Papaya extract as new endodontic irrigant

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Abstract: Introduction: The purpose of the study was to compare the antimicrobial efficacy of crude papaya extract with 5.25% Sodium hypochlorite(NaOCl) and 2% Chlorhexidine(CHX) against Enterococcus faecalis and Candida albicans at 1 min, 5 min and 15 min time intervals. Methods: #72 absorbent paper points were immersed in experimental suspensions (E. faecalis and C. albicans) for 5 mins and placed on test tubes to cover with irrigants (NaOCl, CHX, papaya extract, and distilled water. Distilled water was used as negative control. At 1 min, 5 mins and 15 mins intervals, 18 absorbent points for each irrigants were removed from contact with irrigants, individually transported to immerse in Letheen broth and incubated at 37°C for 48 hr. Microbial growth was analyzed by turbidity of culture medium and measured by spectrophotometer at 560 nm. Inoculum obtained from Letheen broth was serially diluted for spread plate. Colony Count was done for viable cell count. Result: The result of turbidimetric measurement showed crude papaya extract to be effective against E. faecalis after 5 mins whereas it was effective against Candida albicans at all time intervals. The result of viable cell count of crude papaya extract shows gradual decrease of colony count from 1 min to 15 min against E. faecalis and Candida albicans. Conclusion: Papaya extract demonstrated potent antimicrobial activity against E. faecalis and Candida albicans comparable to 5.25% NaOCl and 2% CHX at 5 min and 15 min time intervals.

Keywords - Antimicrobial, C.albicans, E.faecalis, papaya extract

I. Introduction

Success of root canal treatment depends upon complexity of the pulp canal space. Its proper debridement and disinfection is core requisite for the endodontic success. Hence irrigation of root canals with antibacterial solutions is considered as an integral part [1]. Researchers have shown that even after meticulous and advanced instrumentation substantial part of root canal wall is left un-instrumented [2, 3]. Hence, irrigation may be considered as the primary method to clean and disinfect these part of the root canal system [4, 5].

Root canal irrigants should have antibacterial and antifungal properties, in addition it must not be toxic to periapical tissues while in contact with them. To combat the toxicity of synthetic irrigants, use of natural plant extracts as endodontic irrigants is the growing trend of research in dentistry [6, 7]. One of potent natural plant extract with well known antibacterial and antifungal property is Carica papaya [8]. Its leaves extract contains folic acid, vitamins B12, A and C, alkaloids, saponins, glycosides, tannins, and flavonoids [8,9]. These secondary metabolites have bactericidal, bacteriostatic and anti-inflammatory characteristics [10]. Flavonoids provide potential protection against oxidative and free radical damage. They are called as "biological response modifiers" as they modify the body's reactions to allergens, carcinogens and viruses. Hence they have been described as having anti-inflammatory, anti-allergic, anticarcinogenic, antioxidant and antiviral properties [11, 12].

In root canal, Enterococcus faecalis and Candida albicans dominate primary lesion as well as these are recalcitrant and are persistent in periradicular lesions even after root canal treatment. This is because E. faecalis has ability to survive extreme environment, resists different antimicrobial even high pH of calcium hydroxide and can bind to dentin [13, 14]. C. albicans is also versatile microbe, it can adapt to range of pH, changes gene expression according to environmental conditions, adheres to variety of surfaces, produces degradative enzymes and changes morphologic forms to evade the immune system[15]. In addition to these virulence factors, complexity of root canals promotes bacterial growth and limits the action of disinfectants adding more
challenges for obtaining a disinfected state of the root canal system[13,16]. Hence, recent studies have focused on evaluating the effectiveness of different root canal irrigants and medicaments against these virulent microbes.

In the verge of the search for potential ideal endodontic irrigants, different synthetic and herbal irrigants are field of research these days. As papaya extract is used as a potent antibacterial and antifungal agent with proven medicinal property [8], but its efficacy against root canal pathogens is still unclear. Thus, the objective of the study was to evaluate the antimicrobial and antifungal potential of papaya extract and compare with the popular endodontic irrigants i.e. sodium hypochlorite and chlorhexidine.

II. Material And Method

2.1 Preparation of papaya extract: Healthy/ disease free, mature, fresh plant leaves of Carica papaya were handpicked and authenticated by Institute of Agriculture and Animal Sciences (IAAS), Tribhuvan University, Bhairahawa, Nepal. The leaves were surface sterilized in 0.1% mercuric chloride for 5 min and air dried at room temperature for 24 hrs. Sterilized leaves were ground fine by electric blender. The crude extract obtained was centrifuged at 4000 rpm for 30 mins. The supernatant layer obtained was filtered through Whatman filter paper no: 1. The extract was then stored at 4°C.

2.2 Inoculum preparation: Standard strain of E. faecalis (ATCC 29212) and C. albicans (ATCC 10231) was revived from lyophilized state. The inoculums prepared of test isolates were matched with 0.5 MacFarland to get concentration of $1.5 \times 10^8$ cells/mL.

2.3 Experiment: 72 sterile paper points #50 (Dentsply, Maillefer) were taken and divided into 2 groups of 36 paper points for 2 test organisms: E. faecalis and C. albicans. For each irrigants used i.e. 5.25% NaOCl, 2% CHX, crude Papaya extract and Distilled water, 3 paper points were used for specified time interval of 1 min, 5 min and 15 min.

2.4 Direct Contact Test: The paper points were immersed individually into test tubes containing 5 ml of freshly prepared bacterial suspension of E. faecalis and C. albicans respectively for 5 mins. Paper points were transferred to test tubes containing irrigating solution for different time intervals i.e. 1 min, 5 min and 15 min respectively. After definite contact time, paper points were immediately transferred into test tubes containing 5 ml of Leethen broth (neutralizing solution) and incubated at 37ºC for 48 hrs.

2.5 Interpretation of microbial growth: After incubation, test tubes were observed for the presence of turbidity and optical density was measured using Spectrophotometer (Spectrochem-i, Double Beam 2800CE, New Delhi) at 560 nm wavelength. Gram-staining was done to check unwanted microbial growth. Spread plate method was used for the viable cell count. The tubes were serially diluted up to $10^6$ and 10 µl of solution of was spread on to Nutrient agar and Sabourad’s Dextrose agar plate. The plates were incubated at 37ºC for 24-48 hrs. Colony morphology was studied and gram staining was done and biochemical tests were performed to confirm the organism isolated. The colonies grown on the media were counted with the help of Colony counter machine (Optics Technology, New Delhi).

2.6 Data analysis: Experiment was conducted in triplicate form. Data obtained was expressed as mean ± standard deviation. Statistical evaluation of turbidimetric analysis was done using ANOVA test and individual analysis of each group at each time interval was done by Tukey’s test. Analysis of viable cell count was done using independent ‘t’ test.

III. Result

The result of crude papaya extract against E. faecalis showed good antimicrobial property by both colony count and turbidimetric measurement, there is significant decrease in mean CFU count from 112 ± 3 to 1 ± 1 (p< 0.05) from 1 min to 15 min time intervals (Fig 1). Similarly, the mean optical density also decreased significantly from 0.693 ± 0.023 to 0.550 ± 0.03 (p<0.05) in 1 min to 15 min (Table 1). Comparison within each time interval shows 5.25% NaOCl to be most effective irrigants as its shows significant mean difference of optical density at all-time intervals (p< 0.05) whereas 2% CHX and crude papaya extract shows significant mean difference of optical density only after 5 min(p< 0.05) (Table 2).
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Figure 1: Comparison of mean colony forming unit (CFU) of papaya extract and control against E. faecalis at different time intervals.

Table 1: Mean Optical density (OD) (mean ± SD) of different irrigant against E. faecalis and C. albicans

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time intervals</th>
<th>E. faecalis</th>
<th>C. albicans</th>
<th>p-value* of E. faecalis group</th>
<th>p-value* of C. albicans group</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>1 min</td>
<td>0.403±0.006</td>
<td>0.007±0.001</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.347±0.020</td>
<td>0.005±0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>0.008±0.002</td>
<td>0.002±0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX</td>
<td>1 min</td>
<td>0.009±0.001</td>
<td>0.008±0.001</td>
<td>0.008</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.004±0.000</td>
<td>0.005±0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>0.004±0.002</td>
<td>0.002±0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papaya</td>
<td>1 min</td>
<td>0.720±0.023</td>
<td>0.333±0.041</td>
<td>0.008</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.693±0.026</td>
<td>0.217±0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>0.550±0.030</td>
<td>0.000±0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>1 min</td>
<td>0.623±0.015</td>
<td>0.547±0.025</td>
<td>0.355</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>0.580±0.060</td>
<td>0.550±0.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>0.587±0.006</td>
<td>0.550±0.010</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA test was performed to identify the significant groups at 5% level

Table 2: Comparison of mean optical density (OD) of different irrigants within the groups against E. faecalis

<table>
<thead>
<tr>
<th>Irrigants</th>
<th>Contact time (I) groups</th>
<th>Contact time (J) groups</th>
<th>Mean Difference in Optical Density between Contact time groups (I-J)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>1 min</td>
<td>5 min</td>
<td>0.056±0.006*</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>15 min</td>
<td>0.339±0.000*</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>1 min</td>
<td>-0.395±0.000*</td>
<td>0.000</td>
</tr>
<tr>
<td>CHX</td>
<td>1 min</td>
<td>5 min</td>
<td>-0.003±0.003</td>
<td>0.965</td>
</tr>
<tr>
<td></td>
<td>5 min</td>
<td>15 min</td>
<td>-0.005±0.003*</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>1 min</td>
<td>0.005±0.006*</td>
<td>0.012</td>
</tr>
</tbody>
</table>
For *C. albicans* also the papaya extract showed appreciable antimicrobial property as the mean colony count decreased from 2.67 ± 1.15 to 0.00 (p<0.05) in 1 min to 15 min (Fig 2).

Similarly, mean optical density also decreased significantly from 0.333 ± 0.041 to 0.00 (p<0.05) from 1 min to 15 min (Table 1). Comparison within each time interval shows comparable antimicrobial efficacy of 5.25% NaOCl, 2% CHX and papaya extract against *C. albicans* with significant mean difference of optical density at all-time intervals (Table 3).

### Table 3: Comparison on mean optical density (OD) of different irrigants within the group against *C. albicans*

<table>
<thead>
<tr>
<th>Irrigants</th>
<th>Contact time</th>
<th>Mean Difference in optical density between contact time groups (I-J)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>(I) group</td>
<td>(J) group</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>5 min</td>
<td>0.00233333³</td>
<td>0.041</td>
</tr>
<tr>
<td>5 min</td>
<td>15 min</td>
<td>0.0026667³</td>
<td>0.023</td>
</tr>
<tr>
<td>15 min</td>
<td>1 min</td>
<td>-0.0050000³</td>
<td>0.001</td>
</tr>
<tr>
<td>CHX</td>
<td>1min</td>
<td>5min</td>
<td>0.0030000³</td>
</tr>
<tr>
<td></td>
<td>5min</td>
<td>15min</td>
<td>0.0026667³</td>
</tr>
<tr>
<td></td>
<td>15min</td>
<td>1min</td>
<td>-0.0056667³</td>
</tr>
<tr>
<td>Papaya</td>
<td>1min</td>
<td>5min</td>
<td>0.11667³</td>
</tr>
<tr>
<td></td>
<td>5min</td>
<td>15min</td>
<td>0.21667³</td>
</tr>
</tbody>
</table>

* Tukey HSD test was performed to identify the significant groups at 5% level. *The mean difference is significant at 0.05 level.
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<table>
<thead>
<tr>
<th></th>
<th>15min</th>
<th>1min</th>
<th>0.000</th>
<th>0.987</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1min</td>
<td>5min</td>
<td>-0.003333</td>
<td>0.003333</td>
</tr>
<tr>
<td>5min</td>
<td>15 min</td>
<td>0.000000</td>
<td>1.000</td>
<td>0.987</td>
</tr>
<tr>
<td>15min</td>
<td>1min</td>
<td>0.003333</td>
<td>0.987</td>
<td>0.000</td>
</tr>
</tbody>
</table>

# Tukey HSD test was performed to identify the significant groups at 5% level.* the mean difference is significant at 0.05 levels.

IV. Discussion

Till date NaOCl and CHX have gained popularity as most effective irrigants in combating persistent endodontic infection because of their high antimicrobial efficacy. However, unpleasant taste and odor, toxicity, allergic potential, resorption, inability to remove smear layer are the main disadvantages of NaOCl [17]. In addition, Grigoratos D et al. [18], reported decrease in flexural and elastic strength of dentin after 2 hr submersion in NaOCl. The toxic effect of 5.25% NaOCl is postulated to be greater than 2% CHX as reported by Oncag O et al. [19]. CHX has a reasonably wide range of activity against aerobic and anaerobic organisms as well as the Candida species. But the presence of inflammatory exudates and killed microorganisms can inhibit the action of chlorhexidine in root canals and in addition, it is incapable to dissolve tissues [4]. These undesirable characteristics of current synthetic irrigants leads to opt for alternatives like use of natural plant extracts as endodontic irrigant.

For the preparation papaya leaf extract manual method was used as suggested by Oyagade et al [20].This technique provides extraction of enzyme or protein in a more delicate or softer method and minimizes the use of chemical compounds as enzymes and proteins are prone to denaturation and are thermolabile. Time intervals of 1 min, 5 min and 15 min were taken, as minimum irrigation time is 1 min and maximum 15 min while performing endodontic treatment. So the aim was to evaluate whether the irrigants have efficient antimicrobial property in the given time intervals.

In present study, direct contact test (DCT) was used to assess the antibacterial activity as it measures the antimicrobial effect regardless of the solubility and the diffusion of the antimicrobial components. The results of DCT are more quantitative and reproducible when compared to those of the Agar Diffusion Test [21]. Spectrophotometric measurement was used to measure the turbidity as it indirectly measures all bacteria (cell biomass), dead and alive. Hence, to evaluate viable cell count left after antimicrobial activity, colony count method was performed in addition to turbidimetric measurement. Moreover, the test micro-organisms i.e E. faecalis and C. albicans in the methodology are used in planktonic form because the test was conducted in an intention of primary screening of antibacterial and antifungal property of papaya extract.

The result of viable cell count showed that 5.25% NaOCl and 2% CHX had no colony forming unit at 15 min whereas, the study conducted by Retamozo et al showed effective irrigation regimen was 5.25% NaOCl at 40 minutes [25].

The antibacterial activity of CHX was delayed due to the adherence of antiseptic particles to the bacterial cell wall which takes longer than 5 min [26]. Hence, the clinical implication of these findings is that 2% CHX and papaya extract should be in direct contact with the infected dentinal surface for a prolonged time (>5 min) in order to achieve their maximum antibacterial effect against E. faecalis.

The papaya extract showed appreciable antimicrobial property against both E. faecalis and C. albicans. These results are in agreement with the study done by Silva et al. [22] and Retamozo et al showed effective irrigation regimen was 5.25% NaOCl at 40 minutes [25].

V. Conclusion

Within the limitation of the in vitro study, it can be concluded that the antimicrobial efficacy of crude papaya extract was comparable to 5.25% NaOCl and 2% CHX at 15 min time interval for both E. faecalis and C. albicans. Hence, papaya extract can be hypothesized to be more beneficial as in context of patient. The
magnitude of this effect was influenced by the experimental method, characteristics of the microorganisms and the exposure time. For conclusive result further clinical studies with large sample size should be carried out.

Acknowledgements

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References