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Evaluation of Antimicrobial Activity and Cytotoxicity of Biogenic Gold Nanoparticles

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ABSTRACT

Gold nanoparticles are some among the most researched nanomaterials in the field of nanotechnology. They can be easily synthesised and show excellent chemical as well as thermal stability. Biosynthesized nanoparticles offer excellent biocompatibility compared to nanoparticles synthesized by any other physicochemical methods. The main objective of this study was to analyze the antimicrobial activity of the gold nanoparticles synthesised with aspartic acid and to evaluate its cytotoxicity at the embryonic level. The antimicrobial activity of the AuNPs was assessed by the well diffusion method using different clinical pathogenic bacteria namely, E faecalis, S.mutans, Pseudomonas, and S. aureus. Zebrafish were selected for this study to analyze the cytotoxicity of the AuNPs. The zone of inhibition was seen to be the largest in E. faecalis(20mm) and smallest in S.aureus (9mm). When compared to the control antibiotics, Ampicillin (1mg/ml), the zone of inhibition was larger in the S mutans (18mm) at 100µg/mL followed by Pseudomonas (12mm zone) at100µg/mL. It was seen that mortality rate proportionally increased as the concentration of the solution of AuNPs increased. The AuNPs showed considerable antimicrobial property when tested against various pathogenic strains of bacteria. The AuNPs however showed to be dose dependent cytotoxic in nature. More studies need to be undertaken to establish optimum concentrations of AuNPs to have minimum negative effects on the host and best deliver its therapeutic purpose.

Key words: Antimicrobial activity, Cytotoxicity, Gold nanoparticles, Nanoparticles, Zebrafish embryo

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INTRODUCTION

Gold nanoparticles are some among the most researched nanomaterials in the field of nanotechnology [1]. They have attracted a lot of attention due to their unique properties and potential applications in antimicrobial and anticancer drugs, targeted drug delivery and many other applications in nanomedicine [2]. The gold nanoparticles can be easily synthesised and show excellent chemical as well as thermal stability. Conventional methods have been attempted over the past several years for their synthesis. These include methods such as electrochemical, photochemical, sono-chemical and microwave assisted processes [2,3] .

However, these physico-chemical methods were quite complex, cost-intensive, and were toxic in nature. They caused a lot of harm to the environment due to formation of toxic byproducts. Therefore, currently, the use of biological materials such as plants, bacteria, fungi, proteins, and related biomolecules for the synthesis of these nanoparticles has become a popular alternative [4]. Aspartic acid, similarly, is an alpha amino acid and is used by the body for the biosynthesis of proteins. Aspartic acid is one such biomaterial. It is an amino acid containing one amino group and one carboxylic group. The carboxylic group is the free end of the amino acid and can form bonds with other atoms or elements [5].

Biosynthesized nanoparticles offer excellent biocompatibility, which is often superior to the biocompatibility of the nanoparticles synthesized by any other physicochemical methods. This would be mediated by the biomolecules that act as natural stabilizers of the nanoparticles, preventing not only the aggregation over time

but also giving them a particular additional stabilization The biomolecules form the part of the nanoparticle covering that would actively mediate their interaction with other biological molecules. This particular property could be responsible for the antimicrobial activity of the nanoparticles [6] We have numerous highly cited publications on well-designed clinical trials and lab studies [7-22] employing similar biomaterials and applications.

Also, in a previous preliminary study, we have reported a simple and ecofriendly method for the biological synthesis of gold nanoparticles using aspartic acid as a reducing agent. The newly formed gold nanoparticles were stable, uniform, spherical and showed no agglomerations or abnormalities in morphology or configuration. The main objective of this study was to analyze the antimicrobial activity of the gold nanoparticles synthesised with aspartic acid and also to evaluate its cytotoxicity at the embryonic level.

MATERIALS AND METHODS

AuNP biosynthesis

AuCL3 solution (0.266 M) was slowly added to 250ml of aspartic acid with stirring at 45°C. The mixture of the solutions was kept in a long-necked borosilicate flask and continuously stirred on a magnetic stirrer. The formation of gold nanoparticles was confirmed by the change of the colourless solution to a reddish hue. The solution was stirred for approx 9 hours. The synthesized gold nanoparticles were then purified by centrifugation (10,000 rpm: 30 min) at 4°C. The nanoparticles collected were thoroughly washed with deionized water and re-dispersed in Millipore water. Nano particles were formed in approx. 9 hours with peak absorbance at 24 hours at 525nm. The synthesized nanoparticles were spherical in shape, with an average size of 20 nm. The synthesised nanoparticles showed excellent plasmon resonance and optical properties.

Antimicrobial activity

The antimicrobial activity of the Gold nanoparticles was assessed by the well diffusion method using different clinical pathogenic bacteria namely, *E faecalis, S.mutans, Pseudomonas,* and *S. aureus*. The pure cultures of the bacteria were grown in nutrient agar media. Briefly, three different concentrations of (25, 50, and 100 µg/ml) AuNPs were loaded onto the wells of the petri dishes, inoculated with the bacterial isolates individually. The antibiotic ampicillin (1mg/ml) was used as a positive control. The plates were incubated at 37°C for 12–24 h and the zones of inhibition around the wells were measured manually.

Cytotoxicity evaluation

The zebrafish (Danio rerio) has been used as a popular model organism for toxicity testing of chemicals and their cytotoxicity. Zebrafish exhibits an exceptional set of characteristics such as small size and therefore, can be handled without difficulty. Its rapid development, embryonic transparency and acquiescent to genetic as well as chemical screening are other advantages. Hence, the zebrafish were selected for this study to analyze the toxicity of the AuNPs [23]. The AuNPs concentrations 20, 40, 60,80 and 100 µg/ml were selected at convenience.

Initially 10 zebrafish embryos were introduced into individual test tubes containing 25 ml of the AuNP solution of various predetermined concentrations. The solution was then allowed to stand undisturbed. At the end of 24 hours and 48 hours the solutions were taken in separate glass petri dishes and were microscopically examined. The number of viable zebrafish embryos were counted manually and recorded. Percentage mortality was calculated.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activity of the Gold nanoparticles was assessed by the well diffusion method using different clinical pathogenic bacteria namely, *E faecalis, S. mutans, Pseudomonas,* and *S. aureus* at three different concentrations of 25, 50, and 100 µg/ml. Among the three different concentrations that were tested, the zone of inhibition was the highest when the concentration was at 100µg/ml. Furthermore, the zone of inhibition was seen to be the largest in *E. faecalis* (20mm) and smallest in *S. aureus* (9mm).

However, when compared against the control antibiotic, Ampicillin (1mg/ml), the zone of inhibition was larger in the *S. mutans* group with 18mm at 100µg/ml, followed by Pseudomonas group with 12mm at 100µg/ml (Table 1).

Table 1: Table one shows the various zones of inhibition formed when AuNp solution of various concentrations was tested against pathological bacterial strains. The zone of inhibition was the highest when the concentration was at 100µg/mL in all groups of bacteria. When compared against the control antibiotic, Ampicillin (1mg/ml), the zone of inhibition was larger in the *S. mutans* group with 18mm at 100µg/mL, followed by Pseudomonas group with 12mm at 100µg/ml.

Name of Pathogen	Zone of Inhibition (mm)			
	20 µg/ml	50 µg/ml	100 µg/ml	Control (Ampicillin)
E. faecalis	15	18	20	30
S. mutans	12	16	18	11
Pseudomonas	9	11	12	11
S. aureus	9	10	9	31

Cytotoxicity evaluation

Zebrafish were selected for this study to analyze the cytotoxicity of the AuNPs. The AuNPs in concentrations 20, 40, 60, 80 and 100 µg/m were selected in brief. Zebrafish embryos were treated with different concentrations of AuNPs and percentage mortality was observed at the end of 24 and 48 hours, respectively.

Initially 10 zebrafish embryos were introduced into individual test tubes containing 25 ml of the AuNP solution of various predetermined concentrations. The solution was then allowed to stand undisturbed. At the end of 24 hours and 48 hours the solution was taken in a glass petri dish and was microscopically examined. The number of viable zebrafish embryos were counted manually and recorded (Figure 1).

It was observed that at the end of 24 hours all the zebrafish embryos were still viable. However, at the end of 48 hours all groups showed varying numbers of zebrafish embryos that died due to the cytotoxic effects of the AuNPs. The 20µl concentration sample showed a mortality rate of 30%, while the 100µl showed a mortality rate of 90%. In the remaining samples, it was observed that mortality rate was gradual and proportional to the concentration of the solution of AuNPs. The following results demonstrated that the AuNPs in study exhibited a dose dependent cytotoxicity at the embryonic level (Table 2).

There are numerous applications of AuNPs, such as targeted drugs delivery, gene therapy, antitumor cancer therapy, antimicrobial therapy, bio-imaging etc. [24]. Some of the most popular research on AuNPs focuses on their antimicrobial and antimicrobial properties. Gold being one of the oldest metals used by man, has been documented to be used in the medical field for several hundred years. It is particularly known for its superior biocompatibility and antimicrobial properties. Additionally, when gold is made into nanoparticles, they exhibit

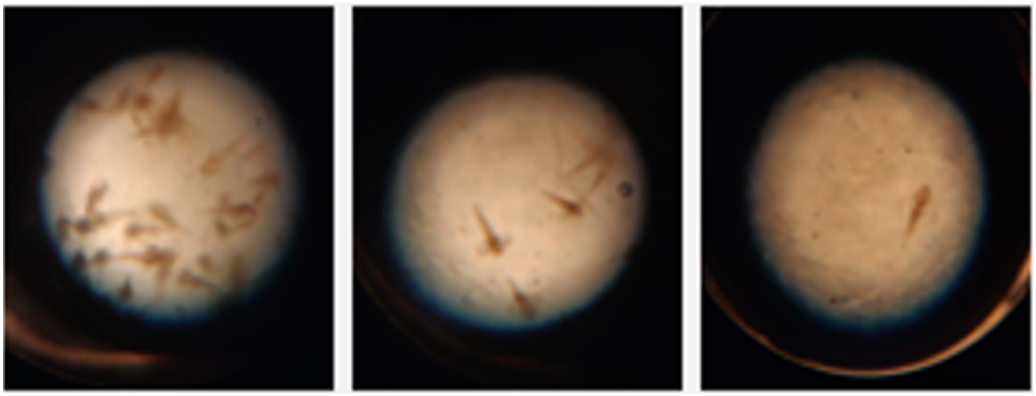

Figure 1: Microscopic view of Zebrafish embryos at different time durations during the study. Images from the left towards right show the decrease in viable zebrafish embryos with time.

Table 2: Table showing the mortality rate of Zebrafish embryos in the AuNP solution at varying concentrations.90% mortality was seen at concentration of 100µl, whereas 30% mortality was seen at concentration of 20µl. The cytotoxicity of the AuNPs was dose dependant and was highest at 100µl.

Concentration (µl)	Number of Zebrafish alive at 24 hours	Number of Zebrafish alive at 48 hours	Mortality rate (%)
20	10	7	30
40	10	5	50
60	10	3	70
80	10	2	80
100	10	1	90

increased surface plasmon resonance. Also, AuNPs are known to have an extremely high surface area ratio and can easily bind to other organic and biological molecules enabling targeted drug delivery and such other purposes [25].

In recent times, several antibiotic resistant bacterial strains are said to have developed and hence the need for finding an alternative to such antibiotics is a prime requisite. Nano particles such as AuNPs have a lot of perspective scope in this regard [26,27]. In the present study we evaluated the antimicrobial properties of AuNPs against four common pathological strains of bacteria in varying concentrations by disk diffusion method to ascertain its antimicrobial property. It was seen that the antimicrobial properties increased with an increase in concentration.

TheAuNP solution when compared against the control antibiotic, Ampicillin (1mg/ml), showed a large zone of inhibition in the *S. mutans* group with 18mm at concentration of 100µg/mL, followed by Pseudomonas group with 12mm at concentration of 100µg/ml. These results are encouraging and proved that there was further scope for further studies on antimicrobial activity of AuNPs synthesized with aspartic acid and could be of clinical importance soon.

Any pharmaceutical drug or chemical that is being administered to an organism should not have a deleterious effect on its functioning or vitality [28]. Hence, it is important to scientifically establish evidence that a particular substance has no or minimal cytotoxic effects. These tests can be done at various levels from cell lines to embryonic levels to animal tests at an advanced stage (In vitro to In vivo). In the present study an embryonic level cytotoxicity test was done with Zebra fish. The zebrafish has been used as a popular model organism for toxicity testing of chemicals and their cytotoxicity. The zebrafish is known to have an exceptional set of characteristics. It is small and therefore, can be handled without difficulty. Its rapid development, embryonic transparency and acquiescent to genetic as well as chemical screening are other advantages. The 20µlconcentration sample showed a mortality rate of 30%, while the 100µl showed a mortality rate of 90%. In the other samples, it was seen that mortality rate gradually and proportionally increased as the concentration of the solution of AuNPs increased. The following data demonstrated that the AuNPs synthesized from aspartic acid exhibited a dose dependent cytotoxicity at the embryonic level.

More research is needed to establish a safe and non-cytotoxic dosage of aspartic acid bio synthesised AuNPs for its future prospective applications. Research can also be undertaken with alternative metal nanoparticles or reducing agents to obtain a non-cytotoxic nanoparticle, to serve the same therapeutic purpose.

CONCLUSION

The AuNPs showed considerable antimicrobial property when tested against various pathogenic strains of bacteria. The AuNPs however showed to be cytotoxic in nature and their cytotoxicity was dose dependent. More studies need to be undertaken to establish optimum concentrations of AuNPs to have minimum negative effects on the host and best deliver its therapeutic purpose.

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