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Evaluation of Antibacterial Effect of Ethanolic Grapes Seed Extract and Sodium Hypochlorite as Root Canal Irrigants using Two Different Techniques in Primary Molars: An in-Vitro Study

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ABSTRACT

Background: It is critical to preserve deciduous teeth near the time of their shedding. Pulpectomy is one therapy option for achieving this goal. The root canal system complexity of the deciduous teeth allows pathogenic microorganisms to spread thorough, making elimination of necrotic tissue using mechanical instrumentation alone unfeasible. The objective of this study was to evaluate and compare the antibacterial effect of 13% Ethanolic grape seeds extract (GSE) versus 5.25% Sodium Hypochlorite (NaOCl) solutions using conventional syringe irrigation(CSI) and passive ultrasonic irrigation (PUI) techniques in primary molars .
Methods: 60 unidentified extracted human primary mandibular first molars with at least 2/3 of root not resorbed. No root caries. No internal or external resorption. (confirmed by periapical X- ray) .Teeth with no signs of visible root cracks confirmed by careful examination . No anatomic abnormalities as fusion or dilacerations. This study focused only on distal roots, so decoronation was performed on all distal roots below the cementoenamel junction until the root length reached 8 mm. 60 prepared distal roots were divided randomly into 2 main groups according to infected bacteria 30 roots infected with E. facials (standard cultures ATCC 29212) and 30 roots infected with S.mutans (standard Cultures ATCC 27351), then each group sub-divided into 5 subgroups according to irrigation technique and types of irrigants: no irrigation (control), CSI technique with 5.25%NaOCl (positive control) ,CSI technique with13% Ethanolic GSE solution. PUI with 5.25% NaOCl. PUI with 13% Ethanolic GSE solution. **Results**: NaOCl 5.25% had a significantly stronger antibacterial impact than GSE 13% ($p<0.05$). In this aspect, no significant differences were observed between the impacts of the CSI and PUI techniques in the E. faecalis and S. mutans groups. **Conclusion:** A 13% GSE solution showed an antibacterial effect against E. faecalis and S. mutans, but to a lesser extent than a 5.25% NaOCl solution. Within the limits of the results of this study, it was found that 5.25% NaOCl solution is the best in antibacterial effect. It was also found that there is no significant difference in the results when using CSI or PUI technique.

Keywords: E.faecalis , S.mutans , Sodium Hypochlorite (NaOCl) , Grape seed extract (GSE) , passive ultrasonic irrigation (PUI) , conventional syringe irrigation (CSI) .

Introduction

Deciduous teeth with pulpal and periapical pathosis should be preserved until their normal physiologic exfoliation time and the eruption of permanent successors. Therefore, Endodontic therapy is important to preserve primary teeth. This technique is regarded as a major therapeutic challenge in pediatric dentistry due to the complex morphology of the root canal system in the deciduous teeth [1].

Complete pulpectomy involves removing the irreversibly inflamed or necrotic radicular pulp tissue by mechanical and chemical means. Subsequently a root canal is filled with a material that can absorb at the same rate as the natural tooth and be quickly absorbed if it were to accidently protrude through the apex .The success of pulpectomy in primary teeth is highly dependent on establishing an acceptable degree of disinfection within the root canals . Unlikely up to 50% of the main root canal surface area keeps untouched by mechanical equipment alone, stressing the significance of using chemical methods to clean and disinfect the entire root canal area. Thus, irrigation plays a critical function in endodontic treatment [2,3,4].

NaOCl is the most widely utilized irrigation solution. When compared to alternative irrigation solutions, it is the best option because it is the only one having the majority of the necessary properties [5]. It is an efficient antibacterial agent that kills most bacteria. It dissolves pulpal remnants and collagen efficiently. Also, NaOCl solutions are cheap, readily available and have a long shelf life. Due to these characteristics aqueous (NaOCl) was first used as the primary irrigant in endodontics in1920 [6].

Grape seeds are the waste products of the grape juice industry is used as a powerful antioxidant for the protection of the body from various diseases. Grape seeds contain lipid, protein , carbohydrates and polyphenols mainly proanthocyanidins [7]. (PAs) are known of their antibacterial, antiviral, anti-allergic, anti-inflammatory and anticarcinogenic properties [8]. (GSE) can be used in endodontics as (PAs) have antimicrobial properties, they act on the cell walls and inhibit the growth of anaerobic bacteria , such as E. faecalis [9].

Prior to the development of passive ultrasonic irrigation , conventional irrigation using syringes has been promoted as an effective means of delivering irrigation. This procedure is still commonly used. The procedure includes application of an irrigation solution inside a canal via needles of different gauges, either passively or with agitation [10]. However, traditional hand-held syringe needle irrigation produces a somewhat mild mechanical cleansing action. Debris and bacteria are likely to retain in inaccessible canal extensions and anomalies after irrigation with conventional syringe needle, making thorough canal debridement challenging [11].

The literature describes two methods of ultrasonic irrigation: one that combines irrigation with simultaneous ultrasonic instrumentation (UI) and one that does not use simultaneous instrumentation, known as passive ultrasonic irrigation. The word "passive" refers to an irrigation situation in which there is no instrumentation, planning, or contact between the canal walls and an endodontic file or instrument due to the "non-cutting" action of the ultrasonically triggered file [12]. It has been demonstrated that PUI is more efficient than conventional syringe irrigation in eliminating necrotic tissue and dentinal debris. This may be related to the fact that ultrasound causes a stronger penetration and flow of the irrigant in the canal during irrigation, hence boosting irrigation dynamics and allowing the irrigant to better reach canal imperfections [13].

E.faecalis, is described as a fermentative, nonspore-forming, Gram-positive, facultatively anaerobic coccus which linked to many periradicular diseases such as primary and secondary endodontic infections [14] . It has been recognized as one of the hardest bacterial species to eliminate from infected root canals, and it is frequently recovered in failed root canal treatment . It is challenging to eradicate theses microorganisms that are located in strategic and privileged areas within the root canal harboring necrotic pulp tissue in deciduous teeth; because of the complexity of the root canal morphology [15].

S.mutans is one of the main causes of tooth decay .which is a facultative anaerobic cocci bacterium that is a non-motile, Gram-positive, catalase-negative , non-spore-forming, and

frequently found in the human oral cavity [16]. When compared to primary infections, the microbiota linked to secondary or persistent endodontic infections differs in terms of species diversity and quantity; S. mutans is frequently present in both kinds of infections [17].

Previous studies have evaluated the antibacterial effect of various irrigation solutions on the primary teeth. Unfortunately, no studies tested the antibacterial effect of NaOCl and GSE using different techniques on the primary molars, therefore this pioneering study investigated the antibacterial effect of 13% Ethanolic grape seeds extract versus 5.25% Sodium Hypochlorite solutions using conventional syringe irrigation and passive ultrasonic irrigation technique in primary molars microbiologically by counting of : (1) E.faecalis. (2) S.mutans. The null hypothesis which stated that there is no differences between the effect of passive ultrasonic irrigation and CSI technique using 13% ethanolic GSE and 5.25% NaOCl solutions on count of the tested micro-organisms.

METHODS

Specimen preparations

Before the experiment, the mandibular first primary molars were stored at room temperature in sterile saline after being soaked for 24 hours in 6% sodium hypochlorite. Caries and previous restorations were removed from all molars. Then the roof of the pulp chamber was removed, using carbide burs (Mani, Japan) in a high-speed handpiece (Linx, China) to gain access to the pulp. The dental crowns were cut with a diamond disc (KG Sorensen,Sao Paulo,Barazil)below the cementoenamel junction in order to maintain an 8mm standard length for each root . Then using a sharp disc, split at the furcation area into two distinct mesial and distal roots. Only distal roots were considered in this investigation.

Two-way analysis of variance was used to calculate the sample size (ANOVA). A total sample size of 60 samples will be adequate to reveal the effect size of 0.2744 [18]. a power (1-β=0.80) of 80% at a significant probability level of $p<0.05$ partial eta squared of 0.07.Calculations of sample size indicated the bacterial-infected groups. There shall be a minimum of six samples per sub-group. The sample size was determined using (G*Power software version 3.1.9.2) Where; f: is the effect size; $\alpha= 0.05$; $\beta=0.2$; Power= 1- $\beta = 0.80$.

By inserting a #10 k-file into the root canal until its tip was visible at the apical foramen, the apical patency was verified. The working length (WL) was then adjusted to be 1 mm less than this measurement. The canals were enlarged by rotary (Kidzo) files to size 30 #6 and 2.5% NaOCl was applied as an intracanal irrigant between each file size in the experimental samples. The samples were finally irrigated with 1 ml of 17% EDTA and 3 ml of 2.5% NaOCl, followed by 3 ml of normal saline. All root apices were covered with resin composite, then placed inside sterilization pouch and autoclaved (Autoclave Crystal, China) at 121℃ and pressure 2 bars for 20 minutes.

Immersion and testing of specimens

The prepared roots were split up into two main groups each consisting of 30 samples. Group (I): Included 30 roots infected with E. facials ,then they were sub- divided into 5 subgroups (6 roots in each) according to irrigation techniques and types of irrigants **Subgroup IA:**(Negative control) were infected with E. faecalis no irrigation. **Subgroup IB:** (positive control) CSI technique with 5.25%NaOCl (CHOLORAX XID 5.25%, Cerkamed medical company , Poland) . **Subgroup IC:** CSI technique with13% Ethanolic GSE solution -Subgroup ID: PUI with 5.25% NaOCl solution. **Subgroup IE:** PUI with 13% Ethanolic GSE solution. Group (II): Included 30 roots infected with S.mutans ,then they were sub-divided into 5

subgroups (6 roots in each) according to irrigation techniques and types of irrigants. **Subgroup IIA**: (Negative control) were infected with S.mutans no irrigation, Subgroup IIB : (positive control group) CSI technique with 5.25% NaOCl, Subgroup IIC : CSI technique with13% Ethanolic GSE solution ,Subgroup IID: PUI with 5.25% NaOCl solution ,Subgroup IIE: PUI with 13% Ethanolic GSE solution.

The extract was prepared at Department of Pharmacognosy , Faculty of Pharmacy , Suez Canal University .One kilogram of grape seeds was purchased from Egyptian market (Harraz Atara). We used an electric grinder to finely grind the seeds .The powder soaked in absolute ethanol to extract existed metabolites . This cold maceration process was repeated three times (3x3L) at ambient temperature with a sonicator aid to guarantee full extraction. The combined ethanolic solutions were concentrated under vacuum to afford 70 gram of ethanolic crude grape seeds extract. The extract was stored at -4 ℃ till used. An ethanolic solution of grape seeds was prepared at concentration of 13% (V/V) by mixing 13gram of extract with 100 ml of ethanol [19].

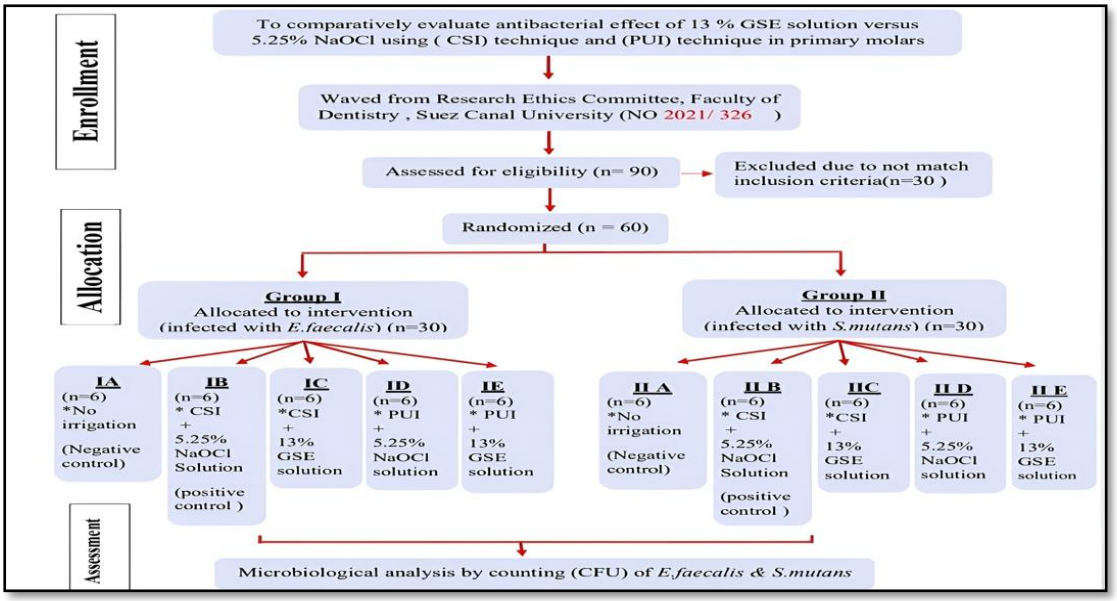

Fig. 1: Diagrammatic illustration of the experimental study design.

Preparation of Grape seeds irrigation solution:

- **Identification of the microorganism**:

1- **E. faecalis:** A standard cultures of E. faecalis (ATCC 29212) grown on plates of KF streptococcus agar that was utilized in our study following incubation for 48 hours at 37 ℃. The organism was identified by culture characteristics, microscopic examination and biochemical.
2- **S. mutans:** A standard Cultures of S. mutans (ATCC 27351) was spread on prepared Mitis Salivarius Bacitracin (MSB) agar surface in concentric motion with minimum pressure then the plates were covered. The plates were incubated for 24 hours at 37℃ anaerobically in a candle jar to identify S.mutans.

Preparation of the bacterial suspension:

Isolated colonies of pure cultures of both E.faecalis (ATCC 29212) and S.mutans(ATCC 27351) were aseptically rubbed off, suspended in a brain-heart infusion (BHI) broth, then disseminated by vortexing under laminar air flow to control the turbidity$3x10^8$ Colony -

Forming Units (CFUs) / ml (equivalent to 1 McFarland standard) So that the amount of bacteria was within a given range to standardize microbiological testing [20].

Inoculation of root canals with E.faecalis:

All 30 prepared roots were placed individually inside Eppendorf tubes. A sterile insulin syringe was used to inject 0.2 mL of bacterial suspension into each root canal under pressure to ensure that it reached to the full working length. 30 Roots that had been infected with E.faecalis (Group I) were incubated at 37 °C for one week. Refreshing broth was added without addition of new inoculum by using insulin syringe to the root canals and around each root in Eppendorf every 48 hours throughout the one week incubation period after initial inoculum [21].

Inoculation of root canals with S.mutans

The other 30 sterilized, prepared roots were inserted individually in Eppendorf tubes. A sterile insulin syringe was used to inject 0.2 ml of bacterial suspension into each root canal under pressure to make sure that it reached to the full working length. 30 Roots inoculated with S.mutans (Group II) were incubated at 37℃ for 7 days in a candle jar .Refreshing broth was added without addition of new inoculum to the root canals and around each root in Eppendorf every 48 hours throughout the one week incubation period after initial inoculum [22].

The first microbial sample (S1) was obtained by inserting three sterile absorbent paper points size #30 into each root canal for 60 seconds to become saturated with the bacterial suspension. The paper point's specimens were retrieved from the canal using sterile tweezers and placed in a sterile Eppendorf with 0.5 ml of BHI broth. For E.faecalis (group I) Blood agar plates were plated with 20 microns of suspension, and the plates were incubated for 48 hours at 37°C. For S.mutans (group II) 20 micron of the suspension was plated into Mitis Salivarius Bacitracin plates and incubated in a candle jar at a 37℃ for 48 hours.

Application of irrigant solution for Group I and Group II :

After incubation of the 30 roots with E. faecalis or S.mutans according to different treatment modalities, roots irrigated with two irrigating solutions with different irrigation techniques and sub-divided into:

Subgroup A: included 6 roots from each group were infected with microorganism with no irrigation(Negative control group).

Subgroup B: included 6 roots from each group (CSI technique with 5.25% (NaOCl) solution) (Positive control group) .2 ml of 5.25% (NaOCl) were used to irrigate the roots by using 3 ml syringe with a sterile 30-gauge side-vented irrigation needle (Fanta ,China) placed 1mm from working length in 20 (sec) . The needle was moved apical- coronal movement in the canal 1mm shorter than the working length After completing these steps three times ,the total irrigant volume was 6 ml , and the delivery time was 60 seconds , and the total irrigant activation time 60 seconds [23]. Followed by 1ml of normal saline to stop the effect of irrigation solution then taking (S2) sample.

Subgroup C: included 6 roots from each group (CSI technique with13% Ethanolic (GSE) solution) roots were irrigated with the same technique (Conventional syringe irrigation) but with 13% Ethanolic GSE solution. The process was repeated thrice such that total irrigant delivered is 6 ml. Total irrigant delivery time 60 seconds. total irrigant activation 60 seconds, then injection of 1ml of normal saline. After the final irrigation procedure, the (S2) sample was taken.

Subgroup D: included 6 roots from each group (PUI with 5.25% (NaOCl) solution.) 2 ml of 5.25% (NaOCl) solution were used to irrigate the roots. Followed by passive irrigation approach with intermittent flush, which consisted of using three cycles of ultrasonic activation of irrigant lasted 20 seconds each, allowing each root to experience 60 seconds of passive ultrasonic irrigation activation. Irrigation with 2 ml of 5.25%(NaOCl) was carried out between each cycle, so the total amount of delivered solution is 6 ml. Using an Irrisafe ultrasonic tip made of stainless steel 25./00 (woodpecker, China), passive ultrasonic activation was carried out . Irrisafe and E2 tips were activated by the use of a 5.5 W, 30 kHz piezoelectric ultrasound unit (SKL,China). Each canal was activated for 60 seconds (1 minute) using passive ultrasonic irrigation and 6 ml of 5.25% (NaOCl) solution (2 ml of solution in each cycle). The file was kept centered in the canal, the ultrasonic tip was placed coronally 1 mm to the working length, and there was 2-3 mm of apical-coronal movement [24]. Then 1 ml of normal saline was injected inside the root canal before taking after irrigation sample (S2) by paper points.

Subgroup E: included 6 roots from each group (PUI with 13% Ethanolic (GSE) solution). Roots were irrigated with 13% Ethanolic (GSE) solution on 3 cycles of ultrasonic activation with total volume 6 ml of solution, for total 60 second irrigation time. Followed by 1ml of normal saline to stop (GSE) solution effect before taking (S2) sample.

The paper point specimens were retrieved from the canal using sterile tweezers and placed in a sterile Eppendorf containing 0.5 ml of BHI broth. For 5 seconds, the Eppendorf were vortexed. For E.faecalis (group I) 20 µl of the suspension was plated onto blood agar plates and incubated for 48 hours at 37℃.For S. mutans (group II) 20 micron of the suspension was plated into Mitis Salivarius Bacitracin plates and incubated in candle jar at a 37°C for 48 hours.

Counting the resulting bacterial colonies of (E.faecalis & S.mutans):

Plates were inspected after the required incubation period, and growth was detected on the plates. To estimate the total number of bacterial colonies on the plate, Each petri dish was divided into four identical squares. Each square's bacterial count was counted and then multiplied by four. The quantity of turbid cultures and whole plate (CFU) counts for each sample at each time point were collected.

Ethical considerations

The Research Ethics Committee (REC) of the Faculty of Dentistry at Suez Canal University approved all the procedures done in the current study with permission number 326/2021. That aligns with the World Medical Association's Helsinki Declaration (Version 2008), as all the patients under 8 years old who came to the Department of Pediatric Dentistry at Suez Canal University for extraction had their parents or legal guardians sign informed consent forms to use their removed teeth for study purposes.

Statistical analysis

To make sure the data were normally distributed, a (Shapiro-Wilk) normality test was performed. The Mean ± Standard Deviation (SD) of the data were displayed. For all variables, an independent sample T-test was used to compare every element of the two groups. The statistical analysis was carried out using SPSS software version 26.0 for Windows (IBM Corp, Armonk, NY, USA).

Results

The mean $\log_{10}$of E.faecalis counts at the base line (S1) (after root preparation and before irrigation) of Negative Control (IA), CSI with 5.25 % NaOCl (IB), CSI with13% GSE solution (IC), PUI with 5.25% NaOCl solution (ID), and PUI with 13% GSE solution (IE) recorded with average±SD of (2.6±2.2),(2.6±2.4), (2.5±2.1), (2.5±2.4),and (2.5±2.1), respectively. The difference between all subgroups for (S1) samples "before treatment" was non-significant (p=0.097). However, the mean $\log_{10}$after treatment (S2) and average (±SD $\log_{10}$of E.faecalis count in previous subgroups (IB),(IC),(ID), and(IE) recorded (0.5±0.3), (1.0±1.0),(0.4±0.1), and (1.4±1.0) ; respectively. The difference between subgroups in (S2) "after treatment" was significant p<0.001 according to a one-way ANOVA **(Table 1)**.

The highest percentage of bacterial reduction was (-85.24%) recorded in sub-group ID (PUI with 5.25% NaOCl solution), followed by (-81.66%) in sub-group IB (CSI with 5.25 % NaOCl), then (-58.41%) in sub- group IC (CSI with13% GSE solution) , and the least percentage of bacterial reduction (-43.55%) recorded in sub- group IE (PUI with 13% GSE solution) **(Table 1)**.

The mean $\log_{10}$of S.mutans counts at the base line (S1) (after root preparation and before irrigation) of Negative Control (IIA) , CSI with 5.25 %NaOCl solution (IIB) , CSI with13% GSE solution (IIC) , PUI with 5.25% NaOCl solution (IID) , and PUI with 13% GSE solution (IIE) recorded and average (±SD) of (3.2± 2.5) , (3.2± 2.3) , (3.1± 2.4) , (3.2 ± 2.6) , and (3.2± 2.5) ; respectively. The difference between all subgroups in "before treatment" (S1) was non-significant (p= 0.819). However, the mean $\log_{10}$after treatment (S2) and average (±SD) $\log_{10}$of S.mutans count in previous subgroups (IIB),(IIC),(IID), and(IIE) recorded (0.7± 0.7), (1.3± 1.1) , (0.1± (-0.3)) ,and (1.0± 1.0) ; respectively. The difference between subgroups in "after treatment" (S2) was significant p<0.001 as revealed by one way ANOVA **(Table 2)**.

The highest percentage of bacterial reduction was (-96.10%) recorded in sub-group IID (PUI with 5.25% NaOCl solution), followed by (-77.06%) in sub-group IIB (CSI with 5.25 % NaOCl), then (-67.88%) in sub-group IIE (PUI with 13% GSE solution), and the least percentage of bacterial reduction (-57.66 %) in sub-group IIC (CSI with13% GSE solution) **(Table 2)**.

Table 1.The mean Log10 Bacterial count of E. faecalis at different treatment group both before (S1) and after (S2) treatment presented as mean and standard deviation

Treatment	*E. faecalis* ($\log_{10}$)						% change	Paired t-test p-value
	Before (S1)			After (S2)				
	Mean	SD	DMRTs	Mean	SD	DMRTs		
Control (IA)	2.6	2.2	a	2.6	2.2	a	0.00	>0.999 ns
CSI+5.25 NaOCl (IB)	2.6	2.4	a	0.5	0.3	b	-81.66	0.008**
CSI+13% GSE (IC)	2.5	2.1	a	1.0	1.0	b	-58.41	<0.001***
PUI + 5.25 NaOCl (ID)	2.5	2.4	a	0.4	0.1	b	-85.24	0.040*
PUI + 13%GSE (IE)	2.5	2.1	A	1.4	1.0	b	-43.55	0.002**
ANOVA	0.097 ns			<0.001***				

, **, *, significant at p<0.05, <0.01, <0.001; ns, nonsignificant at p>0.05. a,b means followed by different letters either vertically (in the same column) or horizontally (in the same row) are significantly different according to DMRTs at 0.05 level.*

Table 2. The mean logbacterial count of S. mutans at different treatment group both before (S1) and after (S2) treatment presented as mean and standard deviation

Treatment	*S. mutans bacterial count* (log_{10})						% change	Paired t-test
	Before (S1)			After (S2)				
	Mean	SD	DMRTs	Mean	SD	DMRTs		p-value
Control (IIA)	**3.2**	2.5	a	**3.2**	2.5	A	0.00	>0.999 ns
CSI+5.25 NaOCl (IIB)	**3.2**	2.3	a	**0.7**	0.7	B	-77.06	0.008**
CSI +13% GSE (IIC)	**3.1**	2.4	a	**1.3**	1.1	B	-57.66	<0.001***
PUI + 5.25 NaOCl (IID)	**3.2**	2.6	a	**0.1**	-0.3	B	-96.10	0.040*
PUI + 13%GSE (IIE)	**3.2**	2.5	a	**1.0**	1.0	B	-67.88	0.002**
ANOVA	0.819 ns			<0.001***				

, **, *, significant at p<0.05, <0.01, <0.001; ns, nonsignificant at p>0.05. a,b means followed by different letters either vertically (in the same column) or horizontally (in the same row) are significantly different according to DMRTs at 0.05 level.*

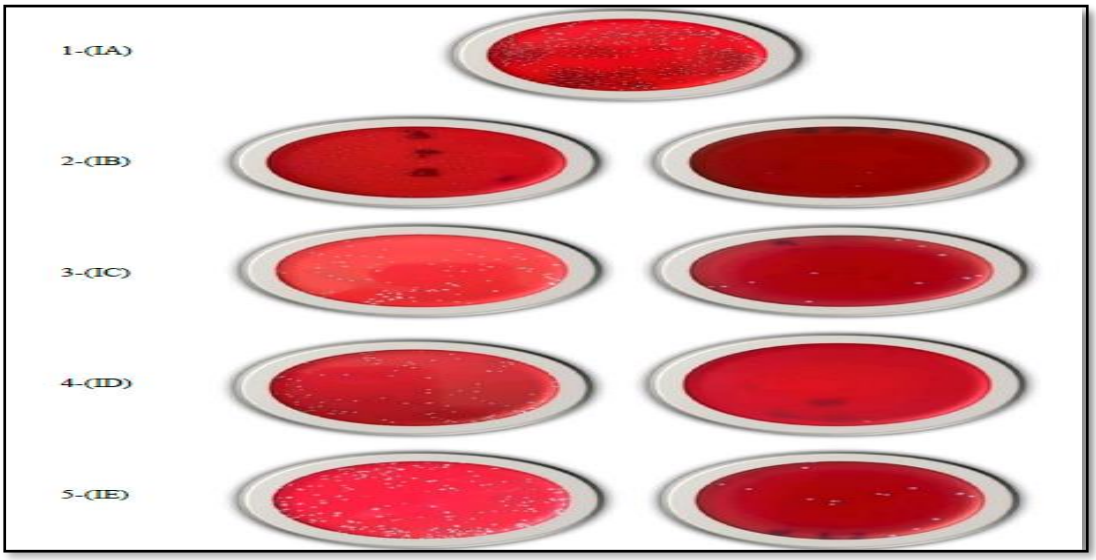

Fig. 2: Microbiological test results for E.faecalis (Group I) shows viable colony growth on blood agar plate before (S1)and after Irrigation (S2).for 1-subgroup IA before and after treatment , 2- subgroup IB before and after treatment, 3- subgroup IC before and after treatment,4- subgroup ID before and after treatment ,5- subgroup IE before and after treatment**.**

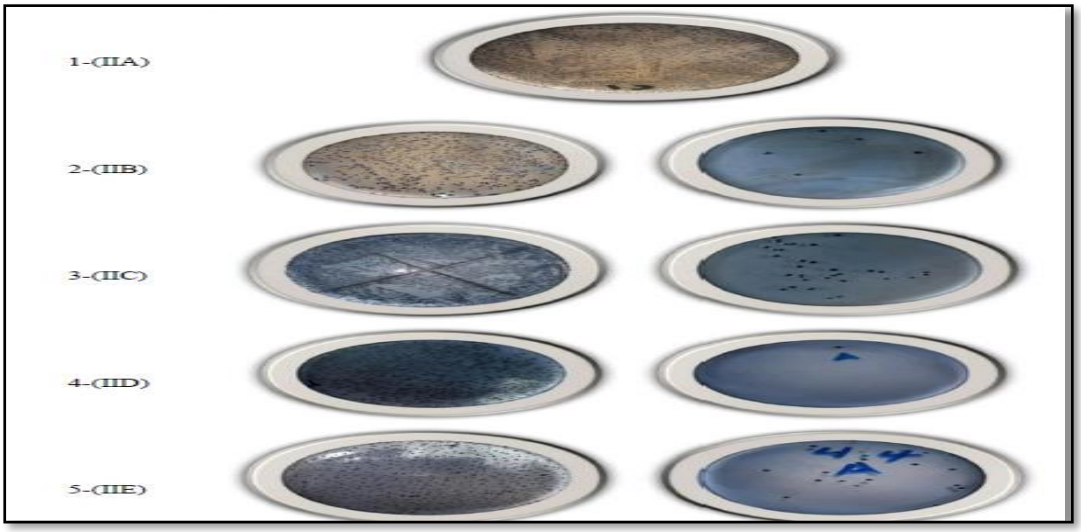

Fig. 3: Microbiological test results for S.mutans (Group II) shows viable colony growth on Mitis Salivarius bacitracin plate before (S1)and after Irrigation (S2).for 1-subgroup IIA before and after

treatment , 2- subgroup IIB before and after treatment, 3- subgroup IIC before and after treatment,4- subgroup IID before and after treatment,5- subgroup IIE before and after treatment**.**

Discussion:

GSE has been determined to be an antimicrobial and antifungal alternative and therapeutically, namely, antioxidants, immunomodulators and antimutagens [25]. Moreover ,(GSE) can be used in endodontics as(PAs) have antimicrobial properties , they act on the cell walls and inhibit the growth of anaerobic bacteria , such as E. faecalis [26].

In the current study, the teeth were chosen according to the following inclusion criteria: have at least two thirds of the root, roots without pathological root resorption (internal or external), no apparent caries in the root, cracks, or fractures (confirmed with periapical radiographs for each molar prior to selection to ensure that the all distal roots had straight and single canals to exclude molars that have internal resorption and / or internal calcification) [27]. Decoronation was done to all distal roots in current study below the cementoenamel junction level till having standardized root length with 8 mm of all selected distal roots samples [28]. All root apices were covered with resin composite in this study to prevent leakage of tested irrigant [29]. Before this experiment, all roots were autoclaved at 121℃ at pressure 2 bars for 20 minutes to ensure complete sterilization [30]. .In order to confirm the complete sterilization of root canals, a sterility test was done to ensure that the sterilized teeth samples were free of bacterial or fungal contamination [31].

The current study's (GSE) concentration was selected based on a prior investigation conducted by Margono et al [32]. who analyzed the ability of (GSE) to clean the smear layer on the apical third area when used as a root canal irrigation solution.. They used different concentrations of GSE (3.25% , 6.5% and 13%) compared to 17% (EDTA) solution, and found that the highest score for removal of smear is on the 13% (GSE) treated group.

In this study E.faecalis and S.mutans bacteria were chosen to evaluate and compare the antibacterial effect of 13% Ethanolic (GSE) versus 5.25% (NaOCl) solutions by passive ultrasonic irrigation and conventional syringe irrigation technique microbiologically since necrotic primary teeth have been found to contain greater quantities of these bacteria [33]. In current study, the pure cultures of E. faecalis (ATCC 29212) and S. mutans (ATCC 27351) were isolated and incubated for 7 days at 37℃ [34].

Refreshing (BHI) broth was added to the root samples of this study in Eppendorf every 48 hours throughout the one week incubation period after initial inoculum of bacteria, to make sure that there were enough nutrients available to the microorganisms [35].And the roots inoculated with S.mutans were incubated in a candle jar as the strains of S. mutans need 5 % (CO2) rich environment to grow [36]. Following the proper incubation period, the plates were examined, and each petri dish was divided into four equal squares, and the number of bacteria for one square was counted, then multiplied by four to determine the total plate colony forming unit count [37].

The results of this study shows that both (NaOCl) and (GSE) solutions have significant antibacterial effect on both bacteria (E.faecalis & S.mutans) ,But (NaOCl) has higher antibacterial efficacy when compared with(GSE) solution, Also The result of this study revealed that, no statistically significant differences in mean $\log_{10}$ of bacterial count observed between the passive ultrasonic irrigation technique and the conventional syringe irrigation technique.

The aim of this study was evaluate and compare the antibacterial effect of 13% Ethanolic grape seeds extract versus 5.25% Sodium Hypochlorite solutions by conventional syringe irrigation and passive ultrasonic irrigation technique in primary molars microbiologically . Additional studies should evaluate the antibacterial effect of 13% Ethanolic grape seeds extract using different techniques .

Conclusions

13% grape seeds extract solution showed a significant "antibacterial" effect with E.faecalis and S. mutans $p<0.005$, but 5.25% sodium hypochlorite solution has higher antibacterial effect . It was also found that there is no significant difference in the results between using conventional syringe irrigation technique and passive ultrasonic irrigation technique.

Abbreviations
E.faecalis: Enterococcus faecalis
S.mutans: Streptococcus mutans
CSI: Conventional Syringe Irrigation
NaOCl: Sodium Hypochlorite
GSE: Grape Seeds Extract
PUI: Passive Ultrasonic Irrigation
PAs: Proanthocyanidins
EDTA: Ethylenediaminetetraacetic Acid

Availability of data and materials: The datasets used and / or analysed during the current study available from the corresponding author on reasonable request .
Conflict of interest: The authors declare no conflict of interest.
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Author contribution: Authors contributed equally in the study.

References:

1. Shamma, B., Arrag, E., Rajab, A., Kurdi, S.(2022): 'Anti-bacterial activity of applying chitosan and propolis dressing against Enterococcus faecalis in primary teeth: in vitro study. Journal of Stomatology, 75(1), pp.36-43.
2. Barcelos, R., Tannure, P. N., Gleiser, R., Luiz, R. R., Primo, L. G. (2012). The influence of smear layer removal on primary tooth pulpectomy outcome: A 24-month, double-blind, randomized, and controlled clinical trial evaluation. *International Journal of Paediatric Dentistry*, *22*(5), 369-381.
3. Tawfiwk, M. A. E., Abdallah, A., Abbas, A. (2022). Evalution Of Turmeric And Thyme As Root Canal Irrigants On Primary Teeth. *Al-Azhar Journal of Dental Science*, *25*(4), 539-546.
4. Siqueira Jr, J. F., Pérez, A. R., Marceliano-Alves, M. F., Provenzano, J. C., Silva, S. G., Pires, F. R., ... & Alves, F. R. F. (2018). What happens to unprepared root canal walls: a correlative analysis using micro-computed tomography and histology/scanning electron microscopy. International endodontic journal, 51(5), 501-508.
5. Topbas, C., & Adiguzel, O. (2017): 'Endodontic Irrigation Solutions: A Review: Endodontic Irrigation Solutions. International Dental Research, 7(3),pp. 54-61.
6. Basrani, B., Haapasalo, M. (2012). Update on endodontic irrigating solutions. *Endodontic topics*, *27*(1), 74-102.
7. Yu, J., Ahmedna, M. (2013). Functional components of grape pomace: Their composition, biological properties and potential applications. International Journal of Food Science & Technology, 48(2), 221-237.
8. Vidhya, S., Srinivasulu, S., Sujatha, M., & Mahalaxmi, S. (2011). Effect of grape seed extract on the bond strength of bleached enamel. *Operative dentistry*, *36*(4), 433-438.
9. Souza, M. A., Dalla Lana, D., Gabrielli, E., Ribeiro, M. B., Miyagaki, D. C., & Cecchin, D. (2017). Effectiveness of final decontamination protocols against Enterococcus faecalis and its influence on bond strength of filling material to root canal dentin. *Photodiagnosis and photodynamic therapy*, *17*, 92-97.

10. Ragul, P., Dhanraj, M., & Jain, A. R. (2018). Irrigation technique used in cleaning and shaping during endodontic treatment-A review. Drug Invent Today, 10(5), 739-43.

11. Thulaseedharan, S. (2017). Comparative Evaluation of Effectiveness of Sodium Hypochlorite with Conventional Irrigation Method Versus Endovac and Ultrasonic Irrigation in the Elimination of Enterrococcus Faecalis from Root Canals: In Vitro (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India).

12. Nithyalakshmi, J. (2017). Evaluation of Debris Removal Efficacy of Different Root Canal Irrigants during Final Irrigation Protocol using Laser Activated Irrigation and Passive Ultrasonic Irrigation: An In Vitro study (Doctoral dissertation, Ragas Dental College & Hospital, Chennai).

13. Middha, M., Arora, M., Khangwal, M., & Kedia, M. (2017). Passive ultrasonic irrigation in endodontics: A literature review. *Journal of advanced medical and dental sciences research*, *5*(5), 73.

14. Rôças, I. N., Siqueira Jr, J. F., & Santos, K. R. (2004). Association of Enterococcus faecalis with different forms of periradicular diseases. Journal of endodontics, 30(5), 315-320.

15. Tulsani, S. G., Chikkanarasaiah, N., & Bethur, S. (2014). An in vivo comparison of antimicrobial efficacy of sodium hypochlorite and Biopure MTAD™ against enterococcus faecalis in primary teeth: A qPCR study. *Journal of Clinical Pediatric Dentistry*, *39*(1), 30-34.

16. El Sherbiny, G. M. (2014). Control of growth Streptococcus mutans isolated from saliva and dental caries. *International Journal of Current Microbiology and Applied Sciences*, *3*(10), 1-10.

17. Rocas, I. N., & Siqueira Jr, J. F. (2018). Frequency and levels of candidate endodontic pathogens in acute apical abscesses as compared to asymptomatic apical periodontitis. *PLoS One*, *13*(1), e0190469

18. Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*, *39*(2), 175-191.

19. Khedr ,A.l.,Goda,M.S.,Farrag,A.F., Nasr , A.M., Swidan,S.A.,Nafie,M.S., Abdel-Kader,M.S., Badr,J.M . andAbdelhameed,R.F. (2022) .Silver Nanaoparticles Formulation of Flower Head's Polyphenols of Cynara scolymus L.:A Promising Candidate against prostate (PC-3)Cancer Cell Line through Apoptosis Activation . Molecules,27(19) , p.6304

20. Arias-Moliz, M. T., Baca, P., Ordóñez-Becerra, S., González-Rodríguez, M. P., & Ferrer-Luque, C. M. (2012). Eradication of enterococci biofilms by lactic acid alone and combined with chlorhexidine and cetrimide. *Medicina Oral, Patología Oral y Cirugía Bucal*, *17*(5), e902.

21. Rodrigues, C. T., De Andrade, F. B., De Vasconcelos, L. R. S. M., Midena, R. Z., Pereira, T. C., Kuga, M. C., ... & Bernardineli, N. (2018). Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. *International Endodontic Journal*, *51*(8), 901-911.

22. Kulshrestha, S., Khan, S., Meena, R., Singh, B. R., & Khan, A. U. (2014). A graphene/zinc oxide nanocomposite film protects dental implant surfaces against cariogenic Streptococcus mutans. *Biofouling*, *30*(10), 1281-1294.

23. Shrivastava, S., Nikhade, P., Chandak, M., & Tibdewal, G. (2015). Comparison of the cleaning efficacy of conventional syringe irrigation, Endovac and Laser in removing smear layer from the root canal wall using scanning electron microscope: An in vitro study. *IOSR Journal of Dental and Medical Sciences*, *14*(5), 45-50.

24. Mozo, S., Llena, C., Chieffi, N., Forner, L., & Ferrari, M. (2014). Effectiveness of passive ultrasonic irrigation in improving elimination of smear layer and opening dentinal tubules. *Journal of clinical and experimental dentistry*, *6*(1), e47.

25. Karygianni, L., Al-Ahmad, A., Argyropoulou, A., Hellwig, E., Anderson, A. C., & Skaltsounis, A. L. (2016). Natural antimicrobials and oral microorganisms: a systematic review on herbal interventions for the eradication of multispecies oral biofilms. *Frontiers in Microbiology*, *6*, 1529

26. Souza, M. A., Dalla Lana, D., Gabrielli, E., Ribeiro, M. B., Miyagaki, D. C., & Cecchin, D. (2017). Effectiveness of final decontamination protocols against Enterococcus faecalis and its influence on bond strength of filling material to root canal dentin. Photodiagnosis and photodynamic therapy, 17, 92-97.

27. Korkut, E., Torlak, E., Gezgin, O., Özer, H., & Şener, Y. (2018). Antibacterial and smear layer removal efficacy of Er: YAG laser irradiation by photon-induced photoacoustic streaming in primary molar root canals: a preliminary study. Photomedicine and laser surgery, 36(9), 480-486.

28. Faiek A., Elmalt M., Ali H. and Mohamed E. (2019) .Evaluation of Antimirrobial Efficacy of Chitosan as Root Canal Irrigant on Enterococcus faecalis , Al-Azhar Dental Journal for Girls; 6(4), 437-443.

29. El Mansy, M., Safwat, E. M., El-Masry, H. M., & Salem, G. A. (2022). Effect of different herbal-based intracanal irrigation solutions on bacterial inhibition and dentin roughness of root canal in primary teeth. Journal of Pharmaceutical Negative Results, 13 (7) , 2198-2211.

30. Neuhaus, K. W., Liebi, M., Stauffacher, S., Eick, S., & Lussi, A. (2016).Antibacterial efficacy of a new sonic irrigation device for root canal disinfection. Journal of endodontics, 42(12), 1799-1803.

31. Faiek A., Elmalt M., Ali H. and Mohamed E. (2019) .Evaluation of Antimirrobial Efficacy of Chitosan as Root Canal Irrigant on Enterococcus faecalis , Al-Azhar Dental Journal for Girls; 6(4), 437-443

32. Margono A., Angellina A. and Suprastiwi E. (2017).The effect of grape seed extraction irrigation solution towards cleanliness the smear layer on apical third of the root canal wall. Journal of International Dental and Medical Research; 10(2), 244-247.

33. Govindaraju, L., & Jeevanandan, G. (2023). Evaluation of the antimicrobial efficacy of different concentrations of a novel root canal filling material for primary teeth-An in vitro study. Dental Research Journal, 20(1), 20-24.

34. Atila-Pektaş, B., Yurdakul, P. I. N. A. R., Gülmez, D., & Görduysus, Ö. (2013). Antimicrobial effects of root canal medicaments against E nterococcus faecalis and S treptococcus mutans. International endodontic journal, 46(5), 413-418.

35. Rodrigues, CT, De Andrade, FB, De Vasconcelos, LRSM, Midena, RZ, Pereira, TC, Kuga, MC, ... & Bernardineli, N. (2018). Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. International Endodontic Journal , 51 (8), 901-911.

36. Kulshrestha, S., Khan, S., Meena, R., Singh, B. R., & Khan, A. U. (2014). A graphene/zinc oxide nanocomposite film protects dental implant surfaces against cariogenic Streptococcus mutans. Biofouling, 30(10), 1281-1294.

37. Abdel Latif, A. A., Katiaa, M. A., Hassan, H. Y., Azab, M. M. (2016). Efficacy of different irrigating systems with a new irrigating solution in reducing intracanal Enterococcus faecalis. Egyptian Dental Journal, 62 (3), 673-682.