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Antimicrobial Effect of Pistacia Atlantica Kurdica as an Intra Canal Medicament in Root Canal Treatment on Enterococcus Fecalis

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ABSTRACT

Background: *Pistachio Atlantica kurdica* (PAK) is an aromatic resin and has many therapeutic effects such as anti bacterial, and anti fungal effects. This study was designed to investigate the antimicrobial properties of PAK resin as an intracanal medicament in failed root canal on *Enterococcus fecalis* in extracting teeth.

Material and Methods: Resin extracted was done by alcoholic ethanol 70% and Dimethyl sulfoxide DMSO 100% respectively. Minimal inhibition concentration identified by spectrophotometer. While the inhibition zone was determined by disc and microwell techniques. Forty-five extracted teeth randomly were divided into three groups of fourteen. They were instrumented sterilized, and then re-infected with standardized Enterococcus Faecalis. After overnight incubation, the intracanal medicament was placed inside the canal. The first group considered as a negative control, Group2 and Grou3 were treated with PAK resin and CHX 2% gel (intracanal medicament) correspondingly.

Result: The minimal inhibitor concentration of resin was $5x10^{-3}$ mg/ml. The resin of PAK dose-dependently increased zone inhibition diameter while the colony-forming unit (CFU) of treated teeth with PAK resin was significantly reduced. However non-significant differences were found in the reduction of CFU between CHX and PAK.

Conclusion: Resin of PAK as intra-canal medicament has an excellent effect to eliminate enterococcus fecalis with no-significant differences to CHX. Minimal inhibition concentration of PAK is ($0.5x10^{-3}$ mg/ml).

KEYWORDS

Anti Microbial Effect, Pistacia Atlantica Kurdica, Root Canal Treatment, Enterococcus Fecalis.

Introduction

The endodontic treatment aim is to remove microorganisms from the infected root canals by mechanical and chemical methods (instrumentation and irrigation). Although, these two methods may incapable of overcome the microorganisms overload. Bacteria, residual pulp tissue, and dentine debris may remain in the irregularity of the root canals. So using intra-canal medicament is mandatory to fights microorganisms. Duration of medicaments inside the canals is the key point for overcome bacteria (1). Enterococcus faecalis among the most resistant types of bacteria. It is a Gram-positive, facultatively anaerobic coccus that can tolerate a high starvation period for up to one year, and temperatures above 45°C. The bacteria selected for this study were enterococcus fecalis which often can be isolated from a failed root canal and necrotic pulp.

Several intra-canal medicaments are being used in the treatment of the root canal. CHX gel as an intra-canal medicament is a type of intra-canal medicament that has excellent antimicrobial properties that can be used during or after complete instrumentation in endodontic procedures for about 3-5 days. It is active against both Gram-positive and Gram-negative bacteria, and yeasts and fungi. It affects the bacterial cell wall by the interaction of the positive charge of CHX and the negative charge of the bacterial cell wall (2).

Due cytotoxic effect of the commercial intra-canal medicaments using herbal plant extraction is introduced in dentistry. WHO defines herbal medicine as a material derived from plants (3). Using the herbal product in dentistry termed phytotherapeutics or ethnopharmacology (4, 5). The use of herbal medicines has a long history in the human community especially in the field of dentistry. According to Encyclopaedia Britannica Mastic is an aromatic resin, obtained as a soft exudation from incisions in mastic trees (6). It has many kinds of pistacia; five more popular types are P. vera, P. Atlantica, P. Terebinthus, P. Khinjuk. P. Lentiscus. Among theme P Atlantica is more common in Kurdistan Mountain. Furthermore, P Atlantica has three subspecies Cabulica, Kurdica, and Mutica) (7). Pistacia tree has different names according to country and the native language of the particular place like, ‘’Mastic, Mastix, Ushgai, Qazwan’’(8). In Kurdistan region it is known as Qezwan or Dare Ban, P.A. kurdica classified as a subspecies of P. Atlantica and in some other study it is classified as P khinjuk (9).

Resin, leave, fruit, and aerial parts of Pestecia had used for different diseases, such as the brain, liver, kidney, heart, stomach, hepatic, and respiratory system disorders (7). The resin of pistacia despite different therapeutic functions contained a high level of Cu, Fe, and Zn (10). Resin has a long history of therapy among Kurdish people (8). The

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number of hunters in kurdistan showed the partridge, that put the resin by its peak in the site of injuring in its body when shoot by the bullet.

In dentistry, it helps oral hygiene by, systematically increasing salivation, which led to prevents plaque collection. It is an anti-bacterial and anti-fungal effect, due to natural antioxidant action; it improves gum disease and protects teeth. Also, it is used as a material for the fillings of the teeth (11-13). Finally, Bneshuta Tala uses as an antiseptic, and relaxing agent due to its eugenol content. It is also used as an ingredient for toothpaste and mouth wash (14).

Material and Methods

This comparative study has been conducted in, Hawler central lab, college of pharmacy, and college of medicine. The process of resin collection has been carried out; in the village of Chewie Saru; in the Kurdistan region of Iraq, from December 2017 to October 2018.

The Extraction Method of Resin *and* Preparation of Resin as an Intracanal Medicament

The first step was rein extraction. Ten g of the resin was mixed with twenty ml of ethanol 70% and Dimethyl sulfoxide, separately. By mixing resin with both solvents, different concentrations of both groups were obtained, from 500-0,5x10^{-5} mg/ml (15, 16). The next step was making a paste from the resin; by adding two ml of Tween 80 as a solvent, to ten grams of the resin, at 100 C^{o}, and under continuous mixing for five minutes.

Antibacterial Evaluation of Resin

- **Inhibition Zone of Bacteria**

The standard strain of Enterococcus fecalis ATCC: 29212 was purchase from the media library/Hawler Medical University. The type of bacteria identified byVitic machine. Serial dilution of bacteria had done to obtain the desired number of 30-300 colonies of bacteria (15, 16).

- **Disc Diffusion Method**

ten µl of different concentrations of resin-impregnated sterile Discs 5 mm in diameter (Wattman paper No.1). discs placed on serialized Petridis at room temperature for 24 h (17). All discs sensitivity checked on nutrient agar, which was surface spread with 0.5 Mac Farland scale of new cultured 24 h of enterococcus fecalis and incubated for 24 h at 37°C. All measurements were repeated three-time, and an average of them has selected.

- **Micro-wells Technique**

The hole was created on the surface of nutrient agar with a cup of the disposable needle. Each hole was filled with ten µl of the antibacterial agent (PAK at different concentrations, CHX, and DMSO negative control). The diameter of the inhibition zone was measured by digital caliper as it did for the disc diffusion method (18).

Determination MBC, MIC, the Optical Density of Resin

For determination of the MIC and the MBC, one ml of enterococcus fecalis 1x10^{-5} CFU mL^{-1} was added to serially diluted extraction of resin. All cultured tubes incubated at 37 °C for 24 h, (19) All samples were observed by spectrophotometer at absorption mode (590 nm). DMSO without bacteria used as a reference guide and DMSO with bacteria used as a positive control (20). Measurement of OD of all samples was repeated three times. The average of them has been selected. For confirmation, the result of all samples cultured on blood agar and incubated overnight at 37 c^{o}.

Teeth Preparation

Forty-five extracted human lower premolar teeth were used. All samples were instrumented by protaper next according to manufactory instruction. Then irrigation by sodium hypochlorite 5.25% and dryness of each root did by size F1 paper point. Each sample steam autoclaved to provide an environment free from bacteria (16). After complete disinfection root samples infected with 0.01 ml of the desired dilution of bacteria (90 X10^{-5}) and, they placed in a special incubator. After 24 h incubation, intra-canal medicament was placed inside each root and incubated an additional 24 h, and the colony formed on each sample were counted by colony counting device.

Group one: 15 samples: negative control does not undergo treatment

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Group two: 15 samples treated with pistacia Atlantica kurdica resin paste
Group three: 15 samples treated by CHX gel 2%

Data Analysis

Data collected and analyzed using the Graph pad prism 7.04. The following statistical test included: descriptive statistics: mean, number of samples, standard deviation, and graphical presentation of data. One way ANOVA analysis of variance. T-test for two independent tests. pos-hoc LSD test, for multiple comparisons.

Results

Table 1. Dilution of different Concentration of P. Atlantica Kurdica Resin Used in the Study

	DILUTION	MG/ML
P. ATLANTICA RESIN	1	500
	10^{-1}	50
	10^{-2}	5
	10^{-3}	0.5
	10^{-4}	$0.5x10^{-1}$
	10^{-5}	$0.5x10^{-2}$
	10^{-6}	$0.5x10^{-3}$
	10^{-7}	$0.5x10^{-4}$
	10^{-8}	$0.5x10^{-5}$

Anti-bacterial Evaluation of Resin against Enterococcus Fecalis

- **Micro wells and Disc Techniques**

Figures 1&2 showed the inhibition zone of Pistacia resin extraction, DMSO, Ethanol, CHX in two different method discs and microwells technique.

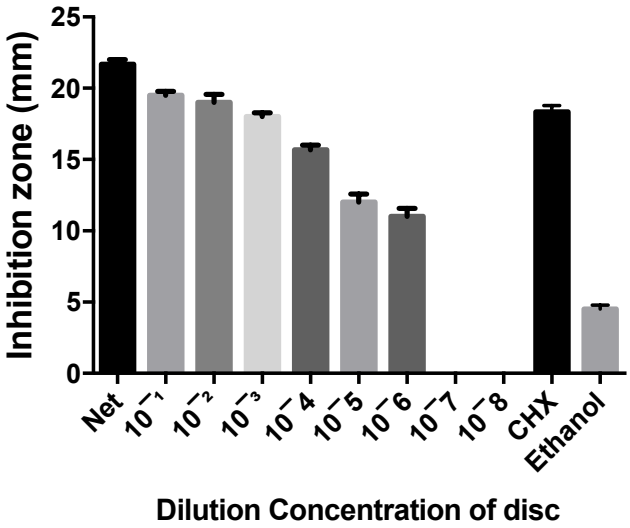

Figure 1. inhibition zone of P.atlantica resin extraction with different concentration against enterococcus faecalis, disc technique

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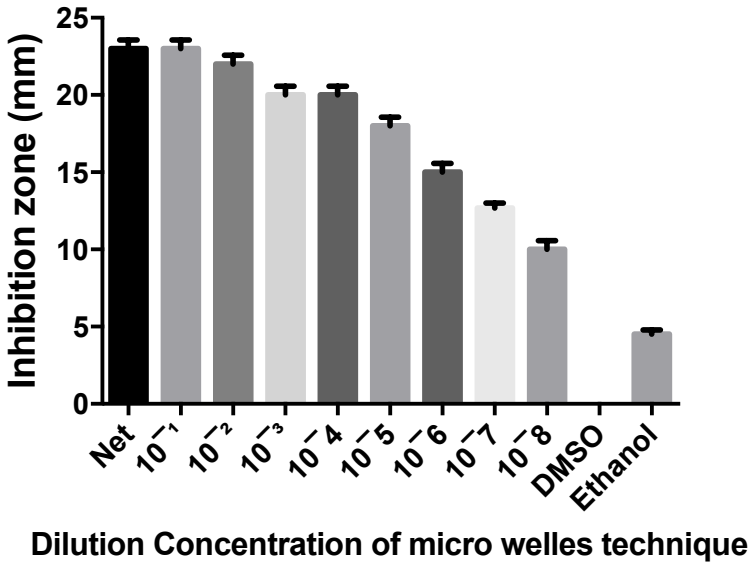

Figure 2. The Inhibition zone of P.atlantica resin with different concentrations against enterococcus faecalis. microwells techniques

Minimal Inhibition Zone, and Minimal Bactericidal Concentration of Pistacia Atlantica Resin Extraction

MIC and MBC of resins' extraction recoreded against enterococcus fecalis. On Figure 3 & table.1 MIC (at dilution concentration10^{-6}), was $0.5x10^{-3}$ mg/ml, while the MBC (at dilution concentration 10^{-5}) was $5x10^{-}$mg/ml.

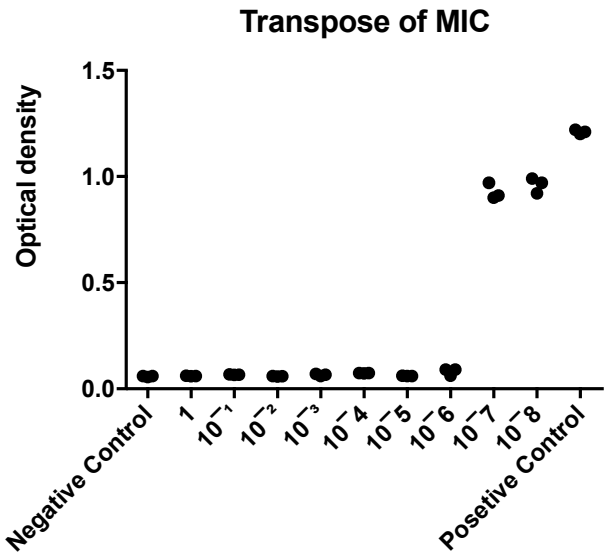

Figure 3. MIC&MBC of resin against enterococcus faecalis

The Optical Density of Transportation of Resin Extraction

Figure 4. shows the optical density of different concentration of extraction of P.atlantica kurdica resin, and DMSO. The optical density of MIC and MBC was 0.08 OD, and 0.061 OD respectively. While the optical density of the positive control was 1.22 OD.

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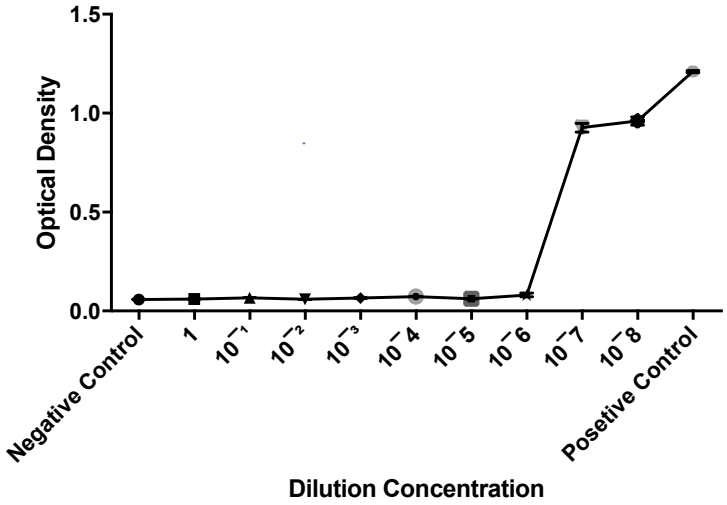

Figure 4: optical density of P.atlantica kurdica resin extraction and positive control.

Pistacia Resin Extraction as an Intracanal Medicament on Teeth Samples

Figure 5 and table 2 showed the mean value and SD of colony-forming units reduction(CFU) of resin intracanal medicaments, CHX, and the control group. The CFU reduction of the control group was (90×10-5 ±0.000). The CFU of both the resin and CHX were (6.667×10-5), (10.87×10-5) consequently.

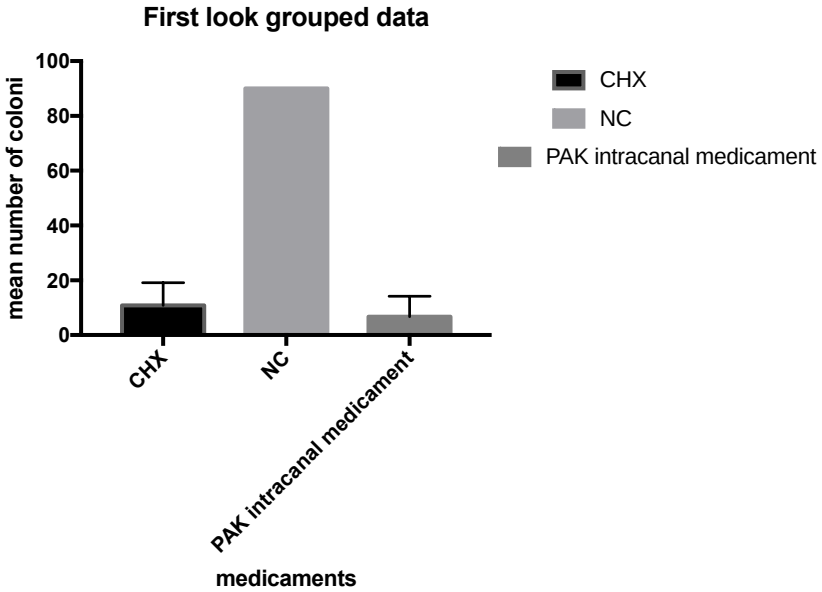

Figure 5. Anti-microbial properties of different intracanal medicaments.

Table 2. Copmarison antimicrobial properties of different intracanal medicaments

Data	Mean& Std	P.valu
P. resin	$6.67\times10^{-5} \pm 7.53\times10^{-5}$	0.1567
CHX	$10.87\times10^{-5} \pm 8.26\times10\text{-}^{5}$	

Discussion

Plants are rich in bioactive compounds that have the benefit to the treatment of various diseases. Therefore, the

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Kurdish people aware benefits of resin. So they used mastic resin for treatments of various diseases. For the first time in this study, pistacia Atlantica resin was studied as an intracanal medicament. The efficiency of it has been tested against enterococcus fecalis on extracted teeth samples. In the current study chlorhexidine as an intra-canal medicament was very effective in decreasing the viability of enterococcus fecalis. It significantly decreased the number of CFUs and the percentage viable of E. faecalis (1). However, this medicament has some undesirable properties. It was capable of causing surface alterations of dentin (21), discoloration of the tongue, teeth, and composite restorations (2). the distraction of viable human stem cells of the apical papilla (22). This study was designed to overcome the shortcomings of the CHX by using the extraction of pistacia resin as a new intra-canal medicament.

Antibacterial Properties of the Resin against Enterococcus Fecalis

In this study antibacterial properties of resin has been evaluated in different technique on enterococcus fecalis. E.faecalis the most resistant bacteria which has the main role in a failed root canal. Disk diffusion, microwell, the optical density of extraction, MIC, and MBC techniques, were used for determining the antibacterial effects of this resin against E.fecalis (23).

In the current study, the serial dilution method was used for the determination of MIC and MBC. the results were MIC 0.5 µg/mL and MBC 5µg/ml. Based on these results, indicated that resin has excellent anti-bacterial properties against E.fecalis. results of both MIC and MBC are close to each other. In the current study, the spectrophotometer was used for determining the optical density of serial dilution of resin. The concentration of MIC and MBC has the same optical density that indicates the resin has bacteriostatic properties. This result is accordant with the findings of other studies (24-26).

Disc diffusion is another method used in the evaluation of the antibacterial activity of resin. The diameter of the inhibition zone in both desc and microwells techniques of the resin extract was directly proportional to its concentration. At the disc method, there was no inhibition zone at a low concentration of 10^{-7}, and 10^{-8} but at the microwell technique, there was an inhibition zone at those concentrations. At disc techniques may be due to the dissolution of resin in ethanol and evaporation of ethanol for making disc some resin constitution evaporate but at microwell due to the dissolution of resin in DMSO and directly contact of resin constitution to the bacteria there was still an inhibition zone of bacteria was seen. The results were consistent with the results of (18).

Some other studies, implemented an antibacterial effect of the resin (Ghalem and Mohamed 2010) in their study, showed resin oil was potent inhibitory activity against E. coli followed by S. aureus and S. pyogenes they used the disc diffusion method for determining antibacterial properties of rein (27). Fathollahi et al. showed in their study that resin has a broad spectrum and essential oil of resin effect on gram-positive bacteria more than gram-negative bacteria (28).

(Ćavar et al, 2019) in there study showed anti-bacterial properties of resin essential oil against nine strains of oral bacteria including E. facalis. 24Z-isomasticadienolic acid, from E.O extraction of resin, has antibacterial activity against the E.faecalis with MIC and MBC values of 78 µg mL^{-1} and 312 µg mL^{-1} respectively (29).

In the current study, both regimens resin and CHX as an intra-canal medicaments compared to each other. CHX is strong antibacterial properties that can kill bacteria in two minutes. resin also showed excellent anti-bacterial properties. The proper antibacterial activities of the extracted resin could be related to the presents of multiple chemical and active constituents such as β-pinene, limonene, α-pinene terpinolene, and antioxidant activity of flavonoid (9, 30, 31).

(Gomes et al, 2013) at their study revealed that CHX is a broad spectrum. It was effective as an intracanal medicament against enterococcus fecalis (2).

Memariani et al. (17) in their study stated that alpha-pinene at high concentration disrupts bacterial cell membrane integrity; this function is the reason for its bactericidal activity.

The resistance of E.fecalis to multiple drugs which is the most causative factor of root canal failure (3) needs an effective intracanal medicament. Bacteria make oxidative stress that damages the cell membrane by unbalancing electrons. Resin neutralizes bacterial activity by donates an electron and prevents oxidative stress. According to the finding of the results of the current study, the resin has strong antibacterial properties due to its high antioxidant capacity against bacteria. The presence of the hydroxyl group is responsible for inhibiting free radicals, therefore, with its antioxidant properties, it disrupts the bacterial cell membrane. For this reason, it can be used in root canals, especially in cases of root canal faiure.

Conclusion

The resin of pistacia Atlantica has excellent antibacterial properties, especially against gram-positive bacteria. It can be used as an intracanal medicament in a failed root canal. It has selective anti-bacterial properties against oral bacteria. It has excellent antioxidant capacity.

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