heated at 55°C for 20 min in a water bath. The absorbance value was recorded at 660 nm after the mixture is cooled at room temperature. An equal amount of *A. racemosus* copper nanoparticles was replaced with dimethyl sulfoxide which is used as a control. The standard that is used for the activity is diclofenac sodium in different concentrations. The experiment is performed in triplicate.

Cytotoxicity Assay (Brine Shrimp Lethality Assay)

Brine shrimp eggs are purchased. Artificial sea water is prepared in a bottle by dissolving 35 g of sodium chloride in 1 L of distilled water and the dried cysts are placed in them. Incubation is made at 37°C for 48 h under strong aeration and illuminations and the nauplii are hatched after the incubation period. The cytotoxicity activity of CuNPs in brine shrimp is evaluated. The experiment is performed in a 6-well plate containing artificial sea water and 10 nauplii. Each well is incubated with different concentrations of *A. racemosus* mediated copper nanoparticles ranging from 5 µl, 10 µl, 15 µl, 20 µl, and 25 µl, respectively. The number of surviving shrimps is counted and taken into account after 24 h. The lethality concentration (LC_{50}) of <100 ppm is considered as potent (active).

RESULTS AND DISCUSSION

The anti-inflammatory activity of *A. racemosus* mediated CuNPs is depicted in Figures 2 and 3. The percentage of inhibition of protein denaturation in bovine serum albumin increases simultaneously along with the increase in the concentration of CuNPs. The maximum percentage of inhibition is found to be 87% at 50 µl which is nearly on par with the standard that is 90%.

Similarly, the cytotoxic activity of *A. racemosus* mediated CuNPs is indicated in Figure 3. As the graph represents, the percentage of lethality increases as there is an increase in the concentration. The maximum percentage of lethality is found to be 40% at 25 µl. Nanoparticles synthesized using plan extracts are actively involved in the anti-inflammatory activity and proved using albumin denaturation assay.[20,21]

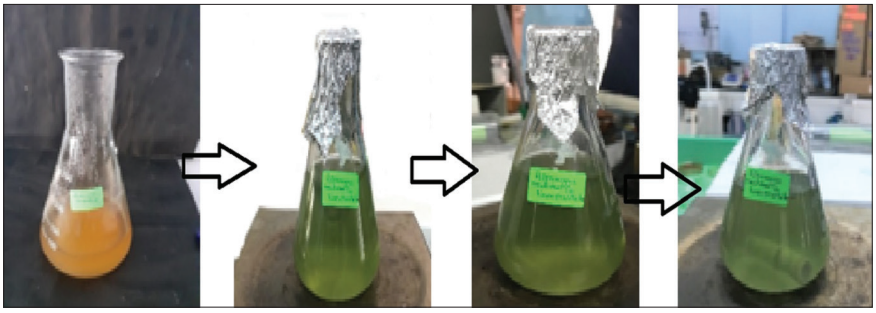

Figure 1: Color change of the extract at different intervals of time

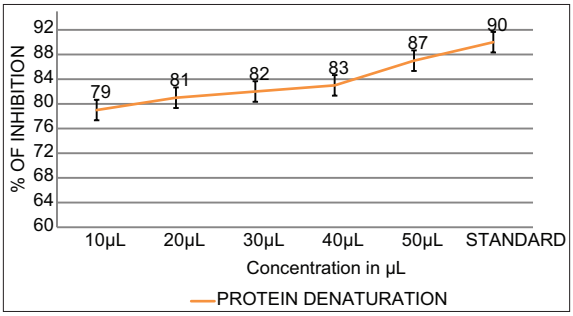

Figure 2: Anti-inflammatory activity of *Asparagus racemosus* mediated CuNPs

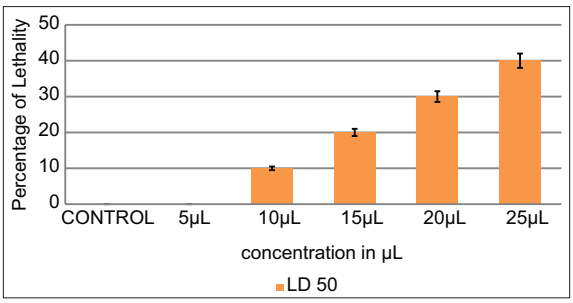

Figure 3: Cytotoxicity activity of *Asparagus racemosus* mediated CuNPs against brine shrimp

CONCLUSION

The use of the herbal medicine is increasing simultaneously with the increase in potential and capability to treat diseases. The significant action of herbal medicine mediated nanoparticles as no side effects and is safe to consume. Based on the results of the current study, it is concluded that *A. racemosus* mediated CuNPs can be used as a potent anti-inflammatory drug to treat inflammations and also as an anticancer drug for the treatment of tumors and cancers.

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