Original Article

An *In vivo* Study to Compare Anti Microbial Activity of Triantibiotic Paste, 2% Chlorhexidine Gel, and Calcium Hydroxide on Microorganisms in the Root Canal of Immature Teeth

Sumit Sabharwal[1], Shresth Kumar Bhagat[2], Kumar Shresth Gami[3], Abhishek Siddhartha[3], Kulwant Rai[1], Yuvika Ahluwalia[4]

[1]Department of Conservative Dentistry and Endodontics, Seema Dental College and Hospital, Rishikesh, Uttarakhand, Departments of [2]Conservative and Endodontic Dentistry and [3]Conservative Dentistry and Endodontics, Sarjug Dental College and Hospital, Darbhanga, Bihar, [4]Department of Conservative Dentistry and Endodontics, Institute of Dental Sciences and Technologies, Ghaziabad, Uttar Pradesh, India

Received : 11-11-18.
Accepted : 21-12-18.
Published : 27-02-19.

ABSTRACT

Aims and Objectives: Intracanal medication plays an important role in disinfecting the root canal from various microorganisms. There are various intracanal medication frequently used by practitioners based on the efficacy of the same, therefore to compare antimicrobial activity of a triple antibiotic paste with chlorhexidine gel and calcium hydroxide against microorganisms in the root canal used as irrigating solution.

Materials and Methods: The present study was conducted on 88 single rooted (central and lateral incisors), asymptomatic, nonvital, and necrotic teeth. Individuals included were between the ages of 12–15 years with no systemic complications. Overall, 88 teeth were divided randomly into four groups on the basis of treatment they were given, with each group having 22 teeth. Each group was assigned to one intervention group namely 2% chlorhexidine gel group, calcium hydroxide group, triantibiotic paste group, and the normal saline group. Triantibiotic paste was prepared from ciprofloxacin Hydrochloride 500 mg+ Metronidazole 400 mg+ Tetracycline 500 mg. Two samples were collected from individual tooth to assess the change in the extent of total colony forming units.

Results: Mean $\log^{10}$ bacterial count among the triantibiotic paste was 5.222 preirrigation which was decreased to 0.653. Maximum percentage of $\log^{10}$ bacterial count was decreased among triantibiotic paste group of 87.20% while control group of normal saline has recorded a decrease of 54.65% in bacterial count. Among all the four groups, there was a decrease of 73.51% of bacterial count.

Conclusion: From the above results, it was concluded that triantibiotic paste demonstrated the highest efficacy against bacterial pathogen when used as irrigating solution.

Keywords: *Antibiotics, immature tooth, root canal*

Introduction

Since centuries, microbial interaction has influenced the outcome of endodontic treatment. Surgical or nonsurgical endodontic treatment essentially is chemomechanical debridement of the canal to disrupt and remove this microbial ecosystem associated with the disease process.[1] Once the root canal is infected coronally, the infection progresses apically until bacterial products or bacteria themselves are in a position to stimulate the periapical tissues, thereby leading to apical periodontitis. Microorganisms and their products in the form of toxins (endotoxins) especially are strongly associated with the etiology of the lesions of the pulp and periapical areas. The toxin from the microorganisms causes pulpal necrosis, and this outcome can be attributed due to consistent persistence of these microorganisms in the root canal system even

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_400_18

Address for correspondence: Dr. Sumit Sabharwal, Seema Dental College and Hospital, Rishikesh, Uttarakhand, India.
E-mail: sumit1sabharwal@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Sabharwal S, Bhagat SK, Gami KS, Siddhartha A, Rai K, Ahluwalia Y. An *in vivo* study to compare anti microbial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on microorganisms in the root canal of immature teeth. J Int Soc Prevent Communit Dent 2019;9:263-8.

after endodontic treatment and these are also capable of inducing periapical inflammatory reaction. Root canals with primary infections contain high bacterial load, chemo-mechanical root canal preparation has proven to reduce bacterial counts by at least 95%.[2,3] Appropriate use of root canal irrigants can be beneficial and has proved to be vital in endodontic treatment as they help in disinfecting and lubricating the root canal, flush out debris from the canal, and dissolves organic and inorganic tissues.[2,4]

Sodium hypochlorite is the most commonly used root canal irrigant.[5,6] It is antimicrobial and effectively dissolves pulpal remnants, collagen, necrotic and vital organic tissue.[5-7] However, it has an unpleasant taste, is toxic and when used in isolation, is unable to remove the smear layer. Root canal system has organic and inorganic material and for the complete cleaning of the system requires complete dissolution of such material, chemicals, like ethylenediaminetetraacetic acid (EDTA), can be of great help to completely remove the smear layer and the dentin debris.[5,7] Chlorhexidine digluconate has antimicrobial activity but has no tissue dissolving capability.[5,7] However, it is biocompatible and does not possess some of the undesired characteristics of sodium hypochlorite, for example, the bad smell.[5] Calcium hydroxide is a slowly working antiseptic. In the literature, it is shown that direct contact experiments *in vitro* require a 24 h contact period for completely killing enterococci microorganism. Calcium hydroxide not only kills bacteria, but it also reduces the effect of the remaining cell wall material lipopolysaccharide. Calcium hydroxide may be mixed with sterile water or saline, but this formula is also available commercially from a number of manufacturers in a sterile single-dose packages. It should be mixed to a thick mixture to carry as much calcium hydroxide particles as possible. This slurry is best applied with a lentulo spiral. Saturated calcium hydroxide solution mixed with a detergent is an effective antimicrobial agent suitable for irrigation of root canal.[8]

Besides drilling and filling of the tooth to control the bacterial infections, the valuable conjunct can be antibiotics as they act as imperative totaling to the armamentarium available to health care practitioners. Undoubtedly, these antibiotics have proved very useful and have saved the life of millions and will continue to do so. For several decades, antibiotics have been prescribed in different disciplines of medicine and dentistry.[8,9] In endodontics and dental traumatology, antibiotics may be applied systemically (oral or parenteral) and locally (intradental).[8]

Irrigation is a vital step of root canal treatment as it disinfects the root canal from all kinds of microorganisms.[9] Various common irrigant solution used in routine practice are citric and phosphoric acids, chelating agent (EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative detergent agents (hydrogen peroxide and Gly-Oxide), local anesthetic solutions, and normal saline. Two new root canal irrigants, mixture of tetracycline, citric acid and detergent and tetraclean, containing antibiotics have recently been introduced.[10]

Varieties of existing antibiotics such as tetracycline, ornidazole, metronidazole, ciprofloxacin, penicillin, and minocycline have recently been used, and there is evidence in the database regarding the antibacterial efficacy of individual antibiotics.[11] The use of these antibiotics as intracanal medicament also been explored in various studies.[12-15] In the Indian scenario, there are no studies done which could explore the use of these antibiotics in combination as an irrigating solution except or one study where few of these antibiotics were used as an irrigant solution.[16] The combination of various antibiotics such as ciprofloxacin, metronidazole, and tetracycline and its use and efficacy has not yet been researched yet. Hence, the aim of the present study was to compare the antibacterial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on microorganisms in the root canal of nonvital teeth.

Materials and Methods

The present study was conducted on 88 single rooted (central and lateral incisors), asymptomatic, nonvital, and necrotic teeth. The sample size was calculated by comparing the mean difference of previous articles with keeping confidence interval (two-sided) 95% with a power of study at 80% along with this group ratio of sample size as 1:1. After calculation, the desired sample size was 88. Individuals included were between the ages of 12–15 years were having necrosed central or lateral incisors and with no systemic complications. Individuals who had undergone antibiotic treatment during the past 6 months were not a part of the study. Furthermore, any other related symptoms such as oral sinus (intraoral or extraoral), presence of any abscess or soft-tissue swelling at a location which involves the tooth of interest were excluded from the study.

Written informed consent was obtained from the participants. Ethical clearance from the Ethical Committee of Seema Dental College (OR SDO98/018) was obtained to carry the study. The teeth of the study participants were divided randomly into four groups of 22 each on the basis of irrigant received. Each group was assigned to one intervention group, triantibiotic paste

group, 2% chlorhexidine gel group, calcium hydroxide group, or the normal saline group.

Triantibiotic paste was prepared from ciprofloxacin Hydrochloride 500 mg (Tablet ciplox-500, cipla Ltd. Kumrek, Rangpo Sikkim) + Metronidazole 400 mg (Tablet Unique's metrogyl 400, J B Chemicals and Pharmaceuticals, Vapi) + Tetracycline 500 mg (Resteclin 500 mg Cap, Abbott Healthcare Pvt. Ltd.). This was mixed with colloidal silicon dioxide 0.5 mg which act as a stabilizer and to give paste-like consistency propylene glycol is added in the ratio of 8:4 powder/propylene glycol. A packet containing 10 g of powder formulation was made to dissolve in 60 mL of distilled water; the resultant mixture was adjusted to 90 mL with the use of distilled water. Now following this the formulation which got prepared was stored in airtight container and used within 24 h of its reconstitution.

A volume of 10 g calcium hydroxide paste (RC Cal, Primeera Healthcare Private Limited Lonikand, Pune, Maharashtra, India) is mixed with 20 mL of detergent solution called Tween 80 (0.5% polysorbate 80 detergent) (Polysorbate 80, Akhil Healthcare Private Limited) which decreases it surface tension and resulting formulation can be used as irrigant solution.

Microbial sample collection

Following the administration of local anesthesia (2% lignocaine hydrochloride with adrenaline 1:200,000), the tooth which was involved was isolated with the help of rubber dam. The region in and around the tooth, clamp and the rubber dam was sanitized using povidone iodine in solution form. After the access, cavity was prepared using sterile burs, minimal instrumentation using K files starting from 10 number file, followed by extirpation of pulp with a sterile broach without the use of any irrigant. To get a preirrigation sample (sample A), sterile paper point of compatible length and diameter with that of the root canal was introduced into the full length of the canal for 60 s for the obtaining the.[10,11] The sterile paper point was removed from the canal and immediately placed in normal saline. The canal was then irrigated with the allotted irrigant ensuring a contact time in the canal of 5 min. Postirrigation sample (sample B) was collected using sterile paper point of appropriate length and diameter similar to collection procedure of sample A. Root canal was not filled from inside as it was empty, but it was temporarily sealed with intermediate restorative material after the placement of sterile cotton pellet. The samples were then incubated at 37°C for approximately 24 h following which individual sample was inoculated on sterile sheep blood agar medium using 0.04 mm sterile inoculation loops. The growth was evaluated after 48 h following aerobic incubation at 37°C and total Colony Forming units (CFU) were counted using colony counter. The count per milliliter was recorded and multiplied with the dilution factor.[15,16] The viable organisms were counted as CFU per milliliter. All the values of CFU were converted to Log^{10} for the ease of comparison.

After postirrigation, samples were collected from the teeth. In the next appointment, intermediate restoration was removed, and biomechanical preparation by crown-down technique with Dentsply ProTaper Universal System NiTi rotary files (SX-F3) were attached to an X-Smart endo motor (Maillefer Corp., Ballaigues, Switzerland).with a speed of 300 rpm and 0.6 N/cm torque was performed. The teeth were obturated with single cone technique using Diadent Gutta Percha Points no. 30 with 6% taper and Resino-seal (Ammdent, New Delhi, India).

The formula used here was:[11]

$$\text{CFU} = \frac{\text{No. of Colonies} \times \text{Dilution Factor}}{\text{Volume Inoculated}}$$

Two samples were collected or each tooth, this is done to evaluate the change in the level of the total CFU.

Sample a – preirrigation (after pulp extirpation but before irrigation.).

Sample b – postirrigation (after irrigation).

Statistical analysis

Log^{10} bacterial count in the sample before the irrigation and after the irrigation among the four groups was measured by applying descriptive analysis. Intragroup comparison between the four groups, in preirrigation and postirrigation samples were measured by using Wilcoxon signed-rank test. Intergroup comparison of pre- and post-irrigation bacterial count of and for saline and chlorhexidine solution, calcium hydroxide, and triantibiotic paste was measured using Mann–Whitney U.

Results

In Table 1, descriptive analysis shows that mean log^{10} bacterial count (CFU) was decreased in each group. Mean log^{10} bacterial count among the triantibiotic paste was 5.222 preirrigation which was decreased to 0.653. Table 2 shows that among microbial count preirrigation and postirrigation among 2% chlorhexidine gel (0.03*) and triantibiotic paste (0.000***) was statistically significant. In Table 3, intergroup comparison showed that statistically significant result was seen between, Group 2 and Group 4 postirrigation, Group 3 and Group 2 postirrigation, Group 4 and Group 1 postirrigation, Group 4 and Group 2 postirrigation,

Group 4 and Group 3 postirrigation (P = 0.000***). The maximum percentage of log^{10} bacterial count was decreased among triantibiotic paste group of 87.20%. While the control group of normal saline has recorded a decrease of 54.65% in bacterial count [Table 4].

Table 1: Log^{10} bacterial count in the sample, before the irrigation and after the irrigation among the four groups

	Samples	*n*	Mean (SD)	SE
Microbial count before irrigation	Saline (control)	22	5.389 (0.378)	0.233
	2% chlorhexidine gel	22	5.681 (1.023)	0.456
	Calcium hydroxide	22	5.410 (1.563)	0.222
	Triantibiotic paste	22	5.222 (0.289)	0.532
	Total	88	5.430 (1.871)	0.348
Microbial count after irrigation	Saline (control)	22	2.445 (0.176)	0.111
	2% chlorhexidine gel	22	1.236 (0.249)	0.510
	Calcium hydroxide	22	1.111 (0.401)	0.200
	Triantibiotic paste	22	0.653 (0.290)	0.156
	Total	88	1.444 (0.230)	0.297

SD=Standard deviation, SE=Standard error

Table 2: The intragroup comparison between the four groups, pre- and post-irrigation

	Microbial count pre- and post-irrigation (normal saline)	Microbial count pre- and post-irrigation (2% chlorhexidine gel)	Microbial count pre- and post-irrigation (calcium hydroxide)	Microbial count pre- and post-irrigation (triantibiotic paste)
Z	4.246	−3.782	−2.398	−5.011
P	0.762	0.03*	1.231	0.000***

*$P \leq 0.05$, ***$P \leq 0.000$

Discussion

Among all the steps of root canal treatment, the most important step for success is proper cleaning and through debridement of the root canal. In search of a perfect irrigating solution, many researches were conducted in the past to discover new uses. In all those studies, those irrigant solutions were broadly, they were designed for detecting bacteria from the root canals of eight teeth, but on long-term, it was seen that the microorganism (bacteria) survived in seven teeth in spite of treatment on five successive occasions.

In the present study, triple antibiotic paste showed a considerable decrease in microbial pathogens from root canal of the sample teeth, the results of the present study were concordance with various studies conducted in the past.[12-14,17-19] In a study conducted on dog teeth (immature) affected with apical periodontitis by Windley *et al*.[19] shows a considerable decrease in bacterial pathogen from root canal.

In the present study, intracanal medicament such as calcium hydroxide and triantibiotic paste were used as irrigant which was done in very less number of studies.[16] As told in study by Chong and Pitt Ford[20] high concentration of these can competently get rid of residual bacteria, the reason could be that penetration of cytotoxic vapor forming medicaments such as formaldehyde penetrates deep into the periodontium and can kill the bacteria, but it is also accompanied by undesirable consequences as it gets distributed widely

Table 3: Inter group comparison of pre- and post-irrigation bacterial count of and for saline and chlorhexidine solution, calcium hydroxide and triantibiotic paste

	Microbial count pre (Group 1/2)	Microbial count post (Group 1/2)	Microbial count pre (Group 1/3)	Microbial count post (Group 1/3)	Microbial count pre (Group 1/4)	Microbial count post (Group 1/4)
Mann–Whitney U	167.03	91.11	173.19	129.56	198.22	213.22
Z	−0.231	−4.344	−0.675	−0.209	−0.167	−0.566
P	1.22	0.03*	0.34	0.89	2.22	1.81
	Microbial count pre (Group 2/1)	**Microbial count post (Group 2/1)**	**Microbial count pre (Group 2/3)**	**Microbial count post (Group 2/3)**	**Microbial count pre (Group 2/4)**	**Microbial count post (Group 2/4)**
Mann–Whitney U	178.02	131.23	123.24	56.04	187.24	23.78
Z	−0.451	−0.230	−0.189	−5.490	−0.845	−6.340
P	2.33	0.24	1.56	0.05*	0.37	0.001**
	Microbial count pre (Group 3/1)	**Microbial count post (Group 3/1)**	**Microbial count pre (Group 3/2)**	**Microbial count post (Group 3/2)**	**Microbial count pre (Group 3/4)**	**Microbial count post (Group 3/4)**
Mann–Whitney U	231.45	24.67	132.56	67.29	234.66	149.56
Z	−0.342	−1.267	−0.349	−3.875	−0.821	−0.431
P	1.56	0.91	0.67	0.000***	1.39	0.40
	Microbial count pre (Group 4/1)	**Microbial count post (Group 4/1)**	**Microbial count pre (Group 4/2)**	**Microbial count post (Group 4/2)**	**Microbial count pre (Group 4/3)**	**Microbial count post (Group 4/3)**
Mann–Whitney U	121.34	12.34	119.41	19.45	190.45	34.21
Z	−0.566	−9.342	−0.233	−3.781	−0.257	−6.788
P	0.45	0.000***	1.24	0.000***	0.53	0.001**

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.000$

Table 4: Percentage decrease in bacterial count among the four groups

Group	Percentage (%)
Saline (control)	54.65
2% chlorhexidine gel	78.25
Calcium hydroxide	79.13
Triantibiotic paste	87.20
Total	73.51

in the body. Therefore, to eliminate this shortcoming the contact time of the antibiotic paste was reduced in the present study and hence can be used as an irrigant in this study.

In the previous studies,[16] it was determined that triantibiotic paste has decreased the pathogenic paste in maximum level. Same was determined in the present study, but the significance level of results was more in the present study as compared to study in the past.

In the present study, triantibiotic paste is prepared from ciprofloxacin Hydrochloride 500 mg (Tablet ciplox-500, cipla Ltd., Kumrek, Rangpo Sikkim) + Metronidazole 400 mg (Tablet Unique's metrogyl 400, J B Chemicals and Pharmaceuticals, Vapi) + Tetracycline 500 mg (Resteclin 500 mg Cap, Abbott Healthcare Pvt. Ltd) as compared to study by Jain *et al*.[16] in which triantibiotic paste was prepared by 1% Ornidazole, 1% Ciprofloxacin and 1% Tetracycline in 100 mL of water. As in the present study, metronidazole was used in the composition of triantibiotic paste due to its effective antibacterial activity against anaerobic cocci as well as Gram-negative and Gram-positive bacilli.[21]

In the present study, triantibiotic paste demonstrated maximum decrease in bacterial pathogen followed by calcium hydroxide followed by 2% chlorhexidine in decreasing bacterial pathogens and least normal saline. In few of the previous to studies,[16,22] triantibiotic past demonstrated maximum efficacy like the present study but 2% chlorhexidine was more effective than calcium hydroxide.

In the present study, on applying Wilcoxon signed-rank test to measure intragroup comparison between the four groups, in preirrigation and postirrigation samples, it was assessed that statistically significant result was shown by triantibiotic paste and 2% chlorhexidine group as compared to study by Jain *et al*.[16] in which statistically significant results were shown by all three groups (normal saline, 2% chlorhexidine and triantibiotic paste).

In the present study, on measuring intergroup comparison of pre- and post-irrigation bacterial count for saline and chlorhexidine solution, calcium hydroxide, and triantibiotic paste, statistically significant results were shown by Group 3 and Group 2 postirrigation, Group 4 and Group 1 postirrigation, Group 4 and Group 2 postirrigation, and Group 4 and Group 3 postirrigation (P = 0.000***) As compared to the present study by Jain *et al*.,[16] statistically significant results were demonstrated between postirrigation Group 1 and Group 2, Group 1 and Group 3 postirrigation (P = 0.001***).

As the present study is conducted in the clinical conditions in the oral cavity of patients, it can give the exact idea of the effect of the irrigant involved in the study.

Limitation of study

As a small amount of mechanical instrumentation has been performed in this study on study samples. The final decrease in the pathologic microorganism can also be attributed to the instrumentation also.

Future implication of the study

As disinfection of the root canal is one of the most important parts of root canal treatment. And with improvement in technology and quality of root canal treatment, by this study, we tried to find newer irrigant with better efficacy of disinfection of root canal which further adds to improvement in success of root canal.

More studies in future should be conducted with other and newer irrigant solutions, and effect of mechanical instrumentation in combination with the use of irrigant with better efficacy should be tried so to the effect of both mechanical instrumentation and chemical agents should be assessed.

Conclusion

From above results, it was concluded that Triantibiotic paste demonstrated the highest efficacy against bacterial pathogen in root canal followed by 2% chlorhexidine and calcium hydroxide. Normal saline had shown the least antibacterial activities among four groups. Future studies are warranted to measure other properties of these irrigant solutions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Dudeja PG, Dudeja KK, Srivastava D, Grover S. Microorganisms in periradicular tissues: Do they exist? A perennial controversy. J Oral Maxillofac Pathol 2015;19:356-63.
2. Borzini L, Condò R, De Dominicis P, Casaglia A, Cerroni L. Root canal irrigation: Chemical agents and plant extracts against *Enterococcus faecalis*. Open Dent J 2016;10:692-703.

3. Blome B, Braun A, Sobarzo V, Jepsen S. Molecular identification and quantification of bacteria from endodontic infections using real-time polymerase chain reaction. Oral Microbiol Immunol 2008;23:384-90.
4. Navarro-Escobar E, González-Rodríguez MP, Ferrer-Luque CM. Cytotoxic effects of two acid solutions and 2.5% sodium hypochlorite used in endodontic therapy. Med Oral Patol Oral Cir Bucal 2010;15:e90-4.
5. British Dental Association. Irrigation in Endodontics: Evidence Summary. Available from: https://www.bda.org/dentists/education/sgh/Documents/Irrigation%20in%20endodontics%202.pdf. [Last accessed on 2018 Oct 15].
6. American Association of Endodontists. Root Canal Irrigants and Disinfectants. Chicago: American Association of Endodontists; 2011.
7. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010;54:291-312.
8. Mustafa M, Saujanya KP, Jain D, Sajjanshetty S, Arun A, Uppin L, *et al*. Role of calcium hydroxide in endodontics: A review. Glob J Med Public Health 2012;1:66-70.
9. Gulabivala K, Patel B, Evans G, Ng YL. Effects of mechanical and chemical procedures on root canal surfaces. Endo Topics 2005;10:103-22.
10. Becker TD, Woollard GW. Endodontic irrigation. Gen Dent 2001;49:272-6.
11. Mohammadi Z. An update on the antibiotic-based root canal irrigation solutions. Iran Endod J 2008;3:1-7.
12. Dhillon JS, Amita, Saini SK, Bedi HS, Ratol SS, Gill B, *et al.* Healing of a large periapical lesion using triple antibiotic paste and intracanal aspiration in nonsurgical endodontic retreatment. Indian J Dent 2014;5:161-5.
13. Mozayeni MA. Antimicrobial effects of four intracanal medicaments on *Enterococcus faecalis*: An *in vitro* study. Iran Endod J 2014;9:195-8.
14. Johns DA, Varughese JM, Thomas K, Abraham A, James EP, Maroli RK, *et al.* Clinical and radiographical evaluation of the healing of large periapical lesions using triple antibiotic paste, photo activated disinfection and calcium hydroxide when used as root canal disinfectant. J Clin Exp Dent 2014;6:e230-6.
15. Mohammadi Z, Jafarzadeh H, Shalavi S, Yaripour S, Sharifi F, Kinoshita JI, *et al.* A review on triple antibiotic paste as a suitable material used in regenerative endodontics. Iran Endod J 2018;13:1-6.
16. Jain P, Yeluri R, Garg N, Mayall S, Rallan M, Gupta S, *et al.* A comparative evaluation of the effectiveness of three different irrigating solution on microorganisms in the root canal: An *in vivo* study. J Clin Diagn Res 2015;9:ZC39-42.
17. Ercan E, Dulgergil T, Yavuz I. The effects of antibacterial solutions on microorganisms isolated from infected root canals *in vivo*. Biotechnol Biotechnol Equip 2006;20:149-56.
18. Devi L, Kamath PM. Antimicrobial efficacy of 0.2 and 2.0% chlorhexidine and sodium hypochlorite as root canal irrigants: An *in vivo* study. Endod 2001;13:57-62.
19. Windley W 3rd, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod 2005;31:439-43.
20. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992;25:97-106.
21. Abou-Rass M, Bogen G. Microorganisms in closed periapical lesions. Int Endod J 1998;31:39-47.
22. Ordinola-Zapata R, Bramante CM, Minotti PG, Cavenago BC, Garcia RB, Bernardineli N, *et al.* Antimicrobial activity of triantibiotic paste, 2% chlorhexidine gel, and calcium hydroxide on an intraoral-infected dentin biofilm model. J Endod 2013;39:115-8.