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Antibacterial activity of Silver Nanoparticles Using Stem bark of Juglans regia against, Streptococcus mutans , Streptococcus. sanguis and ,Porphyromonas gingivalis

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Abstract

Dental careis & periodontal disease ,the most widespread diseases affecting mankind ,involve the adherence of bacteria & development of biofilms on both the natural & restored tooth surface .within this context, a biofilm can be Classed as an aggregate of micro-organisms in which cell adhere to each other & to a surface. Nanotherapeutics offers the possibilitiy to control the formation of these & other oral biofilms through the use of nanoparticles with biocidal ,anti-adhesive & delivery capabilities.

In this study, we investigated the antimicrobial activity of silver nanoparticles (Ag-NPs) using medicinal plant extracts (Stem bark of Juglans regia) against, Streptococcus mutans**,** Streptococcus. Sanguis, and Porphyromonas gingivalis.

Extracellular biosynthesis of silver nanoparticles was carried out by using medicinal plant extracts for the reduction of aqueous silver ions in short period. The silver nanoparticles formation was confirmed by the color change of plant extracts (SNPs) and further confirmed with the help of UV-Vis spectroscopy. Antimicrobial effect of Ag-NPs against, Strep.mutans, Strep. sanguis , and Porph. gingivalis was investigated by using disc diffusion method.

The growth of Gram-positive Strep.mutans, Strept. sanguis (the causative agents of careis) , and Porph. Gingivalis (the causative agents of Periodontitis) were inhibited by Ag-NPs. The highest antimicrobial activity was observed against Strep. mutans (15.6mm SD± 1.6), Strept. sanguis (13.3mm SD±1.4**)** and the least was noticed against Porph. gingivalis (12mm SD± 1.0).

It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against oral bacterial diseases.

Key words: Juglans regia, Silver Nanoparticles, Antimicrobial activity.

Introduction

Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly Strep. mutans and Strep. sanguis), salivary polymers and bacterial extracellular products. Plaque is a biofilm on the surfaces of the teeth. This accumulation of microorganisms subject the teeth and gingival tissues to high concentrations of bacterial metabolites which results in dental disease [(1)] .Various methods have been used in the attempt to

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diminish colonization of these microorganism, from topical application of substances on the dental structure and DNA plasmids resistant to these bacteria to the application of vaccines and antibiotics against this microorganism [2].

Periodontitis is a chronic infectious disease initiated by a group of periodontopathic bacteria, such as Porph. gingivalis. Periodontitis destroys the tooth-supporting tissues and leads to tooth loss if not adequately treated. Of the several hypotheses proposed to account for the effect of chronic infections on the development of atherosclerosis [3].

Porphyromonas gingivalis is a Gram-negative oral anaerobe strongly associated with chronic adult periodontitis. The bacterium produces a number of well-characterized virulence factors and can be manipulated genetically. The availability of the genome sequence is aiding our understanding of the biology of Porph. gingivalis and how it interacts with the environment, other bacteria and the human host[4].

Nanoscience and nanotechnology are a fast growing and dynamic areas, which include novel class of materials that are being developed for various applications. Nanotechnology has immense potential in almost every field of science and technology, primarily due to their size and/or shape dependent intrinsic physicochemical, optoelectronic, catalytic and biological properties and greater surface area [5].

The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silvercontaining materials can be employed to eliminate microorganisms on textile fabrics [5, 6].

Silver and silver ion based materials are widely known for their bactericidal and fungicidal activity. Their antimicrobial effect is due to blockage of respiratory enzyme pathways, alteration of microbial DNA and the cell wall [7]. Therefore, silver and silver ion containing materials are used as prostheses, catheters, vascular grafts and as wound dressings [7, 8].Good dispersion of nanomaterials is a prerequisite for sufficient contact and interaction between nanomaterials and microbial species. Silver nanoparticles have been known to exhibit bactericidal property because of its strong cytotoxicity towards a broad range of micro-organisms, the most powerful antiseptic materials available naturally and posses low toxicity towards mammalian tissue [9]. However they are hardly usable by themselves since they are poor at dispersion stability and tend to aggregate in the medium. At the same time, silver reagent is hardly cost-effective. For the economic and efficient reason, a common challenge is to obtain highly dispersed Ag composite nanoparticles. SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds6. Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it [10, 11] and testing for antimicrobial activities [12, 13, 14].

There is an increasing interest in silver-containing materials due to their antimicrobial properties in applications such as pharmacology, medicine, the food industry and the healthcare environment [15]. Advances have been made in the last decade in medical grade silver technology, from the synthesis of safer and bioavailable silver compounds to new delivery techniques and new environmentally friendly green silver containing

disinfectants. Different studies have established the bactericidal effect of nanosilver against Gram negative and Gram positive bacteria, but the bactericidal mechanism of this compound has not been clearly elucidated [16] defined the antibacterial activity of silver nanoparticles against four types of Gram negative bacteria, E. coli, V. cholera, P. aeruginosa and S. typhus, and suggested that silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions. Furthermore, the antiviral capability of silver nanoparticles against human immunodeficiency virus type 1 and hepatitis B virus [17] has been established.

The present study is an attempt to test the antibacterial and antifungal efficacy of SNPs produced by using the stem barks extract of medicinal plants, which have been using in traditional medicine without any validation.

Materials and methods

Plant material and synthesis of silver nanoparticle:

The dried barks of Juglans regia were ground to a fine powder. 1 mM silver nitrate was added to the plant extract to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min. The supernatants were heated at 50 to 95ºC. A change in the colour of the solution was observed during heating of process within 10-15 minutes. The colour changes indicate the formation of silver nanoparticles (SNPs). The reduction of pure Ag2+ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5 hours after diluting a small aliquot of the sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

Determination of antimicrobial activity by Disk-diffusion method [18, 19].

Mueller Hinton agar plates (Difco) was prepared for testing antibacterial activities. The colony forming units of suspension of the tested isolates were determined and tested inoculums were adjusted to 1×10^5 cells/ml, matching with 0.5 McFarland. Inoculums (100µl) were applied on the surface of the agar plates and spread by using sterile glass spreader. For evaluation of antibacterial activities, Whatman no.1 filter paper disks were sterilized and saturated with volume of Ag nanoparticles 50 µl. On the other hand, others were saturated with 50 µl silver nitrate (5µg/ml). Disks were placed on the inoculated agar plates and left incubated at 37°C for 24 hrs for bacterial isolates. After incubation, plates were observed for antimicrobial activities by determining the zones of inhibition for control, SNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Statistical analysis:

The results were calculated as mean diameter of zone of inhibition in mm ± standard deviation (mean ± SD).

Results

As mentioned above, Ag-Nps have inhibitory effect on several micoorganismes, but the effect of nano-Ag against Porph. gingivalis mostly unknown. To the best of our knowledge, the present study is the first study about the effect of Ag-Nps against this periodontopathic bacterium.

The green synthesis of silver nanoparticles through plant extracts were carried outThe appearances of yellowish-brown colour in the reaction

vessels suggest the formation of silver nanoparticles (SNPs) (Fig-1). UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The absorption spectrum of the pale yellow-brown silver colloids showed a surface Plasmon absorption band with a maximum of 418 nm indicating the presence of spherical or roughly spherical Ag nanoparticles.

Table (1) show the diameter of inhibition zones around each disc with AgNPs against Strep. mutans (15.6mm SD± 1.6), Strept. sanguis (13.3mm SD±1.4**)** and Porph. gingivalis (12mm SD± 1.0).

The antibacterial activity of AgNPs against Strep. mutans , Strept. sanguis and Porph. gingivalis is represented in Figure 2.

Discussion

It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [(20)].

Silver has been used for its well known antimicrobial properties since roman time however the advances in generating AgNPs have made possible a revival of the use of silver as a powerful bactericide (18). Many researchers [(21)] used Escherichia coli as a model for gram negative bacteria and proved that AgNPs may be used as an antimicrobial agent. Other workers [(22)] also opined that the AgNPs have an antimicrobial effect on S. aureus and E. coli.

Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [(23)]. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The antimicrobial mechanism of silver nanoparticales is related to the formation of free radicals and subsequent free radical induced membrane damage. These free radicals may be derived from the surface of silver nanoparticals and responsible for the antimicrobial activity [(24)]. Silver nanoparticles lead to the formation of "pits" in cell wall of the bacteria,and silver nanoparticles could enter into periplasm through the pits and destroyed the cell membrane, then the silver nanoparticles could enter into the bacterial cell, which not only condensed DNA,but also combined and coagulated with the cytoplasm of damaged bacteria. Finally, silver nanoparticles resulted in the leakage of cytoplasmic component [(25, 26)]. Moreover, the silver nanoparticles could increase the decomposability of genome DNA. Silver has an important antimicrobial effect which is depended on superficial contact in these silver can inhibit enzymatic system of the respiratory chain and later DNA synthesis.

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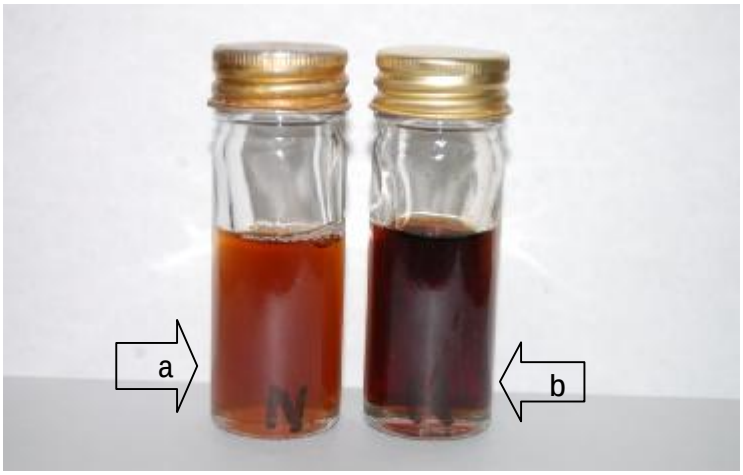
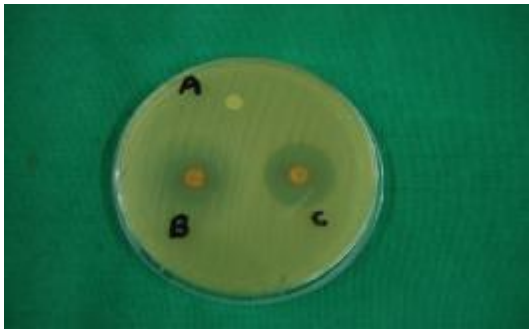


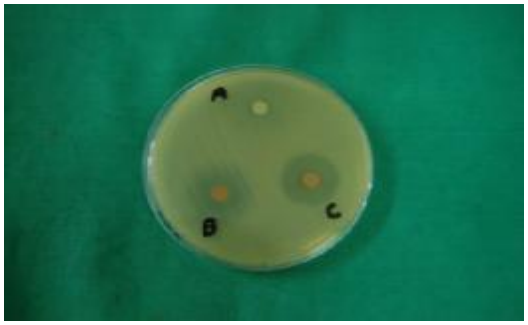
Fig-1: The colour of plant extracts change after addition of silver nitrate
(a) Plant extracts. (b) Silver nanoparticles.

Table 1: Zone of inhibition (mm) of nanoparticles against microorganisms tested

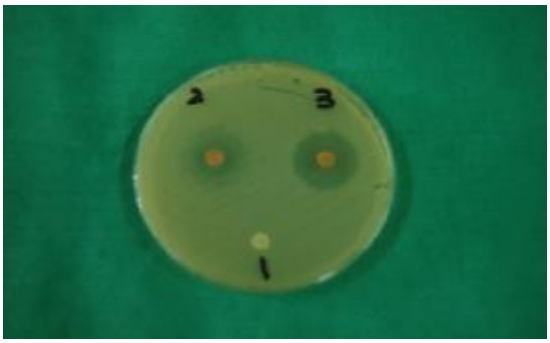
Zone of inhibition (mm)±SD			Organism
Control	Ag(NO3)2	SNPs	
0	6.8±0.6	15.6±1.6	**Strep. mutans**
0	5.9±0.52	13.3±1.4	**Strept. sanguis**
0	5.6±0.5	12±1.0	**Porph. gingivalis**



Strep.mutans



Strept. sanguis



Porph. gingivalis

Fig-2: Antibacterial activity of Juglans regia extract.
(A&1=Control. B&2=Sliver nitrate. C&3= Nanoparticals).