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RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF EFFICACY OF TETRACYCLINE HCL, EDTA & HYALURONIC ACID GEL AS ROOT BIOMODIFICATION AGENTS. -AN IN-VITRO SEM STUDY

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Abstract

Background: Scaling and Root Planing alone leaves a smear layer on the root surface which may interfere with reattachment of cells to root surface during regenerative periodontal therapy. Root bio-modification has been advocated for smear layer removal which may enhance regeneration.

Objective: To evaluate and compare effect of 17% Ethylenediamine Tetra Acetic acid (EDTA), Tetracycline HCL and 1% Hyaluronic Acid gel as root conditioning agents on periodontally involved root surfaces of extracted teeth using a scanning electron microscope (SEM).

Materials And Method: Freshly extracted 10 single rooted human teeth were sectioned into 30 samples and were segregated into 3 groups:

1. **Group Ⅰ -** tetracycline HCl (pH 1.6),
2. **Group Ⅱ –** EDTA 17% (pH 7.3),
3. **Group III –** HA gel 2%. The samples were treated with respective agents and were viewed under 4000X magnification using a SEM. The specimens were assessed for the Residual smear layer score, Number of patent tubules, Total number of dentinal tubules, Proportion of patent to total number of dentinal tubules and Mean Diameter of dentinal tubules.

Results: Group I and Group II were significantly better than Group III for all the measured parameters. However, the number of patent dentinal tubules, total number of dentinal tubules and mean diameter of dentinal tubules were significantly superior in Group II as compared to Group I**.**

Conclusion: Tetracycline HCL was found to be the most effective root conditioning agent amongst the 3 groups, for the assessed parameters.

Introduction:-

Periodontitis affected root surfaces are hyper mineralized and contaminated with cytotoxic and other biologically active substances. Such surfaces are not biocompatible with adjacent periodontal cells, the proliferation of which is pivotal for periodontal wound healing.[1,2]

The undesirable disease induced alterations in and on the root surface includes reduced collagen fiber insertion,[3] alterations in mineral density and surface composition[4], and root surface contamination by bacteria and their

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endotoxins[5]. Pathologically exposed root surfaces undergo substantial alterations and changes, and thus, may not serve as an appropriate substrate for cell attachment and fiber formation.[2]

Mechanical instrumentation of the root surface results in the formation of a smear layer, which acts as a physical barrier, inhibiting new attachment and cell migration. It has been indicated that such root debridement may not completely remove contaminated cementum particularly in more apical areas.[6]

In several in-vitro studies, the removal of the smear layer and dentinal tubules exposure have been described as factors that may favor clot stabilization in the earliest stages of periodontal healing event by increasing blood cells[7,8] and fibrin adhesion to such cleansed root surfaces during wound healing.[9] It is currently believed that stabilization of the blood clot within the wound followed by migration of periodontal ligament cells onto the root surface exposed by the disease process are crucial events in the periodontal regeneration.[10]

A number of agents have been proposed for demineralization procedure which includes EDTA, citric acid, minocycline, tetracycline, doxycycline, fibronectin, phosphoric acid, etc. These agents when applied on the root surfaces remove smear layer, eliminate the cytotoxic material like endotoxins, widen the orifices of the dentinal tubules and expose the dentin collagen matrix. This collagen matrix is thought to provide a substrate which supports the chemotaxis, migration and attachment of those cells involved in wound healing and formation of new connective tissue attachment.[11]

Tetracycline hydrochloride is an effective antibiotic against periodontal pathogens, which is absorbed into the root surface and is slowly released in its active form.[12] Tetracycline HCl may be beneficial in periodontal regenerative therapy.[13, 14]

A supersaturated pH neutral etching solution of Ethylenediaminetetraaceticacid (EDTA) has been found to be effective with respect to smear removal preserving the integrity of exposed collagen fibers and periodontal healing.[15, 16, 17]

Hyaluronic acid (HA)—also known as hyaluronan was originally discovered in 1934 in the vitreous body of the eye and synthesized in 1964.[18] HA is characterized by well-conserved structural properties and linked to several ECM proteins and collagenous fibers responsible for mediating cell adhesion, motility, migration, and proliferation.[19, 20] A variety of biological functions in wound-healing processes including angiogenesis and reepithelialization have been documented both in vitro and in vivo following topical application of HA.[21,22] In vitro studies have demonstrated that HA-induced reduction of periodontal pathogens including Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis.[23] Moreover, HA has been linked with minimizing early bacterial recolonization after and in combination with mechanical debridement.[24,25] In a SEM study conducted by **Mueller [26]**, analysis of the dentin slices treated with cross-linked HA and non-cross-linked HA revealed roughened surface topography of dentin surfaces resulting in improved PDL cell spreading. However, there is limited available literature evaluating HA as a Root conditioning agent using SEM and comparing it with established root conditioning agents.

Thus, the aim of this In-Vitro study was to evaluate and compare the effect of Ethylenediamine Tetra acetic acid (EDTA) 17%, Tetracycline HCL and 1% Hyaluronic Acid gel as root conditioning agents on periodontally involved root surfaces of extracted teeth using a scanning electron microscope.

Materials And Method:-

This In Vitro study was conducted in the Department of Periodontology and Oral Implantology, I.T.S. Centre for Dental Studies and Research, Muradnagar, Ghaziabad and at SAIF, AIIMS, New Delhi. A total of 30 dentin samples were prepared from the periodontally affected region of 10 extracted teeth collected from the patients requiring extraction due to chronic periodontitis. The subjects with no history of root planing, scaling or prophylaxis in previous 6 months were included in the study. Teeth with root surface caries or cervical restorations were excluded from the study.

Following extraction, the teeth were washed and cleaned and were stored in a saline solution to prevent dehydration. Scaling and root planing of root surfaces were done using a sharp Gracey curette with 6 to 8 strokes to obtain a smooth, shiny and hard glass-like surface as shown in Fig 1. Using a high- speed cylindrical bur under copious irrigation, three longitudinal root sections were prepared from each tooth by cutting the cervical two thirds of the

root into two halves first and then splitting one of the halves into two more halves by cutting perpendicular to the first cut, as shown in Fig 2. All the pulpal tissue was removed and an identification notch was made at the pulpal root surface. The three samples from each tooth were stored in individual containers containing PBS for 24 hours as shown in Fig 3.

The prepared tooth root samples were lightly rubbed with the prepared solution and gel saturated cotton pellets that were changed every 30 seconds for a total period of 5 minutes to ensure consistent solution application using passive burnishing method. Following the treatment, samples were rinsed with water for 20 seconds and air dried.

Application Of Solutions:
EDTA 17% - Application of 17% EDTA solution (pH 7.3) was done for 5 minutes.
Tetracycline HCl - Application of Tetracycline HCl (pH 1.6) was done for 5 minutes. (Tetracycline HCl (250 mg/ml) was made by mixing 500 mg in 2 ml of sterile water.)

Sodium Hyaluronan gel- Application of 1% Sodium Hyaluronan gel was done for 5 minutes.

Sample Preparation For SEM
After treatment of the root surfaces, samples were fixed in 2.5 % gluteraldehyde in phosphate buffer (pH 7.3) for 24 hours and washed three times each in phosphate buffer. The specimens were then dehydrated in a graded series of aqueous-ethanol solutions for 10 minutes each. Then the samples were dried overnight in a dehydration jar. Each air dried sample was mounted on aluminium stubs and sputter coated (Agar Sputter Coater) with approximately 20 to 30 nm of gold as shown in Fig-4 & Fig-5 and then examined with a scanning electron microscope (Fig-6) (Leo 435VP, Variable Pressure Scanning Electron microscope).

Parameters Recorded:
SEM photomicrographs were taken, examined and the morphometric measurements were performed. The photomicrographs were examined at 4000X to assess in each treatment group the following-

1. **The degree of smear layer removal** based on the index given by **Sampaio et al** [27](2005)

Score 1: Root surface without smear layer with the dentinal tubules completely open without evidence of smear layer in the dentinal tubules.
Score 2: Root surface without smear layer with the dentinal tubules completely open, but with some evidence of smear layer in the dentinal tubules entrance.
Score 3: Root surface without smear layer with the dentinal tubules partially open.
Score 4: Root surface covered by a uniform smear layer, with evidence of dentinal tubules opening.
Score 5: Root surface covered by a uniform smear layer without evidence of dentinal tubules opening.
Score 6: Root surface covered by an irregular smear layer, with the presence of grooves and/or scattered debris.

2. **The number of patent dentinal tubules** - were identified as the dentinal tubule orifices which were round or oval in shape with sharply defined borders.
3. **The total number of dentinal tubules-** was identified as all the dentinal tubules openings that were visible, of variable shape and size which may be partially occluded.
4. **The mean diameter of dentinal tubules** in each treatment group was calculated on the photomicrographs.
5. **The proportion of the patent tubule to the total number of dentinal tubules** was calculated by dividing Number of patent dentinal tubules by Total Number of dentinal tubules *100

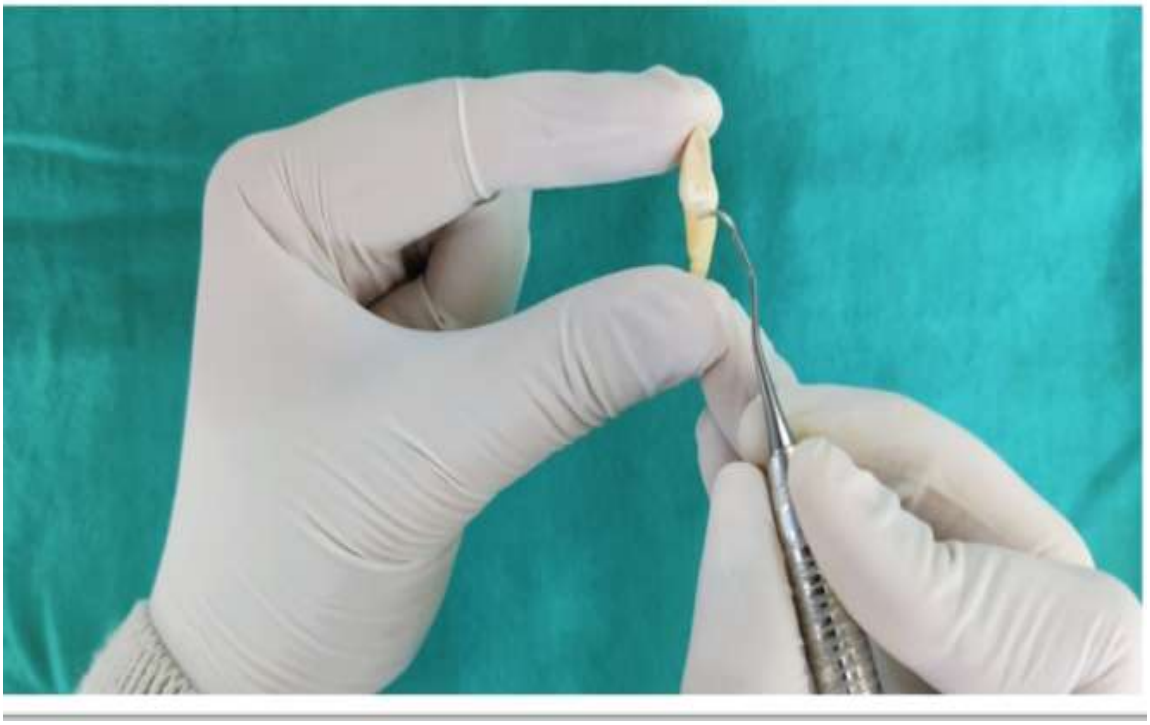

Fig 1:- Root Planing Done Before Preparation Of Samples.

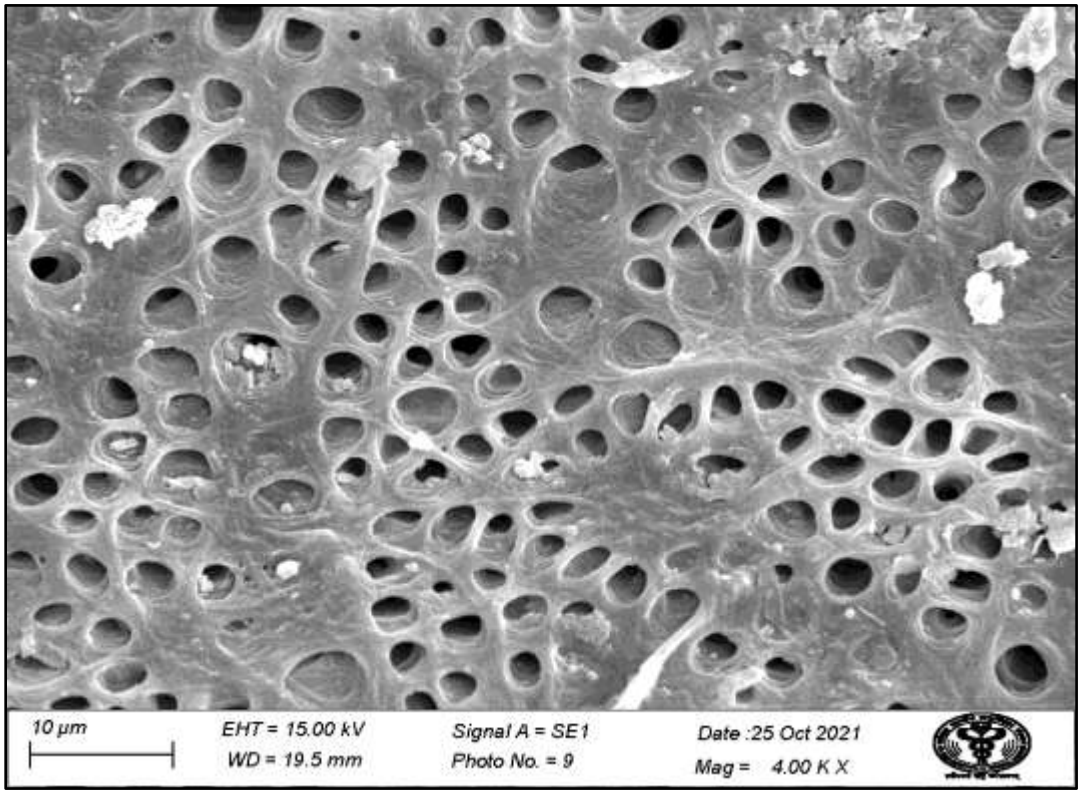

Fig 2:- Sem Photomicrograph Of A Specimen Treated With 17% Edta (Magnification 4000X).

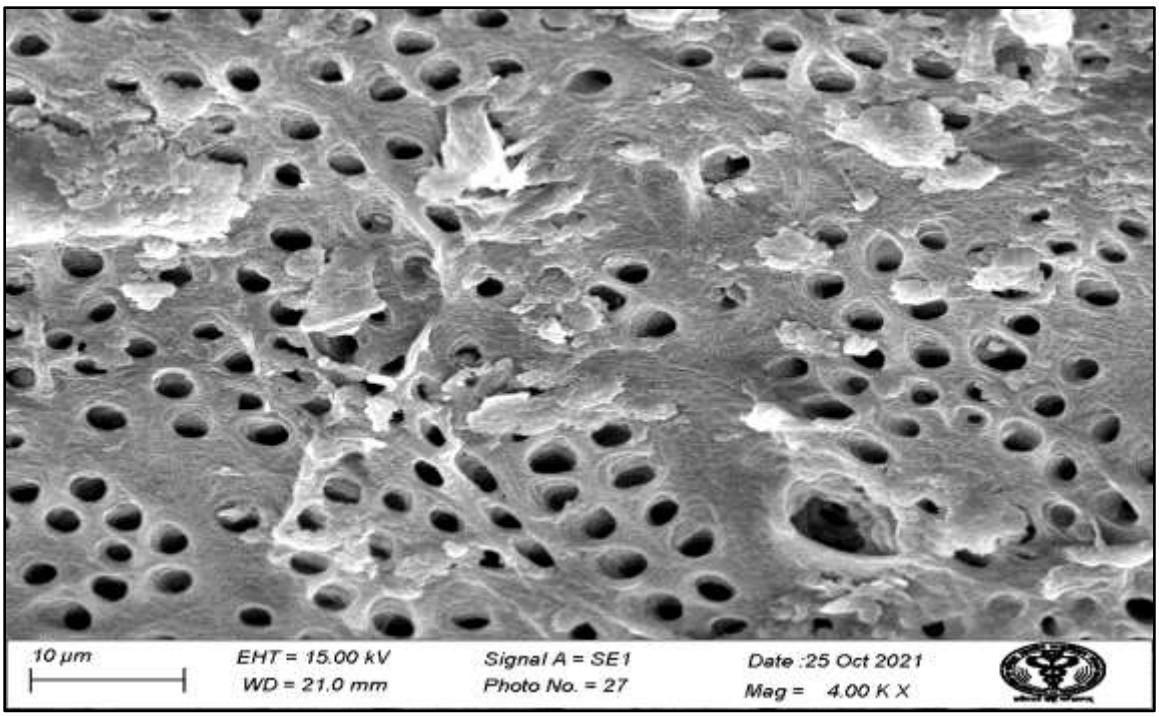

Fig 3:- Sem Photomicrograph Of A Specimen Treated With 25% Tetracycline Hydrochloride (Magnification 4000X).

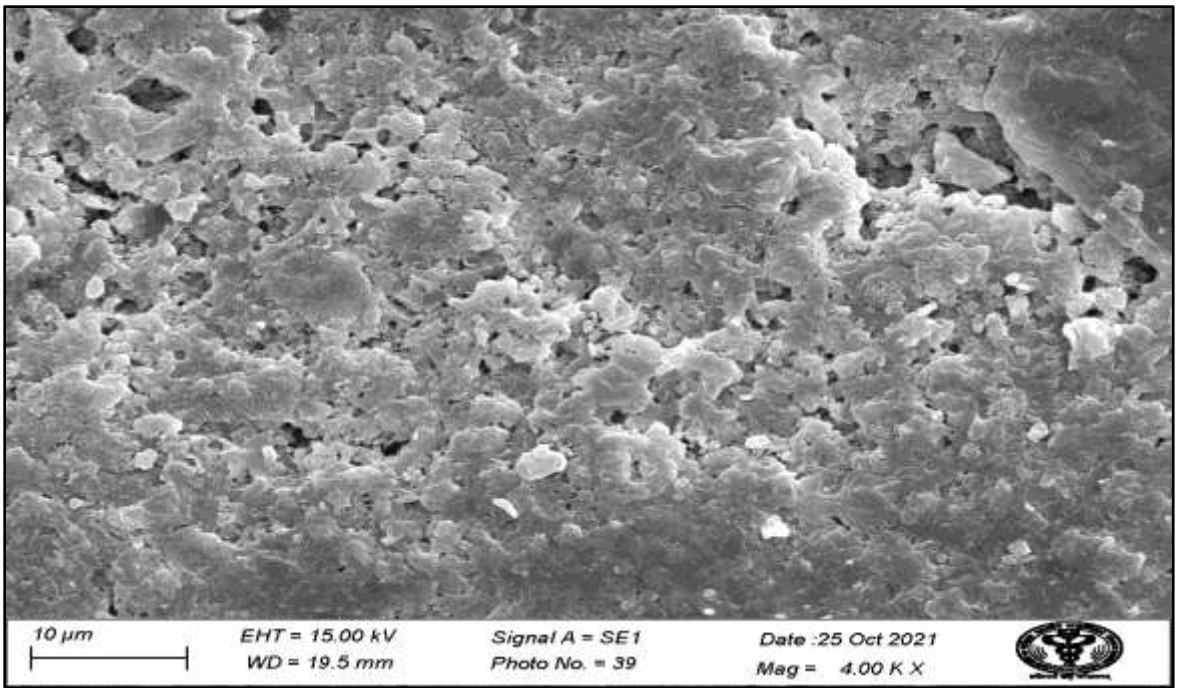

Fig 4:- Sem Photomicrograph Of A Specimen Treated With 1% Hyaluronic Acid Gel (Magnification 4000X).

Statistical Analysis

Statistical analysis was done by software program (SPSS 16 Inc. Chicago IL, USA).The data obtained was statistically analyzed for normality using Kolmogorov-Smirnov test, followed by the one-way ANOVA test for intergroup comparison and Post-Hoc tests for pairwise comparison among the groups. The value of $p<0.05$ was considered to be statistically significant.

Results:-

In this In-vitro study, the root surfaces were conditioned with the following root conditioning agents : Group I (EDTA 17%), Group II (Tetracycline HCl) and Group III (Hyaluronic Gel 2%). The specimens were assessed at 2000x & 4000x magnification for the Residual smear layer score, Number of patent tubules, Total number of dentinal tubules, Proportion of patent to total number of dentinal tubules and Mean Diameter of dentinal tubules.

Residual Smear Layer Score

The mean values of residual smear layers of group I, II and III were 2.40 ± 0.51, 2.40 ± 0.51 and 4.60 ± 0.84 respectively (Table 1), which were statistically significant ($p<0.001$).However, pairwise comparison between group I & II (Table 2) was statistically non-significant ($p>0.05$).Whereas, the pairwise comparison between group II& III (Table 3) and group I & III (Table 4) was statistically significant ($p<0.001$).

Number Of Patent Dentinal Tubules

The mean scores of patent dentinal tubules of group I, II and III were 66.90 ± 10.48, 95.10 ± 12.16, and 26.70 ± 5.29 respectively (Table 1), which were statistically significant ($p<0.001$). The pairwise comparison between group I & II and group II & III and group III & I (Table 2, 3 & 4), was statistically significant ($p<0.001$).

Total Number Of Dentinal Tubules

The mean scores of total number of dentinal tubules of group I, II and III were 99.30 ± 11.73, 130.80 ± 10.42, and 55.80 ± 8.10 respectively(Table 1), which were statistically significant ($p<0.001$). The pairwise comparison between group I & II and group II & III and group III & I (Table 2, 3 & 4) was statistically significant ($p<0.001$).

Proportion Of Patent To Total Number Of Dentinal Tubules

The mean scores of Proportion of patent to total number of dentinal tubules of group I, II and III were 67.33 ± 6.89, 72.76 ± 7.90 and 47.74 ± 5.60 respectively (Table 1), which were statistically significant ($p<0.001$). The pairwise comparison between group I & II (Table 2) was not statistically significant ($p>0.05$). Whereas, the pairwise comparison between group II & III and group III & I (Table 3 & 4), was statistically significant ($p<0.001$).

Mean Diameter Of Dentinal Tubules

The mean scores of diameter of dentinal tubules of group I, II and III were 0.85 ± 0.14, 1.27 ± 0.11 and 0.49 ±0.09 respectively(Table 1), which were statistically significant ($p<0.001$). The pairwise comparison of group I & II, group II & III and group III & I (Table 2, 3 & 4) was statistically significant ($p<0.001$).

Table 1:- Mean & Standard Deviation Of Residual Smear Layer, Number Of Patent Dentinal Tubules, Total Number Of Dentinal Tubules, Proportion Of Patent To Total Number Of Dentinal Tubules And Mean Diameter Of Dentinal Tubules.

PARAMETERS	GROUP-I (EDTA 17%)	GROUP-II (TETRACYCLINE HCL)	GROUP-III (HA)	P-VALUE
	MEAN±SD	MEAN±SD	MEAN±SD	
RESIDUAL SMEAR LAYER	2.40 ±.516	2.40 ±.516	4.60 ±.843	<0.001*
NUMBER OF PATENT DENTINAL TUBULES	66.90±10.482	95.10±12.161	26.70±5.293	<0.001*
TOTAL NUMBER OF DENTINAL TUBULES	99.30±11.738	130.80±10.422	55.80±8.108	<0.001*
PROPORTION OF PATENT TO TOTAL NUMBER	67.339±6.8995	72.762±7.9019	47.741±5.6014	<0.001*

OF DENTINAL TUBULES				
MEAN DIAMETER OF DENTINAL TUBULES	.850±.1454	1.273±.1197	.493±.0974	<0.001*
*Statistically significant as p < 0.05				

Table 2:- Comparisons Between Group I (17% Edta) & Group Ii (Tetracycline HCL).

GROUPS	**RESIDUAL SMEAR LAYER SCORE**	**NUMBER OF PATENT DENTINAL TUBULES**	**TOTAL NUMBER OF DENTINAL TUBULES**	**PROPORTION OF PATENT TO TOTAL NUMBER OF DENTINAL TUBULES**	**MEAN DIAMETER OF DENTINAL TUBULES**
	MEAN DIFF ± STD ERROR	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**
GROUP I & GROUP II	.000±.288	-28.20±4.365	-31.50±4.562	-5.42±3.0705	-.4230±.0547
SIGNIFICANCE	1.000	< 0.001*	< 0.001*	.200	< 0.001*
*Statistically significant as p < 0.05					

Table 3:- Comparisons Between Group Ii (Tetracycline Hcl) & Group III (HA).

GROUPS	**RESIDUAL SMEAR LAYER SCORE**	**NUMBER OF PATENT DENTINAL TUBULES**	**TOTAL NUMBER OF DENTINAL TUBULES**	**PROPORTION OF PATENT TO TOTAL NUMBER OF DENTINAL TUBULES**	**MEAN DIAMETER OF DENTINAL TUBULES**
	MEAN DIFF ± STD ERROR	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**
GROUP II & GROUP III	-2.20±.288	68.4±4.365	75.00±4.562	25.021±3.0705	.780±.0547
SIGNIFICANCE	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
*Statistically significant as p < 0.05					

Table 4:- Comparisons Between Group Iii (Ha) & Group I (17% EDTA).

GROUPS	**RESIDUAL SMEAR LAYER SCORE**	**NUMBER OF PATENT DENTINAL TUBULES**	**TOTAL NUMBER OF DENTINAL TUBULES**	**PROPORTION OF PATENT TO TOTAL NUMBER OF DENTINAL TUBULES**	**MEAN DIAMETER OF DENTINAL TUBULES**
	MEAN DIFF ± STD ERROR	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**	**MEAN DIFF ± STD ERROR**

GROUP III & GROUP I	2.20±.288	-40.20±4.365	-43.50±4.562	-19.598±3.07	-.3570±.0547
SIGNIFICANCE	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
*Statistically significant as $p < 0.05$					

Discussion:-

Root biomodification has been advocated as an effective adjunct to mechanical debridement for removal of the smear layer and condition the roots for better regeneration. Root biomodification uses acid substances or chelating agents to remove the smear layer and further cause the demineralization of the root surface, selective removal of hydroxyapatite, exposure of the collagenous matrix of the root surface and inhibition of collagenolytic activity. Independent of being conditioned, the collagen from the root surface attaches to fibrin present in a clot, preventing epithelial down growth, and forms a scaffold for cell development and mature collagen fiber attachment.[28]

Tetracycline HCl root conditioning may potentially enhance periodontal wound healing. Besides the antimicrobial effect, Tetracycline HCL root conditioning may regulate the adsorption of plasma proteins, enhance adhesion of the blood clot, and stimulate deposition of collagen against the root surface.[29]

Ethylenediamine Tetraacetic Acid (EDTA) is the most widely used irrigant for smear layer removal. In addition to the cleansing function, it acts on inorganic material by reacting with calcium ions in dentin, resulting in calcium chelation, promoting decalcification of dentin.[30]

Hyaluronic acid is a natural component of the extracellular matrix which has anti-inflammatory, antibacterial, anti-edematous, and osteoinductive properties that help in enhancing periodontal wound healing. The application of HA as a root conditioning agent has been seen to modify the surface texture of dentin via increasing the surface roughness, which subsequently enhances cell attachment and spreading onto the dentin surface.[26]

In the present study, an in-vitro attempt was made to evaluate and compare the effect of tetracycline hydrochloride, 17% EDTA and 1% hyaluronic acid gel as root conditioning agents on periodontally involved root surfaces using a scanning electron microscope.

SEM analysis of the conditioned root surfaces revealed that **removal of smear layer** by tetracycline HCL (250 mg/ml) and 17% EDTA was better than 1% Sodium hyaluronan gel. This could be attributed to the lower pH of tetracycline HCL (pH 1.0) and property of EDTA to selectively eliminate minerals from the dentin surface and expose more collagenous structures which create a favorable root surface. Similarly, in a study by **Soares et al** [31] it was reported that root specimens treated with 24% EDTA with neutral pH and tetracycline gel resulted in adequate demineralization without smear layer and smear plug on the root surface following scaling and root planing. In other studies conducted by **Sayin et al**[32]**, Haznedaroglu and Ersev**[33] **and Ahir et al**[34] showed contrasting results that tetracycline HCl solution was effective as smear layer removal, but it was not able to remove it completely.

Tetracycline HCl treated specimens had the highest **number of patent tubules** when compared to EDTA (17%) followed by sodium hyaluronan gel. Similar results were observed in a study by **Nanda et al** [35] found that the number of patent dentinal tubules in the Tetracycline HCL group was more when compared to the EDTA group. However, **Babay** [36] found similar morphological characteristics when comparing EDTA and Tetracycline HCL groups were compared. **Garg et al**[37] showed that EDTA showed higher number of patent tubules as compared to tetracycline HCl, which is in contraindication to our study.

A maximum **mean total number of dentinal tubules** were found in tetracycline HCL treated specimens followed by 17% EDTA specimens followed by 1% Sodium Hyaluronan Gel. The study conducted by **Nanda et al**[35] had similar results as treatment with tetracycline HCL resulted in higher number as compared to EDTA. The difference was probably due to the variation in pH of tetracycline and EDTA, so higher concentration of EDTA may be required to achieve the comparable results. In contrast to the present study, higher number of dentinal tubules were found in EDTA as compared to tetracycline HCl in a study by **Garg et al.**[37]

The **mean proportion of patent to total number of dentinal tubules** were comparable in tetracycline HCl and 17% EDTA. However, both the groups showed better results than 1% sodium hyaluronan gel.**Babay**[36] found similar

morphological characteristics when comparing EDTA and tetracycline hydrochloride. Contrasting results were seen in study conducted by **Garg et al**[37] where the mean proportion of patent to total number of dentinal tubules was high in EDTA as compared to tetracycline HCl.

The **mean diameter of dentinal tubules** was found to be maximum in tetracycline HCl followed by 17% EDTA and 1% Sodium hyaluronan gel. Similarly, **Chahal et al**[38] reported the mean diameter of tetracycline HCL- treated specimens was higher than the citric acid group and the doxycycline group. **Hanes et al** [39] have also shown that in addition to removing the smear layer these agents also enlarge the openings of the dentinal tubules as demonstrated by the increase in tubule diameter following treatment with them. This enlargement or widening of the tubule orifice can be attributed to the preferential demineralization of the peritubular dentin by these agents. On the contrary, **Nanda et al**[35] found that the diameter of the dentinal tubules was more in the 10% EDTA group as compared to the 10% tetracycline HCL group.

In the present study, it was seen that root conditioning by all the three agents used helped in the removal of smear layer, exposure of dentinal tubules, and also widening of dentinal tubule orifices in vitro. But overall, tetracycline HCL group showed significant results with respect to the parameters such as number of patent dentinal tubules, total number of dentinal tubules and mean diameter of dentinal tubules compared to 17% EDTA. Also, both the groups were better than 1% sodium hyaluronan gel group in all the parameters evaluated. This could be attributed to the acidic nature of 17% EDTA and tetracycline HCL resulting in more physical changes in the dentinal tubules. However, in a study by **Babgi et al**[40] the results showed that Hyaluronic acid resulted in higher cell viability compared to scaled roots treated with EDTA. Also, the application of HA increased the fibroblast attachment to the root surface significantly as compared with other root conditioning agents. So, Hyaluronic acid has biological properties which aid in periodontal wound healing but the physical properties of Hyaluronic acid as a root conditioning agent still remains questionable.

Few differences between our results and those of other studies may be related to the disease status of dentin specimens utilized, the concentration and properties of the root conditioning agents used, time and mode of application of these root conditioning agents.

The results of the present study are limited to physical root surface changes seen using SEM and do not present in vivo differences that may result from the physiologic effect of these root conditioning agents. Also, the effect of the root conditioning agents on the movement and attachment of PDL cells was not analyzed which could be another limitation of our study, as HA has been shown to have these biological effects. Thus, further in-vitro and in-vivo studies with different agents with varying concentrations and mode of applications should be conducted to substantiate the findings of the study, for future clinical applications.

Conclusion:-

Thus, it can be concluded that 17% EDTA, Tetracycline HCL and 1% Sodium Hyaluronan gel can be effectively used as root conditioning agents. However, Tetracycline HCL was found to be the most effective root conditioning agent amongst the three groups followed by EDTA and HA.

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