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An In-Vitro Comparative Evaluation in the Effectiveness of Six Different Disinfectant Solutions on Extracted Human Teeth

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Abstract

Aim: In-vitro comparative evaluation of antimicrobial effectiveness of six disinfectant solutions; 10% Formalin, 5.25% Sodium hypochlorite, 3% Sodium hypochlorite, 5% Vinegar, 0.1% Thymol, 3% Hydrogen peroxide on extracted human teeth.

Materials and Method: Sixty extracted human permanent teeth were randomly assigned into six groups, 10 teeth each. Group A: 10% Formalin, Group B: 5.25% Sodium hypochlorite, Group C: 3% Sodium hypochlorite, Group D: 5% Vinegar, Group E: 0.1% Thymol, Group F: 3% Hydrogen peroxide. Specimens were then incubated in the selected disinfectant solutions as per the groups assigned for 7 days at room temperature. Specimens were then transferred in to Peptone water and incubated again at 37°C for 3 days.

Aliquots from each sample were then cultured on Nutrient agar culture media for 48 hours, to check for the microbial growth. Colony-Forming Unit's (CFU's) were then counted using Digital colony counter and data was recorded and tabulated.

Results: Chi-Square test was used to compare CFU's among the six groups. P<0.001, was considered as statistically significant. Complete microbial inhibition was observed in Group A (10% Formalin), Group D (5% Vinegar) and Group F (3% Hydrogen Peroxide) with '0' CFU's. All the other groups; Group B (5.25% Sodium hypochlorite), Group C (3% Sodium hypochlorite), Group E (0.1% Thymol) exhibited substantial microbial growth in all its samples with 10^5 CFU's.

Conclusion: 5% Vinegar, 10% Formaldehyde and 3% Hydrogen peroxide solutions were found to be highly effective as disinfectant solutions on extracted human teeth.

Keywords: Disinfection, Extracted human teeth, Microbial colonies, Vinegar.

Introduction

Extracted human teeth remain as irreplaceable source for learning in dental education and research purpose, especially in pre-clinical training for tooth preparation, root canal treatment, restorative procedures and testing of bonding of restorative materials to tooth structure. Extracted human teeth provides dental students a much needed opportunity to learn and master hand skills, something no artificial teeth can substitute precisely.[1,2] However, these biological specimens (extracted human teeth) pose a substantial risk for disease transmission, necessitating stringent disinfection protocols to ensure the safety of students, faculty members and researchers.[3]

The Centre for Disease Control and Prevention and the American Dental Association mandated that extracted human teeth used for educational or research purposes must be treated as infectious material. Teeth can harbor a variety of pathogens including Hepatitis B, Hepatitis C, HIV (Human Immunodeficiency Virus), Mycobacterium Tuberculosis and a host of aerobic and anaerobic bacteria.[4,5] These pathogens can persist even after extraction due to the organic and inorganic structure of the tooth, which offers niche for microbial survival and biofilm protection. The risk of infection through aerosols, accidental injury or surface contact with extracted human teeth highlights the critical need for validated disinfection techniques.[6]

Over the years, various physical and chemical methods have been employed to completely disinfect extracted human teeth. Physical methods such as autoclaving, boiling and gamma radiation offers excellent microbial elimination, but they compromise physical structure, colour and hardness of these teeth rendering them unsuitable for certain research applications.[7] On the other hand, chemical disinfectants are frequently preferred due to their simplicity, cost-effectiveness and ease of use. Commonly used chemical disinfectant solutions includes; 10% formalin, sodium hypochlorite (NaOCl), hydrogen peroxide, glutaraldehyde, thymol and more recently, natural alternatives like Vinegar (acetic acid).[3,8] Among these, 10% Formalin solution has long been considered as the gold standard for disinfection of extracted human teeth, primarily due to its broad-spectrum antimicrobial activity and tissue fixation properties.[5] However, formalin is known for its toxic, irritant and carcinogenic properties, raising serious concerns over its safe use both in academic and research purpose.[9] Thus, restricting its use and encouraging students, clinicians and researchers to seek other safer, highly effective alternate disinfectant solution.

Sodium hypochlorite in concentrations of 0.5% to 5.25% is widely used as disinfectant solution due to its high

oxidizing ability and tissue-dissolving potential. However, its effectiveness depends heavily on its concentration and exposure time. Moreover, its excessive concentration causes alteration in the tooth dentinal surface and discoloration.[1] 3% Hydrogen peroxide solution is another oxidative agent used as disinfectant solution in many dental research studies. However its standalone disinfecting potential remains debatable and it also causes alteration of dentinal surface.[2]

Thymol is an essential oil with known antibacterial properties, safer, more biocompatible as disinfectant solution. However, limited literature exists validating its antimicrobial efficacy on extracted human teeth.[8] In contrast, Vinegar (acetic acid), a common household product has emerged as a viable alternative due to its potent bactericidal and fungicidal properties. Its non-toxic and ease of availability makes it as an ideal substitute to formalin as disinfectant solution of extracted human teeth in both institutional settings and research purposes.[3]

So, the aim of our In-vitro study was comparative evaluation of antimicrobial effectiveness of six disinfectant solutions; 10% Formalin, 5.25% Sodium hypochlorite, 3% Sodium hypochlorite, 5% Vinegar, 0.1% Thymol, 3% Hydrogen peroxide on extracted human teeth**.**

Materials and Method

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics after obtaining the Institutional Ethical Committee clearance; TIDSHRC/ IEC/TEC No. 2025-26/S001. Sixty extracted human permanent maxillary and mandibular molar teeth were obtained and stored in 0.9% Normal saline (Nivy Remedies Pvt. Ltd Hatbaria, West Bengal) until use. Only the intact and non-caries teeth, extracted due to periodontal disease were included. All specimens were cleaned of superficial debris, calculus, residual tissue tags using ultrasonic instruments and were used in our study within one month of extraction.

All teeth (n=60) were randomly divided into Six Groups, 10 teeth each. Group A: 10% Formalin (Avarice Laboratories Pvt. Ltd., Ghaziabad, India), Group B: 5.25% Sodium hypochlorite (Prime Dental Products, Navi Mumbai, India), Group C: 3% Sodium hypochlorite (Prime Dental Products, Navi Mumbai, India), Group D: 5% Vinegar (Lall's, Arora fruits and Pickles Pvt. Ltd., Jamshedpur, Jharkhand), Group E: 0.1% Thymol (Fisher Scientific, Qualigens Fine Chemicals, Navi Mumbai, India), Group F: 3% Hydrogen peroxide (Pioma Chemtech Inc. Ujjain, India) used as disinfectant solutions (Figure no.1).

Figure 1: Six Disinfectant solutions used in our study; A: 10% Formalin Solution, B: 5.25% Sodium hypochlorite Solution, C: 3% Sodium hypochlorite Solution, D: 5% Vinegar Solution, E: 0.1% Thymol Solution, F: 3% Hydrogen peroxide Solution.

Specimens were then completely immersed in test tubes (Religlas, Jain Laboratory Glassware Company Ambala Cantt, India), each containing 10 ml of the respective disinfectant solution as per the groups assigned, incubated for 7 days at 25°C. The disinfectant solutions were then discarded and teeth were immersed in test tubes containing Normal saline, vortexed on a Vortex

shaker (Remi CM, Scientific Vasai, Maharashtra, India) for 60 seconds. Under fully aseptic conditions, each tooth was again placed in test tubes containing 10ml of 0.1% Peptone water (Himedia Industries Inc. Mumbai India) and incubated (Incubator, Esteem Industries Inc. Baddi, India) at 37 °C for 72 hours. If, No turbidity seen in test tubes, considered as effective disinfection. Whereas, if evidence of turbidity seen, it was indicative of microbial growth, considered as ineffective disinfection.

Aliquots from each test tube were streaked using Inoculation Loop (A1 Engineering Works, Mumbai, India). The loop was first heated red hot, allowed to cool down to room temperature, later dipped into each test tube containing teeth samples and streaked onto the Petri dish (Borosil Scientific, Mumbai, India) containing Nutrient agar culture media (HiMedia Industries Inc. Mumbai, India) as per the groups assigned, incubated at 37°C for 48 hours. The entire procedure was carried under strict aseptic conditions. Nutrient broth was used as control, as the Standard culture media against which the 'test' culture media were compared.

Colony forming units (microbial colonies) were then counted on all agar plates using Digital colony counter (SECOR, Scientific Engineering Corp, India). All the positive samples exhibited microbial growth of more than 10^5 Colony-Forming Unit (CFU)/ml on agar media. The absence of any visible growth of microbial colonies ('0' CFU) on agar media was considered as an indication of highly effective disinfectant solution (Figure no. 2). The obtained data was recorded and tabulated.

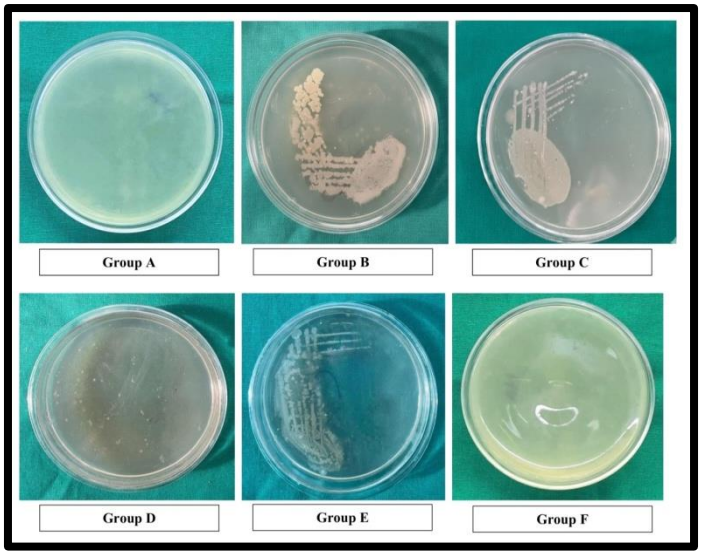


Figure 2: Evidence and Absence of Growth of Microbial Colonies (Colony Forming Units) in Petri dishes containing agar media among the Six groups tested; Group A: 10% Formalin, Group B: 5.25% Sodium hypochlorite, Group C: 3% Sodium hypochlorite, Group D: 5% Vinegar, Group E: 0.1% Thymol, Group F: 3% Hydrogen peroxide.

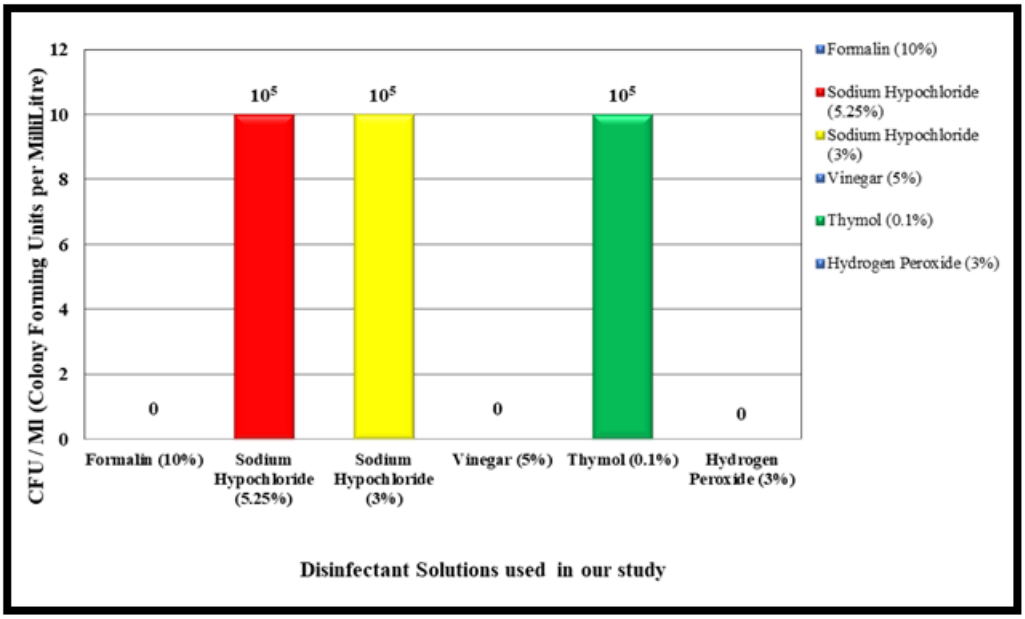
Results

The tabulated data in Microsoft excel was analysed with SPSS (Statistical Package for the Social Sciences) version 22.0 software. Chi-Square test was used for statistical analysis. The $P<0.05$ was considered statistically significant. Three groups showed positive microbial counts (10^5 CFU/ml), whereas, the other three groups showed complete inhibition of microbial growth '0' CFU. The obtained results indicated considerable variation in the effectiveness of six disinfectant solutions used, with three disinfectants totally failed to eliminate microbial growth, while other three showed complete microbial inhibition (Table no.1 and Graph no.1).

Table 1: Chi Square test: Comparison of Colony Forming Units (CFU) among the Six groups

P: Probablity, CFU: Colony Forming Unit, ML: MilliLitre

Groups		No. of Specimens used	CFU/ML		P value
			'0' CFU/ml	10^5 CFU/ml	
Group A	10% Formalin solution	10	10	0	<0.05
Group B	5.25% Sodium hypochlorite solution	10	0	10	
Group C	3% Sodium hypochlorite solution	10	0	10	
Group D	5% Vinegar solution	10	10	0	
Group E	0.1% Thymol solution	10	0	10	
Group F	3% Hydrogen peroxide solution	10	10	0	
Total		60	30	30	



Graph 1: Vertical Bar Graph representing the Number of Colony Forming Units seen among the Six Disinfectant Solutions used in our study.

Of the six Disinfectant solutions used in our study, Group A (10% Formalin), Group D (5% Vinegar) and Group F (3% Hydrogen Peroxide) showed complete disinfection of all specimens, with total microbial inhibition of '0' CFU's (Colony Forming Unit's) seen on the agar media. Whereas, Group B (5.25% Sodium Hypochlorite), Group C (3% Sodium Hypochlorite), Group E (0.1% Thymol) showed incomplete disinfection of the specimens with significant microbial growth of 10^5 CFU's. Statistically significant difference was seen among the six groups tested, $P<0.05$.

Discussion

The findings of our in-vitro study has important clinical implications for laying down future research protocols both in dental teaching and in research methodologies involving the use of extracted human teeth. The 100% efficacy of 10% Formalin in our study aligns with previous reported findings of its highly effective

disinfection of extracted human teeth.[2,3] Formalin acts by protein cross-linking, enzyme inactivation and deep penetration into the tooth structure, thereby eliminating microbial viability.[4] However, due to its potential alteration of physical and mechanical properties of tooth structure, handling risks and complicated disposal, its use was limited as a routine disinfectant solution of extracted human teeth.[3,10]

In our study, 5% Vinegar solution also achieved complete microbial inhibition. Tijare M et al[3] reported that 5% Vinegar solution completely inhibited microbial growth when extracted human teeth were immersed in it for 7 days and stated that it causes acidification of microbial environment leading to microbial membrane disruption, proton gradient collapse and denaturation of microbial proteins. The advantage of 5% Vinegar also lies in its easy availability, low cost, safe handling and relatively low toxicity compared to the use of 10% formalin and 5.25% sodium hypochlorite solutions.[11] Likewise, 3% Hydrogen peroxide solution also achieved complete inhibition of microbial growth in our study. It exerts its antimicrobial effect via oxidative release of free radicals (hydroxyl radicals) damaging microbial lipid membranes and nucleic acids. However, it causes potential alteration of both physical and mechanical properties of teeth.[12] Whereas, Western JS et al[9] reported least effectiveness of 3% hydrogen peroxide as surface disinfectant of extracted human teeth. Thus, 5% Vinegar can be a much safer and highly effective alternative to both 10% Formalin and 3% Hydrogen Peroxide disinfectant solutions.

Interestingly, both 5.25% and 3% NaOCl solutions failed to fully eliminate microbial growth in our study. Lolayekar NV et al[2] reported that 5.25% NaOCl solution showed highly effective disinfectant action on extracted human teeth. However, Western JS et al[9] reported 5.25% NaOCl solution was unreliable for complete disinfection of extracted human teeth. The findings of our study also reinforce caution, that disinfection of extracted human teeth with 3% and 5.25% NaOCl solutions cannot completely guarantee microbial elimination. Whereas, Autoclaving of extracted human teeth or the use of higher concentrations of NaOCl and H_2O_2 solutions negatively affect dentin microstructure, bond strength and mechanical properties of disinfected extracted human teeth and deemed to be less favourable in research studies.[12] Despite the known antimicrobial ability of 0.1% Thymol[11], it did not show any microbial inhibition of specimens used in our study.

In our study, 0.1% Peptone water was used as Culture media, it served as a critical enrichment and recovery media to evaluate any residual microbial viability following disinfection of extracted human teeth.[3] 0.1% Peptone water is a versatile, non-selective media used in microbiology studies for bacterial growth and its enrichment. It contains Peptic digest of animal tissues and sodium chloride, providing essential nutrients and osmotic balance needed for growth of microorganisms.[13]

Limitations of our study include, the fact that we assessed only the microbial colony forming units as the outcome measure for validating disinfection of extracted human teeth. But, we did not evaluate any residual microbial DNA (Deoxyribo Nucleic Acid), viral and spore inactivation. Future research studies should investigate these limitations.

Conclusion

10% Formalin, 5% Vinegar and 3% Hydrogen peroxide solutions showed complete microbial inhibition with highly effective disinfectant ability. However, the use of 5% Vinegar solution as disinfectant could be very beneficial due to its non-hazardous property, easy availability, low cost and most importantly it do not

cause any potential alteration in the physical, mechanical, structural integrity of disinfected teeth and therefore can be safely used in research studies.

References

1. Kumar M, Sequeira PS, Peter S, Bhat GK. Sterilisation of extracted human teeth for educational use. Indian J Med Microbiol 2005;23:256-258.
2. Lolayekar NV, Bhat VS, Shenoy SB. Disinfection methods of extracted human teeth. J Oral Health Community Dent. 2007;1:27–29.
3. Tijare M, Smitha D, Kasetty S, Kallianpur S, Gupta S, Amith HV. Vinegar as a disinfectant of extracted human teeth for dental educational use. J Oral Maxillofac Pathol 2014;18:14-18.
4. Gogineni S, Ganipineni K, Babburi S, Venigalla A, Pinnisett S, Kotti A, et al Evaluation of Vinegar as a disinfectant for extracted human teeth - An In-vitro study. J Clin and Diag Res 2016;10:7:50-52.
5. Dominici JT, Eleazer PD, Clark SJ, Staat RH, Scheetz JP. Disinfection/sterilization of extracted teeth for dental student use. J Dent Educ 2001;65:1278-1280.
6. Lee JJ, Nettey-Marbell A, Cook A Jr, Pimenta LA, Leonard R Ritter AV. Using extracted teeth for research: The effect of storage medium and sterilization on dentin bond strengths. J Am Dent Assoc 2007;138:1599-1603.
7. Sandhu SV, Tiwari R, Bhullar RK, Bansal H, Bhandari R, Kakkar T, Bhushri R. Sterilization of extracted human teeth: A comparative analysis. Journal of Oral Biology and Craniofacial Research 2012;2:3:170-175.
8. Paul T, Desai D, Shahid M, Aziz NZ, Leslie G, Sadhu B. Disinfection of extracted human teeth: A Comparative study. J Adv Med Dent Scie Res 2017;5:1:33-38.
9. Western JS, Dicksit DD. A systematic review of randomized controlled trials on sterilization methods of extracted human teeth. *J Conserv Dent.* 2016; 19:4:343-346.
10. McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, action and resistance. *Clin* Microbiol Rev. 1999;12:1:147-179.
11. Priya A, Selvaraj A, Divya D, Raja R, Pandian S. In-vitro and In-vivo anti-infective potential of thymol against Candida albicans *and* Streptococcus mutans. J Antimicrob Chemother. 2021;1:12:1-16
12. Ofoedu CE, You L, Osuji CM, Iwouno JO, Kabuo NO, Ojukwu M, et al. Hydrogen Peroxide effects on natural-sourced polysacchrides: Free radical formation/production, degradation process, and reaction mechanism—A Critical synopsis. *Foods.* 2021;10:4:699-702.
13. El-Jakee J, Ahmed SF, Sherein I, Samy A, El-Shafii S. Using peptone water enrichment for improved detection of bacterial contaminants in food samples. J Food Saf. 2009;2:92:228–240.