How to Cite:
Harikrishnan, S., Navaneethan, R., & Rajeshkumar, S. (2022). Antibacterial activity and cytotoxic effect of bisphosphonate conjugated gold nanoparticle synthesized using cissus quadrangularis extract. *International Journal of Health Sciences*, *6*(S6), 3997–4007. https://doi.org/10.53730/ijhs.v6nS6.11561

Antibacterial activity and cytotoxic effect of bisphosphonate conjugated gold nanoparticle synthesized using cissus quadrangularis extract

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***Abstract*---**Nanoparticles are being widely used in the field of medicine called Nanomedicine. Biological membranes in the human body are selectively permeable to Nanoparticles. The nanoparticles can be surface modified to attach a variety of ligands which enable them to be biosensors, molecular-scale fluorescent tags, imaging agents, and targeted drug delivery vehicles. Green synthesis of nanoparticles is a type of bottom-up approach in which reaction occurs with the help of reducing and stabilizing agents. Bisphosphonates are increasingly being used to treat a wide range of skeletal issues, including hereditary skeletal disorders in children, cancer-related bone metastases and osteoporosis. Cissus Quadrangularis is a green edible medicinal plant used in Ayurvedic preparations to treat osteoporosis, general inflammatory conditions, ulcers, menstrual disorders, hemorrhoids, and a variety of other ailments. It has been shown to have a variety of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and antiseptic properties. Bisphosphonate conjugated with gold nanoparticle have shown to produce more targeted actionThe purpose of this study was to green synthesize Bisphosphonate conjugated gold nanoparticles with Cissus quadrangularis extract and test their antimicrobial and cytotoxic activity. Green synthesis of Bisphosphonate conjµgated gold

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

nanoparticles using cissus quadrangularis extract was achieved and is characterised using visual colour change, uv spectrophotometer, TEM analysis. Gold nanoparticles have good antibacterial activity against Streptococcus mutans (150 ug/ml – 17 mm zone of Inhibition) & Lactobacillus (150 ug/ml – 20 mm zone of Inhibition) and showed 20% lethality at 50 ul concentration.

***Keywords*---**bisphosphonate, green synthesis, gold nanoparticles.

Introduction

Nanotechnology aims to design, create and control matter in the dimensional range of 1-100nm[1-16]. Nanoparticles possess the unique opportunity of operating at the level of atomic or biomolecules[17].The use of materials in these dimension provides opportunity to modify various properties such as solubility, diffusivity, blood circulation half-life, drug release characteristics, and immunogenicity[18,19]. Nanoparticles are being widely used in the field of medicine called Nanomedicine[20]. Biological membranes in the human body are selectively permeable to Nanoparticles[21,22]. The nanoparticles can be surface modified to attach a variety of ligands which enable them to be biosensors, molecular-scale fluorescent tags, imaging agents and targeted drug delivery vehicles. Nanoparticles synthesis by top down and bottom up method have been described in literature[23–26]. In general both the methods of nanoparticles use high radiation or concentrated reductants and stabilizing agents that are harmful both to the environment and to human health. Green synthesis of nanoparticles is a type of bottom-up approach in which reaction occurs with the help of reducing and stabilizing agents which eliminates the use of expensive chemicals, consumes less energy, and generates environmentally benign products and by products. Hence nanoparticle synthesized by green synthesis is considered far more superior to those manufactured through other methods [27–31].

Bisphosphonates are increasingly being used to treat a wide range of skeletal issues, including hereditary skeletal disorders in children, postmenopausal and glucocorticoid-induced osteoporosis (GIO), and cancer-related bone metastases, osteoporosis (juvenile, postmenopausal or involutional [senile], glucocorticoid-induced, transplant-induced, immobility-induced, and androgen-deprivation–related), Paget disease of bone, osteogenesis imperfecta (OI), hypercalcemia, and metastatic malignancy. Bisphosphonates can provide significant clinical benefit in diseases characterised by an imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption by destroying the osteoclasts by promoting apoptosis, As a result, bisphosphonates have emerged as an effective treatment for osteoclast-mediated bone resorption. Localised Bisphosphonates have been shown to enhance orthodontic anchorage in several studies(32-39)

Various nanoparticles have shown promise in the regeneration of bone tissue. Gold nanoparticles (GNPs) are a popular choice among these nanoparticles because they are effective at both promoting osteo-differentiation and inhibiting the formation of osteoclasts. GNPs have a number of advantages, including the ability to accelerate osteoblast differentiation, inhibit adipose-derived stem cell differentiation, suppress osteoclast formation, and promote bone formation in bone tissue regeneration. GNPs treatment resulted in significantly higher bone tissue regeneration in rabbit calvaria than the control group, according to Heo et al. When injected into the body, however, GNPs can cause toxicity. As a result, the surface of these particles must be modified to specifically target bone tissue [40–43].

Cissus quadrangularis L. is a member of the Vitaceae family, and its common name is Perandai (Tamil). It has been used in Indian folk medicine to treat a variety of pathological conditions such as osteoporosis, general inflammatory conditions, ulcers, menstrual disorders, haemorrhoids, and many others [21]. The plant extract's ability to heal bones has been demonstrated. The phytochemicals present in the plant have been attributed with medicinal properties, and some of the main constituents are ascorbic acid, flavanoids such as quercetin, kaempferol, and luteolin, stillbenes such as resveratrol, quadrangularin A, and pallidol, terpenoids, gallic acid derivatives, and glucosides such as bergenin. These are known to have good antioxidant and reducing properties, which is why the stem extract of this plant was chosen for AuNP preparation. Cissus quadrangularis extracts have previously been used to produce biogenic nanoparticles such as silver, copper oxide, ferric oxide, palladium, and other metals. These nanoparticles have been shown to have antibacterial and cytotoxic properties against a variety of bacteria and cancer cells [22–28]. Bisphosphonate conjugated with gold nanoparticles has been shown to provide a more targeted action [29]. The goal of this study was to green synthesize Bisphosphonate conjμgated gold nanoparticles with cissus quadrangularis extract and to study their antimicrobial and cytotoxic properties.

Materials and Method

Green synthesis gold nanoparticles and conjugation of bisphosphonate

Roots of *c.quadangularis* were dried in an oven at 30 °C and ground to a coarse powder. 1gm of available asparagus racemosus root powder was boiled at 100 degree c with 100 ml of distilled water in a beaker. The extract was then filtered using a filter paper to obtain 75ml. To reduce the Au2+ ions, 30 mL of the extract was added to a reaction vessel containing 70 mL of chloroauric acid solution. The nanoparticles were then centrifuged at 1500 rpm for 10 minutes before being redispersed in 20 mL of distilled water. 2mg/ml of Zoledronic acid with water solubility of 3.27mg added to one part of the sample gold nanoparticle extract and left to stir overnight.

Antimicrobial activity

Biosynthesized nanoparticles are used in a wide range of biomedical applications. Membrane damage is one of the most common causes of nanoparticle antibacterial properties. Using the agar well diffusion method, the green synthesised bisphosphonate conjugated gold nanoparticles were tested against common oral pathogens such as Candida albicans, Enterococcus fecalis, Staphylococcus aureus, and Lactobacillus. The test organisms (S. mutans and

Lactobacillus) were grown in nutrient broth and kept on agar slants for the study. Candida albicans were grown on Rose Bengal agar, which is a yeast-specific medium. Using a sterile cotton swab, the freshly cultured strains were grown and uniformly spread over petri dishes containing MHS agar (Mueller Hinton 2 agar + 5% sheep blood). With the help of a steel borer, agar wells measuring 6.0 mm in diameter were punched into the culture plate containing the test microorganisms 35. A micropipette was used to fill the agar wells with 20µL of different concentrations of nanoparticles (50,100,150g/ml). As a positive control, 20µL of standard antibiotics (Ampicillin) were used. The diameter of the inhibition zone was measured in millimetres after a 24-hour incubation period at 37°C (mm). All of the tests were performed three times.

Cytotoxicity

The eggs of brine shrimp are purchased to perform the cytotoxic assay on brine shrimp. The eggs are then kept at a temperature of 28°C. Artificial seawater and a 37°C light source are used to hatch eggs. This method was tested in 15 well plates The newly hatched Nauplii are selected and transferred to each well using a Pasteur pipette. The Gold nanoparticles with and without Bisphosphonate conjugation were introduced into each of the wells of varying concentrations of 5,10,15,25 µL is added to each well, and the volume is adjusted. After 24 hours, the brine shrimp are removed from the 15 well plates and counted with a magnifying glass. After a 24-hour incubation period, the percentage of dead shrimp in each well is calculated. The number of motile nauplii was calculated to assess the cytotoxicity of the nanoparticles. Viability was calculated per well by:

$$\text{Viability (in \%)} = \frac{\text{live brine shrimp after exposure}}{\text{live brine shrimp before exposure}} * 100\% \quad (46)$$

Result and Discussion

Structural characterization of nanoparticles
Visual colour change

The colour change of the reaction mixtures from light yellow to yellow, dark-purple, and dark brown, respectively, could indicate the biosynthesis of Au nanoparticles in the current experiment. In the current experiment the visual colour change from yellow to dark purple was formed in a period of 6 hours The reduction conformation of Au++ to Au0, as indicated by the solution's colour changing from light brown to dark brown. The brown colour variation indicates an incomplete reduction of less concentration in the plant extract solution, whereas the formation of dark brown colour at high plant extract concentrations revealed a complete reduction reaction.

UV spectroscopy analysis

In the presence of incident photons, Au nanoparticles displayed the surface plasmon resonance (SPR) band as a result of the metal's conduction and free band electrons collectively oscillating. The intensity of the SPR band is primarily determined by the nature of the nanoparticles used in the synthesis, as well as their composition Furthermore, UV-vis spectroscopy is a key tool for determining the nature of synthesised Au. The analysis was carried out every one hour to determine the changes. The analysis showed a consistent peak after 1 hr of preparation at 540 was constantly observed after 2 hours of the preparation of the sample .

TEM and crystallographic analysis

The TEM images and EDX spectra of biosynthesized Au nanoparticles showed that the particles are narrow in size and spherical in shape with a diameter in the range of 10–50 nm However, some froth was noticed on the surface of these obtained nanoparticles, which could be attributed to the different types of phytochemicals present in the plant extract. Therefore, both FTIR and SEM images confirmed the presence of a huge amount of phytochemicals in the plant extract which can prevent the nanoparticles from agglomeration and helps in the production of stable nanoparticles. There was no other defined morphological difference observed in the preparation of Au nanoparticles.

Antibacterial activity of biosynthesized bisphosphonate conjugated gold nanoparticle using asparagus Racemosus root extract on staphylococcus aureus, lactobacillus and candida Albicans

Antibacterial activity of biosynthesized bisphosphonate conjugated gold nanoparticle using asparagus racemosus root extract of staphylococcus aureus, lactobacillus and candida albicans using agar well diffusion method was performed. This method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. It is qualitative, easy to perform, and simple. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer or a tip, and a volume 20 μL of the nanoparticle sample at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganisms. The nanoparticle sample diffuses in the agar medium and inhibits the growth of the microbial strain tested.

In this study, four different concentrations of the nanoparticles were studied (25,50,100,150μg/ml). The diameter of the zone of inhibition increased with increase in concentration of the nanoparticles, against both S.aureus, Lactobacillus and Candida albicans. Whereas, the diameter of the zone of inhibition against Enterococcus faecalis showed no change with concentration of the nanoparticles. The zone of inhibition (in millimetre) of gold nanoparticles of varying concentrations, against S.aureus, Lactobacillus and Candida albicans is represented in Table 1. Au nanoparticles have good antibacterial activity against Streptococcus mutans (150ug/ml – 26 mm zone of Inhibition) , Lactobacillus (150ug/ml – 26 mm zone of Inhibition) and Candida albicans (150ug/ml – 10 mm zone of Inhibition).

Cytotoxicity assessment using brine shrimp lethality assay

Cytotoxicity of the prepared nanoparticles were assessed using Brine Shrimp (Artemia salina) Lethality Assay. It has been demonstrated that early developmental stages of Artemia salina are highly vulnerable to toxins. The lethality was found to be directly proportional to the concentration of the nanoparticles. Gold nanoparticles both with and without Bisphosphonate conjugation showed mortality of 10% at 20 and 25 ug/ml (Table 2).

Table 1
Antimicrobial activity of Bisphosphonate conjugated gold nanoparticles at various concentrations

Nanoparticles	Organisms & zone of inhibition for varying concentrations of nanoparticles (ZOI) in millimeter (mm)														
	staphylococcus aureus					Lactobacillus					Candida albicans				
Bisphosphonate conjugated gold nanoparticles	25µg/ml	50µg/ml	100µg/ml	150µg/ml	control	25µg/ml	50µg/ml	100µg/ml	150µg/ml	control	25µg/ml	50µg/ml	100µg/ml	150µg/ml	control
	9	9	9	26	24	9	9	9	26	26	9	9	9	10	12

Table 2
Calculation of cytotoxicity at various concentrations of nanoparticles

CONCENTRATION(µg/mL)	No. of live Nauplii (Day 1)	No. of live Nauplii (Day 2)	% DEAD
CONTROL	10	10	0
GOLD nanoparticles WITHOUT BISPHOSPHONATE			
5 µg/ml	10	10	0
10 µg/ml	10	10	0
15 µg/ml	10	10	0
20 µg/ml	10	9	10%
25 µg/ml	10	9	10%
GOLD NANOPARTICLES WITH BISPHOSPHONATE			
5 µg/ml	10	10	0
10 µg/ml	10	10	0
15 µg/ml	10	10	0

20 µg/ml	10	8	20%
25 µg/ml	10	9	10%

Conclusion

The present study revealed that green preparation of gold nanoparticles is achievable in a simple and eco-friendly manner using cissus quadrangularis extract. The gold nanoparticles were assessed for their Antibacterial activity against S.mutans, Lactobacillus, and C.albicans. Cytotoxicity of the nanoparticles was assessed using Brine Shrimp Lethality Assay. Gold nanoparticles have good antibacterial activity against Streptococcus mutans (150 ug/ml – 17 mm zone of Inhibition) & Lactobacillus (150 ug/ml – 20 mm zone of Inhibition) and showed 20% lethality at 50ul concentration.

Conflict of interest

There are no conflicts of interest.

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