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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF EFFICACY OF NEO ENDO RETREATMENT FILES BY AUTOCLAVE AND COLD STERILIZATION AFTER EACH USE - AN INVITRO STUDY

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Abstract

Aim: The aim of the study is comparative evaluation of efficacy of neo endo retreatment files after autoclave and cold sterilization.

Materials And Methods: 45 human mandibular premolars with a single straight root canal were selected and decoronated leave 16mm of root.The working length was determined by subtracting 1mm from above measurment. Canal preparation was done with Neo endo flex rotary files (25/6) and were obturated with GP size 25/6% .After 2 weeks of storage in normal saline they were randomly divided into three groups .In Group A (5 sub-groups :A1, A2, A3, A4 and A5 of 3 sets of specimens) Neo endo retreatment files were subjected to sterilization by autoclaving.In group B files were sterilized by cold sterilization with 2.4% Glutaraldehyde solution for 12 hours.In Group C files were cleaned using soap and water . Statistical analysis was done with One-way ANOVA Test followed by Tukey's post hoc test to compare the mean remaining GP & time taken between different groups and usage times.

Result: All samples showed GP remnants. Irrespective of the sterilization methods used, the amount of remaining GP increased sequentially from first to fifth use along with usage time. The remaining GP was lesser in the group A where sterilization was done by autoclave, and remaining GP was highest in Group C where no sterilization was done. The remaining GP of Group B (Cold sterilization) was higher than Group A and lower than Group C.

Conclusion :From the findings of present study it can be concluded that Autoclave sterilization causes least changes to the physical properties of the files when compared to Gluteraldehyde sterilization which adversely effects the cutting efficiency of the files. Unsterilized files exhibits least cutting efficiency resulting in reduced gutta percha removal from the canals. Also the number of use was a determining factor for cutting efficiency. Reduction in the cutting effectiveness of instruments may increase operating time and it could take longer to remove the material from root canal.

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Introduction:-

Endodontic retreatment involves complete retrieval of the root canal obturation material, accompanied by the cleaning and shaping followed by the reobturation .Numerous techniques are available for the removal of the gutta-percha which include thermal, chemical, mechanical and a combination of the above three methods.[1]

Endodontic retreatment can be done either by nonsurgical retreatment (orthograde) or by apical surgery (retrograde).Several techniques have been proposed to remove filling materials from the root canal system, including the use of endodontic hand files, Nickel-Titanium (niti) rotary instruments, Gates Glidden burs, heat, ultrasonic instruments, laser and use of adjunctive solvents.[2] Specialized rotary instruments have been introduced to retrieve the Gutta percha(GP) during retreatment .It has been shown that specialised instruments are better than conventional rotary instruments for removal of Gutta percha. [1]

Most of the endodontic instruments are reused. During cleaning and sterilisation, it is of great importance to provide utmost care to prevent impairment of the instrument which may in turn jeopardize the treatment success. In order to prevent the fracture of the material inside the root canal ,care must be given to monitor and control the number of uses of the endodontic instruments.[3]

On the surface subject to fatigue, surface alterations can give rise to microcrack which can lead to fracture of the instrument, and also reduce the cutting capacity of the blades on the endodontic files.[9] One problem of reusing endodontic instruments that are subject to fatigue is the deterioration they suffer that results from their use in the dental canal for the removal of dentin, as well as the corrosive action by the root canal irrigants such as sodium hypochlorite, and subsequently, the action of the temperature and steam induced by the autoclave sterilization process[9]. The chemical and physical reactions that occur during disinfection and sterilization might cause corrosion and deterioration on the instruments' surface, leading to surface alterations, early fracture, and decreased cutting efficacy. The operational speed, motion principle, metallurgical properties, and surface characteristics are substantial factors that can affect instrument fatigue[10].All these factors will ultimately affect the clinical efficiency of the endodontic files especially in removal of Gutta Percha during the retreatment procedures[10].

This study aims to know the efficacy of NEO ENDO RETREATMENT FILES by autoclave and cold sterilization after each use on GP removal. The efficacy of the retreatment files can be evaluated by measuring the areas of remaining filling material by the stereomicroscopic magnification.

Aim Of The Study:-

The aim of the study is comparative evaluation of efficacy of neo endo retreatment files by autoclave and cold sterilization after each use – an invitro study

Objective Of The Study:-

To compare and evaluate the efficacy of NEO ENDO RETREATMENT FILES by autoclave and cold sterilization after every use by checking the amount of remaining Gutta Percha and the time taken for the retrieval of Gutta percha concomitant to the number of use.

Test Specimen Selection:

A total number of 45 freshly extracted human single rooted mandibular premolar were selected.

Inclusion Criteria:

Single rooted human mandibular premolar with fully formed apices and patent apical foramen. To confirm single root canal of the tooth a preoperative radiograph was taken.

Exclusion Criteria:

a) Teeth having root and root canals with accentuated curves
b) Teeth having calcified canals
c) Teeth with internal and external resorption

Materials And Methods:-

45 human mandibular premolars freshly extracted for orthodontic reasons with a single straight root canal with fully formed apices and patent apical foramen were selected for this study. To confirm single root canal of the tooth a preoperative radiograph was taken. The crowns were removed with diamond disc to leave 16mm of root. A size 10 K-file was introduced into the canal until it was visible at apical foramen. The working length was determined by subtracting 1mm from this measurement. The coronal flaring was done with coronal flaring file of Neoendo Flex Rotary Files(30,8%) . Root canals of each specimen were prepared with NeoEndo Flex rotary files (according to the manufacturers protocol) up to size 25 with 6% taper(25/6%) at apex of the canal. The canals were irrigated between instrumentation with 2.5% of sodium hypochlorite . When instrumentation was completed, 17% EDTA solution was used for 1 min for smear layer removal and the canals were finally irrigated with 5ml of 2.5% Sodium hypochlorite and saline . Following complete debridement, canals were dried with paper points. The canals were coated with AH-plus root canal sealer with the help of lentulo-spirals .GP master cone size 25/6% was placed into the canal upto full working length. The coronal access cavities were sealed with Intermediate Restorative Material (IRM). All teeth were stored in normal saline at 37º C and 100% humidity for two weeks. After two weeks IRM was removed with round bur. The GP was removed from the coronal 2 mm with ISO sizes No. 1,2 & 3 Gates Glidden drills. The teeth were randomly divided into two groups :

1. **Group A: Neo endo retreatment files sterilized by Autoclave**
2. **Group B: Neo endo retreatment files sterilized by Cold sterilization**
3. **Group C : Neo endo retreatment files not sterilized**

Group A: Neo endo retreatment files sterilized by autoclave : Group A was divided into 5 sub-groups (A1, A2, A3, A4 and A5) of 3 sets of specimens each. Neo Endo Retreatment files were used to remove the root canal filling material . N1 was used to remove the GP up to the coronal one third. This was followed by middle third with N2, and then N3 up to the working length. Retreatment was done until no GP or sealer was detected on the instrument flutes or in the irrigating solution. This was done in 3 sets of such specimens and assigned as Subgroup A1. As for sub-group A2 the same Neo Endo Retreatment files was put to second use to remove gutta-percha as in group A1. Thus the files were used sequentially for the third, fourth and the fifth use for the subgroups A3, A4 and A5 respectively. Each group had 3 sets of samples prepared for the respective number of times totaling to 15 for group A. During retreatment the root canals in all groups were constantly irrigated with 2ml of 2.5% sodium hypochlorite and saline between each instrument change. After using the Neo endo retreatment file in each tooth, file were subjected to sterilization by autoclaving. Every files after each use was placed in an endodontic instrument box and was subjected to autoclave at 121°C for 15 minutes at a pressure of 15 pounds.

Group B : Neo endo retreatment files sterilized by cold sterilization : : Group B was divided into 5 sub-groups (B1, B2, B3, B4 and B5) of 3 sets of specimens each. Neo Endo Retreatment files were used to remove the root canal filling material . N1 was used to remove the GP up to the coronal one third. This was followed by middle third with N2, and then N3 up to the working length. Retreatment was done until no GP or sealer was detected on the instrument flutes or in the irrigating solution. This was done in 3 sets of such specimens and assigned as Subgroup B1. As for sub-group B2 the same Neo Endo Retreatment files were put to second use to remove gutta-percha as in group B1. Thus the files was used sequentially for the third, fourth and the fifth use for the subgroups B3, B4 and B5 respectively. Each group had 3 sets of samples prepared for the respective number of times totaling to 15 for group B. During retreatment the root canals in all groups was constantly irrigated with 2ml of 2.5% sodium hypochlorite and saline between each instrument change. After using the Neo endo retreatment file in each tooth, file was subjected to sterilization by Cold sterilization. Every files after each use were placed in a sterile plastic container containing 2.4% Glutaraldehyde solution and were left in it for 12 hours.

Group C : Neo endo retreatment files not sterilized: : Group C was divided into 5 sub-groups (C1, C2, C3, C4 and C5) of 3 sets of specimens each. Neo Endo Retreatment files were used to remove the root canal filling material . N1 was used to remove the GP up to the coronal one third. This was followed by middle third with N2, and then N3 up to the working length. Retreatment was done until no GP or sealer was detected on the instrument flutes or in the irrigating solution. This was done in 3 sets of such specimens and assigned as Subgroup C1. As for sub-group C2 the same Neo Endo Retreatment files were put to second use to remove gutta-percha as in group C1. Thus the files were used sequentially for the third, fourth and the fifth use for the subgroups C3, C4 and C5 respectively. Each group had 3 sets of samples prepared for the respective number of times totaling to 15 for group C. During retreatment the root canals in all groups were constantly irrigated with 2ml of 2.5% sodium hypochlorite

and saline between each instrument change. After using the Neo endo retreatment file in each tooth, files was cleaned using soap and water.

Evaluation:
The specimens were grooved bucco-lingually with a diamond disc and sectioned longitudinally with a chisel and mallet without disturbing the contents in the root canal. Both root halves were photographed(Figure 1 shows the representative images of remaining GP in root canals from first to fifth use subgroups of Group A,B,C). The images of sectioned half were taken with stereomicroscope (Lawrence & Mayo) under 5X magnification. To evaluate the remaining filling material, the images taken were transferred to specific software (Motic Software) which was used to measure the areas of remaining filling material and root canal periphery that were computed and expressed in square microns. The percentage of remaining root canal filling material (RF) was calculated by dividing the sum of total areas of remaining filling material (TARF) to that of total area of the canal (TAC). Mean percentage values were calculated according to the formula below and compared.

$$RF = \frac{TARF}{TAC} \times 100$$

Statistical Analysis
Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

DescriptiveStatistics
Descriptive analysis includes expression of remaining GP & time taken in terms of Mean & SD for each group.

Inferential Statistics:
One-way ANOVA Test followed by Tukey's post hoc test was used to compare the mean remaining GP & time taken between different groups and usage times.

The level of significance was set at $P<0.05$.

Results:-

The mean of remaining GP in different groups and the time taken for GP removal for the 1st, 2nd , 3rd, 4th & 5th use of Neo endo retreatment files after autoclave, cold sterilization and no sterilization were calculated . All the specimens showed the presence of remaining GP irrespective of the technique employed. The remaining GP was lesser in the group where files were sterilized by autoclave and maximum amount of remaining GP was found in group were sterilization was not done.

Comparison of mean Remaining GP (%) between 3 groups at different usage times using One-way ANOVA test followed by Tukey's post hoc Test. This showed A1 was significantly lesser as compared to A3, A4, A5 groups; A2 was significantly lesser as compared to A4, A5 groups; A3 was significantly lesser as compared to A4, A5 group; A4 was significantly lesser as compared to A5 group. Similarly B1 was significantly lesser as compared to B3, B4, B5 groups; B2 was significantly lesser as compared to B4, B5 groups; B3 was significantly lesser as compared to B4, B5 group; B4 was significantly lesser as compared to B5 group. C1 was significantly lesser as compared to C3, C4, C5 groups; C2 was significantly lesser as compared to C4, C5 groups; C3 was significantly lesser as compared to C4, C5 group; C4 was significantly lesser as compared to C5 group.

Comparison of mean Remaining GP (%) between different usage times in each group using One-way ANOVA Test. The remaining GP in Neo endo retreatment files(NER) first use (A1 group) and NER second use (A2 group) was significantly lesser as compared to B1, B2, B3, B4 and B5,C1,C2,C3,C4,C5 groups. The remaining GP in NER third use (A3 group) was significantly lesser as compared to B3, B4, B5,C3,C4,C5 groups. The remaining GP in NER fourth use (A4 group) was significantly lesser as compared to B4 ,B5,C4,C5 groups. The remaining GP in NER fifth use (A5 group) was significantly higher as compared to B1 , B2,B3,C1,C2,C3 group. The remaining GP in Neo endo retreatment files(NER) first use (B1 group) was significantly lesser as compared to C1,C2,C3,C4,C5,A3,A4,A5. The remaining GP in B2 was significantly lesser than C2,C3,C4,C5,A4,A5 groups. The remaining GP in B3 was significantly lesser than C3,C4,C5,A4,A5 groups. The remaining GP in B4 was significantly lesser than C3,C4,C5,A5 groups. The remaining GP in B5 was significantly lesser than C5 groups. The remaining GP in C1 was higher than A1,B1. The remaining GP in C2 was higher than A1,A2,A3,B1,B2. The

remaining GP in C3 was higher than A1,A2,A3,A4,B1,B2,B3,B4. The remaining GP in C4 was higher than A1,A2,A3,A4,A5,B1,B2,B3,B4. The remaining GP in C5 was higher than A1,A2,A3,A4,A5,B1,B2,B3,B4,B5. Both in group-A, group-B and group–C the mean of remaining GP was seen to steadily increase with increase in use. The mean time required for the GP removal increased with an increase in number of use.

Discussion:-

The sterilization of endodontic instruments represents an important process for proceeding with the reuse of endodontic instruments. Sterilization has the purpose of breaking down and eliminating all microorganisms, viruses, and spores and preventing cross infections[11]. Endodontic instruments can be influenced by sterilization and disinfection processes at various times. The analysis of the literature shows how these alterations can come from two phases: The disinfection phase, by physical chemical action by decontaminating agents . The sterilization phase, by autoclave for temperature action[12]. The effects can be summarized as follows: Corrosive effects both from disinfectant agents (sodium hypochlorite) with the phenomenon of micropitting, and from oxygen, with the formation of NiTi oxides. Sterilization caused an increase in surface roughness, reduction in the cutting angle (endodontic instruments in steel and partially for NiTi) and reduction in cutting efficiency of endodontic files[13-20].

In the present study files sterilized by autoclave (Group A) showed the least amount of remaining GP . Morrisoon et al. tested K steel files following the use of extracted teeth and autoclave sterilization. The authors came to the conclusion that there is no reduction in the depth of the cut following five autoclave cycles and that the reduction is due only to clinical use . From the metallurgical point of view, the temperature used in sterilization may not be high enough to cause significant changes in the alloy structure[21]. Autoclave sterilization changed the surface and subsurface microstructure of the machined parts of the instruments, rendering these regions more susceptible to fatigue crack nucleation and propagation, but no change in the bulk or on the non machined surfaces took place. Since the autoclave sterilization do not cause significant changes in the alloy structure , cutting efficiency of the files was not reduced and hence Group A showed least amount of remaining GP.

In Group B (cold sterilization) the remaining GP was observed to be greater than group A (autoclave) and lesser than Group C (non sterilization) . Glutaraldehyde was classified as a high level of disinfection. It is the most commonly used disinfectant in the dentistry. A study conducted by Ozalp et al. analyzed that to achieve perfect sterilization with glutaraldehyde solution, endodontic files need to be submersed for 8–12 hours[22]. When the endodontic files are kept overnight in the solution, it can be detrimental to their efficiency during procedures[22]. According to Busslinger et al , Stokes Et al localized defects on the surface of the instrument undergoing chemicals for sterilization showed possible evidence of corrosion of the surface caused by chemicals like Naocl , Gluteraldehyde etc[23]. These corrosion defects causes surface alterations and deformities to the files and acts as areas for debris accumulation during the use.

Setu R. Bavaria 2016 conducted a study in which he compared 6 groups of files of 3% hydrogen peroxide, 2% glutaraldehyde solution, 0.2% chlorhexidine gluconate, 3% sodium hypochlorite, ultrasonic bath with an enzymatic solution, control group unused instruments [24]. He observed that among experimental groups, highest percentage maximum contaminatiom values were found for instruments that were immersed in 2% glutaraldehyde . 2% Glutaraldehyde,(Gravenmade & Dankert. 1975) was used in this study, as it is a strong disinfectant, fixative . Disadvantages of these agents are: Vapors are irritating and must be neutralized by ammonia, have poor dispersion, leave non-volatile remains, action is reduced in the presence of debris in the file flutes. This could be because glutarldehyde being a bactericidal, fixative does not loosens the adherent debris. Alterations in the physical properties of the files caused by gluteraldehyde adversely affects its cutting efficiency . All these factors could reduce the GP removal resulting in increased remaining GP .

The remaining GP was found to be maximum in group C were no sterilization was done. For unsterilized files it has to be considered that surfaces of the files itself might be a site of contamination with metallic spurs and debris as they are not pre sterilized (Suhad 2021). As these files undergo reuse the amount of debris clogged in the flutes of the file also increases resulting in the decreased cutting efficiency. In the study conducted by Guandalini et al (2014) comparative analysis of four cleaning methods of endodontic files, he compared four groups of flexofiles : control group(without cleaning), group 1 (enzymatic detergent + manual brushing with nylon bristle brush), group 2 (ultrasound + enzymatic detergent), group 3 (ultrasound + water) and group 4 (gauze embedded in 70%alcohol) he found that percentage of dirty spirals(debris) were 91.1% and percentage of clean spirals were 8.9% for the control group (the uncleaned group)[25]. This group exhibited the maximum debris

accumulation compared to other groups. In another study conducted by DA Van Eldik in 2004 , he observed that the mean percentages of clean surface area demonstrating the effect of rotary file showed the least value for the group of non sterilized files of 13.09 % for 4% taper files and 7.94 % for files with 6% taper respectively when compared to rest groups[26]. Hence from the above findings it could be confirmed that unsterilized files exhibits least cutting efficiency resulting in least GP removal when compared to other groups.

In the present study it was observed that irrespective of the sterilization methods used, the amount of remaining GP increased sequentially from first to fifth use. Hanan etal 2016 in his study observed that the quality of both the cutting surfaces and head surfaces of rotary NITi files after repeated use detected blunt cutting edges, microcracks[26]. The repeated use of NiTi files may cause the plastic deformation of the material[26]. According to Oliveiria et al 2006, Weine et al 1998 , the action of the file is to scratch the debris from root canal walls to detach it and remove it towards outside the canals[25]. Accordingly, debris are left along their spirals upon repeated use because of the design , presenting angles between the spirals and the long axis of the files [25] . Hence the plastic deformation, surface alterations and clogged debris on the flutes of the files (Linsuwanont et al 2004) on repeated use could cause reduced efficiency in removing the GP resulting in increased remaining GP. Reduced efficiency of the files ultimately effects the time taken for the procedure and increases the usage time sequentially from first to fifth use. According to Arunajatesan et al (2020) the increase in amount of remaining GP and also the time taken for retrieval of GP concomitant to number of use clearly shows that there is a decrease in efficiency of the retreatment file with repeated use . But manufactures recommends discarding instruments after a specified number of uses or whenever visible deformation is observed[28].

Studies proves that there is necessity of mechanical removal of debris prior to chemical removal of organic materials. Linsuwanont et al 2004[27] in his study with SEM evaluation he observed that unless the instruments are cleaned under dust free condition using filtered distilled water , it may be impossible to achieve complete removal of dust or fine silica particles from tap water which are universally distributed along the files. He also mentioned that mechanical cleaning alone could not totally clean the instrument . Murgel et al (1990) reported that various mechanical cleaning methods like guaze soaked in alcohol , a sponge soaked in alcohol and ultrasonic bath was unable to clean the instruments totally[27]. On the other hand , Reiss-Araújo et al. alert that the manual cleaning itself is subject to human error, such as omission or failure in cleaning itself[25]. The authors still emphasized that even nylon bristles did not penetrate in the angle formed by the spirals and body of most of the endodontic files. In the present study all the groups of files were cleaned with cotton soaked in water , but the amount of debris removed from the files were questionable.

Only few studies have investigated the effectiveness of cleaning methods for endodontic instruments(Segall et al 1977) . To date no cleaning method have been demonstrated to clean NITI endodontic instruments totally (Linsuwanont et al 2004) .Even SEM studies is limited in its ability to determine the nature of contaminants and it is impractical as a scoring technique (Linsuwanont et al 2004)[27]. Based on the knowledge from scientific literature this is the first study to compare the efficiency of sterilization methods of endodontic files by comparing the amount of remaining gutta percha . Hence there is only limited information regarding the area of subject and future studies should be carried out with large samples to validate the findings from our study.

Limitations

The root canal volumes of human mandibular premolars differ from the root canal volumes of other teeth. This may interfere with the amount of remaining GP and the results may also vary accordingly.

Conclusion:-

Within the limitations of the present in vitro study , it can be concluded that autoclave sterilization causes least changes to the physical properties of the files when compared to gluteraldehyde sterilization which adversely effects the cutting efficiency of the files. Unsterilized files exhibits least cutting efficiency resulting in reduced gutta percha removal from the canals. Also the number of use was a determining factor for cutting efficiency. Reduction in the cutting effectiveness of instruments may increase operating time and it could take longer to remove the material from root canal. Scientific studies report conflicting opinions on the alterations affecting endodontic instruments subject to sterilization procedures. This divergence of opinions and results could depend on the countless instruments and protocols used in endodontics and on the different sterilization and disinfection methods.

Table 1:-

GROUPS	GROUP A (Average of 3 sets of samples 3*5=15)		GROUP B (Average of 3 sets of samples 3*5=15)			GROUP C (Average of 3 sets of samples 3*5=15)		
SUB GROUPS	Remaining GP (%)	Time Taken (min)	SUB GROUPS	Remaining GP (%)	Time Taken (min)	SUB GROUPS	Remaining GP (%)	Time Taken (min)
First use(A1)	18.57	5.01	First use(B1)	30.32	5.13	First use(C1)	32.90	5.08
Second use(A2)	25.64	5.23	Second use(B2)	34.87	5.37	Second use(C2)	38.11	5.47
Third use(A3)	30.46	5.41	Third use(B3)	40.25	5.50	Third use(C3)	47.83	6.23
Fourth use(A4)	41.22	5.56	Fourth use(B4)	47.49	6.22	Fourth use(C4)	54.38	6.51
Fifth use(A5)	48.51	6.04	Fifth use(B5)	56.05	6.42	Fifth use(C5)	62.24	7.11
Average	32.88	5.45	Average	41.79	5.72	Average	47.09	6.08

Mean of remaining GP and time taken in both groups and subgroup [original]
Group A : Neo endo retreatment files sterilized by Autoclave
Group B : Neo endo retreatment files sterilized by Cold sterilization
Group C : Neo endo retreatment files not sterilized (cleaned with soap and water)

Table 2:-

Comparison of mean Remaining GP (%) between 3 groups at different usage times using One-way ANOVA test followed by Tukey's post hoc Test							
Usage	Groups	N	Mean	SD	p-value [a]	Sig. Diff	p-value [b]
First	Group A	3	18.57	0.13	<0.001*	A vs B	<0.001*
	Group B	3	30.32	0.18		A vs C	<0.001*
	Group C	3	32.90	0.70		B vs C	0.001*
Second	Group A	3	25.64	0.14	<0.001*	A vs B	<0.001*
	Group B	3	34.87	0.72		A vs C	<0.001*
	Group C	3	38.11	0.97		B vs C	0.003*
Third	Group A	3	30.46	0.81	<0.001*	A vs B	<0.001*
	Group B	3	40.25	0.63		A vs C	<0.001*
	Group C	3	47.83	0.19		B vs C	<0.001*
Fourth	Group A	3	41.22	0.73	<0.001*	A vs B	<0.001*
	Group B	3	47.49	0.31		A vs C	<0.001*
	Group C	3	54.38	0.36		B vs C	<0.001*
Fifth	Group A	3	48.51	0.66	<0.001*	A vs B	<0.001*
	Group B	3	56.05	0.54		A vs C	<0.001*
	Group C	3	62.24	0.46		B vs C	<0.001*

* - Statistically Significant
Note: a. p-value derived by One-way ANOVA Test; b. p-value derived by Tukey's post hoc Test

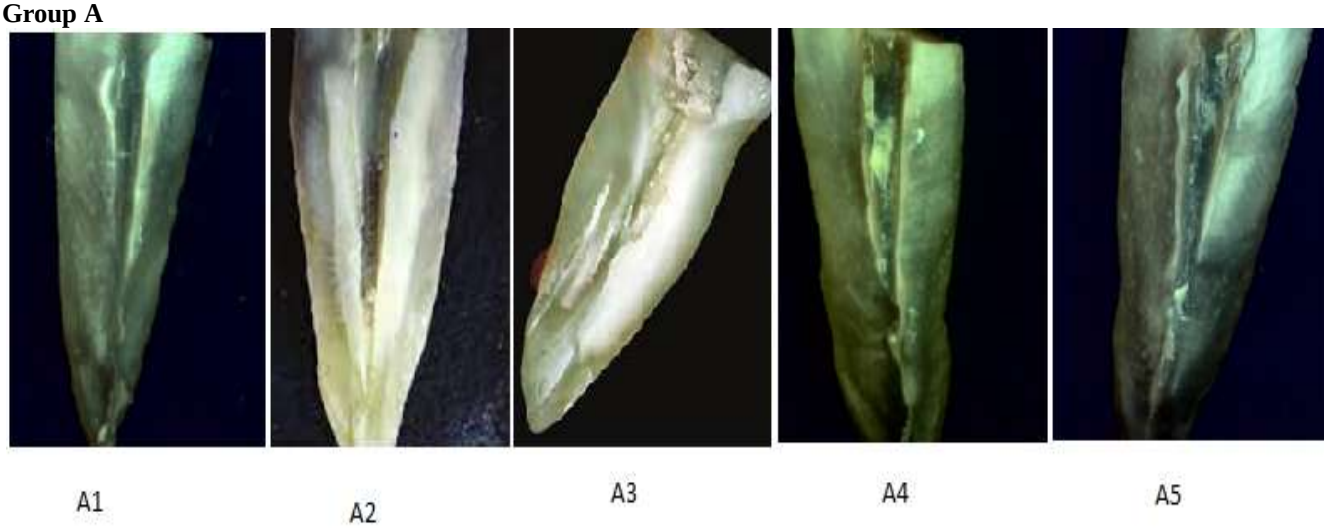
Table 3:-

Comparison of mean Time Taken (in mins) between 3 groups at different usage times using One-way ANOVA test followed by Tukey's post hoc Test							
Usage	Groups	N	Mean	SD	p-value [a]	Sig. Diff	p-value [b]
First	Group A	3	5.01	0.40	0.82	A vs B	..
	Group B	3	5.13	0.03		A vs C	..
	Group C	3	5.08	0.04		B vs C	..
Second	Group A	3	5.23	0.12	0.04*	A vs B	0.24

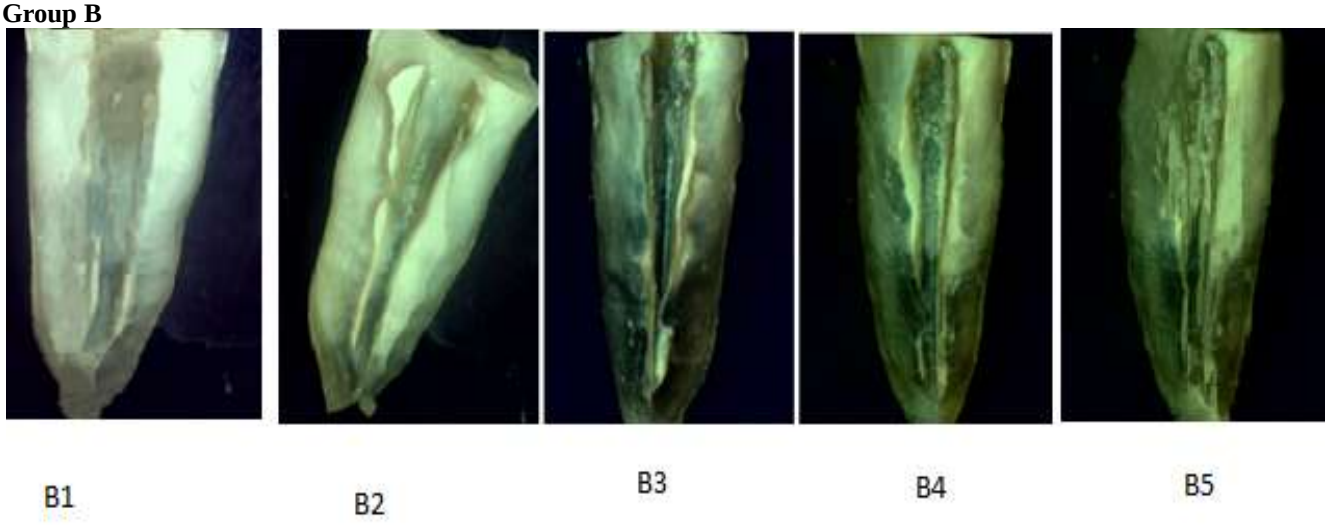
	Group B	3	5.37	0.10		A vs C	0.04*
	Group C	3	5.47	0.04		B vs C	0.44
Third	Group A	3	5.41	0.10	<0.001*	A vs B	0.30
	Group B	3	5.50	0.03		A vs C	<0.001*
	Group C	3	6.23	0.06		B vs C	<0.001*
Fourth	Group A	3	5.56	0.01	<0.001*	A vs B	<0.001*
	Group B	3	6.22	0.10		A vs C	<0.001*
	Group C	3	6.51	0.01		B vs C	0.003*
Fifth	Group A	3	6.04	0.04	<0.001*	A vs B	<0.001*
	Group B	3	6.42	0.01		A vs C	<0.001*
	Group C	3	7.11	0.02		B vs C	<0.001*

Representative Steriomicroscopic Images (Figure 1)

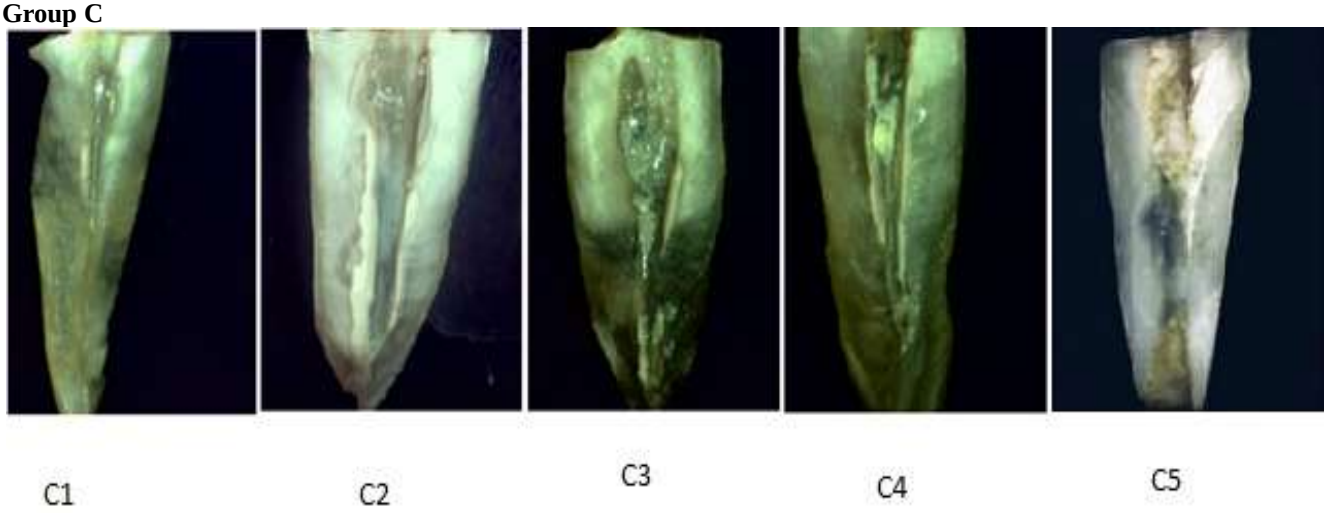
Group A



Group B



Group C



Conflict Of Interest:
Nil.

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