

**EVALUATION OF ANTIBACTERIAL EFFICACY OF CHITOSAN, CHLORHEXIDINE, APPLE CIDER VINEGAR AND SODIUM HYPOCHLORITE ON ENTEROCOCCUS FAECALIS BIOFILM IN PRIMARY TEETH: AN IN VITRO STUDY.****Insha Showkat<sup>\*1</sup>, Seema Chaudhary<sup>2</sup>, Ashish Sinha<sup>3</sup>, Mohd Ghaus Ali<sup>4</sup> and Neha Priya<sup>5</sup>**<sup>1</sup>Postgraduate Student, Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad, India.<sup>2</sup>Head of Department and Professor, Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad, India.<sup>3</sup>Professor, Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad, India.<sup>4</sup>Postgraduate Student, Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad, India.<sup>5</sup>Postgraduate Student, Pedodontics and Preventive Dentistry, Kothiwal Dental College and Research Centre, Moradabad, India.**\*Corresponding Author: Insha Showkat**

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**ABSTRACT**

Endodontic infections are polymicrobial, so disinfecting the root canal system is one of the major objectives in endodontics. Enterococcus faecalis has unique capacity to survive with limited means and form a biofilm. **Aim:** This study aims to compare the efficacy of two herbal ingredients with sodium hypochlorite and chlorhexidine. **Materials and Method:** 20 freshly extracted deciduous teeth were collected and decoronated. The autoclaved specimens placed in tissue culture wells exposing the root canal surface to *E. faecalis* to form a biofilm. At the end of 3rd week, all groups were irrigated with 3 ml of test solutions and control for 10 minutes. The samples were then scraped with a scalpel, inoculated on tryptone soy agar plates and incubated for 24 hours at 37°C. The plates were then subjected to digital colony counter and evaluated for *E. faecalis* growth. The growth was statistically analysed by ANOVA & paired t test. **Result:** Chitosan was found to be as efficacious as sodium hypochlorite. The use of natural alternatives as root canal irrigation solutions might prove to be advantageous considering several unfavorable properties of NaOCl.

**KEYWORDS:** chitosan, sodium hypochlorite, apple cider vinegar, photomedicinal solutions.**INTRODUCTION**

Biofilm form when planktonic bacteria in a natural liquid phase are deposited on a surface containing an organic conditioning polymeric matrix or conditioning film. Biofilm formation in root canals is probably initiated at sometime after the first invasion of the pulp chamber by planktonic oral organisms after some tissue breakdown.

Chemical debridement in primary teeth becomes of utmost importance because the canals are ribbon shaped.<sup>[2]</sup> Enterococcus faecalis is documented to be the most dominant species that persist in endodontically treated teeth.<sup>[3]</sup> Several irrigants have been universally used in combination with mechanical instrumentation.

Sodium hypochlorite (NaOCl) has been widely used for long time as the irrigant of choice for nonsurgical endodontic procedures due to its powerful antimicrobial action and effective tissue dissolution property.

However, it has some drawbacks such as being corrosiveness to devices, unpleasant taste, and cytotoxicity to the periapical tissues.<sup>[4]</sup> Also chlorhexidine exhibits sustained antimicrobial activity in the root canal for some time after being used as an endodontic irrigant. Therefore, Chlorhexidine has been suggested as a root canal irrigant owing to its unique ability to bind to dentin, its effectiveness as an antimicrobial agent, and its substantivity in the root canal system.<sup>[5]</sup> It has certain disadvantages like taste perturbation, tooth discoloration, oral ulcerations, unilateral, or bilateral parotid swelling.

Therefore, the search for ideal root canal irrigants continues with the development of new materials and methods.

Materials being investigated include chitosan and apple cider vinegar, which has been considered for many

dental applications.<sup>[6]</sup> Chitosan is a cationic biopolymer that possesses lasting antibacterial properties and low production costs.<sup>[7]</sup>

Apple cider vinegar has proven antimicrobial action, reduces dentinal microhardness, in addition to removing the smear layer. It is a combination of acetic, citric, formic, lactic, succinic and tartaric acids with a lesser amount of alcohol, helps in reducing the surface tension of the solution.<sup>[8]</sup>

The aim of this study is to compare the antimicrobial efficacy of various endodontic root canal irrigants in primary teeth.

## MATERIALS AND METHOD

In the present study 20 extracted carious primary teeth with intact roots or with at least 2/3rd roots were included in the study. Teeth with curved roots, less than 1/3rd roots and those that are endodontically treated were excluded from the study.

A pure culture of *Enterococcus faecalis* (ATCC 29212) [MTCC, Chandigarh] was inoculated on tryptone agar [Himedia, Mumbai] incubated at 37°C overnight. A suspension of *E. faecalis* in distilled water was prepared with dilution of 10<sup>12</sup> cells/ml.

The bacterium was inoculated in 1ml of tryptone soy agar broth in tissue culture wells and the root canals were contaminated with bacterium and broth and placed in incubator at 37°C for 30 days.

At the end 30 days of inoculation, all specimens were then placed in sterile petridishes and the biofilm on root canal surface was taken with a paper point and inoculated on tryptone soy agar plates and incubated for 24 hours at 37°C. The plates were then analysed for colony forming units by a digital colony counter.

The samples were then divided into four experimental groups with 5 samples (after vertical sectioning) each and irrigated with 3 ml of each irrigant for 10 minutes. The irrigants include.

Chemical irrigating solutions

- ✓ 5.25% Sodium hypochlorite
- ✓ 2% chlorhexidine gluconate

Phytomedicinal irrigating solutions

- ✓ Apple cider vinegar
- ✓ 0.2% Chitosan

Sterile paper points were inserted into root canals again and kept for 1 minute. Then, inoculated on tryptone soy agar plates and incubated for 24 hours at 37°C. The plates were then analysed for colony forming units by a digital colony counter.

## RESULT

From table 2 it is evident that the ANOVA is significant as  $p < 0.05$ , and thus there is significant difference between the means of the different groups. Paired t test evaluation showed that there is significant difference between the different groups of irrigants used against *E. faecalis*, however we can condense the above results in the following order

NaOCl = Chitosan > Chlorhexidine > Apple Cider Vinegar

## DISCUSSION

*E. faecalis* is commensal Gram-positive facultative anaerobic, non-spore forming cocci that occur singly, in pairs, and in short chains. *E. faecalis* has been commonly isolated in primary and secondary root canal infections.<sup>5</sup> *E. faecalis* possess different virulence factors that avail their adhesion to host cells and extracellular matrix, which in turn facilitates tissue incursion, causes immunomodulation and engenders toxin mediated damage. That is why *E. faecalis* has been grown in this study.

The choice of irrigants play a very important role in the removal of this biofilm.

The higher success rate with NaOCl can be attributed to its double action, i.e. it causes dissolution of necrotic tissues (due to its high pH) and is also germicidal. In this study NaOCl showed antimicrobial efficacy almost similar to 2% chitosan.

Chitosan's antibacterial effect exerts as a result of the interaction between positively charged chitosan and a negatively charged bacterial cell which alters the bacterial cell permeability and may lead to bacterial death. Yadav et al., demonstrated the anti-bacterial efficacy of chitosan nearly equivalent to 3% NaOCl, which may well be used as endodontic irrigant to overcome the deleterious effects of the conventional irrigants like NaOCl and chlorhexidine on dentine.<sup>[7]</sup> Mechanism of action of chitosan is thought to be that cationically charged amino group may combine with anionic components such as N-acetyl muramic acid, sialic acid, and neuramic acid on the cell surface and suppresses growth of bacteria by impairing the exchanges with medium, chelating transition metal ions, and inhibiting enzymes.<sup>[4]</sup>

Delany et al. (1982) evaluated 0.2% CHX-gluconate in infected root canals. Bacteriologic samples were obtained before, during, immediately after and 24 h after instrumentation, irrigation and medication either with CHX-gluconate or with sterile saline. There was a highly significant reduction in the number of microorganisms in the CHX-treated specimens after instrumentation and irrigation.<sup>9</sup> Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls thereby altering the cells' osmotic equilibrium. This

increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria.<sup>[10]</sup> In our study chlorhexidine has been used and good antimicrobial efficacy was achieved.

Apple cider vinegar is used in a wide number of health-related issues such as in cancer, cardiovascular diseases, body and joint pains, diabetes, and weight loss. Its

antimicrobial action is mainly due to the presence of acetic acid in it, that is, it causes loss of cell integrity. This also can be used in dentistry as a potent root canal irrigant. Very few studies have been carried out using apple cider vinegar as a potential root canal irrigant. In this study apple cider vinegar showed poor antimicrobial efficacy.

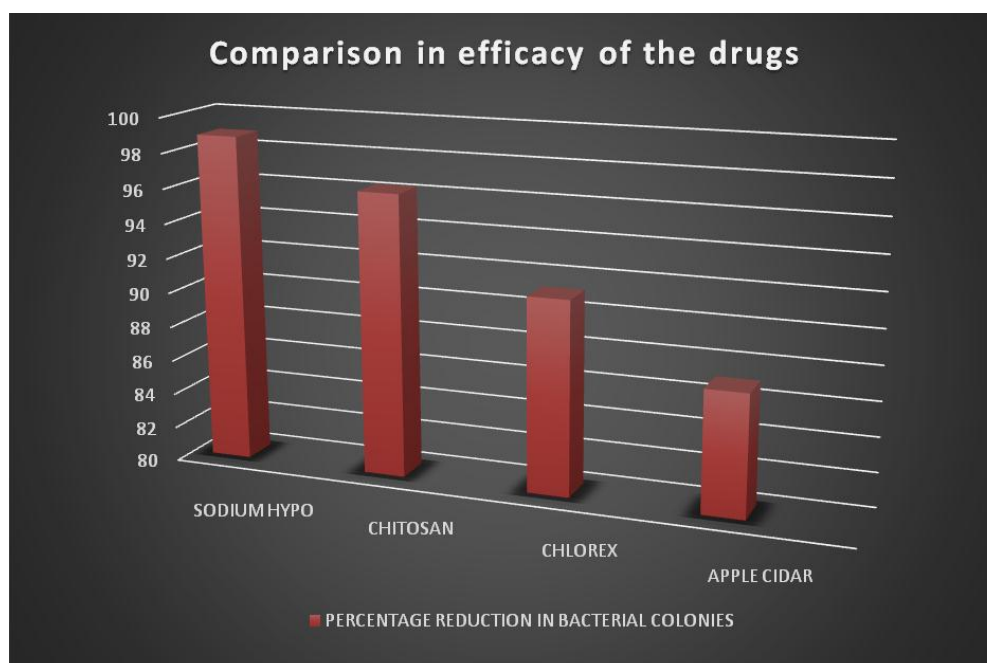
**Table 1: The mean and standard deviations obtained for all groups.**

	N	MEAN	STD. DEVIATION	STD. ERROR	95% CONFIDENCE INTERVAL FOR MEAN		t Stat	P value (two tailed)
					LOWER BOUND	UPPER BOUND		
SODIUM HYPO	5	-1977	480.292	214.793	-2573.361	-1380.639	-9.204	0.001
CHITOSAN	5	-1453.200	406.705	181.884	-1958.191	-948.209	-7.990	0.001
CHLORHEX	5	-1278.8	413.284	184.826	-1791.960	-765.640	-6.919	0.002
APPLE CIDAR	5	-1304.4	552.829	247.233	-1990.828	-617.972	-5.276	0.006
TOTAL	20	-6013.4	1853.11	828.736	-8314.34	-3712.46	-	-

\*Since  $p < 0.05$ , the result is significant

**Table 2: One-way ANOVA.**

	Sum of Squares	df	Mean Square	F ration of mean square	p value (two tailed)
Between Groups	23772340.6	3	7924113.5	36.08	0.00001*
Within Groups	3247391.2	16	202961.9		
Total	27019731.8	19			



**Figure 1: Percentage Reduction in Bacterial Colonies.**

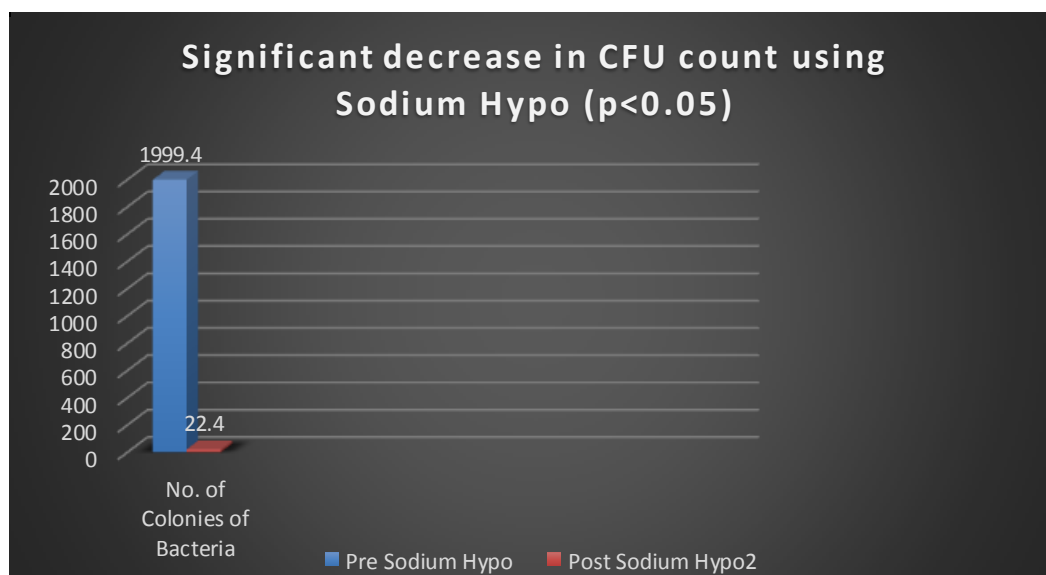
**Table 3: CFU counts before and after treating with Sodium hypo.**

Sodium hypo Pre Treatment CFU	Sodium hypo Post treatment CFU
1562 X 10 <sup>12</sup>	12 X 10 <sup>12</sup>
2138 X 10 <sup>12</sup>	18 X 10 <sup>12</sup>
1620 X 10 <sup>12</sup>	9 X 10 <sup>12</sup>
1871 X 10 <sup>12</sup>	32 X 10 <sup>12</sup>
2761 X 10 <sup>12</sup>	41 X 10 <sup>12</sup>

**Table 4: Paired T-Test.**

	Sodium hypo Pre Treatment	Sodium hypo Post treatment
Mean	1999.4	22.4
Variance	241373.3	186.3
Observations	5	5
Pearson Correlation	0.811	
Hypothesized Mean Difference	0	
df	4	
t stat	9.204	
P (T<=t) one-tail	0.000387	
T critical one-tail	2.132	
P (T<=t) two-tail	0.000774*	
T critical two-tail	2.776	

\*Since  $P < 0.05$ , the result is significant

**Figure 2: Significant Decrease in CFU counts using Sodium Hypochlorite ( $p < 0.05$ ) using Paired t test.****Table 5: CFU counts before and after treating with Chitosan.**

Chitosan Pre Treatment CFU	Chitosan Post treatment CFU
$1655 \times 10^{12}$	$22 \times 10^{12}$
$1097 \times 10^{12}$	$81 \times 10^{12}$
$1231 \times 10^{12}$	$74 \times 10^{12}$
$1456 \times 10^{12}$	$40 \times 10^{12}$
$2109 \times 10^{12}$	$65 \times 10^{12}$

**Table 6: Paired T-Test.**

	Chitosan Pre Treatment	Chitosan Post Treatment
Mean	1509.60	56.40
Variance	157787.8	610.3
Observations	5	5
Pearson Correlation	-0.357	
Hypothesized Mean Difference	0	
df	4	
t stat	7.990	
P (T<=t) one-tail	0.000665	
T critical one-tail	2.132	
P (T<=t) two-tail	0.00113*	
T critical two-tail	2.776	

\*Since  $P < 0.05$ , the result is significant

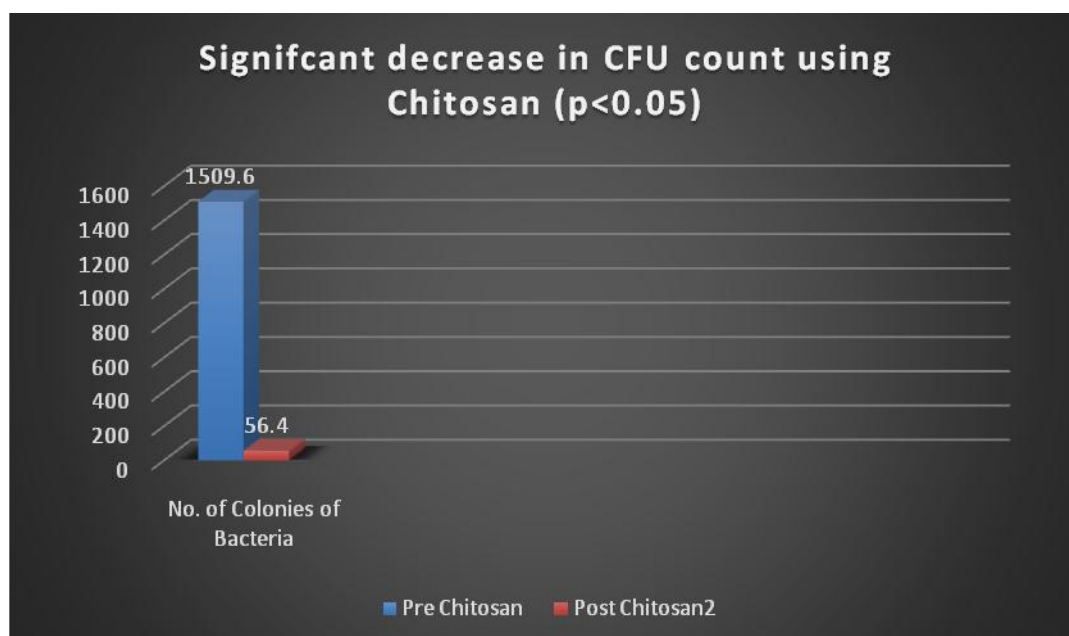


Figure 3: Significant Decrease in CFU counts using Chitosan ( $p < 0.05$ ) using Paired t test.

Table 7: CFU counts before and after treating with Chlorhex.

Chlorhex Pre Treatment CFU	Chlorhex Post treatment CFU
$1968 \times 10^{12}$	$16 \times 10^{12}$
$1096 \times 10^{12}$	$163 \times 10^{12}$
$1413 \times 10^{12}$	$281 \times 10^{12}$
$1447 \times 10^{12}$	$68 \times 10^{12}$
$1088 \times 10^{12}$	$90 \times 10^{12}$

Table 8: Paired T-Test.

	Chlorhex Pre Treatment	Chlorhex Post Treatment
Mean	1402.40	123.60
Variance	254244.8	8430.7
Observations	5	5
Pearson Correlation	-0.429	
Hypothesized Mean Difference	0	
df	4	
t stat	6.919	
P (T<=t) one-tail	0.00114	
T critical one-tail	2.132	
P (T<=t) two-tail	0.00229*	
T critical two-tail	2.776	

\*Since  $P < 0.05$ , the result is significant

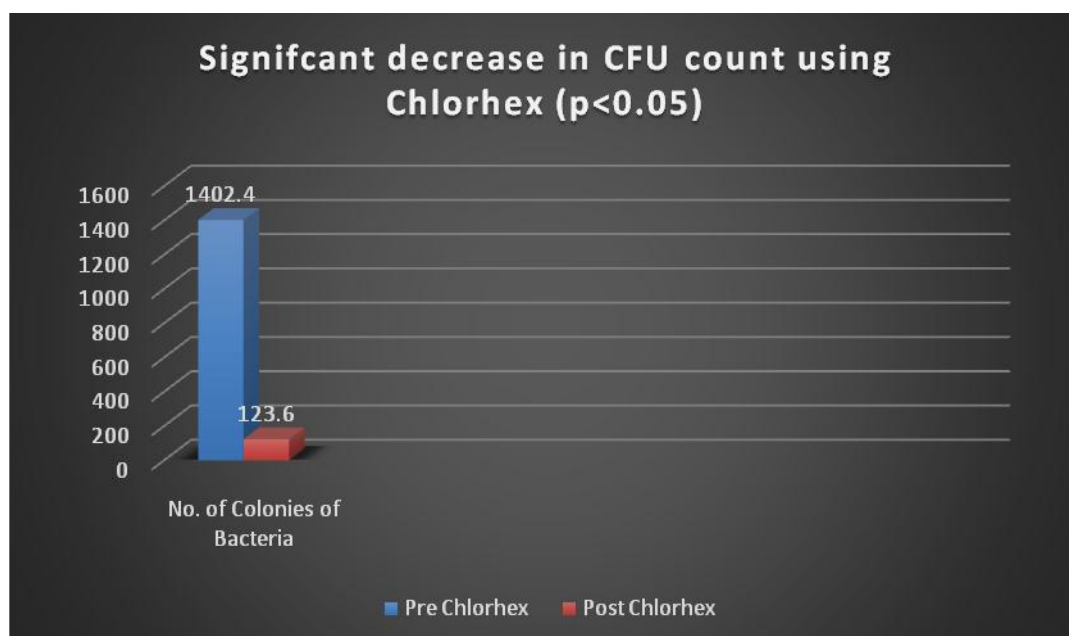


Figure 4: Significant Decrease in CFU counts using Chlorhexidine ( $p < 0.05$ ) using Paired t test

Table 9: CFU counts before and after treating with Apple Cidar.

Apple CidarPre Treatment CFU	Apple Cidar Post treatment CFU
901 X 10 <sup>12</sup>	123 X 10 <sup>12</sup>
1199 X 10 <sup>12</sup>	322 X 10 <sup>12</sup>
1559 X 10 <sup>12</sup>	174 X 10 <sup>12</sup>
2275 X 10 <sup>12</sup>	101 X 10 <sup>12</sup>
1559 X 10 <sup>12</sup>	251 X 10 <sup>12</sup>

Table 10: Paired T-Test.

	Apple CidarPre Treatment	Apple Cidar Post Treatment
Mean	1498.6	194.2
Variance	128683.3	10531.3
Observations	5	5
Pearson Correlation	-0.349	
Hypothesized Mean Difference	0	
df	4	
t stat	5.276	
P (T<=t) one-tail	0.003093	
T critical one-tail	2.132	
P (T<=t) two-tail	0.006187*	
T critical two-tail	2.776	

\*Since  $P < 0.05$ , the result is significant



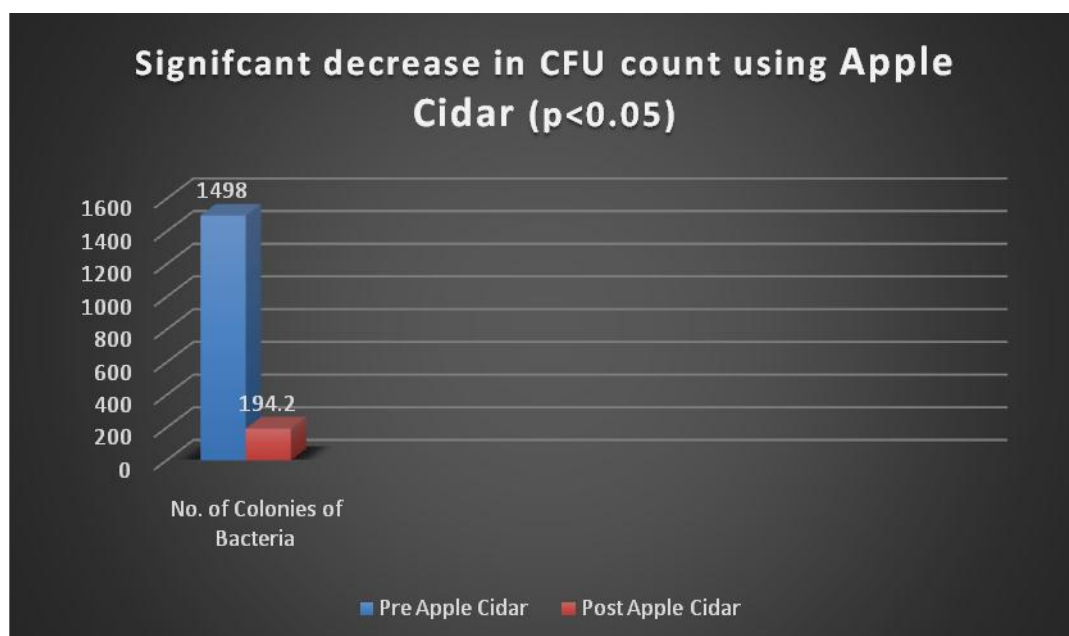


Figure 5: Significant Decrease in CFU counts using Apple Cidar Vinegar ( $p < 0.05$ ) using Paired t test.

## CONCLUSION

Under the limitations of this study, it can be concluded that chitosan can be used as root canal irrigating was shown to have better antimicrobial efficacy.

Chlorhexidine is equally efficacious against *E. faecalis* biofilm.

NaOCl performed equally well as that of 2% chitosan.

Thus, from the results of the study, it can be suggested could be used as an alternative to NaOCl for endodontic infections although, further *in-vivo* long term studies are warranted.

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