

Comparative evaluation of antimicrobial efficacy and cytotoxicity of nanohydroxyapatite reinforced particles and Vitamin D incorporated nanohydroxyapatite particles: An *in vitro* study

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ABSTRACT

Introduction: The increasing prevalence of dental problems necessitates the development of novel preventive dental materials with supreme antimicrobial efficacy to reduce global disease burden. Hence, the aim of this study was to comparatively evaluate the antimicrobial efficacy and cytotoxicity of nanohydroxyapatite reinforced particles and Vitamin D incorporated nanohydroxyapatite particles. **Materials and Methods:** An *in vitro* experimental study was carried on three groups, namely, Group I: Nanohydroxyapatite prepared using black tea as reducing agent, Group-II: Commercially available Vitamin D powder using ethanol as solvent, and Group III and Group IV: Nanoformulation which comprised a combination of nanohydroxyapatite reinforced particles with 100 and 500 mg of Vitamin D dissolved in 3 ml of ethanol. **Results:** It was found that Group-II which comprised pure Vitamin D exhibited superior antimicrobial effect but was observed to be having cytotoxic effect. However, Group IV – nanoformulation with 500 mg of Vitamin D dissolved in 3 ml of ethanol showed a superior antimicrobial effect than Group I and inferior such effect when compared to Group II without demonstration of cytotoxic effects. Group IV is statistically significant at all different concentrations with $P < 0.01$. It was also observed that Group III and Group IV showed higher antimicrobial effect at a concentration of 100 than any other groups against *Streptococcus mutans* with statistical significance. **Conclusion:** The nanoformulation prepared which contained nanohydroxyapatite reinforced particles with 500 mg Vitamin D had supreme antimicrobial efficacy with no cytotoxic effects than any other groups.

KEY WORDS: Antimicrobial, Black tea, Cytotoxicity, Nanohydroxyapatite, Preventive dentistry, Vitamin D

INTRODUCTION

Dental caries is the most common oral diseases affecting a majority of the community which is caused as a result of imbalance between remineralization and demineralization process. Hence, control of demineralization and addition of effective remineralization agents are the key to preventive dental programs.^[1]

Nanohydroxyapatite is well known for its unique characteristics such as solubility, higher surface energy, and more biological activity than larger particles compared to hydroxyapatite. It is also popularly regarded for its guided tissue, nerve, and pulpal regenerative features and in tissue engineering.^[2]

Vitamin D is a fat soluble vitamin which prevents rickets in children and osteomalacia in adults.^[3] The recommended daily intake is 400 IU/day for 0–12 month's infants, 600 IU a day for 1–70 years old individuals, and 800 IU each day for those belonging to an age beyond 70 years.^[4] Vitamin D contributes to dental health by helping in preservation of the oral structures such as teeth and supporting structures. Vitamin D plays a key role in tooth development and protects it from the risk of getting dental caries.^[3]

Several researches have concluded that low Vitamin D levels or intakes are associated periodontal disease. The previous studies have suggested that Vitamin D has antimicrobial potential.^[5]

Black tea powder is well known for its antioxidant property. Researches have concluded that black tea increases mental alertness and reduces the risk of heart

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attack, strokes, postural postprandial hypotension, ovarian cancer, brittleness of bones, and Parkinson's disease. It also has anticancer and antimicrobial roles.^[6]

Lack of adequate information exists regarding the exact potential of new dental materials when used in combinations. With the emergence of success rate on using nanohydroxyapatite in the field of dentistry, this *in vitro* study is conducted with an aim to comparative evaluate nanohydroxyapatite reinforced particles with Vitamin D incorporation.^[7]

The increasing prevalence of dental problems necessitates the development of novel preventive dental materials with supreme antimicrobial efficacy to reduce global disease burden.^[8] The aim was to comparatively evaluate the antimicrobial efficacy and cytotoxicity of nanohydroxyapatite reinforced particles and Vitamin D incorporated nanohydroxyapatite particles and the findings of this study will pave way to the formulation of new remineralizing agents with efficacious antimicrobial property and biocompatibility.

MATERIALS AND METHODS

Study Place

This study was carried out at the Nano Bio Science Laboratory, Saveetha Dental College and Hospitals, Chennai.

Study Design

This was an *in vitro* experimental study.

Sample Size

Four groups (I, II, IIIA, and IV).

Group Segregation

Group I: Nanohydroxyapatite prepared using black tea as reducing agent, Group-II: Commercially available Vitamin D powder using ethanol as solvent

and Group III A: Nanoformulation which comprised a combination of nanohydroxyapatite prepared using black tea as reducing agent with 100 mg of Vitamin D dissolved in 3 ml of ethanol. Group III B: Nanoformulation which comprised a combination of nanohydroxyapatite prepared using black tea as reducing agent with 500 mg of Vitamin D dissolved in 3 ml of ethanol.

Armamentarium

Chemical balance, micropipette, measuring flask, glass beaker, separating funnel, magnetic stirrer, magnetic bead, calcium hydroxide powder, orthophosphoric acid (88%), deionized water, calcium chloride, sodium phosphate, acetic acid, potassium hydroxide, hard tissue microtome, pH meter, centrifuge, hot air oven, disposable centrifuge tubes, test tubes and conical flask, surgical gloves, mouth mask, commercially available black teas extract powder, commercially available pure Vitamin D and ethanol were used in this project.

Flowchart of Adopted Methodology

Table 1 shows the comparison of antimicrobial activity between groups.

Table 2 shows the cytotoxic effect observed among the three groups on day 1 and 2.

DISCUSSION

The factors affecting this study were the environmental storage condition of the media used for checking antimicrobial efficacy. This finding is similar to the observations in a study conducted by Chowdhury *et al.*, which revealed that media enriched with Vitamin D3 can exert a toxic effect on *Hymenolepis microstoma*, leading to degenerative changes in the worm.^[9] As this study has utilized Vitamin D as a component for reinforcement and detected its antimicrobial potential, the findings of this study suggest its use to prevent viral infections which is in accordance with the recommendations given in a study done by Edlich *et al.*, Bikle *et al.*, and Sabetta *et al.*^[10-12] The formulation in this study chiefly consists of hydroxyapatite showing antibacterial potential which is in accordance with the findings of the study done by Ragab *et al.*^[13] The preparations in our study utilized black tea as a reducing agent and ethanol as dissolving agent which

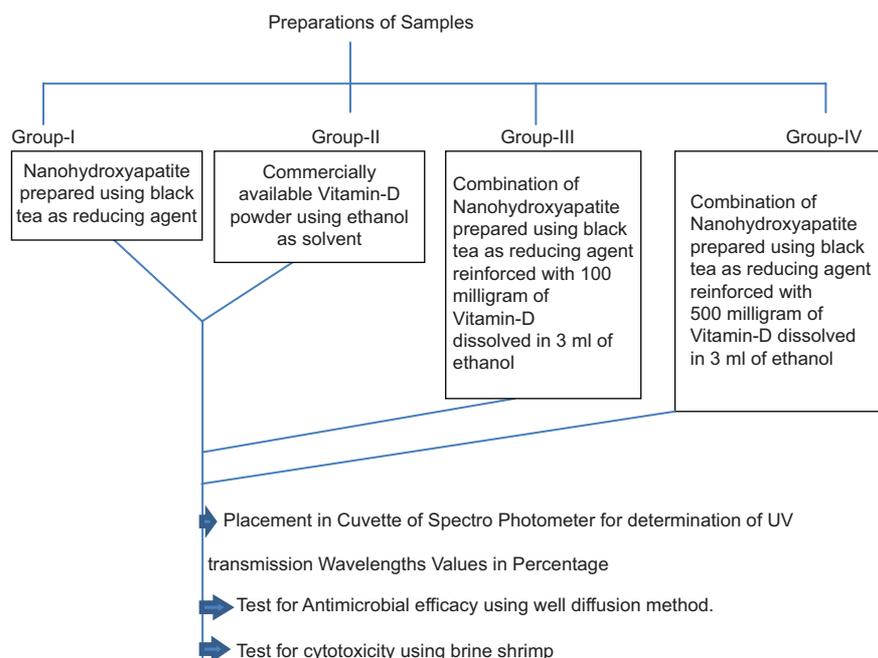
Table 1: Comparison of antimicrobial activity between groups

Comparisons	U-value	Z-ratio	P-value
Group I versus Group II	46	-2.73	0.006*
Group III versus Group IV	17	-3.94	0.000*

*Mann-Whitney U-test; $P < 0.05$

Table 2: The cytotoxic effect observed among the three groups on day 1 and 2

Groups	Day-1 (Total number of live nauplii)					Day-2 (Total number of live nauplii)				
	5 μ l	10 μ l	15 μ l	20 μ l	25 μ l	5 μ l	10 μ l	15 μ l	20 μ l	25 μ l
Group-I	10	10	10	10	10	3	7	4	6	6
Group-II	10	10	10	10	10	4	6	3	6	6
Group-IIIA	10	10	10	10	10	10	10	10	10	10
Group-IV	10		10	10	10	9	9	9	10	10



could be the possible contributory constituent for antimicrobial activity against *Streptococcus mutans* which is in accordance with the findings of the study done by Mughal *et al.*^[8] The study has measured only the short-term effect of the materials used under *in vitro* setting.

CONCLUSION

The evidence presented in this study led to a conclusion that the nanoformulation prepared which contained nanohydroxyapatite reinforced particles with 500 mg Vitamin D had supreme antimicrobial efficacy with no cytotoxic effects than any other groups.

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