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Evaluation Of the Antibacterial Effect of MTAD And Vanillin Against Root Canal Bacteria

Running Title: MTAD And Vanillin Against Root Canal Bacteria

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Abstract

Objective: Comparison of the effectiveness of Bio pure MTAD (mixture of Doxycycline, citric acid and a detergent) versus vanillin(V) as an endodontic aqueous solutions for tested canals infected with *E. faecalis* and *S. Mutans*. Materials and methods: Fourty human extracted lower bicusped teeth (single-rooted) unit at the cementoenamel junction (CEJ) were decoronated. Thereafter, the teeth were prepared with Protper NiTi rotary files till (F3 [30/.06). Then samples are often divided into "2" main groups (18/each), infected by bacteria and incubated in brain heart infusion (BHI) for forty-eight hours at "37°C". Then the teeth were sub-divided into three groups(N=6) according to the irrigation protocol (MTAD, V, and Distilled water"DW", which was the control group "Gp.").-ve control(N=4). Dentin chips were removed from the canals by sterile low-speed handpiece spherical bur, transfered to BHI, then cultured to count the growing colonies which were recorded as colony forming unit (CFU).Results: Siginficant differences were found in the bactericidal efficacy of (Bio pure MTAD, V and DW) irrigations. Conclusion:Bio pure MTAD had the maximum antibacterial efficacy on *E.faecalis* and *S. mutans* as compared to vanillin and DW. Significance and impact of the study: Understanding the mode of action of natural antimicrobials(V) on endodontic therapy may facilitate their application as natural endodontic solutions, particularly for their potential use in endodontic irrigation protocol employing multiple hurdles.Additionally compared these natural compound with artificial one like MTAD and so on.

Introduction:

Root canal irrigants performance a basic job for the whole disinfection of the root canal system, specifically unavailable areas for instrumentation. In addition to the presence of microorganisms in dentin tubules, are also present in the anatomical anomalies of the canals. Microscopic organisms present in dentin tubules caused

steady endodontic sickness after root canal treatment. Various regions of the dental canal left entirely unaffected by current procedures of instrumentation(1).

An expansive variety of substances are utilize as a root canal irrigation solution, including acids (citrus, phosphoric), chelating operators (Ethylenediaminetetraacetate) EDTA, compounds (proteolytic), soluble arrangements (sodium hydroxide, sodium hypochlorite, potassium hydroxide, and urea), aerobic specialists (Gly-Oxide and hydrogen peroxide), local anaesthetic solutions and normal saline. These irrigants facilitate the debridement of the canals. Biopure MTAD (Dentsply, Tulsa, OK) is blended of an acid (citrus extract), tetracycline isomer (doxycycline), and a cleanser (Tween80), it has antimicrobial efficacy, evacuates the smear layer, dissolves pulpal tissue, biocompatible, has some dentin conditioning properties, a useful effect on the root canal seal, and does not give a negative effect of the physical properties of the tooth(2).

Vanillin

(4-hydroxy-3-methoxybenzaldehyde) is that the real constituent of vanilla beans and is produce commonly by multistep treatment techniques, the method of activity of almost all antimicrobials can react with the cell membrane, inactivation of basic enzymes, pulverization or inactivation of hereditary material (3-11).

One among the most resistant pathogens adverse antimicrobial irrigants and intra canal medicament is (*E.faecalis*), which can bear nutritional impoverishment situations and has been largely associated with persistent periapical infections in necrotic endodontically treated teeth (12). Additionally,(*S. mutans*), a "Gram- +ve" microorganism in the oral cavity, it's survived at low pH environment (aciduricity), produced organic acids by different carbohydrate metabolism processes (acidogenicity) that lead to the development of dental decay(13-15).

The motivation behind examination investigated the comparison between the antimicrobial adequacy of MTAD versus vanillin as irrigantion for dental root canals tainted with "*S. Mutans* and *E.Faecalis*".

Materials and Methods

Ethical Statement

The protocol of this study was scientifically approved by Higher Scientific Researches Committee at Faculty of Dentistry, Mosul University, Iraq at clearance number (REC reference no. UoM.Dent/H.L.27/20 in 1-December 2019.

Persistent selection

From the clinical examination the data were collected by one of the authors, the periodontal pocket depth (PD) had been measured with a periodontal probe to the nearest highest point in all six representative surfaces of each tooth in the patient mouth, and the clinical attachment loss had also been registered. To determine the periodontal health status, patients with 1 or more deep pockets (≥5 mm) and local bleeding (recorded with "Bleeding on Probing" index) were considered periodontitis patients ("Perio"), while the others were periodontitis free ("No perio") (16).

Then utilizing radiography taken, select to patients who have apical periodontitis.

Determination of samples

This analysis is an experimental laboratory one. It had been carried on at the Dental Conservative Specialist Clinic and Basic science unit within the Faculty of Dentistry, University of Mosul. This analysis has worn out in January-July 2020.

An all-out sample of 36 removed freshly single, human -established lower premolar teeth older 16-multi-year from periodontal patients were drenched refined H_2O. Excluded teeth with root resorption, extensive carious lesion, incomplete root formation.Piezon Master 400scaler (EMS, Swiss) used to expel the delicate tissue remainders and analytics on the outer root surface precisely. Under stereomicroscope (x20 amplification), all samples were reviewed to distinguish any imperfectness or root cracks and to affirm the entire development of root apices.

A low-speed, water-cooled, diamond sectioning disc (Brasseler, Germany) was used to decoronated every tooth of tested samples at the (CEJ). Pulp tissue was evacuated with a a barbed broach, at that

point root canal patency was affirmed with No.15 K-type document (Mani Co, Tokyo, Japan).

Instrumentation

No-15 K-type file was utilized to decide the working length of each root, which inserted inside the root canal under stereomicroscope. All teeth were prepared with Protper NiTi rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland), utilizing the crown-down method. The apical parts of the root canals were finished at F3 [30/.06].These instruments were used with contra-angle rotary hand piece at a rotational speed of "300 rpm" and torque" 3 "N cm. Protaper files were used according to manufacturer's recommendations.(4,5,17)

Root canal irrigation was performed at the beginning of the instrumentation and after each instrument size with (2 ml) of "2.5%"sodium hypochlorite (NaOCl) for about (1 minute) (Safe Plus, Neelkanth, India), after that (2 ml) of "17%" ethylenediaminetetraacetic acid (EDTA) (Dent Wash , Prime Dental products private limited, India) was utilized for (1 minute) to remove the dentinal smear layer, finally (3ml) of distilled water was used in order to remove the remaining of irrigating solutions (4,5).

All teeth should be autoclaved to be sterile and killed the remaining micro organisms form in dentinal tubules of the teeth root canals.

The apical foramen of the teeth was coated with light cure Glass-Ionomer (GI) (GC, corporation, TOKYO. JAPAN),while the roots surfaces were coated with nail-varnish.

In the negative control specimens (N=4), the roots orifice was also sealed with GI to avoid external microbial contamination during the study.

Seclusion of microorganisms

Isolation of (*Enterococcus faecalis*) and (*Streptococcus mutans*)

Isolated process of microorganisms (*E. feacalis*), by utilizing a cotton swab, a loopful of inoculated "BHI broth agar" was spread on the surface of *Enterococcus* specific media. Incubated the inoculation media anaerobically by utilizing an anaerobic flask at "37 ˚C for 48 hrs". *E. feacalis* states appear as "reddish-pink color" on the outside of the media.

After that we began to do isolation process of *S.mutans*, a cotton swab of a loopful of inoculated "BHI" was spread on the surface of *Mutans* selective media. At that point by utilizing anaerobic jar at "37℃ for 48 hrs". Aerobically incubated the inoculated medis. *Mutans* colonies showed up as "bluish" shading.

The specimens were randomly divided into "2" main groups(Gps.), (18/ each) according to the type of contaminated bacteria.

Pollution of Samples with the *E. feacalis* and *S. mutans*.

From isolated of both kinds of bacteria, take a colony from the selective media transferred to "BHI" by sterile syringe interject in the canals, after that Incubated an aerobically utilizing anaerobic container at "37 ° for 48 hrs".(19)

After completion of the incubation period, every specimen was subdivided into three subgroups according to the disinfection protocol used,(N= 6).

In this progression, the "irrigation solutions" can be utilized (MTAD, vanillin, and DW "control Gp").

- Toward the starting, the samples flooded with the (normal saline), at that point in the MTAD team inundated with the MTAD, leafed for around 5 min. as per producer guidelines, at that point canals ought to be dried with the "paper point".
- In vanillin(V.) Gp. utilized (5 ml) of this "solution as the last irrigate" and dried the root canals.
- Additionally, similar advances have done in the DW Gp.as "a control Gp.".

A sterile handpiece "round bur" entered into the tested samples to cut the internal wall of the canal and afterward collected the dentin chips, transferred to BHI, "broth agar", after thirty min. Draw (0.1 ml) from this "bro.agar", and addition to "0.9 ml" ("new" not sullied stock "agar"). Draw 0.1 ml from this new broth, at that point add to 0.9 ml to deliver 1 ml from this agar. On "selective media" inoculated the microorganism, incubated at (37 °C) for forty-eight hrs., lastly checked the

colonies forming unit "CFU", which is a unit generally utilized to gauge the concentration of pathogens in "a test sample". (CFU) that exist on an agar plate can be reproduced by the dilution factor providing a "CFU/ml" result.The statistical data were performed according to the simple experiment system, complete random design, and the variance test (F-test) "ANOVA",samples means were compared using Duncan's Multiple Range Test

Results:

To clarify experimentation, performed statistical analysis of the data from the test groups was performed initially using non-parametric statistical methods (Kruskal-Wallis ANOVA) on ranks in Tables 2 and 5, and Duncan test in Tables 3 and 6 for both microbes. For further confirmation, Tables 1 and 4 were clearly explained the descriptive statistic of three sample groups.

Discussion:

In current study, the bacterial growth in tested samples from infected root canals irrigated with antibacterial irrigation solutions was compared with normal bacterial growth (+ve control Gp.), The usage of distilled water as an irrigant within the positive control Gp. gave the concept that the microbes stayed viable inside the canals and also this irrigant was unfit to expel this bacterium from the trial tests.[6] On the other hand the bacterial growth from when the sterilized roots were incubated (-ve control Gp.). During the incubation period, it was clearly determined in which period the reproduction occurred, slowed down and regressed. [18]

Within the current study, we analyzed the influence of both (different types of bacteria and different kindes of irrigation protocol), when two types of bacteria was applied on the tested samples using different kinds of irrigants, the outcomes revieled that BioPure MTAD gives off an impression of being preferable in antimicrobial action in compared to V. and DW(control Gp.)

MTAD is biocompatible, antimicrobial movement, capability to evacuate smear layer, BioPure MTAD additionally contains doxycycline hyclate in powder structure and is broad spectrum antibiotic(7)

V. is a phenol exacerbate whose antimicrobial action has been utilized in the eradication of pathogens, V. is mainly a membrane-active compound, resulting in the dissipation of ion gradients and the inhibition of respiration, the extent to which is species-specific. These effects initially do not halt the production of ATP. Bezerra et al. (8) (2017) founded that V. was selectively modulated the activity of antibiotics against multi-resistant bacteria and might be useful in the development of new therapies against resistant microorganisms.

Descriptive statistics incorporate mean of MTAD and vanillin has appeared in Table (1,4) these tables exhibited that there was a significant difference between MTAD and other test teams. MTAD gived the best outcomes among the other, these outcomes corresponds to other studies (Ghada et al., (1) 2014; Sandeep D.(9) 2016, havani Srikrishna et al [20]) who performed a a comparative study among MTAD and other test Gps., founded that MTAD Gp. was significantly varied at ($P \leq 0.05$) from other trial Gp. Contrarly, there were different examinations conflicted with our analyses (10), they founded that another irrigation solution like (NaOCl) had more efficacy than the MTAD irrigations.

The outcomes of our experiment were in accordance with Pillai et al. (11). who stated that there was a statistically significant difference in the zone of bacterial inhibition against "*Enterococcus faecalis*" within entire tested materials (Tetracycline, Acid, and Detergent (MTAD) and Chitosan) when compared to other materials. Based on the mean diameters, MTAD had the maximum zone of inhibition (11).

Our study outcomes were not in accordance with Sandeep et al. (13) who was clarified that metronidazole was established to be the most potent root canal irrigant against B. fragilis and P. acnes among BioPure MTAD, aztreonam. (13)Manikandan et al.(14)

reported that, although chlorhexidine (CHX) performed better than MTAD™, it provided to be bacteriostatic in action at lower concentrations. NaOCl and iodine potassium iodide (IKI) was founded to be inferior and were excluded. Surfactants such as cetrimide(CTR) were founded to be better than MTAD™; while sodium dodecyl sulfate (SDS) showed insignificant results(14).

In the future, this study needs more other enchancement studies that study the other effects of vanillin solution and study its effect on human health. Some of the tested combinations may be increase the antimicrobial effect and would allow the effective doses to be reduced when it used as endodontic irrigation solution.In addition the usage of ultra-sonic device may motivate the efficiency of natural irrigation solutions.

Conclusion:

The consequences of this examination expressed that MTAD has a superior antibacterial efficacy than V. against *S. mutans* and *E.faecalis*.

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- **Conflct of interest:**

There are no conflict of interests

- **Financial support and sponsorship**

Nil

- **Authors contributions**

The researcher contributed the coordination and work of all parts of the manuscript.

- **Ethical policy and institutional review board statement**

Higher Scientific Research Committee at Faculty of Dentistry, Mosul University, Iraq approved this study.

- **Data availability statement**

The data of the study results are available from the author

Table (1): Illustrated the descriptive statistics of different irrigantion solutions that display mean, variance (SD), (SE), minimum and Maximum values for (Streptococcus mutans)

Material	"N"	"Mean"	(SD)	(SE)	"95% confidence interval for mean"		"Minimum"	"Maximum"
					(Lower Bound)	(Upper Bound)		
MTAD	6	2.83	1.33	0.54	1.44	4.23	1.00	4.00
Vanillin	6	4.83	1.17	0.48	3.61	6.06	3.00	6.00
Distilled water	6	27.17	0.98	0.40	26.13	28.19	26.00	28.00
Total	18	11.61	11.40	2.69	5.94	17.28	1.00	28.00

N=Number of samples
SD=Standard deviation
SE=Standard error

Table (2): (One way ANOVA) illustrated the comparison among the materials displayed a statistically significant difference between Gps. (*P*≤0.000)

	Sum of Seqares	Df	Mean Square	F	Sig.
Between groups	2189.78	2	1094.89	801.13	0.000
Within groups	20.50	15	1.39		
Total	2210.28	17			

Df=Degrees of Freedom
F=F calculated
Sig.=Significance probability

Table (3): Demonstrated the bactericide activity of *Strep. mutans* were analyzed by Duncan's Multiple Range Test, as post hoc comparison that exposed (a significant difference) among tested irrigantion solutions.

Material	**mean**
MTAD	2.83a
Vanillin	4.83b
DW	27.17c

Different letters mean significant results.
* There is a significant difference at the level of probability.
** mean of antibacterial activity.

Table (4): Show that the descriptive statistics of irrigantion solutions(is) that display (mean, SD, SE, minimum and high value for {*E. faecalis*}).

Material	N	Mean	SD	SE	"95% confidence interval for mean"		"Minimum"	"Maximum"
					(Lower Bound)	(Upper Bound)		
MTAD	6	5.17	1.94	0.79	3.13	7.20	2.00	7.00
vanillin	6	7.83	1.17	0.48	6.61	9.06	6.00	9.00
Distilled water	6	63.50	2.35	0.96	61.03	65.96	60.00	66.00
Total	18	25.50	27.73	6.54	11.71	39.29	2.00	66.00

N=Number of samples
SD=Standard deviation
SE=Standard error

Table (5) :(One way ANOVA) displayed the comparison among the materials diclosed a statistically significant difference among groups (*P*≤0.000)

	Sum of Square	Df	Mean Square	F	Sig.
Between groups	13017.33	2	6508.67	1836.30	0.000
Within groups	53.17	15	3.54		
Total	13070.50	17			

Df=Degrees of Freedom
F=F calculated
Sig.=Significance probability

Table (6): Showed the bactericide activity against *Strept. mutans* were analyzed by Duncan`s test, as post hoc comparison that exposed a significant difference among there.

Material	mean
MTAD	5.17a
Vanillin	7.83b
DW	63.50c

Different letters mean significant results. * There is a significant difference at the level of probability. ** mean of antibacterial activity.

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