Antibacterial effects of mineral trioxide aggregate and Biodentine ™ after the addition of different concentrations of black seed aqueous solutions

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ABSTRACT

Background: Mineral Trioxide Aggregate (MTA) and Biodentine™ cements are new materials with numerous exciting clinical applications. Both have appreciable properties which include good physical properties and the ability to stimulate tissue regeneration as well as good antibacterial effects. The aim of this study was to investigate and compare the antibacterial effects of MTA and Biodentine™, when they were mixed with different concentrations of aqueous solutions of Black Seed extract, against Enterococcus faecalis.

Materials and methods: MTA and Biodentine™were prepared according to the manufacturer's instructions. The method of Mawlood was followed to prepare the Black Seed aqueous solution. Agar diffusion method on Brain Heart Infusion agar was employed.Twenty, 9 cm diameter, petri-plates with 25 ml of Muller Hinton agar media were prepared. A sterile spreader was used to inoculate the microorganisms. With a micropipette 0.1 ml of the Enterococcus faecalis suspension was added to the surface of the plates. Within 15 minutes, after inoculation of the plates, 4 cavities, each one measuring 5 mm in diameter and 4 mm in depth, were made in each agar plate. A total of 20 agar plates were divided into 2 groups consisted of 10 plates each; Group A: each plate contained 4 wells filled with MTA alone and MTA mixed with 10%, 30% and 50% of Black Seed aqueous solutions respectively.Group B: each plate contained 4 wells filled with Biodentine™alone and Biodentine™mixed with 10%, 30% and 50% of Black Seed aqueous solutionsrespectively. Next day after incubation, the agar plates were examined for bacterial inhibition zones. With a scientific ruler the diameter of the antibacterial inhibition zones were measured. The data were recorded and statistically analyzed, by the ANOVA and the Student's t-test.

Results: Both cements had antibacterial effects, which were increased with the addition of the aqueous solutions of Black Seed extract. The increase in the diameter of Enterococcus faecalis inhibition zones was directly proportional with the increase in the concentration of the added Black Seed aqueous extract.

Conclusion: Adding aqueous solutions of Black Seed extract to both MTA and Biodentine™ increased their potential to inhibit the growth of Enterococcus faecalis.

Key words: Mineral Trioxide Aggregate, Biodentine ™, Black Seed. (J Bagh Coll Dentistry 2015; 27(1):48-53).

INTRODUCTION

It is well known that the microorganisms have the most important role in endodontic treatment failures.(1,2) The prognosis of any treatment will depend on the successful elimination of the microorganisms and infected tissues as well as effective sealing of the root-end to prevent future recontamination.(3,4) Studies have shown that certain microorganisms are recovered fromthe infected teeth. These are chiefly Enterococcus, Actinomyces, Propionibacterium, yeasts, Candida albicans, Streptococcus and other types.(5) However, advances in techniques and materials have increased the success rate of the conventional root canal treatment or retreatmentcases.(3)

Mineral trioxide aggregate (MTA) is a mechanical mixture of 3 powder ingredients; it contains fine hydrophilic particles of 75% Portland cement clinker, 20% Bismuth oxide and 5% gypsum by weight,italso contains trace amounts of SiO_2, CaO, MgO, K_2SO_4, and Na_2SO_4.MTA is a powder that, in the presence of moisture, forms a colloidal gel that solidifies, after approximately 4 hours, to form a hard cement. (6)

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It was used to seal off the pathways of communication between the root canal system and the external surface of the tooth.(7-10). Moreover, it was concluded that the mechanism of action of MTA, may encourage hard tissue deposition (11).

Biodentine™ was reported as a safe and useful material, sinceit possesses specific properties, including the promotion of significant intratubular calcium diffusion,(12,13) biocompatibility,(14,15) and expansion to reduce the dentin/-material interface to a minimum, allowingstable micromechanical intratubular attachment.(12,14) Biodentine ™ is presented in a capsule contain-ing the predetermined ratio of powder and liquid. The powder consists of Tricalcium silicate ($3CaO.SiO_2$), Calcium carbonate ($CaCO_3$), and Zirconium dioxide (ZrO_2); the liquid consists of Calcium chloride ($CaCl_2.2H_2O$), water reducing agent, and water. (14,15)

Nigella Sativa (NS) commonly known as the Black Seed (BS); this mild annual aromatic herb is indigenous to the Middle East and Southeast Asia where it has been used as a traditional remedy for over 2000 years. It was used so extensively that it became known as the seed of

blessing. [16] It was reported that, a marked decrease in the number of intracanal microbes occurred when different concentrations of NS extract were used.[17] Most of the NS pharmacological actions are due to its antioxidant activity which is mainly due to its ability to scavenge free radicals and/or inhibit lipid peroxidation. The seeds of NS contain a yellowish volatile oil (0.5-1.6%), a fixed oil (35.6-41.6%), proteins (22.7%), amino acids, minerals and vitamins.[17]

The purpose of this,in vitro,study was to investigate and compare the antibacterial effects of MTA and Biodentine™,when they were mixed with different concentrations of aqueous solutions of Black Seed extract, against Enterococcus faecalis (E. faecalis).

MATERIALS AND METHODS

The tested materials, MTA (Dentsply, Tulsa dental, OK, USA) and Biodentine ™ (ZiZine, France), were prepared strictly according to the manufacturer's instructions. The antimicrobial activity of the endodontic cements, mixed with three different concentrations of the aqueous solutions of BSwas evaluated bythe agar diffusion method.

E. faecalis microorganisms were identified in the Central Health Laboratories, Ministryof Health in Baghdad by a combination of colonial pigmentation, colonialmorphology, haemolysis on BHIBA, cell morphology (microscopicmorphology) and biochemical tests. Grouping Beta haemolytic streptococci test was done on these colonies by using Pastorex strep test (Bio-Rad/Japan).

The method of Mawlood (1996) was followed to prepare the BSaqueous solution. NS seeds (100g) were grinded and dissolved in distilled water bath for boiling, and then filtrated. The filtrated suspension was placed in an incubator at 37ºC, for drying. To obtain the first concentration (100 mg/ml), one gram of the collected dried powder was re-dissolved in 5 ml of distilled water and the volume was completed to 10 ml, from this concentration the concentrations of 50%, 30%, and 10% were prepared by the dilution technique.

Agar diffusion method on Brain Heart Infusion agar was employed. Twenty, 9 cm diameter, petri-plates with 25 ml of Muller Hinton agar media were prepared.A sterile spreader was used to inoculate the micro-organismsfrom the prepared normal saline tubes inoculated with microorganismswhich had been fit to 0.5 McFarland standards. With an adjustablemicropipette, 0.1 ml of the bacterial suspension was added to the surface ofthe plates which were inoculated by spreading the suspension in threedirections, and a final spreading was done over the outer rim of the plate.The plates were allowed to dry for 3-5 minutes. Within 15minutes, after inoculation of the plates, 4 cavities each one measuring 5 mm in diameter and 4 mm in depth were made in each agar plate using corkpoorer.The arrangements of the wells werenot close tothe outer edges of the plates and far enough apart to prevent overlapping of the zones of the microbial inhibitions.

A total of 20 agar plates were used in this study; the samples were divided into 2 groups consisted of 10 plates each;

Group A: each plate contained 4 wells filled with MTAalone and MTA mixed with different concentrations of BS aqueous solutions (10%, 30% and 50% respectively).

Group B: each plate contained 4 wells filled with Biodentine ™ alone and Biodentine ™ mixed with different concentrations of BS aqueous solutions (10%, 30% and 50% respectively).

The plates were pre-incubated in the culture media at the environmental temperature,for two hours before incubation, to allow dissociation and diffusion of thetested materials. Thenthe plates were incubated at 37ºC for 24 hours.Next day, the agar plates were examined for bacterial inhibition zones.Witha scientific ruler the diameter of theantibacterial inhibition zones were measured by passing the scientific ruler through the center of the wells. Data were statistically analyzed, by the ANOVA and the Student's t-test, to compare the differences of the anti-E.faecalis effects of MTA and Biodentine ™ cements when mixed with different concentrations of BS aqueous solutions.

RESULTS

Regarding the antimicrobial activity of MTA, which was mixed with different concentrations of aqueous solutions of BS extract, the summary of the recorded results are presented as means, standard deviations (SD), minimum and maximum values of E. faecalis inhibition zones (in mm); these results are shown in Table 1, Figure 1.

Table 1.Descriptive statistics of MTA, in mm.

Testes Groups	Min.	Max.	Mean	SD
MTA	2.62	2.77	2.72	± 0.105
MTA+10% BS	2.72	2.87	2.82	± 0.104
MTA+30% BS	2.92	3.07	2.98	± 0.100
MTA+50% BS	3.32	3.57	3.48	± 0.103

Figure 1: A sample MTA groups.

As seen in Table 1, MTA has antibacterial effect which was increased with the addition of the aqueous solutions of BS extract. The increase in the MTA antibacterial effect, against E. faecalis, was directly proportional with the increase in the concentration of the added BS extract.

The ANOVA test results of the MTA groups showed a highly significant difference (P <0.000) (Table 2). To compare the paired groups, Student's t-test was performed, (Table 3).

Table 2. The ANOVA test of MTA groups

S.O.V	SS	DF	MS	F	Sig.
Between Groups	6.00	7	0.857	77.143	0.000 HS
Within Groups	0.80	72	0.11		
Total	6.80	79			

Table 3. Student's t-test of the MTA groups

Compared Groups	M.	M. Diff.	P	Sig.
MTA vs. MTA+10% BS	2.72 2.82	- 0.10	0.000	HS
MTA vs. MTA+30% BS	2.72 2.98	- 0.16	0.000	HS
MTA vs. MTA+50% BS	2.72 3.48	- 0.76	0.000	HS
MTA+10% BS vs. MTA+30% BS	2.82 2.98	- 0.16	0.000	HS
MTA+10% BS vs. MTA+50% BS	2.82 3.48	- 0.66	0.000	HS
MTA+30% BS vs. MTA+50% BS	2.98 3.48	- 0.50	0.000	HS

The statistical analysis results, concerning the E. faecalis inhibition zones, revealed highly significant differences between all the compared groups (P<0.000). It is clear that MTA had produced clear antibacterial inhibition zones. The collected data demonstrated highly statistically significant increases in the inhibition zones with the increase in the concentration of the added aqueous solutions of BS extract (P<0.000).

On the other hand, the effect of Biodentine™, which was mixed with different concentrations of aqueous solutions of BS extract, the summary of the descriptive statistics (means, standard deviations (SD), mini-mum and maximum values of E. faecalis inhibition zones, in mm, are presented in Table 4, Figure 2.

Table 4: Descriptive statistics of Biodentine™ (in mm)

Group	Min.	Max.	M.	SD
Bio	3.90	4.10	4.02	± 0.109
Bio+10% BS	4.10	4.30	4.10	± 0.103
Bio+30% BS	4.20	4.40	4.32	± 0.107
Bio+50% BS	4.40	4.60	4.52	± 0.109

Figure 2: A sample of Biodentine™ groups.

The antimicrobial action of Biodentine™ on E. faecalis microorganisms was superior to that of MTA. Biodentine™ showed a remarkable antibacterial effect, which was increased with the addition of the aqueous solutions of BS extract. Also, the increase in the Biodentine™ effect, against E. faecalis, was directly proportional with the increase in the concentration of the added BS aqueous extract.

The analysis of variance (ANOVA) test results of the Biodentine™ group scored a highly significant difference (P<0.000) (Table 4), therefore; to compare the paired groups, the Student's t-test was done, concerning the inhibition zones of E. faecalis as shown in Table 5.

Table 4: The ANOVA test of Biodentine™ groups

S.O.V	SS	DF	MS	F	Sig.
Between Groups	0.676	3	0.225	18.778	0.000 HS
Within Groups	0.192	16	0.120		
Total	0.868	19			

Table 5: Student's t-test of Biodentine groups

Compared Groups	M.	M. Diff.	P	Sig.
Bio vs. Bio+10% BS	4.02 4.10	- 0.08	0.000	HS
Bio vs. Bio+30% BS	4.00 4.32	- 0.30	0.000	HS
Bio vs. Bio+50% BS	4.02 4.52	- 1.50	0.000	HS
Bio+10% BS vs. Bio+30% BS	4.10 4.32	- 0.22	0.000	HS
Bio+10% BS vs. Bio+50% BS	4.10 4.52	- 0.42	0.000	HS
Bio+30% BS vs. Bio+50% BS	4.32 4.52	- 0.20	0.000	HS

Concerning Biodentine ™ the statistical analysis, regarding the E. faecalis inhibition zones, had showed highly significant differences between all the compared groups (P<0.000). It is clear that Biodentine™ has produced bacterial inhibition zones that were increased in diameter with the increase in the concentrations of the added BS aqueous extract.

One final interesting statistical analysis was performed to compare both groups of MTA and Biodentine™; the recorded results are seen in Table 6.

Table 6: Student's t-test results of both MTA and Biodentine™.

Compared Groups	t-test	P	Sig.
MTA vs. Bio.	55.52	0.000	HS
MTA+10% BS vs. Bio+10% BS	57.56	0.000	HS
MTA+30% BS vs. Bio+30% BS	60.82	0.000	HS
MTA+50% BS vs. Bio+50% BS	71.03	0.000	HS

In this study the antimicrobial activity of Biodentine™ when used alone was highly statistically better than that of MTA alone (P<0.000). The same thing was detected when different concentrations of aqueous solutions of BS extract were added; all the tested Biodentine™ groups were highly statistically superior in their antimicrobial activity than their respective groups of MTA.

DISCUSSION

Certain herbs and plants found to have different antibacterial effects; Nigella Sativa (the Black Seed) is one of them. This aromatic, well known herb has been used in Middle East, as a traditional remedy, for over 2000 years.[18,19]

The results of this investigation showed that, all the tested samples possess antibacterial properties against E. faecalis. The antibacterial effect of MTA, when used alone, was clear in the study. This comes in agreement with some studies which reported that MTA is an effective material against microorganisms including E. faecalis [20-23]. While other studies showed limited antimicrobial activity of MTA.[24-26] The conflicting results regarding the antibacterial activity of MTA against E. faecalis may be attributed to the available nutrients, level of oxygen tension, incubation period, methods of evaluation, and different laboratory set-ups employed.

The antimicrobial effect of MTA, against E. faecalis, was increased with the addition of different concentrations of the aqueous solutions of the BS extract. It worth's to mention that the increase in the MTA antibacterial effect was directly proportional with the increase in the concentration of the added BS extract. This could be attributed to the presence of thymohydroquinone in the chemical composition of the BS [27]; moreover, it could be due to the presence of other materials including nigellone, thymoquinone, thymol, carvacrol, α & β-pinene, d-limonene, d-citronellol, p-cymene and 2-(2-methoxypropyl)-5-methyl-1,4-benzen-ediol in the chemical composition of the BS extract which could be the responsible factors of its antimicrobial effects.[28]

In this study, Biodentine™ showed an antibacterial activity that is more than MTA, since the antibacterial inhibition zones were larger in diameter than that around MTA specimens. This material exhibits an efficient and durable protection of the pulp from bacterial invasion as a dentine substitute. Biodentine™ is similar to MTA in its basic composition; the powder mainly contains tricalcium silicate, calcium carbonate, and dicalcium silicate. The liquid consists of calcium chloride in aqueous solution with an admixture of polycarboxylate [29], therefore; they have comparable antibacterial effects.

In this investigation, it was found that, all the tested samples of Biodentine™ possessed antibacterial properties. When Biodentine™ was used alone, there was an obvious antibacterial effect against E. faecalis. This comes in agree-

ment with another study which reported that Biodentine™ has an antibacterial activity that is comparable to that of Ca-based cement. [29, 30]

The antibacterial effect of Biodentine™, in the present study showed that the inhibition zones of E. faecalis, on the experimental agar plates, were increased with the addition of different concentrations of the aqueous solutions of the BS extract. Furthermore, the increase in the diameter of E. faecalis inhibition zones was directly proportional with the increase in the concentration of the added BS extract. This finding, as mentioned before, may be due to the chemical composition of the BS extract [28]; which statistically increased the anti-E. faecalis properties of Biodentine™ even more than that of MTA.

Within the framework of this research, it could be concluded that MTA, as well as, Biodentine™ are promising materials since they have the potential to inhibit the growth of E. faecalis. Moreover, the adding aqueous solutions of BS extract increased their antibacterial activity against E. faecalis.

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