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ORIGINAL ARTICLE

DISINFECTION OF EXTRACTED HUMAN TEETH: A COMPARATIVE STUDY

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ABSTRACT:

Background: Extracted teeth are used commonly by dental students as well as researchers to improve their technical skills for future dental practice as well as to conduct various studies. Extracted teeth are a potential source of infection and should be disinfected/ sterilized before use. **Aim:** To assess the efficacy of different materials for disinfection/sterilization of extracted human teeth. Settings and Design: The study was conducted in A.J Institute of medical/dental sciences, ethical clearance was obtained from the institutional ethical committee. **Material and Methods:** A total of 90 extracted non carious human teeth were kept in nine disinfectant media-10 % formalin, 99% acetic acid, vinegar (commercially available), 6% hydrogen peroxide, neem extract, curcumin extract, aloe vera extract, lime extract and normal saline. Teeth were kept in different groups for one week, samples were collected and streaked over the surfaces of Mc-Conkeys and Blood agar medium. The media were later incubated at 37^{o}C for 48 hours. No growth in the culture media was considered an effective method of sterilization/disinfection. **Statistical analysis:** Chi Square Analysis. **Results:** 10% formalin, 6% hydrogen peroxide and 99% acetic acid were totally effective. The result was statistically significant with a Chi square value of 80.957 and $P < 0.001$ for 7 days and X^2 value of 180.00 and $P < 0.001$ for 3 days. **Conclusions:** 10 % formalin, 6% hydrogen peroxide and 99% acetic acid can be used as an disinfectant medium for extracted human teeth

Key-words: Culture media, Disinfection/sterilization, Infection.

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INTRODUCTION:

Natural teeth are an important teaching tool for dental students which can be used to develop their preclinical skills especially when it comes to cavity preparations and other endodontic techniques. They are also used to test and develop various restorative materials. Freshly extracted teeth are known to be a potential source of infection which may contaminate the laboratory equipments as well as the personal handling them. The American dental association (ADA 1988) and the Centre for disease control and prevention (CDC 1986) have adopted certain guidelines which are to be followed to prevent any transmission of blood borne pathogens while dealing with these extracted teeth.[1] Diseases like AIDS and Hepatitis B pose a high risk of cross infection and transmission during medical or dental procedures. The occupational safety and health administration (OSHA) blood borne pathogens considers extracted human teeth to be a potential source of blood borne pathogens. Several chemicals and methods have been tried for sterilization/disinfection of extracted teeth, all have their own merits and de merits. Methods like microwave radiation, autoclaving have been proved to be effective but are not that practical to be used on a routine basis.[2] The effectiveness of any method of sterilization is dependent upon a number of factors like time, contact, high pressure, temperature with steam sterilization, the type and number of microorganism present, the type of organic materials that protects the microorganisms and also the surface defects on the instruments which might provide a habitat for these microorganisms to survive. Agents like formalin, sodium hypochlorite, glutaraldehyde and thymol have been used for sterilization/disinfection of extracted teeth. The Centre for disease control and prevention (CDC), USA recommends storing extracted teeth in 1:10 household bleach, while studies conducted by Tate and White showed that household bleach was not efficient when used as a disinfectant. Ethylene dioxide has also been used as a sterilizing agent, with efficiency of about 20-36% on the spores of Bacillus subtilis on extracted teeth. Newer methods have been adopted like Gamma radiation which sterilizes without any involvement of any chemical, gases, high temperature, having minimum effect on the tooth structure and more effective sterilization. Sodium hypochlorite which is also used as a root canal irrigant has also been proved to be

effective in reducing the bacterial load in root canals especially when combined with agitation. At present there is no precise method of sterilization used in dentistry for extracted teeth.[3]

Extracted teeth being of so much importance especially in educational setups should be sterilized and made free of any pathogens before considering it for any use. The sterilization methods should be effective without causing any alterations to the normal enamel and dentinal structures.

The present study was conducted to determine the efficacy of different methods of disinfection/sterilization on freshly extracted human teeth.

SUBJECTS AND METHODS:

A total of 90 freshly extracted human teeth were collected from the Department of Oral and Maxillofacial Surgery of our institute. The teeth included were intact, without the presence of any carious lesions and extracted as a part of orthodontic or periodontal therapy. The extracted teeth were initially stored in normal saline. They were randomly divided into eight study groups having ten teeth in each group. 100 ml of each group was taken and divided into 10 ml for each group. The study group included 10% formalin, 6% hydrogen peroxide, vinegar(commercially available), 99% acetic acid, lime juice, curcumin extract, neem extract, aloe vera extract. Normal saline was taken as control. (figure 1)

One kg of fresh neem leaves were taken, washed in distilled water and grinded using sterile mortar and pestle, distilled water was added during the grinding process, the final product was put in a sterile muslin cloth and squeezed, the filtrate was made to 100 ml by adding distilled water and stored in a sterile container. Fresh lime was taken and 100 ml juice was extracted and stored in a sterile container. Curcumin legumes were washed in distilled water and were grinded using a sterile mortar and pestle, distilled water was added to give the product a liquid consistency, the extract was then put in a sterile muslin cloth and squeezed, distilled water was added to make the final product to 100 ml. 250 gms of fresh aloe vera was cut and the fleshy part was scrapped and transferred to a sterile mortar and pestle, distilled water was added, the product was grinded and distilled water was added to make the final volume up to 100 ml. Commercially available vinegar was taken as one of the study groups. Ten teeth of each group were immersed individually in test tubes and incubated at 37°C for seven days. (figure2)

COLLECTION OF SAMPLES

To obtain the microbial sample, metal transfer loop was used, which was first heated till red hot and then allowed to cool. The loop was later dipped into the test tubes containing the teeth samples. The collected sample was then streaked over the surfaces of Mc-conkeys and blood agar medium. and incubated at 37°C for 48 hours.

DETERMINING THE STERILIZATION LEVEL

Sterilization was determined based on the microorganism growth on the culture media. The presence of growth was observed after 48 hours of inoculation. The absence of any visible growth on the culture media was considered as an indicator of effective sterilization.

RESULTS:

Before sterilization was done, sample was taken from the normal saline and streaked over the two medium, bacterial growth was observed on the two culture medias (figure 3). The results have been displayed in (Table 1 and Table 2). The graphical representation is shown (figures 4 and 5). It was observed that no growth of microorganism was seen on the culture media inoculated with samples taken from 10% formalin, 6% hydrogen peroxide and 99% acetic acid (figure 6), thus indicating 100 % efficacy in sterilizing extracted teeth when compared to other methods. Vinegar which is a household product showed 30% efficiency when used as a disinfectant. The other methods which included the natural products were not found to be effective in sterilizing/disinfecting the extracted teeth specimen (figure 7). After incubating the teeth samples in all the natural groups for 3 days, there was still growth observed on the agar media inoculated with the natural groups. The results were statistically significant with a X^2 value of 80.957 and $P<0.001$.

Figure 1: Natural products used for the study a) neem leaves, b) aloe vera, c) curcumin legumes and d) commercially available vinegar

Figure 2: Teeth specimen immersed in formalin

Figure 3: Microbial growth seen on mac-conkeys agar medium when streaked over with sample taken from normal saline

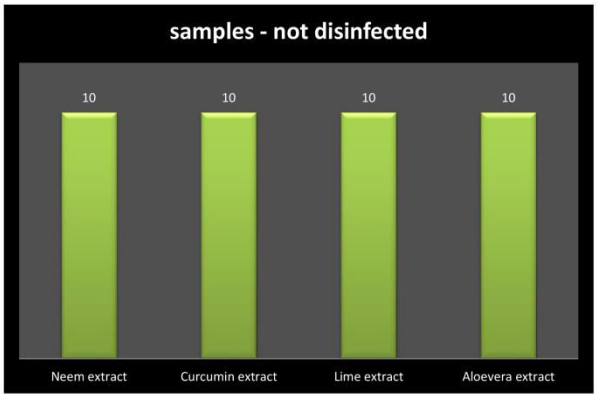

Figure 4: Showing samples not disinfected (natural group)

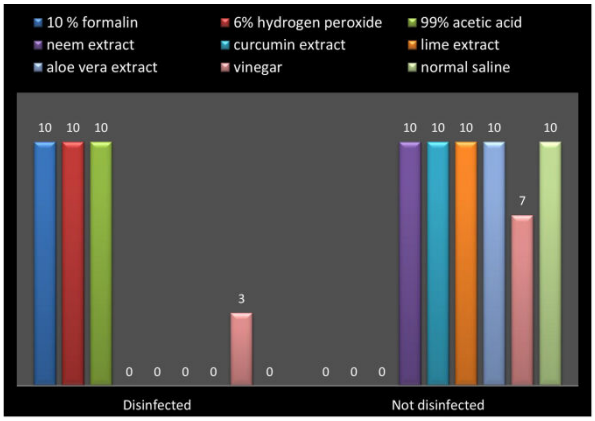

Figure 5: Showing disinfected and not disinfected sample in all groups

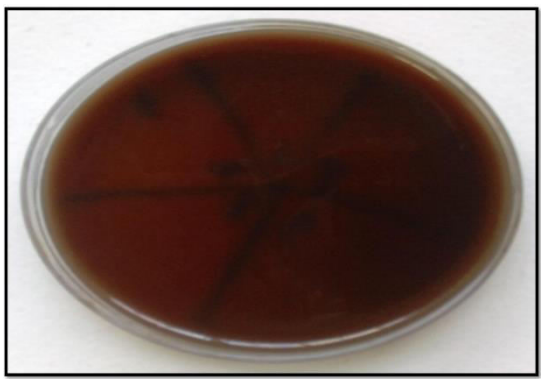

Figure 6: No microbial growth seen in samples from formalin on blood agar media

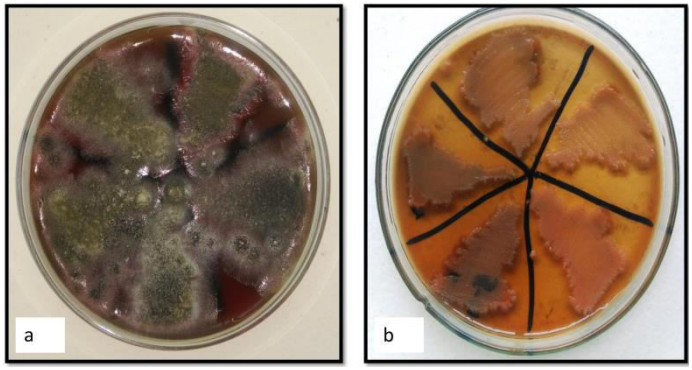

Figure 7: a) Microbial growth observed on blood agar when streaked over with sample from lime.
b) Growth observed in mc-conkeys agar when streaked over with sample from neem extract

Table 1: Comparison of efficacy of different methods used and teeth sterilised/ disinfected

Disinfectants used	N (No. of samples)	Disinfection		P value, χ^2 value
		Disinfected (%)	Not disinfected (%)	
10 % formalin	10	10 (30.3)	0 (0)	
6% hydrogen peroxide	10	10 (30.3)	0 (0)	
99% acetic acid	10	10 (30.3)	0 (0)	
Neem extract	10	0 (0)	10 (17.5)	P value < 0.001
Curcumin extract	10	0 (0)	10 (17.5)	(HS),
Lime extract	10	0 (0)	10 (17.5)	80.957
Aloevera extract	10	0 (0)	10(17.5)	
Vinegar	10	3 (9.1)	7(12.3)	
Normal saline	10	0 (0)	10 (17.5)	
Total	90	33 (100)	57(100)	

Table 2: Efficacy of the natural products used and the teeth disinfected/ sterilised

Disinfectants used	N (No. of samples)	Disinfection	
		Disinfected (%)	Not disinfected (%)
Neem extract	10	0 (0)	10 (25)
Curcumin extract	10	0 (0)	10 (25)
Lime extract	10	0 (0)	10 (25)
Aloevera extract	10	0 (0)	10 (25)
Total	40	0 (0)	40 (100)

➢Extracted teeth used for teaching dental health care workers should be considered infective and classified as clinical specimens as they contain blood.
➢All persons who collect, transport or manage extracted teeth should handle them with the similar precautions as a sample for biopsy.
➢Before extracted teeth are manipulated in dental educational training, the teeth first would be cleared of adherent patient material by scrubbing with detergent and water or by using an ultrasonic cleaner.
➢Teeth should then be stored, immersed in a fresh solution of sodium hypochlorite (household bleach 1:10 with tap water) or any liquid chemical germicide for clinical specimen fixation.
➢Persons handling extracted teeth should wear gloves. Gloves should be disposed off properly and hands washed after completion of work activities.
➢Additional personal protective equipment e.g. face shield or surgical mask and protective eyewear should be worn if mucous membrane make contact with debris or spatter is expected when the specimen is handled, cleaned or manipulated.
➢Work surfaces and instruments should be cleaned and decontaminated with a suitable liquid sterilizer after completion of work activities.

Table 3: Infection control measures

DISCUSSION:

Sterilization is defined as the process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.[16] Sterilization of extracted teeth is of great concern to all the individuals who come in direct contact with them. Potentially pathogenic microorganisms have been recovered from extracted teeth after drilling procedures done in dental laboratories.[4] It has been found that many blood borne pathogens which includes viruses like HIV, HBV, HCV and bacterial pathogens are present in the pulp chamber as well as the radicular and periradicular tissue of the extracted human teeth.[3] Tooth preparation is generally done without a liquid coolant especially in the laboratories, students in many dental institutions also follow the same technique which make them more vulnerable to infections. The spread of infection can be in the form of aerosols released during tooth preparation or from the injuries caused during the handling of the instruments used during the procedures.[3] Extracted teeth are a potential source of infection, To prevent the spread of infection from the extracted teeth a proper infection control method is required (Table 3).[3]

Recently various methods of sterilization has been used that shows minimum effect on the integrity of the tooth structure and are also efficient in sterilization.

Gamma radiation is a method of sterilization without the involvement of high temperature, pressure, chemicals or gases [3] Autoclaving for 30-40 min at 240^{o}C and 15-20 psi, soaking the teeth in 10% formalin for one week and 5.25%. sodium hypochlorite have demonstrated to be an effective method of disinfection.

Sandhu V S et al conducted a study which showed that immersion of teeth in 10% formalin, 5.25% sodium hypochlorite for 5 days and autoclaved at 121^{o}C at 15 lbs psi pressure for 20 min were efficient in disinfecting extracted human teeth. The other chemicals taken in the study like 0.1 % thymol in distilled water, 3% hydrogen peroxide, 2% glutaraldehyde were not found to be effective in sterilization. In our study, 10% formalin was found to be 100% efficient in disinfecting the extracted teeth.[3] Tijare M et al conducted a study which showed that teeth immersed in 3% hydrogen peroxide, 10% formalin and vinegar (acetic acid) for 7 days were found to be completely disinfected.[2] In our study we found that 6% hydrogen peroxide, 99% acetic acid proved to be efficient in disinfecting extracted teeth whereas commercially available vinegar showed 30 % efficacy. Apart from all these chemicals used, many naturally occurring substances have also been proved to possess antibacterial and antifungal properties. Even though the chemicals and other methods like autoclaving have been proved to be efficient in sterilizing/disinfecting the extracted teeth, most of them have some or the other drawbacks. Formalin is found to be a potent carcinogen so it has to be handled carefully.[3] It also affects the dentin bonding strength of the teeth.[2] Sodium hypochlorite may increase enamel porosity by deproteinization and alter dentin structure by removing or modifying the protein matrix.[2,3] Extracted teeth with amalgam restorations cannot be autoclaved as the mercury vapors might be released in the air and the residual mercury can also contaminate the autoclave, it also has an effect on the dentine bond strength.[2,3] To overcome these problems, natural products can be used as a substitute for the chemical methods of sterilization. Many studies have been conducted which proved that many naturally occurring substances have antimicrobial, antifungal properties. In our study we have used many natural product extracts like neem, curcumin, aloe vera and lime and tried to study whether they could be used as substitutes for sterilization/disinfection.

Owhe-Ureghe U B et al conducted a study to detect the antibacterial property and found that a paste made by blending garlic and lime could be used as a mouth wash in the treatment of dental caries, mouth-sore, sore-throat and also, be incorporated into toothpaste to prevent dental caries.[17] Aibinu I et al conducted a study using different forms of lime like the juice, burnt rind and the oil to check for the antibacterial property on strains of bacteria, the result showed that all the extracts at the stock concentration had antimicrobial activity.[6] Sudhir K et al conducted a study to evaluate the antibacterial activity of plant extracts against against bacterial pathogens which showed that lemon when used possessed antibacterial properties.[18] In the present study we observed that the teeth immersed in lime extract for one week did not show any disinfection property.

Praveen Kumar S et al conducted a study to investigate the anti-bacterial potential of curcumin, against standard strains of common endodontic bacteria which showed that curcumin had antibacterial activity against all the test organisms used except E. Faecalis.[19] Hegde V, Kesaria P D conducted a study to evaluate of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E.faecalis and C.albicans which showed that turmeric showed good antimicrobial efficacy against C.albicans.[20] Neelakanthan P et al conducted a study to evaluate the antimicrobial efficacy of curcumin against Enterococcus faecalis biofilm formed on tooth substrate which showed that curcumin had considerable anti bacterial property.[21] In the present study we found that there was no disinfection action showed by curcumin.

Pareek et al conducted a study to evaluate the effect of aloe vera as a disinfectant in reducing the microbial growth in the dental unit water lines, The study showed that aloe vera was found to be most effective as a disinfectant in reducing the microbial colonies.[12] AthibanP et al conducted a study to evaluate the antimicrobial efficacy of Aloe vera in decontaminating guttapercha cones which showed that aloe vera was effective in disinfecting guttapercha cones.[22] In the our study aloe vera showed no disinfectant property.

Many studies have been conducted using vinegar which is a form of acetic acid as a disinfectant.

Sloss M J et al conducted a study on live patients using acetic acid for elimination of Pseudomonas aeruginosa from burn and soft tissue wounds which showed that acetic acid was efficient in achieving the goal.[23] In the present study we used the pure form of acetic acid 99% and found that it was 100% efficient as a disinfectant and there was no morphological changes which observed on the tooth surface.

In the present study none of the natural products were found to be effective in disinfecting the extracted teeth samples, there could be a possibility that these natural products were in the pure form without any added preservatives, the duration could be a factor for their failure. In our study we found that the teeth immersed in lime were coated with fungus like growth after one week, there was also growth observed on the rim of the test tubes in which the teeth were immersed. To rule out the duration factor for the failure, fresh teeth samples were immersed in all the natural groups for a period of 3 days, there was neither growth observed on the immersed teeth samples, nor on the test tubes. After inoculation on the same agar media used in the study, growth was still observed on the agar media inoculated with the natural groups showing that duration could not be the only contributing factor. There could be many technical and environmental errors involved, the extracts would have got contaminated during the grinding procedure either from the environment or from the mortar and pestle which were investing some microorganisms. It could also be from the gloves while handling the solutions during the methodology. The groups used in the research did not cause any morphological changes to the tooth structure which reveals that the teeth can be used for research purposes post sterilization with these methods of disinfection.

CONCLUSION

Based on the present study we came to a conclusion that even though the natural products were efficiently used as antimicrobials and also possessed anti septic properties, they failed to act as a preservatives for long term storage of extracted human teeth. Formalin is a potent carcinogen which has been stated by many authors and has to be handled carefully, in our study it showed 100% efficiency when used as a disinfectant. Hydrogen peroxide is most commonly used in clinical set ups and also in educational institutes as a storage medium for extracted teeth specimens. It was also found to be 100% efficient in disinfecting the tooth specimens and not much literature is present stating that they are harmful. Glacial acetic acid when used in the pure form i.e. 99% was also found to be 100% effective as a disinfectant, it being a strong acid should be also handled very carefully and also the fumes evolved from the acid should also be taken into consideration with necessary precautions taken. Further researches are needed with these natural products to use them as disinfectants, preservatives can be added to prolong their duration of disinfectant property-to be added followed by keeping in mind the errors also keeping in mind the errors. The positive finding in this study was that none of the disinfectant agents used, caused any morphological alteration to the tooth structure, hence showing that these teeth samples can be used for research purposes. The present study adds to the literature of the various studies conducted in the same field.

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