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Comparative Evaluation of Tissue Dissolution Efficacy of Azadirachta indica Irrigant and Sodium Hypochlorite – An in vitro Study

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Abstract

Objectives: This study aimed to evaluate the tissue dissolution efficacy of Azadirachta indica (A. indica) irrigant and sodium hypochlorite. **Materials and Methods:** 40 bovine muscle tissue specimens of equal dimensions were used in the experiment. Based on tissue specimen treatment, they were divided into four test groups - 3%NaOCl (Group I), 7.5% A. indica irrigant (Group II), alternative use of 3%NaOCl and 7.5% A. indica irrigant (Group III) and control group as distilled water (Group IV). Tissue specimens were weighed before and after placing in test solutions at 2 different time intervals (30,60 minutes). **Results:** The results revealed that 3%NaOCl showed highest tissue dissolution followed by alternative use of 3%NaOCl. and A. indica irrigant, A. indica alone and distilled water (Group I >III>II>IV). The larger the duration of contact with irrigant better is the tissue dissolution. **Conclusion:** 7.5% A. indica irrigant alone does not have any tissue dissolving property but in combination it acts as a neutral solution neither increasing nor decreasing the capability of 3%NaOCl.

Keywords: Azadirachta indica, NaOCl, Smear Layer, Tissue Dissolution

1. Introduction

During chemo mechanical preparation, root canal complexities results in approximately 40-50% of uninstrumented pulp space leading to incomplete debridement of pulp tissue. Therefore, irrigation with a tissue dissolving antimicrobial solution is a prerequisite for reaching inaccessible pulp spaces. NaOCl has set up a standard in tissue dissolving activity and antimicrobial efficacy. However, it has certain drawbacks like unpleasant taste, highly irritant to periapical tissues, corrosive action and inability to remove the smear layer. All that man needs for a healthy living has been provided in nature and it is a challenge for science to find it. Development in alternative medical research has led to development of herbal irrigants. Invitro studies showed successful use of ethanolic neem leaf extractas root canal irrigant with demonstrable antimicrobial efficacy. However, no scientific data is available regarding tissue dissolution capability of neem leaf extract. So, the present study aimed evaluate tissue dissolution efficacy of A. indica irrigant and sodium hypochlorite.

2. Methodology

2.1. Sample preparation

Fresh bovine meat was used for the tissue samples in this experiment. It was obtained from a slaughterhouse. Before use tissue was rinsed thoroughly with distilled

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water to remove excess blood and was placed in the refrigerator for 1 week at 4^{0}C. This study did not in any way influenced the premortal fate of the animals or the slaughtering process. Therefore, this investigation was not classified as an animal study and the institutional ethics committee has given ethical clearance number (IES/VDC/MDS 16/ENDO 07). Frozen tissue was then cut into equal-sized pieces of 4×4×2 mm using a surgical blade no.15. Tissue specimen mass was then measured before treatment using an analytical balance (ELB-300, Shimadzu Corporation.) in airtight container. There was no significant difference in the sample mass used for each group, with an average initial mass of 50±10 mg[1–3].

2.2. 7.5% Azadhiracta indica extract preparation

Fresh leaves were washed with distilled water and dried under shade for 10-12 days. All the material was ground in an electric grinder to produce a powder. Neem extract was prepared by macerating 7.5gms of dry powder of neem leaves with 100ml of 70%(w/v) ethyl alcohol for a week in flask with occasional shaking. The flask was kept in the dark to avoid effect of light on the active ingredients of the neem. The extract was then filtered and stored in a container[4]. The filtration was done by tightly packing the cotton in the funnel which was placed in the conical beaker. The cotton acts as a multiple compartment to filter the debris.

Irrigation solutions were grouped as follows:

Groups

Group I: 3% NaOCl

Group II: 7.5%Azadirachta indica (A. indica) irrigant

Group III: Alternative use of 3%NaOCl and 7.5% A. indica irrigant

Group IV: Distilled water

2.3 Dissolution procedure

Group I, II, IV: Specimens of single solution irrigation were placed in respective test tubes containing 5ml of solution for 30 and 60 minutes and weighed in respective intervals.

Group II: In this group, test solutions 2.5ml of 3%NaOCL and 2.5ml of 7.5% A. indica irrigant were taken. The sample was placed alternatively for every 7.5 minutes in respective solutions.

2.4 Immersion of samples in testing solutions

40 tissue samples were divided randomly into four experimental groups (n = 10). Bovine tissue specimens in each group were placed in individual test tubes containing the test solution, and all the test tubes were placed on an agitator (REMI RS-12 plus) to agitate the test solution for 60 minutes. All the specimens were washed with distilled water to remove precipitate, blotted on a filter paper, dried and then weighed after 30 and 60 min. All the samples were weighed by a single investigator who was unaware of the test solutions. The difference in weight of the tissue sample before and after exposure to the test solution was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss or gain[2,3].

Mean loss of weight of tissue specimens was calculated as follows =

Difference in the weight of tissue sample before and after exposure to the solution × 100/Original tissue weight (gms)

2.5 pH measurement

Before the procedure pH of fresh solutions were noted by using pH indicating papers (MERCK company). The test solutions were collected in beakers after tissue treatment and pH was noted.

3. Results

Statistical analysis of all the obtained data was done using one-way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) tests using the SPSS software version 16.0 (SPSS Inc.., Chicago III).

The ANOVA test (Table 1) revealed significant difference among various segments of groups. The results of Tukey's HSD test (Table 2) showed that, there was statistically significant difference between all the subgroups except in the situation related to group II and III. There was no significant difference at 30 minutes but there was significant difference at 60 minutes.

Table 1: Comparison of percentage lost tissue weight of different study groups using one-way ANOVA

Groups	Mean percentage lost tissue weight after 30 minutes (in %)	Mean percentage lost tissue weight after 60 minutes (in %)	p value
Group I	41.5	59.8	0.00
Group II	12.3	16.3	
Group III	17.9	33.3	
Group IV	0.00	0.00	

*The mean difference is significant at the 0.05 level.

Table 2: Multiple comparison percentage lost tissue weight in between study groups using Tukey HSD test

Groups comparison	Mean difference in percentage lost tissue weight after 30 minutes	p value	Mean difference in percentage lost tissue weight after 60 minutes	p value
Group I Group II Group III Group IV	29.2 23.6 41.5	0.00 0.00 0.00	43.5 26.5 59.8	0.00 0.00 0.00
Group II Group III Group IV	-5.6 12.3	0.204 0.00	-17 16.3	0.00 0.00
Group II Group IV	17.9	0.00	33.3	0.00

*The mean difference is significant at the 0.05 level.

4. Discussion

The success of endodontic treatment depends on the eradication of microbes from the root-canal system and prevention of reinfection. According to Schilder cleaning involves emptying of root canals of vital pulp or necrosed pulp. This sanitization process is determinant of success. But, the complexities of root canal system may not allow mechanical debridement of the pulp tissue with mere endodontic files.

The most commonly used root canal irrigant is NaOCl in concentrations between 0.5% and 6%. Despite the excellent tissue-dissolving capacity and antimicrobial activity, the drawbacks of NaOCl include the unpleasant taste, toxicity, and are incapable of removing the smear layer, as it dissolves only organic material.

NaOCl presents a relatively higher surface tension (48.90 mJ/m2). This high surface tension limits the penetration of the solution into canal irregularities and deep into the dentinal tubules, reducing the quality of the debridement. The presence of inactivating substances like dentin collagen, pulp tissue, periapical exudate and microbial biomass limit the NaOCl effectiveness and also has detrimental effect on dentin elasticity, flexural strength.

Hence the quest for searching ideal biocompatible root canal irrigant is on. Since antiquity, herbs and spices derived from medicinal plants rich in minerals and organic matter remain the mainstay of about 75–80% of the world's population for health care and gaining popularity in developed and developing countries.

A. indica is a useful traditional medicinal plant in India known as "Indian neem'. The tree still regarded as traditional "village dispensary" in India. Each part of the tree has some medicinal property. From this crude principle some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Nimbidin and sodium nimbidate possess significant dose dependent antibacterial activity because of inhibition of cell wall synthesis and anti-inflammatory activity[5].

According to Nayak Aarthi et al., alcoholic extract of neem leaf showed significant antibacterial activity against S. mutans, E. faecalis and significant antifungal activity against C. albicans. The minimum inhibitory concentration of neem extract is 7.5% w/v which was used in the present study[4].

Ethanolic neem leaf extract has low toxic potential and its safety tolerance limit is 176 mg/kg body weight. 5 mL of the Ethanolic neem leaf extract irrigant was used in this study, which was within the safety criteria[6,7].

Since antimicrobial efficacy of A. indica irrigant has been proved, there is a need to evaluate other ideal requirements of irrigant i.e. tissue dissolution, to use it in routine clinical practice.

Bovine muscle was used in the present study and tissue dissolution was evaluated by using percentage loss of tissue specimen weight. In the present study, use of muscle allowed us to standardize the surface area of tissue samples. If we use pulp tissue, it would have been difficult not only to obtain sufficient tissue and also to standardize the surface area of samples.

Many authors reported that concentration, time, and pH play important roles in determining the amount of tissue dissolution. So, In the present study surface area, volume of solution, time and agitation were taken into consideration and were standardized[1,8–11].

The tissue-solution complex was agitated by using a vibrator throughout the test period to simulate fluid movement during root canal instrumentation. It was shown that the influence of slow mechanical agitation of tissue systems on the dissolution of tissue gave striking results and change in the pH was recorded[11,12].

Group I: 3%NaOCl

pH of 3%NaOCl was 13. According to the results, 3%NaOCl showed highest mean tissue dissolution among the Groups at both intervals with 41.5% at 30 minutes and 59% at 60 minutes followed by Group II, III and Group IV. It showed statistically significant difference with all the Groups at both intervals (Table 1, 2) ($p<0.05$). The possible reason for tissue weight loss with NaOCl might be free chlorine ions which turned pH of the solution acidic resulting in tissue dissolution and the more the time, more will be the free chlorine and the dissolution was more in case of present group. These results are in accordance with previous studies[10].

Group II, Group III:

In Group II pH of the solution was 7-8. The mean tissue dissolution for Group II were 12.3%, 16.3% and at 30, 60 minutes respectively.

In Group III the pH of 3% NaOCl was 13 and 7.5% A. indica irrigant was 7-8. The mean tissue dissolution for Group III were 17.9%, 33.3% at 30 and 60 minutes respectively. The results showed that there was statistically significant difference in percentage loss of tissue weight at both intervals when compared with Group I and IV (Table 1, 2) ($p<0.05$). In case of alternative use, there was no statistically significant difference at 30 minutes but there was statistical significant difference at 60 minutes ($p>0.05$). There was no change in pH in 7.5% A. indica irrigant and the change in pH of 3%NaOCl was 10-11.

In case of Group II, minor change in the percentage loss of tissue weight was observed. There was no change in pH with A. indica irrigant. On eliminating the possible reasons, it was hypothesized that the change in the tissue weight might be due to ethanol which is a major component of the extract. It is a non-additive precipitant fixative, which fixes the tissue by dehydration and precipitation of proteins and dissolves some of fatty acids[13].

According to previous studies Ca(OH)2 causes the tissue to swell and thus become more accessible to the NaOCl. But in the present study alternative use of 3% NaOCl and 7.5% A. indica irrigant, the use of 7.5% A. indica irrigant might have caused shrinkage of tissue specimen in combination that might have prevented further action of 3%NaOCl. In Group IV the results showed there was no change in tissue weights at both time intervals. It supports the previous reports that distilled water does not have capability of tissue dissolution. The results of the present study correlate with other in vitro studies[9].

5. Conclusion

Within the limitations of the study

1. Group I - 3%NaOCl showed highest tissue dissolving ability followed by alternative use of 3%NaOCl, 7.5% A. indica irrigant and 7.5% A. indica irrigant alone.
2. 7.5% A. indica irrigant alone does not have any tissue dissolving property but in combination it acts as a neutral solution neither increasing nor decreasing the capability of 3%NaOCl.
3. As the time increases, there was increased dissolution and decreased pH.

As the major quantity of pulp would be removed by extirpation and mechanical instrumentation, only the tissue in inaccessible area need to be addressed by irrigating solution. According to the present study the

combination of 3%NaOCl and 7.5% A. indica irrigant was able to dissolve the pulp tissue up to 40% but lesser than 3%NaOCl. Newer irrigating solutions like MTAD, Qmix etc., ethanolic neem leaf extract can be used in combination with hypochlorite for synergistic action.

Upon considering the advantage of combination of 3%NaOCl and 7.5% A. indica irrigant i.e. antimicrobial, anti-inflammatory the combination can be recommended in the clinical practice.

The ethanol which is a major component of A. indica irrigant showed its action as a surfactant. It might increase the flow properties, enhancing tissue dissolution of NaOCl in root canal dentine and however the tooth model was not considered in the present study. This is one of the limitation of the study.

So, further studies should be done to evaluate the effect of A. indica on removal of smear layer, flexural modulus of dentin, ease of removal from the root canal prior to obturation and its effect on the obturating material in in vivo conditions.

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