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Cytotoxicity And Antimicrobial Activity Of Papain And Bromelain Gel 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: Two separate caries removal gels were prepared using papain and bromelain as their key ingredients. The cytotoxicity of the gels were tested by Brine Shrimp Lethality Assay. Antimicrobial activity was evaluated against S mutans, E faecalis, S aureus and C albicans oral bacteria.

Results: Antimicrobial properties of bromelain gel for Candida albicans, Enterococcus faecalis and Streptococcus mutans was comparable with that of control group antibiotic amoxicillin. Antimicrobial properties of papain gel for all the tested four oral microbes was not very much comparable with that of the control amoxicillin antibiotic group.

Bromelain showed a lesser cytotoxic effect with 80% 0f nauplii being alive after 24 hours compared to papain gel as 70% of the nauplii were alive after 24 hours.

Conclusion: Bromelain gel showed better antimicrobial and lesser cytotoxic effects compared to Papain gel.

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

INTRODUCTION

Dental caries is one of the most common diseases affecting the oral cavity. The treatment modality followed conventionally was drilling using dental burs and then caries was removed. In the past, the widely accepted concept of caries removal was " Extension for Prevention" by G V Black. This concept has changed in modern times with the invention of adhesive dentistry [(1)].

Caries removal with traditional dental burs is known to cause adverse biological effects and can cause pulpal reactions. It causes fear and anxiety in patients, especially children(2).There has always been a quest for painless caries removal methods like lasers, air abrasion, and chemico mechanical caries removal.(3) Chemico mechanical caries removal makes use of a proteolytic enzyme that digests and removes the carious tissues [(4,5)]

Papain is a proteolytic enzyme extracted from the raw fruit of the papaya plant. Proteolytic enzymes help break proteins down into smaller fragments. Papain contains bactericidal, bacteriostatic, and anti-inflammatory properties. Bromelain is another proteolytic enzyme that belongs to a group of protein-digesting enzymes obtained commercially from the fruit or stem of pineapple [(6)]. The function of proteases is to catalyze the hydrolysis of proteins to give amino acids. Both of them can be used as caries removal agents as an alternative to conventional Drilling methods.

Papain gel has been studied in literature to some extent [(7,8)] but the evidence on bromelain gel is very minimal. In our present study, papain and bromelain gel were self-prepared in the laboratory and were evaluated as caries removal agents. (9)But the cytotoxicity and antimicrobial activity of these two gels have not been checked.

The aim of the study is to evaluate the cytotoxicity and antimicrobial activities of self-made Papain gel and Bromelain gel against oral microbes

MATERIALS AND METHODS

Method of preparation of Papain/Bromelain Gel

Papain gel was self-prepared in the laboratory in our College using the following ingredients.

TABLE 1: Composition of Papain/Bromelain gel

Ingredients	Concentration For 5 Grams	Function
Papain/ Bromelain powder	250mg	Proteolytic agent
Alpha-d-tocopherol	0.5ml	Antioxidant
Carbopol+distilledH2O	200mg+2ml	Gelling agent
Methyl paraben	100mg	Preservative

0.8 gram of carbopol is homogeneously mixed with 8ml of water. To that 32 ml of water is added.1 gram of papain powder is mixed with 5ml of water separately and added to the carbopol solution. 2ml of alpha-d-tocopherol is added to the solution directly. Mix 0.2gram of methyl paraben separately in 5ml of distilled water and add to the above solution. Finally, mix using a homogeneous stirrer for 30 minutes to get freshly prepared papain gel.

Similarly using the same concentrations, bromelain gel can be prepared. So, papain and bromelain gels were prepared separately and stored in airtight containers. They have a shelf life of 1 year and can be stored at room temperature.

Figure 1: Carbopol solution

Figure 2: Papain Solution

Figure 3: Bromelain solution

Figure 4: Methyl paraben solution

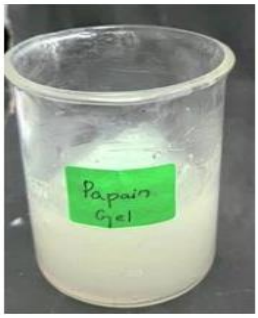

Figure 5: Papain Gel

Figure 6: Papain Gel

Figure 7: Bromelain Gel

Brine Shrimp Lethality Assay(Cytotoxicity Test)
Saltwater preparation

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water.

6 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

FIGURE 8: Twelve well tissue culture plate used for cytotoxicity test

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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.

RESULTS

Cytotoxicity Test of Papain Gel

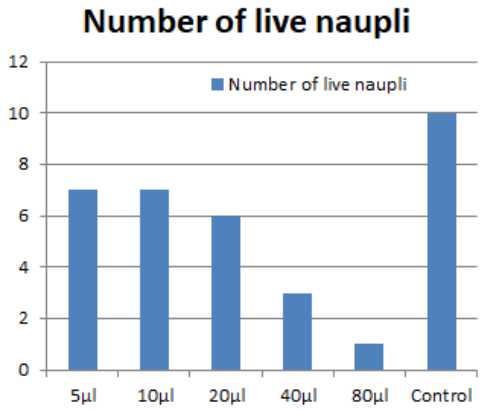

FIGURE 8: Graph showing the number of live nauplii for papain gel after incubation

TABLE 2: Table showing live and dead nauplii after incubation for papain gel

Concentration	Number of live nauplii	Number of dead nauplii
5µL	7	3
10µL	7	3
20µL	6	4
40µL	3	7
80µL	1	9
Control	10	0

Cytotoxicity Test for Bromelain Gel

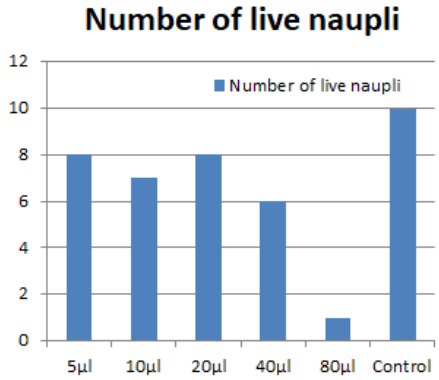

FIGURE 9: Graph showing the number of live nauplii for bromelain gel after incubation

TABLE 3: Table showing live and dead nauplii after incubation for bromelain gel

Concentration	Number of live nauplii	Number of dead nauplii
5µL	8	2
10µL	8	2
20µL	7	3
40µL	6	4
80µL	1	9
Control	10	0

Antimicrobial Activity of Papain Gel

FIGURE 10: Zone of inhibition of papain gel against C. albicans

FIGURE 11: Zone of inhibition of papain gel against E. faecalis

FIGURE 12: Zone of inhibition of papain gel against S. aureus

FIGURE 13: Zone of inhibition of papain gel against S. mutans.

TABLE 4: Zone of inhibition(in mm) of papain gel

Concentration	C albicans	E faecalis	S aureus	S mutans
25µL	9	9	9	9
50µL	9	9	9	12
100µL	10	9	10	13
Control(commercial product)	10	27	21	16

Antimicrobial Activity of Bromelain Gel

FIGURE 14: Zone of inhibition of bromelain gel against C. albicans.

FIGURE 15: Zone of inhibition of bromelain gel against E.faecalis.

FIGURE 16: Zone of inhibition of bromelain gel against S. aureus.

FIGURE 17: Zone of inhibition of Bromelain gel against S mutans.

TABLE 4: Zone of inhibition(in mm) of bromelain gel

Concentration	C albicans	E faecalis	S aureus	S mutans
25µL	9	10	10	13
50µL	11	25	14	15
100µL	13	26	15	16
Control(commercial product)	13	34	23	22

DISCUSSION

Dental caries by far affects the majority of the population. Traditionally, for over many years the concept followed for treatment was " Extension for Prevention" given by G V Black(10).Dental burs were the aid for such a treatment. But in modern times, this concept is considered destructive to the healthy remaining tooth structure. With the invention of adhesive dentistry, the concept is now on " Minimally Invasive Dentistry"(11).

Due to this concept and also the adverse effects caused by dental burs drilling like microcrack formation, adverse biological response to the pulp, and most importantly fear and anxiety among the patients especially children,(12) there has been interest in search of newer and lesser invasive methods for caries removal. Such methods include the use of lasers (13) air abrasion, atraumatic restorative treatment, Chemico mechanical caries removal agents etc.Herbal formulations have been used for different purposes in dentistry.(14,15)

Our study here is about chemico mechanical caries removal agents. The use of papain gel is not new to the field as it is a known proteolytic agent. Papain is a proteolytic enzyme derived from the raw fruit of papaya plants (16). They cause proteolysis,digest the proteins and break

them down into simpler amino acids.Papacarie is a commercially available papain gel that is used as a caries removal agent.(17)

Bromelain is a newer addition to the proteolytic enzymes [18]. They are derivatives of fruit or stem of pineapple. They perform similar functions to the papain enzyme and are also known to have bactericidal, bacteriostatic, and anti-inflammatory functions.(19)

The papain and bromelain gel in this study are self-prepared in our laboratory(20) and were evaluated as caries removal agents on extracted human permanent molar teeth. The efficacy on caries removal, time taken for caries removal, and surface characteristics was checked against drilling burs using polarized light microscopy(21).(22) This study evaluates the cytotoxicity and anti microbial activity of these papain and bromelain gels.

The cytotoxicity was evaluated by Brine Shrimp Lethality Assay (23). The cytotoxicity of papain gel showed that at the end of the incubation period, at lower concentrations of 5µL and 10µL, seven nauplii(shrimp larva) were alive after 24 hours. At a concentration of 20µL, six nauplii were alive, at 40µL three were alive and at a higher concentration of 100µL, only one live nauplii was found. This was compared with the control group in which no solution was added, and all the 10 nauplii were alive in brine solution. Survival of nauplii at lower concentrations and average survival at moderate concentrations shows that papain gel prepared was less cytotoxic at lower and moderate concentrations and can be considered safe for usage on living tissues.

In the case of bromelain, at lower concentrations of 5 and 10 µL, eight nauplii survived and at 20µL concentration also seven nauplii were alive. At a higher concentration of 40µL, six nauplii survived and at the highest concentration of 100µL only one survived. These results favor the bromelain gel compared to papain gel. Bromelain gel is less cytotoxic and safer to use on living tissues than papain but both the products were within the safe level of cytotoxicity.

In our study, Antimicrobial activity of papain and bromelain gel was tested against the most common four oral microbes, C albicans, E faecalis, S mutans, S aureus. In a study conducted by Bansal et al, antimicrobial activity pf neem and clove extract was done(24).Zone of inhibition was the indicator used for antimicrobial activity. Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. This can be used to measure the susceptibility of the bacteria towards the antibiotic. Larger the zone is, more sensitive the bacteria is to that antibiotic, smaller the zone, more resistant is the bacteria to that particular product.The antibiotic used in the study was amoxicillin.

In our study,papain gel at lower concentration showed a zone of inhibition of about 9mm for C albicans which was comparable with the control group(10mm).At higher concentration(100µL), zone of inhibition for S mutans was 13mm which was comparable with that of the control group(16mm). The prepared papain gel did not prove to be very effective against E faecalis and S aureus at both lower and higher concentrations.A study conducted by Bharadwaj et al. in 2012, compared the antimicrobial activity of papain gel,aloe vera gel, chlorhexidine gel(25)

Antimicrobial activity was tested for self-prepared bromelain gel as well. The zone of inhibition for C albicans at a concentration of 25µL was 9mm,at a higher concentration of 100µL , it was 13mm which was similar to that of the control group. For E faecalis, the zone was 10mm at 25µL concentration and 26mm at 100µL concentration, the control being 34mm. For S aureus, at 25µL ,the zone of inhibition was 10mm and 23mm at 100µL,control being 23mm. For S mutans, at 100µL ,16mm was the zone of inhibition ,control being 22mm. These values clearly indicate that bromelain gel exhibited very good anti microbial activity and was comparable with the commercially available product. Also, bromelain exhibited better antimicrobial properties against oral microbes than papain gel. Both papain and bromelain gels tested for toxicity showed values within the allowed limitation of toxicity. However, bromelain showed better properties compared to papain.

Our team has extensive knowledge and research experience that has translate into high quality publications(26–35)

CONCLUSION

Within the limitations of the study, we can conclude the following.

- Antimicrobial properties of bromelain gel for Candida albicans, Enterococcus faecalis and Streptococcus mutans was comparable with that of control group antibiotic amoxicillin.
- Antimicrobial properties of papain gel for all the tested four oral microbes was not very much comparable with that of the control amoxicillin antibiotic group.
- Bromelain showed a lesser cytotoxic effect with 80% 0f nauplii being alive after 24 hours compared to papain gel as 70% of the nauplii were alive after 24 hours.
- Overall, Bromelain gel showed better antimicrobial and lesser cytotoxic effects compared to Papain gel.

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CONFLICT OF INTEREST

No conflict of interest

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