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RESEARCH ARTICLE

Uv-Vis Spectroscopy of Silver Nanoparticles and Toxicology Evaluation of Silver Nanoparticle Based Oral Rinse on Embryonic Development of Zebrafish

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ABSTRACT

Aim: In this study we aim to formulate nanoparticles by green synthesis using miswak, and evaluate the formed particles using UV spectroscopy. The formed silver nanoparticles are then incorporated into a mouthwash. To evaluate the cytotoxic evaluation of the mouthwash on embryonic development of zebra fish.
Materials and methods: Miswak mediated silver nanoparticles were synthesised and incorporated along with sodium lauryl sulphate, sodium benzoate, xylitol and peppermint oil along with water to formulate a mouthwash. The nanoparticle formulated was evaluated using UV-vis spectroscopy. For toxicology evaluation silver nanoparticle, the oral rinse was added in various concentration to medium containing developing embryonic zebrafish egg.
Results and Discussion: The broad peak recorded in the UV vis spectrophotometer of 350-400nm indicated that the silver nanoparticles were synthesised. The hatching rate of the zebrafish was only affected only in high concentrations and the viability of the zebrafish embryos was above 50 % in all concentrations. The embryo development that when visualised under microscope revealed that the mouthwash containing media didn't cause any structural and developing abnormality during its development.
Conclusions: The results of our study reveal that the silver nanoparticle-based mouth rinse is not toxic to the developing zebrafish in lower concentrations. From the results of our study, we can reveal that smaller green synthesised nanoparticles at the right concentration is not toxic and could be used for purpose of oral hygiene products such as mouthwash.

KEYWORDS:
Embryonic development, silver nanoparticle, mouth rinse , zebrafish , UV visible spectroscopy

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INTRODUCTION

Silver nanoparticles can be synthesised using various methods and the most common being its reduction from silver nitrate that is available in low cost and is highly stable. (1)There is often an inability to control the size of the silver nanoparticle formed by such chemical reduction reaction, green synthesis avoids the toxic reducing agents required. (2)Plant extracts are very significant in the green synthesis due to their antimicrobial property.(3) Silver nanoparticles are widely being accepted and are being using in medical fields due to their antibacterial activity and have strong broad spectrum action against gram+ and gram- organisms.(4,5) The

antibacterial property can't be attributed to its action that interacts with the DNA, peptidoglycan cell wall or the protein , a combined effects provides for a broad spectrum antibacterial activity.(6,7)

Silver nanoparticle has already been in use in dentistry in materials like composites and fillings due to its antibacterial effect.(8) They have previously been incorporated in mouth wash as an alternative to ethanol and for that it had equal antimicrobial effect even at low concentration but the silver nanoparticle used was obtained by a chemical process.(9) Silver and zinc nanoparticles in mouthwash has also been compared along with chlorhexidine and has shown excellent antibacterial effect thus indicating it can be an alternative.(10) Silver nanoparticle of size 20-25nm has shown to have antimicrobial activity without causing cytotoxicity and nanoparticle of size 10-20 nm has shown to be non-toxic in animal studies.(11,12) Cytotoxicity of silver nanoparticles to the mitochondria has shown to increase when there is an increase in concentration.(13)Nanoparticle toxicology have previously been carried out on shrimp nauplii, Drosophila eggs on exposure to the particles and these studies indicated that the silver nanoparticle of size 20-30 nm didn't cause toxicity .(14,15)

The use of developing zebra fish larvae as an animal model for evaluation of toxicity has been popularised in recent times.(16) Zebrafish is a model fresh water vertebrae and can be used to evaluate developmental disturbances on exposure to any chemicals that they are exposed to.(17) It has high similarity to human genome, cost effective and its high rate of reproducibility makes it highly efficient to use in toxicology studies.(18) The aim of this study is to do an UV-vis spectroscopy of silver nanoparticles and toxicology evaluation of silver nanoparticle based oral rinse on embryonic development of zebrafish.

MATERIALS AND METHODS

Synthesis of silver nanoparticle-based mouth rinse

Shredded pieces of the bark of miswak tree were obtained and boiled in order to obtain the plant extract. The extract was added with 60ml of silver nitrate solution which was prepared using 0.016mM of silver nitrate. The solution was subjected to continuous dissolution in the magnetic stirrer. The solution was taken and later centrifuged in 6 test tubes to obtain pellets. The size of the nanoparticle thus formed were of size upto 25nm. For the formulation of mouthwash 6 formed solution was added with xylitol along with 0.001 sodium benzoate , 0.1gm sodium lauryl sulphate and 10ml of water. The flavouring agent used was peppermint oil and sodium benzoate 0.001gm was added in order to act as acid buffer. Figure 1 is pictorical representation the

Characterization using UV-vis spectroscopy

The formed solution after being obtained from dissolution by magnetic stirring. In the collected solution the reduction of silver was then recorded using to UV – vis spectroscopy at the intervals of 1, 3,6, 12 ,24, 48 and 72 hours. The wavelength assessed were between 300-700nm. Color change was also observed and recorded.

Embryonic toxicology analysis of mouth wash

Zebrafish (Danio rerio) were obtained from the vendors in Chennai and maintained in individual tanks in the a temperature of 28 ± 2°, light for 14 h and dark cycle for 10 hand the pH maintained between 6.8- 8.5 . The fish were fed two times a day using shrimp and dry flake. Males and females were manually separated using a transparent block an entire night and was then removed the following morning light cycle and allowed for reproduction. The fish embryos were collected by crossing one female fish for two male ratios. Viable eggs were collected and were rinsed using E3 medium (5 mmol/ L Sodium chloride, 0.18 mmol/L Potassium chloride, 0.33 mmol/L Calcium chloride, 0.33 mmol/L Magnesium sulfate). The fertilized eggs were transferred to culture plate. Experimental and control groups were isolated. The concentration of mouth rinse were 1, 2, 4, 8 and 16 µL that were used to incubate the fertilized embryos for 24hpf to 96hpf.

Different concentrations of mouth rinse were exposed to the experimental group containing 5 fishes for a period of 96 h. The hatching rate and viability were recorded every 24 hours In addition 5 fishes were maintained as control group. If any of the fishes were dead, they were recorded and removed.

At periodic intervals during the exposure to the mouth rinse, the embryonic development of the zebra fish embryo was observed under a stereo microscope. The end point of the experiment was to assess the developmental toxicity including mortality, embryo hatching rate and larva viability. The photographs of the developing embryos were taken using a stereomicroscope.

RESULTS

UV – Vis analysis of silver nanoparticle based mouthwash

The nanoparticles formed turned white colour after follow up observation upto 12 hrs. The silver nanoparticles were determined by UV-vis spectrophotometer at a range of 250-600nm. The broad peak was formed between 300-450nm confirmed that the formed particles were silver nanoparticles that were formed using miswak by green synthesis. Figure 2 gives us the graphical representation of the recordings using the UV spectrophotometer.

Embryonic toxicology analysis of mouth wash

Figure 3 shows the hatching rate of fertilised eggs in mouthwash containing medium. 1 µL showed that there was hatching rate of 80-90% whereas in higher concentrations of 16 µL the hatching rate was reduced to 10-15% demonstrating an embryonic hatchability. In concentrations of 2 and 4 µL the hatching rate were mildly reduced but still above 60%. Even at a hatching rate of 8 µL the hatching rate was reduced to 30%.

Figure 4 is a graphical representation of the viability of zebra fish embryos. At all the concentrations the viability was demonstrated to be higher than 50%. At 1 µL concentration the viability of the embryos were 80 percent. The highest concentration tested at 16 µL reduced the viability at less than 60.

Figure 5 shows images taken via stereomicroscope at regular intervals demonstrating embryo development in the control and silver nanoparticle-based mouth rinse. There was no malformation observed in the various stages of development. This indicated that the mouth rinse used in the test sample didn't cause any harmful effect on the development of zebrafish embryo.

DISCUSSION

The results of our study confirm that silver nanoparticles were synthesised by green synthesis using miswak and were confirmed by the colour change and reduction recorded using UV vis spectrophotometer. The mouth rinse synthesised in this method didn't cause any severe adverse effects in embryonic development of zebrafish.

Silver nanoparticle has shown bactericidal effect against gram negative micro-organisms. Silver is an unreactive component and has low ionization potential and is, thus also stable in aqueous solution, physiological fluids and solids. Silver nanoparticles have previously been coated onto hexagon dental implants and has shown positive antimicrobial activity against S.aureus and Candida. (15,19) Lynch et al has observed in an overview of studies about xylitol and dental caries suggests potential clinical dental applications for xylitol. Short-term consumption of xylitol is associated with decreased Streptococcus mutans levels in saliva and plaque.(20) Miswak has shown strong antifungal and antibacterial properties when extracts were previously examined.(21,22) Thus a possible synergistic action was assessed when in this study we have used a green synthesised silver nanoparticles that was formulated along with xylitol as sweetener for the formulation of this mouth rinse.

UV- visible spectroscopy is a well-known methodology for characterization to determine the particle formation and properties. The electrons are usually limited to having specific vibrations based on the size and the shape of the particle. Thus metal based nanoparticles will have specific vibration modes having a characteristic optical observation spectrum when recorded under UV–vis spectrophotometer. (23)The peak recorded in the UV vis spectrophotometer was between the range of 300-450nm. This confirmed that silver nanoparticles were synthesised by green synthesis using miswak. The results of this study was in accordance to over studies that demonstrate similar peak where silver nanoparticles were synthesised.(24-26)

The factors like size, shape and presence of chemical residues from chemically synthesised silver nanoparticles have a causative role in the toxicology of nanoparticles. (27)Green synthesis is a type of methodology where the biological agents like bacteria, fungi and plant extracts are used for the formulation of nanoparticles; green synthesis has become a revolutionary in the world of nanoparticles. There is a vast reserve of plant extracts that can be used for used for safe formulation, which has a wide variety of metabolites and minimises the cost of production and reduces chemical weightage. This method is not only a method that reduces the cost but is also an efficient method to reduce the toxicity of nanoparticles (28)

Zebrafish (Danio rerio) has been a prominent model vertebrate in a variety of biological disciplines. Substantial information gathered from developmental and genetic research, together with near -completion of zebrafish genome project, has placed zebrafish in an attractive position for use as a toxicological model. (29)Although still in its infancy , there is clear potential for zebrafish to provide valuable new insights into chemical toxicity, drug discovery and human disease using recent advances in forward and reverse genetic techniques coupled with large-scale, high throughput screening.

Toxicity of nanoparticles is the major reason of doubt in nanotechnology that often makes us wonder if it is a boon or bane.(30) Nanoparticles of larger size are more toxic than the samller ones ranging from a size of 20-30nm. This was proven when tested onto Drosophilia eggs where particle silver was more toxic that the nanoparticle of silver tested.(31) Green or biologically synthesised silver nanoparticles have shown to be less toxic than the chemically synthesis that occurs due to the biomolecules on the surfaces of the nanoparticles.(27) Previous studies using earthworms revealed that silver ions caused decreased growth but no significant mortality or bioaccumulation of silver. (32) One such study on adult zebrafish where they had assessed the toxicity of silver nitrate versus nanoparticle of silver also revealed the dissolved silver nitrate was more toxic.(33)

The limitation of the study is that cellular , nuclear changes, liver marker enzymes or reactive oxygen species were not performed. More exhaustive long term studies are needed for the nanoparticle based mouth rinse that can be brought in to wide spread pharmacological application.

CONCLUSION

The indigenous mouthwash prepared from miswak plant mediated silver nanoparticles could be used as an alternative to commercially available mouthwashes for reducing plaque load as an adjunct to tooth brushing. The silver nano particles can be synthesized using green synthesis from miswak stem. Green synthesis is very affordable, environment friendly, nontoxic as it uses phytochemicals as reducing agents to reduce the silver ions. In vitro evaluation undertaken to check the biocompatibility by using zebra fish egg and observing for its hatching and development for 5 days, the excellent biocompatibility was shown by the developing eggs at concentrations of mouthwash up to 10 microliters.

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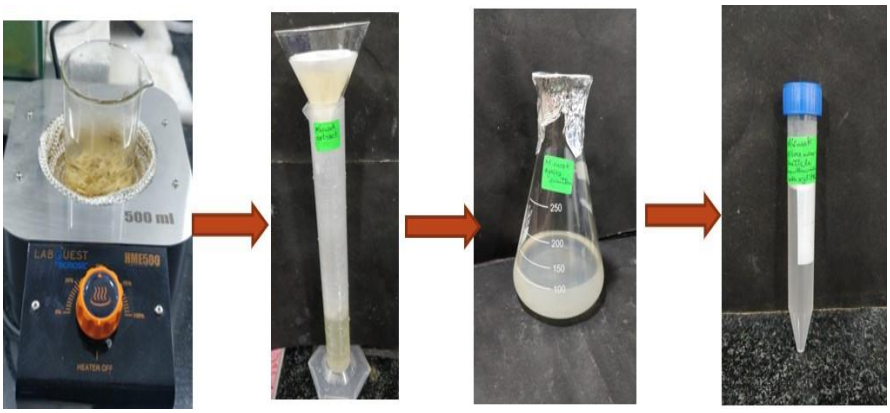

Fig.1: Miswak synthesized silver nanoparticle mouth rinse

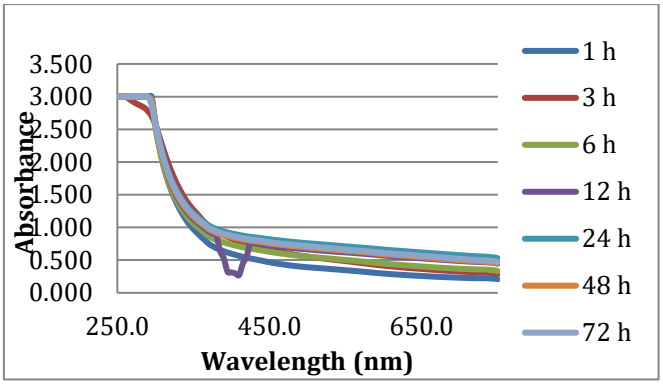

Fig.2: Result of UV - vis spectroscopy

EMBRYONIC TOXICOLOGY ANALYSIS OF MOUTH WASH

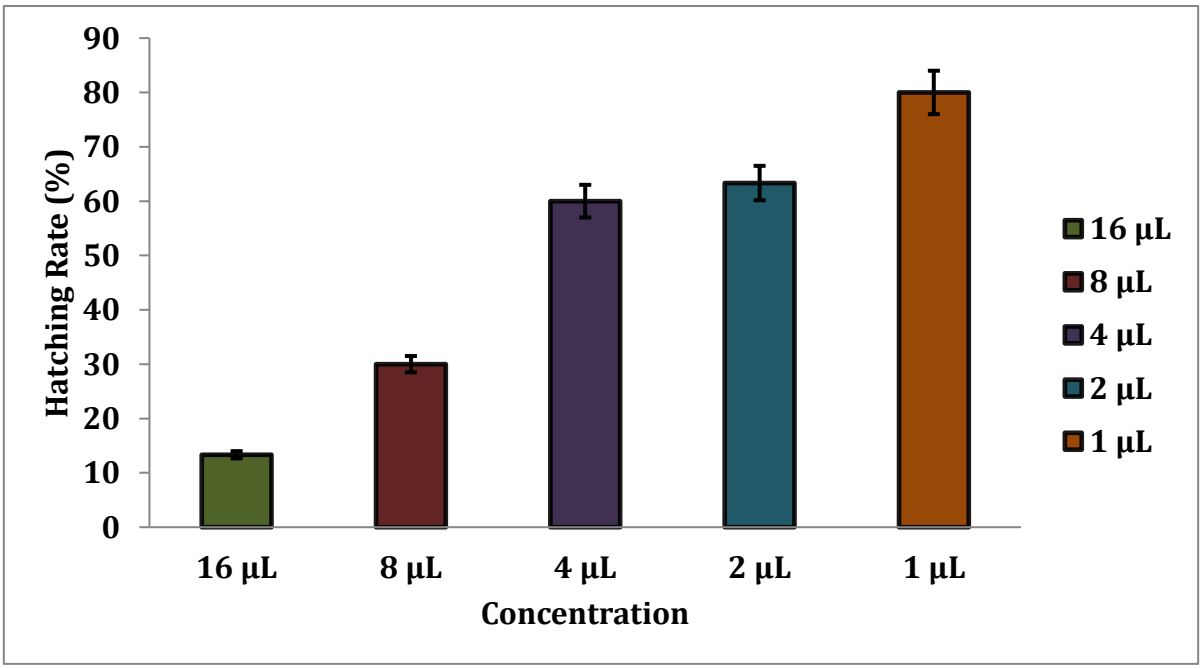

Fig.3: Hatching rate of zebra fish embryos in mouth wash containing medium

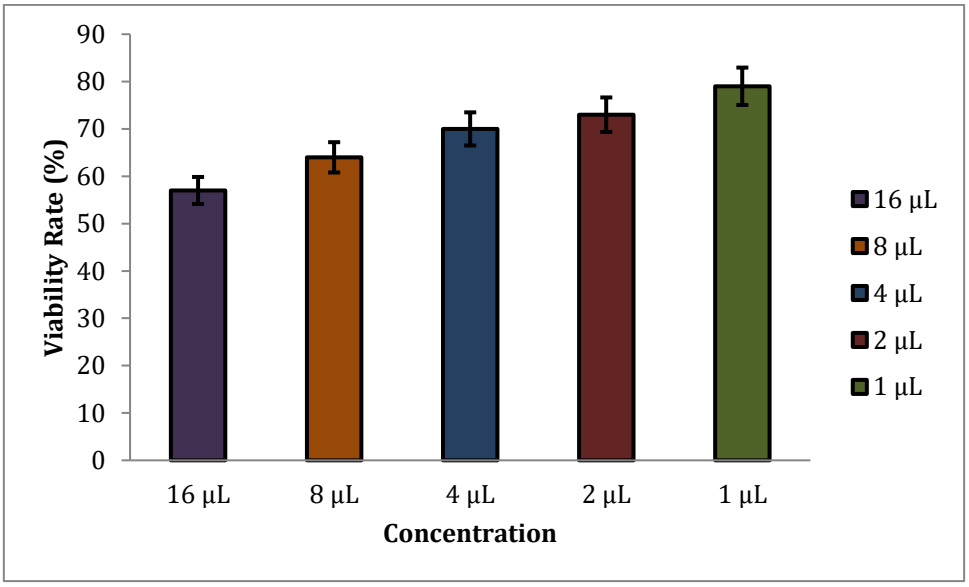

Fig.4: Viability of zebra fish embryos

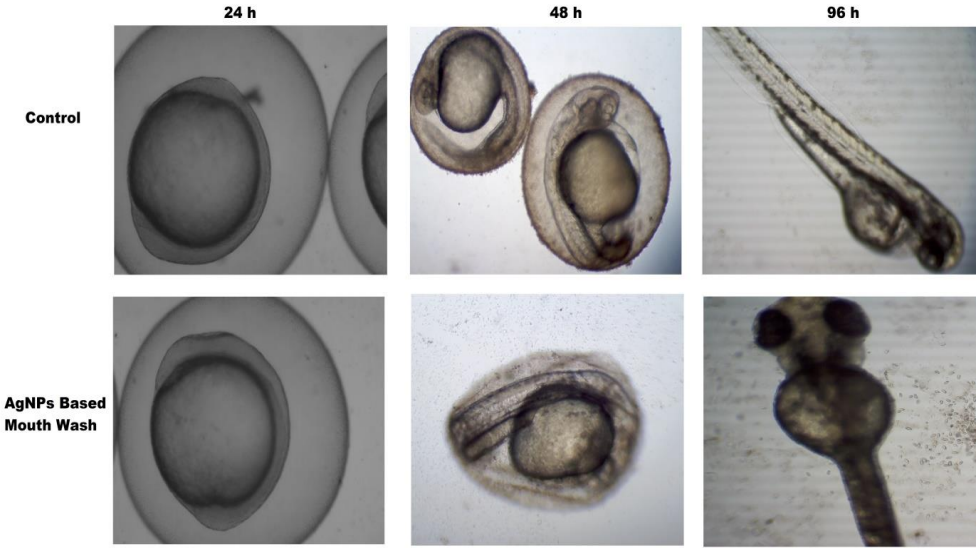

Fig.5: Embryo development in different time interval with mouth wash and control