Enhanced Antibacterial Effect of Titanium Dioxide Nanoparticles Mediated Grape Seed Extract on Oral Pathogens - *Streptococcus mutans* and *Lactobacillus*

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ABSTRACT

BACKGROUND

Streptococcus mutans is the primary pathogen responsible for initiating dental caries and decay. Lactobacilli present in caries lesions is a major contributor to caries progression. Titanium dioxide nanoparticles have been found to have a large spectrum of activity against microorganisms. The grape seed extract has been shown to have inhibitory effects against several clinically important microbes. This study was conducted to assess the enhanced antibacterial effect of titanium dioxide nanoparticles mediated grape seed extract against oral pathogens, *S. mutans* and *Lactobacillus*.

METHODS

This experimental study was done by preparing the grape seed extract, synthesising titanium dioxide (TiO₂) nanoparticles, preparing the nanoparticles powder, and then evaluating the antibacterial activity of nanoparticles against *Lactobacillus* species and *Streptococcus mutans*.

RESULTS

The peak found in the spectroscopy indicates the formation of titanium dioxide nanoparticles. The zone of inhibition for antibiotics enhanced with 50 µl, 100 µl, 150 µl of grape seed mediated TiO₂ nanoparticles were 16 mm, 18 mm and 19 mm in *Streptococcus mutans*. In *Lactobacillus* species the zone of inhibition for standard antibiotics enhanced with 50 µl, 100 µl, 150 µl of grape seed mediated TiO₂ nanoparticles remained constant at 35 mm.

CONCLUSIONS

The titanium dioxide nanoparticle mediated grape-seed extract showed excellent antimicrobial activity against *Lactobacillus* and *Streptococcus mutans*. This proves that in future it can be used for treating these bacterial infections in the oral cavity after further in vivo studies.

KEY WORDS

Nanotechnology, Titanium Dioxide Nanoparticles, Grape Seed Extract, *Lactobacillus*, Enhanced Antibacterial, *S. mutans*

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BACKGROUND

Nanotechnology is an interdisciplinary field that is undergoing rapid development and materialising, it has brought about enormous changes in the field of dentistry especially. Nanotechnology has demonstrated to have different applications in dentistry, including dentition renaturalisation, treatment for dentin hypersensitivity, complete orthodontic realignment in a solitary visit etc.¹⁻³

The adjunct 'nano' originated from the Greek word nanos, meaning dwarf, and describes substances whose molecular size lies in the range of 0 to 100 nanometres. The concept of nanomedicine was first introduced by Robert A Frietas, Jr. in 1993 and was defined, as follows: Nanomedicine is the preservation and improvement of human health using molecular tools and molecular knowledge of the human body. It has diversified actions ranging from target drug delivery to creating tissue scaffolds based on nanoscale molecules.⁴⁻⁸

Streptococcus mutans is a microbe that has been strongly associated with the initiation of dental caries, studies also show that lactobacilli may be more significant in the progression of caries lesions and have found it to be responsible for the initiation of a low percentage of coronal caries.⁹⁻¹¹ Lactobacilli adheres poorly to oral tissue and is normally present either in low numbers or absent in healthy mouths, but increased in sites of active caries, particularly carious dentine.¹²

Grape seeds due to their phenolic components and good antioxidant properties possess the ability to benefit the health of an individual in various ways including treating tooth decay, protecting against pathogens, improving night vision, treating diabetic retinopathy and improving blood sugar control, reducing oedema, reducing iron levels in people with haemochromatosis, reducing inflammation.¹³ Grape seed extract is a readily available plant-based supplement that, due to its concentrated levels of proanthocyanidins, has promising characteristics that may assist in dental caries prevention via its antibacterial and antioxidant properties.^{14,15}

The widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains. The search for new antimicrobial substances has been engaged toward metal oxide nanoparticles.^{16,17} Specifically, titanium oxide has been considered as an attractive antimicrobial compound due to its photocatalytic nature and because it is a chemically stable, non-toxic, inexpensive and recognised as a biocompatible substance.¹⁸⁻²⁰ Several studies have revealed this metal oxide demonstrates excellent antifungal and antibacterial properties against a broad range of both gram-positive and gram-negative bacteria.^{21,22} These properties were significantly improved by titanium oxide nanoparticles synthesis.

Nanotechnology has gigantic potential, yet issues, for example, open acknowledgment, morals, guidelines, and human well-being must be considered before subatomic nanotechnology can be viewed as a chance of giving great dental care.^{23,24}

The aim of the study was to assess the enhanced antibacterial effect of TiO₂ nanoparticles mediated grape seed extract against oral pathogens, *S. mutans* and lactobacillus.

METHODS

The study was done under laboratory setup and in the premises of Saveetha Dental College. It was an experimental study conducted from December 2019 to January 2020.

Preparation of the Grape Seed Extract

The collected grape seeds were rinsed for 4 - 5 times in distilled water and dried for 8 - 15 days. The well dried grape seeds were powdered. The powdered grape seed was stored in air-tight containers. In a beaker, 0.5 g of grape seed powder was added to 100 ml of distilled water and mixed well. Then boiled for 10 mins at 70 - 80° C. The solution was allowed to cool and then filtered by using Whatman no. 1 filter paper and funnel. Freshly prepared grape seed extract was thus obtained.

Synthesis of TiO₂ Nanoparticles

65 ml of 0.4 g TiO₂ was prepared using distilled water. It was added to the freshly prepared grape seed extract and made into 100 ml solution with distilled water. It was mixed well by placing in a magnetic stirrer in an orbital shaker for 10 hours. The absorbance was measured using ultraviolet UV-visible spectrometer on regular intervals to confirm the presence of TiO₂ nanoparticles formed. The colour change was noted and reading was taken every 2 hours. The final reaction mixture was centrifuged for 10 mins at 4000 rpm using Lark refrigerated centrifuge. It was then collected and stored in an air-tight Eppendorf tube (Figure 1).

Enhanced Antibacterial Activity

The agar well-diffusion method was used to determine the enhanced antibacterial activity of TiO₂ nanoparticles. Different TiO₂ concentrations were tested against lactobacillus species and *Streptococcus mutans* (gram-positive). Concentrations of 50 µl, 100 µl, 150 µl of grape seed mediated TiO₂ nanoparticles were placed inside the wells. Then the agar plates were incubated at 37° C for 24 hours. The standard antibiotics and the prepared particles were together used to test the enhanced effect of the synthesised grape seed mediated TiO₂ nanoparticles on the bacterial species. In each plate, the zone of inhibition was recorded (Figure 2, 3).

RESULTS

From the table, the zone of inhibition for antibiotics enhanced with 50 µl, 100 µl, 150 µl of grape seed mediated TiO₂ nanoparticles were 16 mm, 18 mm and 19 mm in *Streptococcus mutans*. In lactobacillus species the zone of inhibition for standard antibiotics enhanced with 50 µl, 100 µl, 150 µl of grape seed mediated TiO₂ nanoparticles remained constant at 35 mm. (Table 1).

Figure 1.
Collection of the Synthesised TiO₂ Nanoparticles after Centrifuging, in the Eppendorf Tube

Figure 2.
Agar Plate Showing the Zone of Inhibition of Enhanced Activity of Antibiotics along with Different Concentrations of Grape Seed Mediated TiO₂ Nanoparticles (50 µl, 100 µl, 150 µl) against Streptococcus mutans

Figure 3.
Agar Plate Showing the Zone of Inhibition of Enhanced Activity of Antibiotics along with Different Concentrations of Grape Seed Mediated TiO₂ Nanoparticles (50 µl, 100 µl, 150 µl) against Lactobacillus Species

Figure 4. Graph Depicting the Enhanced Antibacterial Activity of Grape Seed Mediated Titanium Dioxide Nanoparticles against *Streptococcus mutans* and *Lactobacillus Species* as Observed in This Study

Enhanced Antibacterial Activity of Grape Seed Mediated Titanium Dioxide Nanoparticles

Concentration of the Prepared Extract.	Zone of Inhibition in mm.	
	<i>Lactobacillus Species</i>	<i>Streptococcus mutans</i>
50 µl	35	16
100 µl	35	18
150 µl	35	19

Table 1. Table Depicting the Zone of Inhibition as Observed in This Study

periodontics, endodontics, implantology, dental prosthesis and orthodontics.^{38,39} This can prevent the activity of caries brought about by the microbial demineralisation and erosion activities.⁴⁰ It can likewise be utilised as antibacterial irrigant or sealer in endodontics.⁴¹ There are many strategies in dentistry in which the antibacterial nanoparticles can be integrated.⁴²

DISCUSSION

Titanium dioxide has become part of our everyday lives. It is found in various consumer goods and products of daily use such as cosmetics, paints, dyes and varnishes, textiles, paper and plastics, food and drugs and even paving stones.²⁵⁻²⁷ The great versatility of titanium dioxide is owing to its various forms and sizes.²⁸ Titanium dioxide NPs have been explored for a variety of biomedical applications.

Studies performed previously by Maryam revealed that metal oxide nanoparticles such as titanium dioxide nanoparticles also show significant antimicrobial activity through connection to microbial deoxyribonucleic acid (DNA) and proteins, and are caused to prevent bacterial duplication, avoiding metabolic enzymes of the bacterial electron transport chain leading to their inactivation.²⁹⁻³¹ According to Zhang, TiO₂ nanoparticles can be selected as an antibacterial material because of their superior properties, such as high specific surface area and high activity to block a wide scope of pathogenic agents.^{32,33} Although titanium dioxide nanoparticles improve mechanical and antibacterial properties of conventional glass ionomer cements, their biocompatibility is still controversial.³⁴

The oral cavity is continuously exposed to a lot of microorganisms. Plaque biofilm is a significant reason for caries, periodontitis and other dental problems.³⁵ It comprises a complex network of microorganisms that cause various diseases.³⁶ *Lactobacillus* species and *Streptococcus mutans* are the most significant oral microbes experienced in the arrangement of plaque biofilm causing dental caries and periodontal issues.³⁷ This investigation advances the turn of events and utilisation of antibacterial nanoparticles in dentistry which incorporates therapeutic dentistry,

CONCLUSIONS

The antibacterial activity of grape seed mediated TiO₂ nanoparticles was studied. *Lactobacillus* species and *Streptococcus mutans* were studied as they are closely associated with dental problems. Grape seed mediated TiO₂ nanoparticles have enhanced antibacterial activity against *Lactobacillus* species and *Streptococcus mutans*.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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