Antimicrobial Efficacy of various concentrations of silver nanoparticles used in canal disinfection: an in-vitro analysis

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Abstract

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Introduction: to determine the antimicrobial Efficacy of various concentrations of silver nanoparticles used in canal disinfection.

Materials and methods: The separation of Silver nanoparticles from Silver was carried out by the method of UV-Spectroscopy The antibacterial activity of ethanolic extract of Mimosa pudica was studied by agar well diffusion method in vitro.

Results: The results of the disk diffusion method, in this study, showed that all disks containing different weight percentages of nanosilver formed growth inhibition zones. However, the diameters of the growth inhibition zones around 200, 400, 600, and 800 µg concentrations of nanosilver at 24hours considerably increased with the increase in the concentration of the particles.

Conclusion: Silver nanoparticles incorporated extorted from Mimosa Pudica against E.Faecalis showed considerable effect in increasing concentrations.

Keywords: Silver nanoparticles, Well diffusion, Mimosa Pudica, Antimicrobial Efficacy

INTRODUCTION

Nano material denotes an incidental, natural, or manufactured materials containing particles in an unbound state [1] .Nano materials as unique physicochemical properties, such as large surface, ultra small size, and increased chemical reactivity, compared with their bulk counterparts [2,3] .Physicochemical and biologic properties of nano materials, especially the ones containing silver, attracted investigators in recent decades (4). Furthermore, silver nanoparticles show advantageous properties in biocompatibility and antimicrobial activity when compared to the salt precursors (5). Silver plays a pivotal role as an antimicrobial agent. Nevertheless, after the introduction of antibiotics in 1940, the use of silver salts decreased and silver compounds were used in different biomedical fields, especially in treatment of burns [6]. Silver when compared to other metals, it's more toxic to microorganisms in the following descending sequence: Ag > Hg > Cu > Cd > Cr >Pb> Co > Au > Zn > Fe >Mn> Mo >Sn with low toxicity to mammalian cells. Another advantage of silver is the low propensity to induce microbial resistance than many other antimicrobial materials [7].

Antibacterial properties of AgNPs can be shown against a large spectrum of microorganisms including gram-positive and gram-negative bacteria [8], fungi [9] and viruses [10]. Endodontic infections are mainly caused by bacterial etiology. Therefore, this study investigated the preparation, characterisation and evaluation of the antimicrobial properties of silver nanoparticles aiming to develop root formulations for endodontic therapy [11]. Several studies show that bacteria are the main etiologic agent of pulpal infection and periradicular lesion formation [21–23] The microbiota of infected root canals is polymicrobial and is dominated by Gram-negative anaerobes [24, 25]. It has been demonstrated that the presence of residual bacteria in root canal is connected with significantly higher rates of treatment failure [26].Since elimination of bacteria in root canals is the key to treatment success [27], endodontic materials should ideally provide some antimicrobial activity [28,29].From many previous studies conducted it has been seen that AgNPs, had demonstrated significant effect against Enterococcus faecalis, Staphylococcus aureus, Candida albicans, and Escherichia coli hence making it an extremely suitable material for Endodontic root canal disinfectant .The bactericidal effect of AgNPs depends on different parameters including size, shape, and the surface charge of the particles. In this respect, smaller particles are found to have greater antibacterial activity and shown to have benefits. Firstly, they can easily reach the nuclear content of bacteria due to the structure of the bacterial cell wall, especially in gram-negative ones [12]. Secondly, they can provide a greater surface area and therefore stronger bactericidal interactions [13, 14].

Plants have been used for generations in the treatment of bacterial and fungal infections for its wide range of bioactive molecules. Photochemicals are applied as natural anti pathogenic which can be derived from leaves, stems, barks and flowers of plants [30].

The traditional plant medicine in the present is getting back with modern science. The extracts from medicinal plants are used in the treatment of different diseases of humans, plants and animals [31]
About 80% of the world's population rely on traditional plant medicines for the treatment of common illness [32, 33]. The bacterial strains developed its genetic ability to various pharmacological antibiotics [34]. The synthesized drugs associated with adverse effects which lead to immunosuppression and allergic reactions [35]. The formulation of appropriate and efficient antimicrobial drugs to the patient is ultimate goal in this decade.Plants are the traditional helpers having alkaloids, flavonoids, saponins, tannins, protein and amino acids as its chemical constituents [36].
Mimosa pudica Linn. (Family: Mimosaceae) is used as an ornamental plant due to its thigmonastic and nyctinastic movements. M. pudica is also used to avoid or cure several disorders like cancer, diabetes, hepatitis, obesity, and urinary infections. M. pudica is famous for its anticancer alkaloid, mimosine, along with several valuable secondary metabolites like tannins, steroids, flavonoids, triterpenes, and glycosylflavones. A wide array of pharmacological properties like antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antinociceptive, anticonvulsant, antidepressant, antidiarrheal, hypolipidemic activities, diuretic, antiparasitic, antimalarial, and hypoglycemic have been attributed to different parts of M. pudica. [37].
Control of microbial infection in periodontal and endodontic tissues is essential for the successful management of endodontic–periodontal lesions and external root resorption due to root canal perforation [15-17] .The objective of this study is to determine and assess the effect of various concentrations of nanoparticles incorporated with herbal substitutes against endodontic micro organisms.

MATERIALS AND METHOD

Preparation of Extract

The Mimosa pudica extract was extracted with ethanol at room temperature for 72 hours using a shaker. After filtration with Whitman filter paper No. 1 using a vacuum pump, the residue was re-extracted again with ethanol solvent. The solvent was completely removed using a rotary vacuum evaporator at 40°C. The concentrated extract was then kept in dark bottles at 4°C until used.

Preparation of silver nitrate and synthesis of Silver Nanoparticles

The ethanolic extract of Mimosa pudica (1 g) was added to distilled de-ionized water (100 mL) with vigorous stirring for 1 h. A hundred milliliters of Ag NO_3 (1×10^{-2} M) was then added and mixed at room temperature (25 °C) for 48 hours. Ag-NPs were gradually obtained during the incubation period.

UV -Vis spectroscopy analysis

UV-visible spectroscopy analysis was carried out by using UV-Visible absorption spectrophotometer between 200 to 700 nm. The reduction of silver ions in to metallic silver nanoparticle was monitored by UV–Visible spectra of silver nanoparticles in aqueous solution. The interactions of these particles with light occur as electrons on the metal surface undergo oscillations when excited by light at specific wavelengths. The silver nanoparticles obtained exhibit a unique peak in the range of 470 nm.

Uv-Vis spectroscopy of silver nanoparticles synthesized from Mimosa pudicaextract after 48hrs of incubation

Antibacterial activity using Agar well diffusion method

1. The samples were screened for antibacterial activity against Enterococcus faecalis using agar well diffusion method[1-4].

Agar well diffusion method

Sample Preparation:
The extract was mixed with ethanol and vortexed for maximum dissolution.
Procedure: Luriabertani Agar (LBA) plates were inoculated with test organism. The plates were evenly spread out. Then wells were prepared in the plates with a cork borer. Each well was loaded with 20,40,60,80 µl. The plates were incubated for 24h at 37°C. The development of inhibition zone around the well was measured (diameter) and recorded. Tetracycline was used as a positive control.

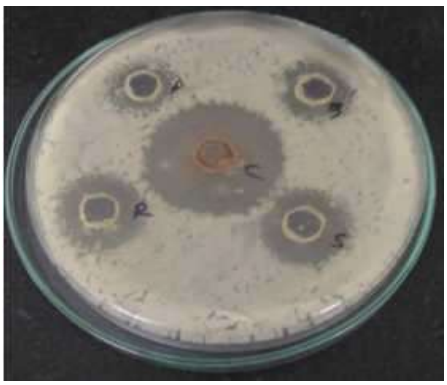
RESULTS

Table 1 shows Antibacterial activity of samples

Sample	Concentration (µg)	Zone of inhibition (mm)	
		Sample	Control (400 µg)
Extract	200	11	30
	400	13	
	600	15	
	800	16	

The antimicrobial activity of silver Nano particles with Mimisa Pudica extract was moderate compared to that of conventional Tetracycline. Tetracycline which was the positivecontrol group showed an antibacterial activity of 30µg..This shows that on increasing the concentrations of the extract incorporated along with Silver nanoparticles we were able to see that the antimicrobial activity of the extract against the bacteria increases along with the increase in concentration.

Figure 1 shows antibacterial activity of the sample Enterococcus fecalis



DISCUSSION

In this in vitro study the antimicrobial efficacy of silver nano particles with Mimosa Pudica extract and Tetracycline against E. faecalis. The antimicrobial activity of silver nano particles with Mimosa Pudica extract was moderate when compared to that of conventional Tetracycline. Tetracycline was rapidly killed the bacteria in the petredish, only partial disinfection can be achieved in the surface wall of the root canal and Tetracycline was relatively ineffective. E. faecalis resists high pH levels. It maintains a pH level by the buffering capacity of its cytoplasm. It also has a proton pump in which it provides additional homeostasis. However, studies have reported that this microorganism cannot resist pH levels over 11.5 [12].Especially microorganisms recovered from the persistent periradicular infection or the secondary infection are found to have biofilm that have high resistance against the commonly used intracanal medicaments [40, 41]. Studies have suggested that most common bacteria isolated from the secondary root canal infection are the E. faecalis [42].

Hence the present study was conducted with the objective of checking the antimicrobial efficacy of silver nanoparticles at different concentrations against E. Faecalis. This study evaluated the antibacterial efficacy of 200, 400, 600, and 800 µg concentrations of nanosilver combined with mimosa pudica extract against E. faecalis using the disk diffusion method [43-45]. The results of the disk diffusion method, in this study, showed that all disks containing different weight percentages of nanosilver formed growth inhibition zones. However, the diameters of the growth inhibition zones around 200, 400, 600, and 800 µg concentrations of nanosilvers at 24hours considerably increased with the increase in the concentration of the particles.16mm being the highest zone of inhibition in the disk at 800 µg.

Most of the nanoparticles were tested for root canal disinfection depends on time-dependent and contact-mediated antibacterial activity. Adding of various nanoparticles into root filling materials sealers or significantly improved the antibacterial efficacy by inhibition of biofilm formation on the surface as well as the resin-dentin interface. Mimosa pudica also called Sensitive Plant is a perennial herb often grown for its curiosity value. The leaves of the plant are used in the treatment of biliousness, leprosy, dysentery, vaginal and uterine complaints, inflammations and burning. Leaf contains an alkaloid mimosine. Root contains tannin, ash, calcium oxalate crystals and mimosine [1]. Considering the various antibacterial, antifungal, antimicrobial action of Mimosa Pudica against end odontic organisms proves it to be a good source of extract to be used in curing endodontic infections.

Mimosa Pudica as a plant is extremely useful and its individual parts have separate antimicrobial, antibacterial, anti-inflammatory and wound healing properties. As seen from the literature we were able to see that Mimosa Pudicas s antimicrobial properties can be mainly seen from extracts derived from the Leaves, roots, aerial parts, and whole plant extracts (Vadlapudi and Naidu (2010),Tamilarasi and Anathi (2012),Sukanya and others (2009),Sukanya and others (2009),Mohan and others (2011),Marimuthu and others (2011),Gandhiraja and others (2009),Genest and others (2008),Chowdhury and others (2008))anti inflammatory properties can be mainly seen from extracts derived from theLeaves extract (Kumar and Kumar (2011), Vikram and others (2012)) wound healing properties seen from root an leaf extract (Kannan and others (2009),Paul and others (2010),Vinothapooshan and Sundar (2010),Kokane and others (2009)) and antioxidant properties seen from leaves (Genest and others (2008); Chowdhury and others (2008); Haripyaree and others (2010); Rekha and others (2010); Arokiyaraj and others (2012))

Utilisation of silver NPs is in root canal irrigation solution which directly target pulp canal microbes. According to a study, the nanosilver-based irrigant is as potent as 5.25% NaOCl in eradication Enterococcus faecalis and Streptococcus aureus [39]. Secondary caries is the main cause of failure of dental restorations. Several efforts have been made to improve the longevity of dental restorations by incorporating bioactive agents having antimicrobial activities. Slow-release of various low molecular weight antibacterial agents such as antibiotics, zinc ions, silver ions, iodine, and chlorhexidine was evaluated in glass ionomer cement [38]. 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. Silver has more microbial efficacy and more effective in the presence of proteinaceous material.Many studies have shown that silver, in its nanoparticulate form, possesses an inhibitory effect against many bacteria and fungi, including S. mutans, C. albicans, P. aeruginosa, E. faecalis, and S. aureus, among others, which could decrease the occurrence of secondary caries, fungical infection, fails on endodontic treatment, and dental implant loss. AgNP has also been proved to be biocompatible with mammalian cells, suggesting that its application on dental materials does not represent a threat to human health. Despite the antibacterial effectiveness of Silver Nanoparticles in dentistry, the possible side effects such as cytotoxicity to host cells and dentin

staining made it a controversial aget for in vivo applications[46] .Although previous studies have confirmed that the cytotoxicity of Silver nanoparticles is concentration dependent [47]However, more studies are required to determine the optimal concentration of this silver compound, in order to guarantee the antimicrobial effect without increasing its cytotoxicity. As from the above study we can prove that Silver nanoparticles has an extremely well antimicrobial effect against E.Faecalis and by increasing the concentrations of the same and incorporating it with the extract of Mimosa Pudica gave us better results.

CONCLUSION

The results from the current study indicate the potential antimicrobial efficacy achieved when silver nanoparticles were combined with Mimosa Pudica extract against E. faecalis at different concentrations .Nanoparticle incorporated treatment strategies have the potential to improve antibacterial/antibiofilm efficacy in endodontics. From the present study we can conclude that Silver nanoparticles incorporated extorted from Mimosa Pudica against E.Faecalis showed considerable effect in increasing concentrations.. However, more studies are required to determine the optimal concentration of this silver compound, in order to guarantee the antimicrobial effect without increasing its cytotoxicity.

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