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RESEARCH ARTICLE
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Antimicrobial activity, Cytotoxicity and Anti inflammatory activity of Papain and Bromelain based caries removal gel decorated with Silver Nanoparticles against oral microbes

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ABSTRACT

Background: Traditionally caries removal has been always done using dental burs. There are other methods of caries removal as well. Chemico mechanical method of caries removal is one of the well known methods. This study is papain gel and bromelain gel which have been self prepared and used as caries removal agent.

Materials and methods: The cytotoxicity of the self prepared caries removal gel was tested by Brine Shrimp Lethality Assay. Antimicrobial activity was evaluated against S mutans, E faecalis, Lactobacillus and C albicans oral bacteria. Anti inflammatory activity was evaluated using albumin denaturation analysis.

Results: Self made caries removal gel is more effective against oral microbes S mutans, Lactobacillus and C albicans. Also it shows mora anti inflammatory and less cytotoxic effect than commercial caries removal gel.

Conclusion: Self made caries removal gel is more effective than commercially available gel.

Keywords: *Papain, Bromelain, cytotoxicity, antimicrobial,Tree species, Children*

INTRODUCTION

Dental caries is the disease that is most prevalent in the world population. Conventional caries removal and cavity preparation entail the use of burs. Disadvantages of this system include (i) the perception by patients that drilling is unpleasant, (ii) local anesthesia is frequently required, (iii) drilling can cause deleterious thermal effects, (iv) drilling can also cause pressure effects on the pulp, and (v) the use of a handpiece may result in removal of softened, but uninfected dentin, resulting in an excessive loss of sound tooth tissue(1). As a result, there is a growing demand for procedures or materials that facilitate caries management. Chemico mechanical caries removal is one of the other alternative methods known for caries removal.

Initially, sodium hypochlorite was introduced as a caries removal agent but as it was caustic, other agents were tried(2). N-monochloroglycine and amino butyric acid were the other compounds tried as caries removal gel. Caridex, Carisolv, Carie care etc. were the commercially available caries removal gel(3).

Though different formulations were introduced and tried in clinical practice,there is still a quest for herbal and natural formulations(4). In 2003, a research project in Brazil led to the development of a new formula to universalize the use of chemo-mechanical methods for caries removal and promote its use in public health(5,6). The new formula was commercially known as Papacarie(7–9). Papain is an enzyme derived from the papaya fruit. The enzyme works by causing proteolysis of carious tissue thereby helping in easy removal of caries. Bromelain is a similar enzyme derived from the plant of pineapple(10).

Nanotechnology is the branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at nanoscale level(11,12). Nanotechnology is being applied in every field of dentistry(13). Similarly biosynthesis of nanoparticles from papain and bromelain would increase the efficacy of papain and bromelain(14,15).

The present study is about a novel self made caries removal gel which is based on papain, Bromelain decorated with silver nanoparticles used for removal of caries. Bromelain is an enzyme derived from the plant of pineapple. It is said to be a potent proteolytic enzyme compared to papain. Silver nanoparticles were biosynthesised using papain and Bromelain and formulated as a gel.Silver nanoparticles are well known for their antimicrobial action and increasing the efficacy of the prepared formulation.

The aim of this study was to evaluate the cytotoxicity, anti microbial activity and anti inflammatory activity of self made papain and bromelain based caries removal gel decorated with silver nanoparticles.

MATERIALS AND METHODS

Preparation of caries removal gel

0.5 gram of Papain and bromelain powder which was readily available in the market was taken. To this 100 ml of distilled water was added. To this solution, 0.1gram of silver nitrate powder was added and placed in the shaker for 24 hours. After 24 hours, a change in color of the solution was seen indicating that biosynthesis of silver particles had occurred. The supernatant was removed and residue was further centrifuged to obtain papain and bromelain based silver pellets.

The silver pellets were added to a gel preparation which contained carbopol, carboxymethyl cellulose and sodium benzoate to obtain the final caries removal gel.

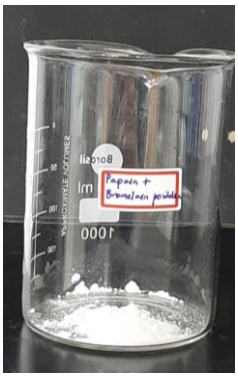

FIGURE 1: Papain, Bromelain Powder

FIGURE 2: Papain, bromelain powder with silver nitrate

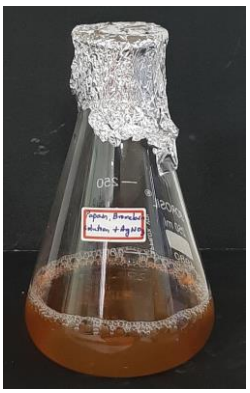

FIGURE 3: Biosynthesis nanoparticles

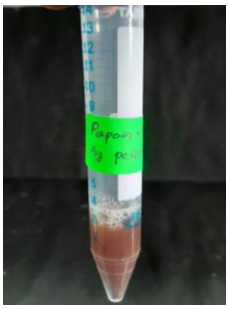

FIGURE 4: Silver pellets

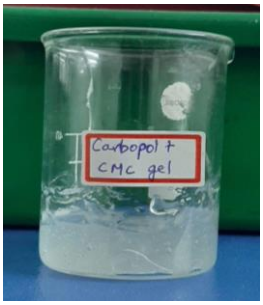

FIGURE 5: Gelling agent

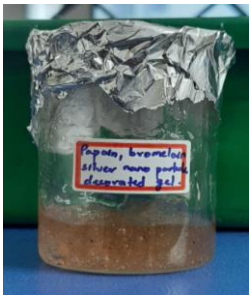

FIGURE 6: Caries removal gel

Brine Shrimp Lethality Assay(Cytotoxicity Test)
Saltwater preparation

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water. Six well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL, 10µL, 20µL, 40µL, 80µL).Nauplii is the early larval stage of shrimps.This organism is one of the important test organisms for toxicity test because of its characteristics such as easy culturing (hatching from eggs gives organisms of similar age, genotype and physiological condition), short life cycle, and resistance to manipulation, wide geographic distribution, simplicity and cost-effectiveness of performed tests.

Then papain gel and bromelain gel were added to separate wells according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for the number of live nauplii's presence and calculated by using the following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100

Antibacterial Activity

Antibacterial activity of the prepared papain and bromelain gel solution was assessed against the strains of oral microbes staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis and Candida albicans. Muller Hilton Agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and was let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The papain and bromelain gels of different concentrations were loaded onto plates and were incubated for 24 hours at 37 ° C. After the incubation time, the zones of inhibition were measured.

Anti inflammatory activity

Albumin denaturation Assay was carried out to assess the anti-inflammatory activity of the prepared gel. 0.05ml of prepared gel was added to 0.45ml bovine serum albumin. pH of mixture maintained at 6.3. The mixture was incubated at room temp for 20 minutes and heated to 55 degree celsius. This was followed by Spectrophotometric analysis. Absorbance was estimated at 600nm.

RESULTS

Brine Shrimp Lethality Assay(Cytotoxicity Test)

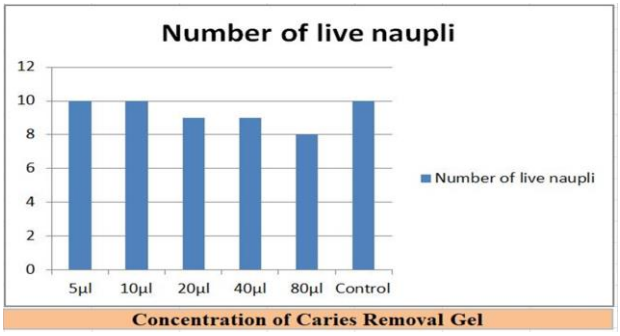

FIGURE 7: Graph showing number of live nauplii

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The number of live nauplii at concentration of 5µl and 10µl were ten, at 20 and 40µl concentration number of live nauplii were nine, at higher concentration of 80µl, 8 nauplii were alive.

Anti microbial activity

Zone of inhibition

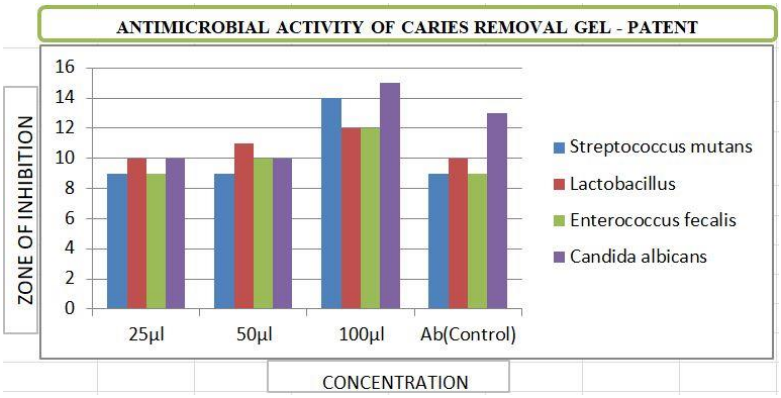

FIGURE 8: Graph showing Zone of inhibition

FIGURE 9: Zone of inhibition against S mutans

FIGURE 10: Zone of inhibition against E faecalis

FIGURE 11: Zone of inhibition against C albicans

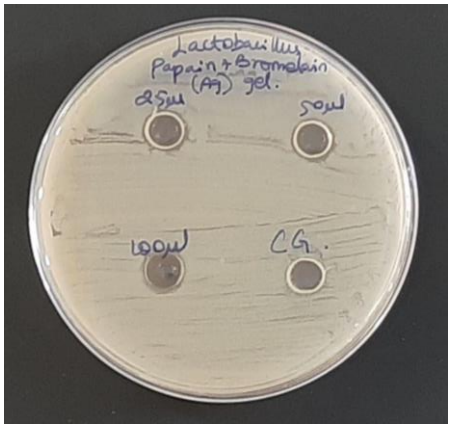

FIGURE 12: ZOI against Lactobacillus

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Anti inflammatory activity

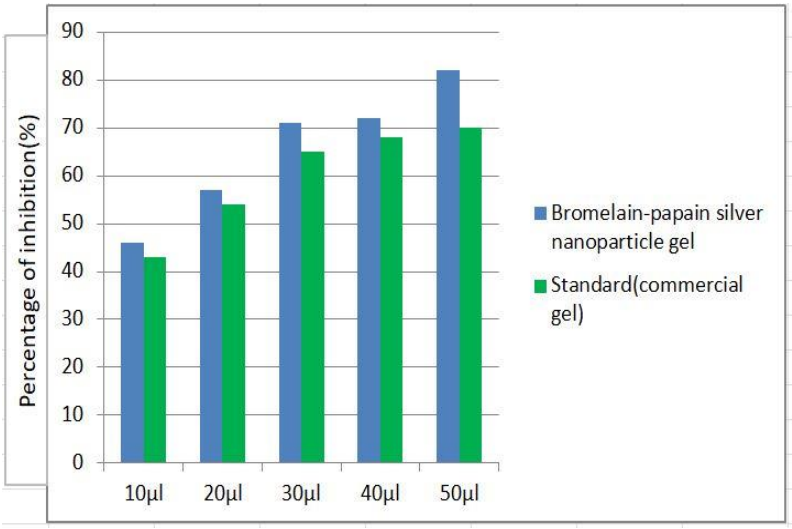

FIGURE 13: Graph showing anti inflammatory activity

DISCUSSION

Dental caries is the disease that is most prevalent in the world population(16). Conventional caries removal and cavity preparation entail the use of burs. Conventional burs cause anxiety and fear in patients. Alternate method of caries removal includes chemico mechanical method of caries removal(17). Different caries removal gels are available in the market but majority of them do not show much success. The key ingredient in such gels is papain, a proteolytic enzyme obtained from the plant of papaya(18). In the present study a novel caries removal gel has been formulated which is based on papain, Bromelain which is a newer and more potent, effective proteolytic enzyme(19). Effectiveness and specificity of a papain-based chemo-mechanical caries-removal agent in providing minimum residual caries after cavity preparation has been studied by Neves et al.(20). Both the ingredients have been compared by Vamsi et al. It was concluded that bromelain was more effective in amount of caries removal than papain(21). Alkhouli et al. compared 2.25% sodium hypochlorite gel and brix 3000.The author concluded that the use of a 2.25 % sodium hypochlorite gel can be an effective and well-tolerated method of removing decay from primary teeth and reduce the trauma associated with conventional rotary caries removal(2). Kumar et al. compared the clinical efficiency of chemomechanical caries removal using Carisolv and Papacarie. According to the author, both carisolv and papacarie showed similar effects(22)

Silver nanoparticles have been used for different purposes in dentistry. (23)To this, silver nitrate has been added for biosynthesis of silver nanoparticles. Papain and bromelain are the proteolytic enzymes that cause proteolysis of carious tissue. Biosynthesis of silver nanoparticles from papain and bromelain adds to the efficacy of the caries removal gel. Biosynthesis of silver nanoparticles have been tried in wound dressing by Sanaa et al(24). Also silver is a compound which is antimicrobial in nature, thereby promoting increased caries removal efficacy along with antimicrobial activity(25). The cytotoxicity was evaluated by Brine Shrimp Lethality Assay. Brine shrimp lethality assay has been exclusively used for analysis of cytotoxicity of compounds. Fitoterapia at al evaluated the cytotoxicity of biflavanois(26). The cytotoxicity of caries removal gel showed that at the end of the incubation period, at lower concentrations of 5µL and 10µL, ten nauplii(shrimp larva) were alive after 24 hours. At a concentration of 20µL and 40µL, nine nauplii were alive, and at higher concentration of 80µL seven live nauplii were found. This was compared with the control group in which no solution was added, and all the 10 nauplii were alive in brine solution. The high survival of nauplii indicates that the compound is less cytotoxic.

The results of an antimicrobial test show the zone of inhibition. The greater is the zone of inhibition compared to the control group, better is the antimicrobial action of the compound against oral microbes S mutans, E faecalis, Lactobacillus and C albicans. At 25µL, 9mm of zone of inhibition is seen against S mutans and E faecalis, 10mm of zone of inhibition was seen against Lactobacillus and C albicans. At 50µL concentration, 9mm of zone of inhibition was seen against S mutans, 10mm of zone of inhibition was seen against E faecalis and C albicans, 11mm was seen against Lactobacillus. At 100µL concentration, 12 mm of zone of inhibition was seen against Lactobacillus and E faecalis, 14mm was seen against S mutans, 15mm was seen against C albicans. The results of the test show that the gel is effective against S mutans, Lactobacillus and E faecalis compared to the commercial product.

The results show that the prepared gel anti-inflammatory test showed that at 10µL concentration, the percentage of inhibition was 45% for self made caries removal gel and 40 % for commercially available caries removal gel. At 20µL concentration, the percentage of inhibition for self made caries removal gel and commercially available caries removal gel were both 55%. At 30µL concentration, it was 70% and 65% for self made gel and commercially available gel respectively. At 40µL, 70% for self made gel and 68% for commercially available gel. At 50µL concentration, 85% and 70% respectively for self made and commercially available gel. This indicates that the percentage of inhibition was much higher for self made gel than that of commercially available gel. Our team has extensive knowledge and research experience that has translate into high quality publications (Neelakantan et al. 2013; Aldhuwayhi et al. 2021; Sheriff et al. 2018; Markov et al. 2021; Jayaraj et al. 2015; Paramasivam et al. 2020; Li et al. 2020; Gan et al. 2019; Dua et al. 2019; Mohan and Jagannathan 2014)

The results of the study favor self made papain bromelain decorated caries removal gel over commercially available gel. The gel is less cytotoxic, more anti-inflammatory and more antimicrobial against oral microbes S mutans, Lactobacillus and C albicans.

CONCLUSION

Self made caries removal gel is more effective against oral microbes S mutans, Lactobacillus and C albicans. Also it shows more anti inflammatory and less cytotoxic effect than commercial caries removal gel.

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CONFLICT OF INTEREST

No conflict of interest

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