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COMPARATIVE EVALUATION OF FRACTURE RESISTANCE OF ENDODONTICALLY TREATED TEETH IRRIGATED WITH FOUR DIFFERENT IRRIGATING SOLUTIONS: AN IN-VITRO STUDY

Endodontics

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ABSTRACT

Introduction: Various solutions have been used for chemo mechanical preparation during endodontic treatment. However there have been reports suggesting that there might be some detrimental effects of these irrigants on the fracture resistance of root canal treated dentin.
The aim of this study was to comparatively evaluate the effects of different irrigants, on the fracture resistance of endodontically treated teeth.
Methods: Eighty freshly extracted, single rooted human mandibular premolars were selected. Access cavities were prepared, working lengths were measured and root canals were prepared till #45 K-file (MANI, INC., Japan). 5ml Normal Saline was used for irrigation during preparation. After preparation, they were divided into following groups:
Group I-Normal Saline,
Group II- ASEP-RC (Anabond Stedman pvt. ltd. Chennai, India),
Group III-2.5% NaOCl (Sodium Hypochlorite, PRIME Dental Products, Maharashtra)
Group IV- Q Mix 2-in-1 (® Dentsply, Tulsa Dental Specialities)
Obturation was done using Gutta Percha with cold lateral compaction. Teeth were incubated at 37°C and 100% humidity for a week. Fracture resistance was tested for the teeth under a Universal Testing Machine (UTM). One-way ANOVA and one-way Tukey HSD test were used for the comparative analysis of the groups and significance level was set at 0.001.
Results: Group IV (QMix 2-in-1) showed significantly higher values of fracture resistance compared to other groups.
Conclusion: Roots that were irrigated using QMix 2-in-1 demonstrated higher fracture resistance compared to the other groups.

KEYWORDS

ASEP-RC, Endodontics, Final Irrigation, Fracture resistance, Irrigants, NaOCl, Qmix 2-in-1.

INTRODUCTION:

The success of endodontic treatment depends on three main steps: efficient access cavity preparation and caries removal, followed by thorough chemo-mechanical preparation and lastly proper obturation that provide a complete sealing of the root canal systems.[1] Among these, the chemo-mechanical preparation is a vital step as it results in the elimination of microorganisms by using various irrigating solutions and intracanal medicaments.

Ideally, an irrigating solution should promote debridement, lubrication, dissolve organic tissue and eradicate the microorganisms from the root canal system.[2-3]

Sodium Hypochlorite (NaOCl) is one of the traditional irrigants which is used most commonly. It was first produced in 1789 in Javelle, France, by passing chlorine gas through a solution of sodium carbonate. The resulting liquid was known as "Eau de Javelle" or "Javelle water". *Coolidge*later introduced NaOCl to endodontics.[4]

It is an excellent antibacterial agent, capable of dissolving necrotic tissue, vital pulp tissue, and the organic components of dentin and biofilms.[5] NaOCl possesses a broadspectrum antimicrobial activity against endodontic microorganisms and biofilms, including microbiota difficult to eradicate from root canals, such as *Enterococcus, Actinomyces,* and *Candida* species.[6]

During endodontic therapy, NaOCl solutions are used at concentrations ranging from 0.5% to 6%. However, the most commonly used concentrations are 2.5% and 5.2%.

Even though NaOCl is the most widely used irrigant, studies have related sodium hypochlorite irrigation with dentin microhardness reduction.[7] Furthermore, final irrigation with NaOCl negatively affects various physical properties of root canal dentin including elasticity and flexural strength.[8] Hence, fracture resistance of the teeth may be affected by the use of sodium hypochlorite as an irrigating solution.

Keeping in mind these possible adverse effects of sodium hypochlorite, there has been constant advancement and discovery of new irrigating solutions that might have lesser adverse effects than sodium hypochlorite.

ASEP-RC is a new irrigating material that was introduced in 2015 which consists of 2% Chlorhexidine as its constituent.

Chlorhexidine (CHX) is a chemical that was developed more than 50 years ago at Imperial Chemical Industries in England and first marketed in the United Kingdom in 1953 as an antiseptic cream.[9] Since 1957, it has been used for general disinfection purposes and the treatment of skin, eye, and throat infections in both humans and animals.[9] Subsequently, CHX began to be used as an endodontic irrigant as well as an intracanal medicament.

The action of CHX is due to the fact that it is a wide-spectrum antimicrobial agent, active against gram-positive and gram-negative bacteria as well as yeasts.[10] Owing to its cationic nature, CHX is capable of electrostatically binding to the negatively charged surfaces of bacteria,damaging the outer layers of the cell wall and rendering it permeable.[11-12]

Depending on its concentration, CHX can have both bacteriostatic and bactericidal effects. At high concentrations, by damaging the cell membrane, it causes precipitation of the cytoplasm and thereby exerts a bactericidal effect. At low sublethal concentrations, CHX is bacteriostatic, causing low molecular-weight substances (i.e., potassium and phosphorous) to leak out without the cell being irreversibly damaged.[13]

Another very important, unique property of CHX is substantivity. CHX has the ability to bind to proteins such as albumin, present in serum or saliva, pellicle found on the tooth surface, salivary glycoproteins, and mucous membranes.[14].It can also be adsorbed onto hydroxyapatite and teeth.[14] This reaction of uptake and release of CHX leads to substantive antimicrobial activity and is referred to as *substantivity.* This property makes it adsorb onto dentine and prevent microbial colonization on the dentine surface for some time beyond the actual medication period.

CHX in liquid and gel form is commonly used as an irrigating solution. Many investigators have conducted studies on the effect of CHX used as irrigant on fracture resistance of root filled teeth. They concluded that 2% CHX irrigation following EDTA/NaOCl irrigation enhanced the fracture resistance of roots filled with AH Plus[15]

Since NaOCl is still the most commonly used irrigant, CHX has been used alongside NaOCl and the fracture resistance values of teeth treated with both were compared. Results showed that Final irrigation with NaOCl reduced vertical root fracture resistance of root canals filled with MTA-Fillapex compared to CHX.[16]

Qmix-2-in-1 is another irrigating solution which was developed in 2011 that contains both 17% EDTA and 2% CHX in a single formulation, along with cetrimide detergent that helps to reduce the surface tension and increases the efficacy of the other components.

EDTA (Ethylenediaminetetraacetic acid) was first described in 1935 by Ferdinand Munz, who prepared the compound from ethylenediamine and chloroacetic acid. Today, EDTA is mainly synthesized from methylenediamine (1, 2-diaminoethane), formaldehyde (methanal), and sodium cyanide.

EDTA works as follows: On direct exposure for extended time, EDTA extracts bacterial surface proteins by combining with metal ions from the cell envelope, which can eventually lead to bacterial death. When all available ions have been bound no further dissolution takes place;therefore, EDTA is self-limiting. [4]

EDTA came into use in endodontics in 1957 and since then it has become a vital constituent in endodontic treatment. Chelators such as EDTA act by creating a stable calcium complex with dentinal mud, smear layers, or calcific deposits along the canal walls. This may help prevent apical blockage and aid disinfection by improving access of solutions through removal of the smear layer.[4]

EDTA is normally used in a concentration of 17% and can remove the smear layers when in direct contact with the root canal wall for less than 1 minute. However, EDTA alone normally cannot remove the smear layer completely and effectively; a proteolytic component (e.g., NaOCl or CHX) must be added to remove the organic components of the smear layer.[17]Moreover, it has a negative effect on the strength of dentin when used alone. Since it is capable of decalcifying up to 50 µm, it can open up an occluded, very fine canal[4]

These findings have been proven by a comparative evaluation of the effects of different concentrations of EDTA for varied amounts of time on the fracture resistance of endodontically treated teeth. It was concluded that it is safer to use higher concentration for a shorter application time or a low concentration with a longer application time and that prolonged use of high concentrations of EDTA might increase the risk of root fracture.[18]

Hence, Qmix-2-in-1 was introduced in order to combine these benefits of EDTA and aforementioned actions of CHX in a single formulation as a root canal irrigant.

According to the manufacturer's guidelines, QMix 2in1 irrigating solution offers a fast working time of about 1 to 1.5 minutes for complete effectiveness. It is premixed and ready to use straight from the bottle with easy chair-side handling. And because QMix 2in1 irrigating solution removes smear layer and disinfects in one easy step, it saves time over using EDTA and chlorhexidine sequentially and separately.[19]

Even though the chemo-mechanical preparation is a very important step in the endodontic treatment and is pivotal in deciding the fate of a root canal treatment, still it has its fair share of adverse effects on the tooth itself which cannot be totally avoided. For instance, it is one of the causative factors that weakens the tooth during endodontic treatment and results in a reduction of the fracture strength of the tooth.[20]

Thus, it is necessary to keep on looking for newer and better irrigants that can have all or at least most of the desirable properties and at the same time have little or no adverse effects on the fracture resistance of the tooth itself. Also, it is imperative to keep on conducting studies and evaluating the various old and new materials and solutions in order to find out the best irrigating solutions that can be used to achieve the best results of endodontic treatments.

In view of this, this study was aimed at evaluating the effect caused by these irrigating solutions, (NaOCl, ASEP-RC, and QMIX-2-in-1) on fracture resistance of root canal treated teeth.

MATERIALS

- 80 freshly extracted, single rooted human mandibular premolars,
- Normal Saline, (GJ Surgical and Pharma),
- Diamond cutting disk,
- #10-#45 K-files (Mani Inc.),
- 17% EDTA (AvuePrep),
- ASEP RC® Anabond Stedman,
- QMix 2-in-1® Dentsply, Tulsa Dental Specialities,
- Sodium Hypochlorite, NaOCl (PRIME DENTAL),
- AH-26 Sealer (Dentsply),
- Gutta percha (META),
- Glass Ionomer Cement (GC),
- Incubator,
- Copper mould,
- Baseplate wax,
- Self-cure acrylic resin (DPI),
- Universal Testing Machine (ASIAN testing equipment),

METHODOLOGY:

The present study was conducted in the Department of Conservative Dentistry and Endodontics of Inderprastha Dental College and Hospital.

Inclusion criteria in the study were:-The non-carious mandibular premolar teeth with single root and a single canal, intact mature root apices.

Exclusion criteria in the study were:- The teeth with caries, more than one root, immature or open root apices, calcified canals, pulp stones, or apical resorption.

Specimen Preparation:

For this study, 80 human mandibular premolar teeth that were extracted for periodontal and prosthodontic reasons were collected from the Department of Oral Surgery, Inderprastha Dental College and Hospital. After collection, the specimens were decoronated with diamond disc and water as a coolant to a standardized length of 16 mm. Preoperative radiographs were taken before decoronation. They were cleaned of any debris and stored in normal saline until further use.

Instrumentation of Root Canal Systems:

After location of the root canals, determination of the working length was done, and root canals were instrumented with hand K-files upto apical size #45. During preparation, irrigation was performed using 5ml saline and 17% EDTA after each change of instrument.

Division into Groups and distribution of samples:

The samples were then divided into 4 groups, and irrigated for 1 minute each with the respective irrigating solutions (fig.1) as follows:

Table 1: Division into Groups and distribution of samples

GROUPS	DISTRIBUTION OF SAMPLE	N
Group-1	Normal Saline	20
Group-2	ASEP-RC	20
Group-3	2.5% NaOCl	20
Group-4	QMix 2-in-1	20

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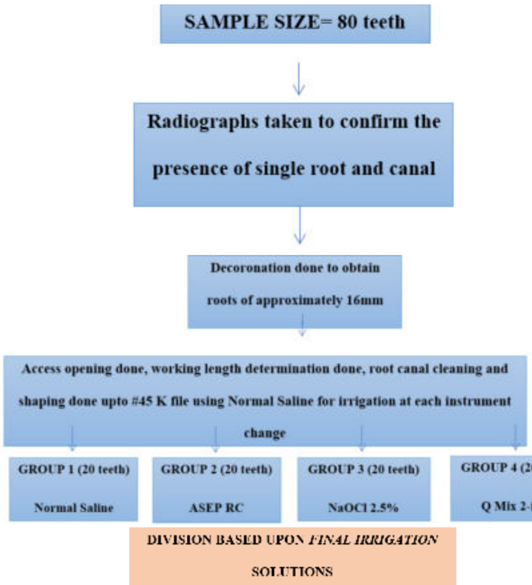
Final rinsing was done with 2ml saline in all groups.



FLOWCHART: DISTRIBUTION OF SAMPLES


Normal Saline


ASEP-RC


2.5% NaOCl


Qmix 2-in-1

Fig. 1: Irrigants used in the study

Obturation of Root Canal System:

Specimens were obturated using gutta-percha with cold lateral condensation technique and AH-26 sealer. Excess gutta-percha protruding out of the root canal was seared off with a hot burnisher to 1-2mm below CEJ. Specimens were then sealed and restored using Glass Ionomer Cement and then stored in an incubator at 37°C for 8 h to allow complete set of the sealer.

Mounting and Testing of Specimens:

Root surfaces were covered using 0.2 to 0.3 mm modelling wax upto 1-2mm below CEJ to simulate periodontal ligament. Specimens were mounted vertically using self-cured acrylic resin. They were placed on the lower plate of Universal Testing Machine (Fig. 2,3); upper plate included a steel spherical tip with a diameter of 5mm. Forces were applied at a crosshead speed of 1mm/min until fracture occurred and the force required to fracture each specimen was recorded in Newtons(N).


Fig. 2: Universal Testing Machine

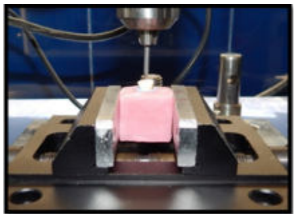


Fig. 3:Specimen Tested In Universal Testing Machine

OBSERVATIONS AND RESULTS

This present in-vitro study was carried out to evaluate the *in vitro* fracture resistance of endodontically treated teeth irrigating solutions, (Saline, ASEP-RC, NaOCl and QMIX-2-in-1), when used as final irrigants, on fracture resistance of root canal treated teeth. The specimens were subjected to a compressive load until they fractured individually in a universal testing machine. Peak load to fracture (N) was measured for each specimen. The statistical software SPSS 16.0 for windows (SPSS Inc, Chicago, IL, USA, 2001) was used for analysis of data. The mean difference of fracture resistance between four groups were evaluated using one-way ANOVA. Tukey HSD TEST was used to determine the multiple comparison of mean difference of fracture resistance between groups. Level of statistical significance was set at p- value less than 0.001.

The fracture resistance means (N) and standard deviation for four groups were as follows-

1. **GROUP I** – Normal Saline: 312.35±7.819
2. **GROUP II** – ASEP RC: 350.78±11.857
3. **GROUP III** – NaOCl: 414.26±11.973
4. **GROUP IV** – QMix 2-in-1: 528.80±14.312

Evaluation of Fracture Resistance (N)

Graph 1:A box plot graph showing the fracture resistance values of the four groups: Saline, ASEP-RC, NaOCl and QMIX-2-in-1 groups.

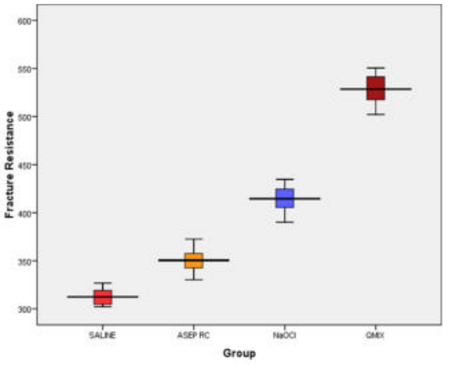
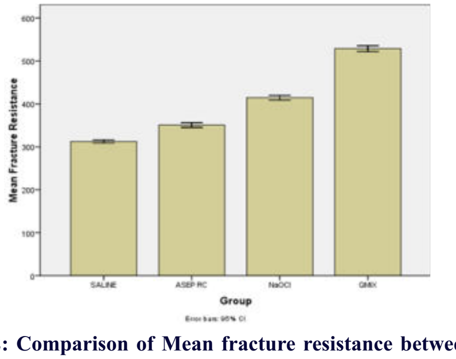


Table 2:Distribution of mean ±S.d. Fracture resistance of four groups

	N	Mean ±Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
SALINE	20	312.35 ±7.819	1.748	308.69	316.01	302	327
ASEP RC	20	350.78 ±11.857	2.651	345.24	356.33	330	373
NaOCl	20	414.26 ±11.973	2.677	408.65	419.86	390	435
QMIX	20	528.80 ±14.312	3.200	522.10	535.50	502	550

Table 2: Shows that fracture resistance of group 4 had highest average N followed by group 3. Group 1 had the lowest average N value.



Graph 2: Comparison of Mean fracture resistance between the four groups

Table 3: Multiple Comparisons of Fracture resistance of four groups by one-way Tukey HSD test

Group	Group	Mean Difference	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
SALINE vs	ASEP RC	38.433	3.708	<0.001*	-48.17	-28.69
SALINE vs	NaOCl	101.904	3.708	<0.001*	-111.64	-92.16
SALINE vs	QMIX	216.446	3.708	<0.001*	-226.19	-206.71
ASEP RC vs	NaOCl	63.471	3.708	<0.001*	-73.21	-53.73
ASEP RC vs	QMIX	178.013	3.708	<0.001*	-187.75	-168.27
NaOCl vs	QMIX	114.542	3.708	<0.001*	-124.28	-104.80

Table 3 shows, the multiple comparison of mean difference of fracture resistance between groups by Tukey's HSD test, the mean difference of fracture resistance between SALINE vs ASEP RC (38.433), SALINE vs NaOCl (101.904), SALINE vs QMIX (216.446), ASEP RC vs NaOCl(63.471), ASEP RC vs QMIX (178.013), NaOCl vs QMIX (114.542) are highly significant, p<0.001.The fracture resistance of QMIX group is significantly higher than other groups.

DISCUSSION

The present study evaluated the effects of different final irrigation regimens on the fracture toughness of freshly extracted human mandibular premolar teeth subjected to endodontic therapy, and showed that resistance to fracture varied according to different irrigants. Mandibular premolars were chosen for the study because of the reason that they are easily available, and in nearly intact condition due to the reason that they are frequently extracted from patients requiring orthodontic correction. Hence, proper endodontic therapy can be performed on them and testing for fracture resistance can be subsequently done.

The present study compared the fracture resistance of endodontically treated teeth irrigated using normal Saline, ASEP RC, 2.5% NaOCl, QMix 2-in-1, when used as final irrigants. The results of the study showed that QMix 2-in-1, which is a mixture of 17% EDTA solution, 2% CHX solution and a surfactant, Cetrimide, demonstrated better results when used as a final irrigating solution as compared to the other three used solutions.

Root Canal irrigants cause a change in the composition of dentin, both chemically and structurally, by altering its characteristics like permeability and solubility [22,23]. Moreover, preparing the root canals using any of the hand, rotary or reciprocating instruments also negatively affects the strength of the tooth and leads to a decrease in its fracture resistance, as shown by Shantiaee Y et al.[21]However, it should be mentioned that the authors in this study found out that samples instrumented using hand files were better significantly in terms of fracture resistance as compared to those instrumented using ProTaper Universal rotary files, OneShape rotary files and the WaveOne reciprocal system. Likewise, in another study done by Mohan G M et al., [24] hand K files made the teeth more resistant to fracture compared to WaveOne files and Rotary NiTi ProTaper files.

Therefore, hand filing was preferred over rotary or reciprocating mode of instrumentation in the current study.

According to Voilich D R et al., as dentin is cut, the mineralised tissues are not shredded but are crushed to produce significant amounts of debris, a large part of which is composed of very tiny particles of mineralised collagenous matrix that spreads evenly over the entire surface to form what is called the smear layer.[25] Studies like those of Prado M.et al. have shown that the root canal irrigants influence the bond strength of resin sealers to dentin either by removing the smear layer or affecting the surface properties of the dentin of root canals.[26]

There has been report by Johnson ME et al. that stated that endodontic sealers should bond to the dentin to reinforce root fracture resistance.[27] The strength of the roots increases via the removal of smear layer, which allows root canal sealers to contact the root canal wall more efficiently by penetration into the dentinal tubules.[27]

As shown in a study done by Patil P H et al., normal Saline solution basically has a flushing action. It can wash out debris generated from instrumentation. However, it has no effect on the smear layer.[28] Hence as stated before, due to the action by the hand files, the smear layer produced was not removed by the saline solution.[28] Therefore, there was ineffective and inefficient wetting and bonding of the sealer to the dentin in the root canals irrigated only using normal saline. This led to a low fracture resistance of teeth treated with saline.

CHX, which is the chief ingredient of ASEP RC, has also been advised as a final irrigating solution due to its antimicrobial properties as shown by Bhandari S et al. [29]. Moreover, according to Prado M. et al., CHX has also shown to have a positive effect on the surface free energy of dentin. [30]An increase in the surface free energy means that there is a better bonding ability of the root dentin with the sealer. However, Mohammadi Z et al. have shown that just like saline, CHX is also unable to remove the smear layer formed from instrumentation.[31] Hence roots irrigated using ASEP-RC, also had low values of fracture resistance, even though statistically more when compared to those irrigated using saline (p<0.001).

Grigoratos D et al. studied about Sodium Hypochlorite and concluded that Sodium hypochlorite is one of the most popular and probably the single most widely used irrigating solution in endodontics.[8] It has got excellent antimicrobial properties and remarkable pulp tissue dissolving abilities. However, as outlined by Grigoratos et al., it also has several drawbacks, important among them being its inability to remove the inorganic portion of the smear layer.[8] Sodium hypochlorite has also been shown to have a concentration dependentreduction of elastic modulus and flexural strength in human root dentine, though this might have played minimal role in our study. Thus, the ability of NaOCl when used as a final irrigant to remove the organic portion of Smear layer leads to better bonding ability with the sealer as compared to final irrigation done using saline or ASEP-RC. This accounts for significantly higher values of fracture resistance of this group as compared to the previous two groups (p<0.001).

QMix(Dentsply Tulsa Dental Specialties, Tulsa, OK) is a new antibacterial agent designedfor smear layer removal; it contains a combination of 17% EDTA solution, 2% CHX solution, and cetrimide, which is a surfactant. Ethylene diamine tetra aceticacid (EDTA), one of the chief contents of QMix, is a chelating agent which reacts with the calcium ions of dentine and thus forms calcium chelates that dissolve easily. However there have been studies (Bezerra da Silva et al.) that have stated that this chelating effect is nearly negligible in the apical one-thirds of the root canal spaces. QMix 2-in-1 also consists of surfactants that help in enhancing the chelating action by reducing the surface tension. This leads to an increase in the dentin wettability.[32]ZhangK et al. [33] found out that EDTA when used as the final irrigant leaves a thin layer consisting of demineralized collagen fibrils on the surface of dentin. In the absence of an adjuvant surfactant, the presence of this residual layer of collagen fibrils is the reason for the poor wettability of root canal sealers on the dentin (especially in the apical third) when irrigated using EDTA without a surfactant. In addition, CHX, the other constituent of QMix, has been shown to increase the surface-free energy of dentin[30,34], as outlined before. This is highly beneficial as it leads to better bonding of the root canal sealer used with the dentin of the root.[30]In addition, Chlorhexidine digluconate(CHX) exhibits broad-spectrum antimicrobial activity against endodontic bacteria[36] and substantivity to dentin [35,37]. So, final irrigation using QMix 2- in-1 might have led to a stronger bonding between the sealer and root dentin, leading to significantly higher values of fracture resistance among all the groups (p<0.001).

So, a one-minute irrigation protocol using saline had no effect on the smear layer, thereby reducing the bonding capability of sealer to root canal dentin. Thus, saline group accounted for the lowest values of fracture resistance. ASEP-RC, by virtue of its CHX content, was able to increase the surface free energy of dentin, leading to better fracture resistance values compared to Saline. However, it also had no effect on the smear layer. Final irrigation using NaOCl for one minute led to a significantly better fracture resistance compared to saline and ASEP-RC mainly due to the fact that it was able to remove the organic portion of smear layer present on instrumented roots. QMix 2-in-1 was the only irrigant able to remove the smear layer out of all the groups due to the chelating action of its constituent i.e. EDTA. In addition, presence of surface tension lowering surfactant i.e., cetrimide lead to overall significantly better performance as a final root canal irrigant. Apart from this, the other main constituent namely CHX (2%) present in QMix 2-in-1 was able to increase the surface free energy of root dentin, thus causing better wetting of the root dentin with the root canal sealer used. Hence, it showed maximum (statistically significant) values of fracture resistance among all the four groups.

Limitations of the study include the fact that the compressive test alone cannot simulate clinical conditions. Forces acting on teeth inside the oral cavity are different as compared to those that are tried to simulate in the study. Further, in the clinical setting, often there is a need to use NaOCl extensively in order to get rid of live/dead pulp tissue or other organic tooth debris during the instrumentation. However, in the study, only Saline was used in all the groups as an irrigant during instrumentation. This was done in order to maintain uniformity in the groups and NaOCl was only used in one of the groups (Group IV) as a final irrigating solution.

CONCLUSION

Within the limitations of this study, the following conclusions can be drawn:

- Fracture resistance values of teeth irrigated using different solutions used as final irrigants after root canal cleaning and shaping were different.
- Fracture resistance values of teeth irrigated using 2.5% Sodium Hypochlorite were significantly greater as compared to those irrigated using saline and using ASEP RC.
- Fracture resistance of teeth irrigated using QMix 2-in-1 solution as a final irrigant was the highest and significantly greater as compared to all other groups.

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