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Original Article

# Effect of Rosmarinus Officinalis Versus 2.5% Sodium Hypochlorite as Root Canal Irrigant on The Intensity of Postoperative Pain and Bacterial Reduction in Asymptomatic Mandibular Premolars: A Randomized Clinical Trial.

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## Abstract

**Aim:** The present study assessed the effect of Rosmarinus officinalis extract solution versus 2.5% NaOCl as root canal irrigant on the postoperative pain intensity and the bacterial reduction after root canal treatment of necrotic mandibular premolars.

**Subjects and methods:** Thirty patients with asymptomatic necrotic single-canalled mandibular premolar were included. Diagnosis had been confirmed clinically and radiographically then patients had been allocated randomly in two equal groups. Teeth in the intervention group were irrigated using 25mg/ml Rosmarinus Officinalis extract solution and those in the control group were irrigated by 2.5%NaOCl. Root canal treatment was done in a single visit by M-Pro files during chemo-mechanical preparation and modified single cone technique using resin sealer for obturation. Assessment of Postoperative pain level was done at 6, 12, 24, and 48 hours using Numerical Rating Scale (NRS). Culturing was used for the assessment of intracanal bacterial levels before and following chemo-mechanical preparation. Statical analysis was done for demographic, baseline and all the outcome data.

**Results: :** Rosmarinus officinalis extract solution caused less postoperative pain to 2.5% NaOCl at 6 hours with a significant difference (p=0.007), at 12 hours with a significant difference (p=0.11), and at 24 hours with significant difference (p = 0.029). Both irrigants significantly reduced the amount of intracanal bacterial load**.**

**Conclusion: :** Rosmarinus Officinalis extract solution could be more effective than 2.5% NaOCl in terms of reducing intensity of postoperative pain. Both irrigations significantly reduced intracanal bacterial levels of necrotic mandibular premolars.

**Keywords: :** Rosmarinus officinalis, Bacterial reduction, postoperative pain, Irrigation, anerobic bacteria, Numerical rating scale (NRS).

## Introduction

Postoperative pain tops the main problems in root canal treatment [1] Some patients complain of moderate to severe pain after root canal treatment that affects their life quality such in eating, sleeping, also general functioning, and leads to a negative attitude towards root canal treatment [2]. According to reports, the average of its incidence is 40 ± 24% of cases, although it can rise to 80% in the first 24 hours [3].

Many factors may cause pain postoperatively after root canal treatment as improper instrumentation, Irrigant extrusion or apical debris or intracanal medication extrusion, high occlusion, missed canals, preoperative pain, and presence of periapical pathosis [4].

The primary goal of endodontic treatment is the bacteria eradication with its byproducts from the root canals of infected teeth. Thus, it is a very challenging task because of the complexity of the root canal system anatomy which makes the complete eradication of bacteria difficult [5].

The main causative factor of the postoperative pain is the presence of microorganisms because of failure to properly disinfect the canal. Several studies declared a direct relationship between the infection of root canals and the postoperative pain level [6].

Root canal instrumentation alone failed to remove all microorganisms, tissue remnants and debris because of the complexity of the root canal anatomy, Therefore, irrigants pose a crucial role in having complete endodontic disinfection and overcoming the shortcomings of instrumentation [7].

Numerous irrigants have been recommended to be used in endodontic treatment. Sodium hypochlorite (NaOCl) is considered the gold standard and the irrigant of choice for all endodontists owing to its high level of antimicrobial activity and its ability to dissolve soft tissue. But on the other hand, it has unacceptable properties as: It's known to be toxic to the tissues, when extruded beyond the root apex and getting in contact with vital tissues. It causes acute immediate symptoms which are known as (the hypochlorite accident). The hypersensitivity reactions to NaOCl have been also reported. Also, its unaccepted taste, odor, failure of smear layer removal, and its proteolytic effect cause a reduction in dentine strength. All these events have led to further investigation for more biocompatible irrigant which poses no harms to dentin with better taste and odor to overcome NaOCl ramifications [8].

Herbal irrigants and natural products have drawn attention from researchers all over the world in recent years because of their therapeutic qualities, which include biocompatibility nature, antimicrobial activity, anti-inflammatory actions, and antioxidant effects [9].

Rosmarinus officinalis (R. officinalis) as it is commonly called, is a member of the Lamiaceae family and is native to the Mediterranean region. It's a fragrant perennial plant. It is also a versatile plant that can be utilized as food spices, a decorative and medicinal herb, and helps in food preservation naturally. It has antioxidant, antiviral, antifungal, antimicrobial, and anti-inflammatory properties[10]. R. officinalis phytocompounds and plant extracts can also hinder the colonization of the bacterial species. Plant-derived microbes have demonstrated a reduced ability to adhere, forming an easily removable biofilm of adherent cells popularly[11,12].

So, this study aims to detect the impact of R. officinalis extract solution against 2.5% NaOCl as an irrigation for root canal treatment on the level of postoperative pain intensity and the of bacterial reduction level after root canal treatment of premolars with necrotic pulp. The null hypothesis was that there was no difference in post-operative pain levels and amount of bacterial load reduction between 20mg/ml Rosmarinus Officinalis extract solution and 2.5% NaOCl as root canal irrigant, in asymptomatic necrotic mandibular premolars.

## Subjects and Methods

### Study design, settings and sampling:

The present study design was a parallel, prospective, triple blinded, RCT with an allocation ratio 1:1 and framework superiority. The protocol was registered at www.clinicaltrials.gov (ID: NCT05289843) and it was approved by ethics committee at Faculty of dentistry, Cairo University (Approval Number 4-6-22).

**Eligibility criteria**

The criteria for participants inclusion were age range from 20 to 45 years, The participants had asymptomatic mandibular premolars suffering from pulp necrosis with or with no apical periodontitis. Patients who consumed analgesics or antibiotics during the past 12 hours, pregnant women, teeth associated with acute periapical abscess or phoenix abscess, and previously accessed teeth were excluded from the study.

A final diagnosis of single rooted asymptomatic mandibular necrotic premolars was confirmed by the following: Both clinical and radiographic examinations. The chief complaint's history of reporting no discomfort in response to heat or cold, negative response to an electrical pulp tester (Denjoy dental Co, LTD,China), where the contralateral tooth served as a control to verify the previous diagnostic tests. Radiographic examination (Digora, Optime DXR-60, Soredex, Finland) showed lower premolar with slight widening in the periodontal membrane space or a periapical radiolucency within 2 mm. chief complaint's history of reporting no discomfort in response to heat or cold, negative response to an electrical pulp tester, where the contralateral tooth served as a baseline to verify the previous diagnostic tests.

**Sample size:**

Based on a previous study by **(Hosny et al. 2021)**[9] Using power 80% and 5% significance level, 13 patients were allocated in each group (total 26 patients in the two groups) were studied. To compensate for loss during follow-up, the number was then raised once more to be 15 patients per group (drop-out rate 15%).

**Randomization and blinding**

A random sequence generator software (http://www.random.org/) was used to randomly place patients in each group. A single clinician executed all root canal treatments in one visit. This study was triple-blinded (the participant, the operator and outcomes assessors were blinded). The numbers of patients were written on 8 folded papers and placed in opaque sealed envelopes. When an outpatient met the eligibility criteria, the patient was asked to draw from the concealed envelope containing a number which determined his assignment to one of the 2 groups according to the randomized sequence, then the operator contacted the assistant supervisor, who used the produced random sequence to assign the patient to either the intervention or control group. The assistant supervisor oversaw preparing the irrigation solutions and delivering them to the operator while the operator oversaw all the clinical procedure steps and evaluating the patients' outcomes.

**Preparation of Rosmarinus Officinalis:**

R.Officinalis extract had been prepared following (Hosny et al 2021)[9]. Collected fresh mature R.Officinalis leaves from the Faculty of Pharmacy's medicinal garden, Cairo University were cleansed in sterilized distilled water and then were dried. One hundred-fifty grams of dry-weight powdered R. Officinalis leaves were macerated to create the ethanolic extract, which was then extracted with ethyl alcohol of 95% (w/v) for seven days in a flask with round bottom, while shaking the flask periodically. Light exposure was avoided to the prepared solution to prevent its harmful effect on the active groups of the extract. Muslin cloth was used to filter the extract for coarse residue then filter paper (Whatman filter paper number one) (Figure1) was utilized, and Rota-vapor was used to remove alcohol under reduced pressure

conditions and stored for one night in the desiccators (Figure2). A 300 ml of 9.99% Dimethyl Sulfoxide (DMSO) was then used to dissolve the residue to make the final 50% concentrations then solution was kept in the refrigerator till needed.

**Figure (1):** Ethanolic R. Officinalis extract filtration.

**Figure (2):** Rotary evaporator system**.**

**Root canal treatment procedures and sampling**

Single-visit endodontic treatment was accomplished, after signing an informed consent by the patients. 1.8 mL of 2% Mepivacaine HCl with Levonordefrin 1:100,000 (Alexandria, Egypt) was administered after which the operating field was isolated, disinfected with 3% $H_2O_2$, and 5.25% NaOCl. Round bur & Endoz bur (Dentsply Maillefer, Ballaigues, Switzerland) were used for preparation of the access. Disinfection of the operative field was done once more then NaOCl was neutralized using 5% sodium thiosulfate. Sterility checking was done for every tooth through culturing of paper point samples taken from the access cavity and the external crown surface. Positive sample indicates a breach in the aseptic conditions so, the case was excluded from the study. Patency of the canals was checked using k-file #15(MANI, INC., Industrial Park, Utsunomiya, Tochigi, Japan), 2% and canals were irrigated with 1 ml Saline solution (Nasr Group, Egypt).

Regarding the pre-instrumentation bacterial sample (S1), Three consecutive sterile paper points #15 (Meta Biomed, Chungbuk, korea) were inserted and left to absorb the fluid for 60 seconds each with pumping motion. Then, transferred to tubes containing 2 ml of sterile brain heart infusion broth (Oxoid microbiology product, LTD, England England).

After contacting the assistant supervisor, the patients were allocated randomly to one of 2 groups according to the irrigation protocol used:

Group 1: irrigation with R.Officinalis.

Group 2: irrigation with 2.5%NaOCl.

Electronic apex locator (Root ZX, J. Morita) was used to determine the working length for both groups. Mechanical preparation was done using M-Pro file system (IDM, 25, 6% and 35, 6% reaching an apical China) set on X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland) with adjusted torque 2.5 Ncm and speed 350 rpm in continuous rotary brushing motion; Rotary files were used: file 18, 4%, file 20, 4%, file size preparation of 35 or 40 according to the size of the canal, and

ensuring the establishment of definite apical stop and sufficient cleaning.

For group 1(interventional group) thorough irrigation by Rosmarinus Officinalis extract solution using disposable plastic syringe with size 30-needle gauge (Diadent, Chungcheongbuk-do, Korea) placed at distance 1 mm from the confirmed working length. The same irrigation volume was received by all teeth (5 ml before instrumentation, 5 ml between each file and 5 ml as a final flush reaching total volume of 25 ml).

For group 2 (Control group): thorough irrigation by 2.5%NaOCl solution was done using disposable plastic syringe with size 30 needle gauge (Diadent, Chungcheongbuk-do, Korea) placed at distance1 mm from the confirmed working length. The same irrigation volume was received by all teeth as mentioned in the intervention group.

The canals were finally flushed with sterile saline then 17% EDTA solution (Prevest Direct, India) for 1 min. The post-instrumentation sample (S2) was collected as mentioned. Both microbiological samples were transferred for culturing within 20 minutes. Modified single gutta-percha cone technique together with a resin-based root canal sealer (AdSeal:Meta Biomed CO., LTD, Korea) were used for obturation and a temporary restoration (Cavit, Germany) was utilized to seal the access cavity. Patients were referred to the conservative dentistry department, faculty of Dentistry, Cairo University for final restoration.

**Postoperative pain assessment:**

Both incidence & intensity of the postoperative pain were recorded using NRS at 6, 12 hours, 1 day and 2 days after obturation.

**Intracanal bacterial count**

Culture technique was used for the microbiological analysis. Samples in 1 ml of BHI broth were dispersed with vortex in the mixer for 60 secs. Then preparation of serial 10 folds dilution (1/10 and 1/100) was made. For aerobic bacterial culture, 50 µl were cultured over BHI agar plates and incubated at 37ºC for 24 hours. Regarding anaerobic bacteria, another 50 µl were cultured on BHI agar plates, were placed in a sealed anaerobic jar containing GasPak & an anaerobic indicator (England) then incubated at 37ºC for 48 hours. Visual quantification of the resultant growth was made under the microscope through counting the number of colony forming units per ml (CFUs/ml) of each dilution [13], then multiplied by the dilution factor to obtain the actual bacterial count. Log bacterial load reduction was calculated using the equation: Log bacterial load reduction = log10*(Mean CFU at S1 / Mean CFU at S2) [14].

**Statistical analysis**

Data were tested for normality using Shapiro Wilk test. Continuous data was presented as mean, standard deviation (SD), median and range values. Independent *t* test was the test of choice for independent samples comparison of normally distributed data. Mann-Whitney U test was used for independent samples comparison of non-normally distributed data. While Wilcoxon signed-rank test was used for related samples comparison. Categorical data was presented as frequencies (N) and percentages (%). Independent group comparison of categorical data was done by Chi-square test. Significance level for all tests was set as 0.05. Statistical analysis was performed using SPSS software.

Primary outcome was the intensity of postoperative pain: It was measured by the Numerical Rating Scale (NRS) was categorized as None 0 ,Mild 1-3, Moderate 4-6 and Severe

7-10.The primary outcome was measured at 6, 12, 24, 48- hours post-treatment.

Secondary outcome was amount of intracanal bacterial load reduction: It was measured through the bacterial counting using agar Culture technique in CFUs/ml. First sample (S1) is taken before chemo-mechanical preparation and the second sample (S2) is taken after completion of chemo-mechanical preparation.

**Results**

The process of patient enrolment and progress through each phase of the trial is shown in Figure 3. Forty-five 45 patients were assessed for meeting the eligibility criteria, Thirty patients were enrolled and included in the analysis. Demographic data showed no significant difference was detected among the two groups in terms of gender distribution (P= 1.), age (P= 0.23) and tooth type (P= 0.45).

The mean and standard deviation values of pain intensity at 6. 12, 24 and 48 hours postoperatively is reported at table 1. The postoperative pain intensity for both groups is reported in figure 3. A significant difference was found the two groups' intensity of postoperative discomfort at 6, 12, and 24 intervals, (P>0.05) while no significant difference was found at 48 hours postoperatively.

No bacterial growth was detected in all sterility control samples; therefore, all cases were included in the analysis. Mean and standard deviation (SD) values of bacterial count reduction between both groups are presented in table 2. Both irrigants showed considerable bacterial load reduction following chemo-mechanical preparation with no detected significant difference between the two irrigants regarding both aerobic (P=0.412) and anaerobic bacteria (P= 0.512)

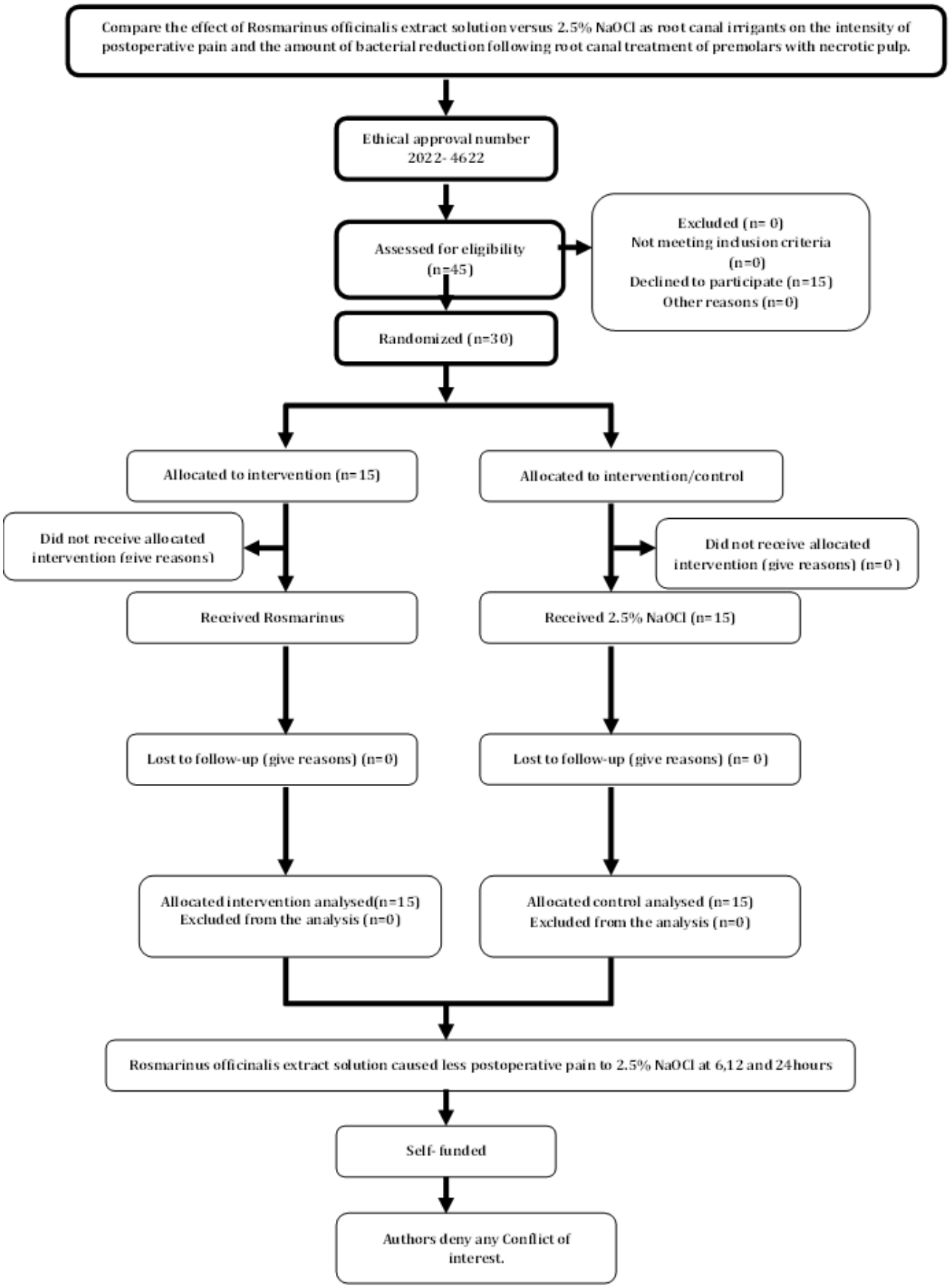


**Figure (3):** PRIRATE 2020 flowchart

**Table (1):** Descriptive statistics and the results of Mann-Whitney U test for comparison of pain intensity at different postoperative time intervals between the two groups:

| | | Intervention | Control | *p*-value |
|---|---|---|---|---|
| **6 Hours** | **Mean (SD)** | **0.8 (2.6)** | **2.6 (2.3)** | **0.007*** |
| | **Median (Range)** | **0 (0 - 10)** | **2 (0 - 8)** | |
| **12 Hours** | **Mean (SD)** | **0 (0)** | **2.3 (3.2)** | **0.011*** |
| | **Median (Range)** | **0 (0 - 0)** | **1 (0 - 10)** | |
| **24 Hours** | **Mean (SD)** | **0 (0)** | **1.6 (2.3)** | **0.029*** |
| | **Median (Range)** | **0 (0 - 0)** | **0 (0 - 7)** | |
| **48 Hours** | **Mean (SD)** | **0 (0)** | **0.7 (1.2)** | **0.217*** |
| | **Median (Range)** | **0 (0 - 0)** | **0 (0 - 3)** | |

***Significant at p=0.05***

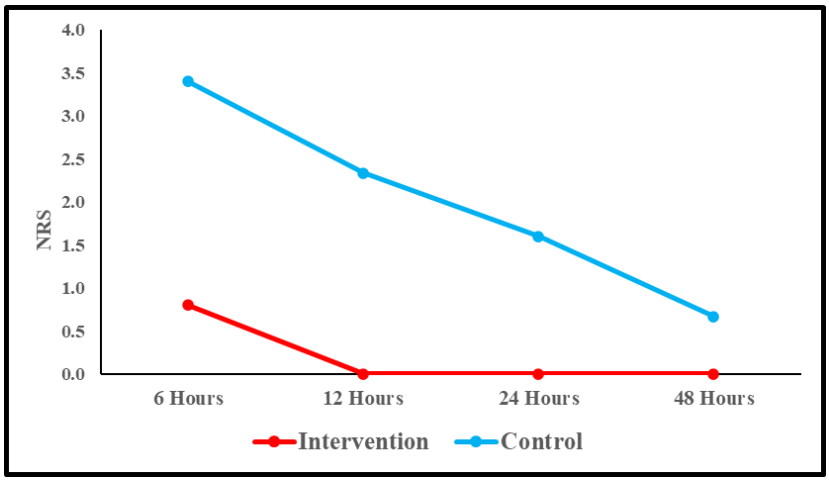


**Figure (4):** line chart representing the changes in mean pain intensity at different postoperative time intervals in the two groups.

**Table (2):** descriptive statistics and the result of Mann-Whitney U test for comparison of aerobic and anaerobic bacterial percentage reduction between the two groups:

| | | **Intervention** | **Control** | ***p*-value** |
|---|---|---|---|---|
| ***Aerobic*** | ***Mean (SD)*** | 86.0% (12.6%) | 87.1% (16.1%) | 0.412 |
| | ***Median (Range)*** | 90% (50% - 99%) | 92.5% (43.3% - 99.7%) | |
| ***Anaerobic*** | ***Mean (SD)*** | 86.3% (13.2%) | 89.3% (10.9%) | 0.512 |
| | ***Median (Range)*** | 94.7% (60% - 99.5%) | 90% (65.7% - 99.8%) | |

## Discussion

Natural products and herbal irrigants have gained great interest from worldwide researchers owing to their medicinal properties including anti-inflammatory, antimicrobial, and antioxidant properties and their excellent biocompatibility which is the most important property. Furthermore, they also showed lower postoperative pain which favored their usage as endodontic irrigants[9]. Rosmarinus officinalis has been known for a long time for its anti-inflammatory, antioxidant, antiviral , anti-bacterial, and anti-fungal activity[10,15]. In This context this study assessed the effect of Rosmarinus Officinalis extract solution against 2.5% NaOCl as an irrigation for root canal treatment on the postoperative pain level and the amount of bacterial reduction after root canal treatment of asymptomatic mandibular premolars with necrotic pulps treated in single visit in a prospective, randomized, double-blinded, parallel clinical trial.

Thirty patients having asymptomatic single canalled necrotic mandibular premolar were included. Only asymptomatic patients were included in this study as pre-operative pain has been reported to be significant predictor for post-treatment pain[16]. Teeth having large periapical lesions have been excluded because this indicates long-standing infections, cystic transformation or extraradicular infection negatively affecting the outcome [17].

Single visit approach was adopted in this investigation. A well conducted systematic review stated that single visit approach causes less postoperative complications by reducing mechanical and chemical injuries to the periradicular tissues and omitting the risk of inter-appointment leakage[18]. Additionally, success rates are comparable in both single- multiple-visit root canal treatment.

2.5% NaOCl was used as it has less cytotoxicity than 5.25% NaOCl. Furthermore, previous studies showed that low NaOCl concentrations caused less postoperative discomfort than higher concentrations[19,20].

In the current study, 50% ethanolic extract of Rosmarinus officinalis in dimethyl sulfoxide solvent was used as a root canal irrigant. Ethanol 95% was used because it has better dissolving capabilities. Rosmarinus officinalis active ingredients are made out of phenol groups that has the ability to dissolve in an organic solvent while has no ability to dissolve in water, Thus, the extracts dissolved in ethanol pose higher concentrations/amounts of bioactive

compounds than that found in the dried powder of the plants compared to water, methanol[9]. 9.99% of Dimethyl sulfoxide (DMSO) was used in the study because it is clear, has no toxic properties, colorless, organic hygroscopic solvent with proven safety in humans, also doesn't have any actions against microbes, especially in low concentrations. Its viscosity is comparable to water (1.19 gm/mL as compared to water at 1 gm/mL) to facilitate using the extract as an irrigant by allowing better penetration in the root canal space and potentially eliminate the vapor-lock effect in the narrow [21].

In the current study, M Pro rotary files was used to make rotary instrumentation. M Pro system is made out of an X-wire with special treatment that make the rotary files have the ability to be pre-bent, have more flexible properties, high resistant to cyclic fatigue failure, it poses a convex triangle cross section, cutting edges with increased pitch , with less screwing effect. M Pro files manufacturer stated that it poses high resistance for fracture and can remain centralized in the [22]. Numerical Rating Scale (NRS) was used in this study. It is a 11-point rating scale that offer better patient compliance and response, ease of use, and better applicability than the Verbal Rating Scale (VRS) or the Visual Analogue Scale (VAS) [23].

A follow up period of 48 hours was selected as pain prevalence decreases substantially during the first 2 days following obturation [3]. Pain assessment began at 6 hours postoperatively, to be sure that the local anesthetic effect was totally worn off, then continued to be assessed at 12, 24, and 48 hours postoperatively [9].

Statistical analysis demonstrated that the two groups exhibited comparable baseline data indicating successful randomization. The intervention group showed statistically significant lower pain at all tested times except at 48 hours compared to the control group. For the 48 hours that did not significantly differ between both groups. No clinical studies investigated the effect of Rosmarinus Officinalis solution as a root canal irrigant on post-operative endodontic pain to date. However, the results of this investigation were in conjugation with the results of the findings of Hosni et al (2021) on postoperative pain after irrigating with neem (Azadirachta indica), which was like 2.5% NaOCl at all follow-up periods except at 24 hours where neem caused lower intensity of pain[9.]

The significant difference in the incidence and the intensity of the level of the postoperative pain with the intervention group is owing to the nature of the R.officinalis as it has antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties[10]. It can perform several pharmacological impacts because of the interaction between molecules of the plant with the organic systems[11]. Microbes grown together with plant products have shown less adhesion capacity, resulting in a biofilm formed by adhered cells that can be easily removed causing less postoperative pain than NaOCl, Microbes that have grown together along the plant products have shown capacity of less adhesion, which results in that the biofilm which is formed by adhered cells that could be easily removed [12].

Both irrigants showed a considerable aerobic and anaerobic bacterial load reduction following chemo-mechanical preparation reaching 90 % and 94.7% respectively in the intervention & 92.5% and 90% in control group respectively. These results agreed with previous investigations stating that chemo-mechanical preparation caused significant disinfection and bacterial reduction that could reach 100% [24,25].

No clinical trials investigated the anti-bacterial efficacy of Rosmarinus officinalis aerobically & anaerobically in vivo. However, our results are comparable with

the results of Nasr et al. (2021)study that found chitosan nanoparticles were as effective as 2.5% NaOCl against aerobic bacteria but exhibited higher efficacy against anaerobic bacteria.

The clinical significance of the present investigation showed that Rosmarinus officinalis extract solution seems to be a promising root canal irrigant with no postoperative pain compared to 2.5_NaOCl and comparable antibacterial efficacy.

## Conclusion

Within this study limitations, it can be concluded that Rosmarinus Officinalis extract solution could be used as an alternative irrigant to NaOCl with less postoperative pain. Both irrigating solutions significantly reduced intracanal bacterial levels.

## Conflict of Interest:

The authors declare no conflict of interest.

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## Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 28-6-2022, approval number:4622

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