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Effect of Nigella Sativa (Habbatul Baraka) Water Extract on Microhardness of Initial Carious Lesion of Permanent Teeth Enamel Compared to Sodium Fluoride (An in Vitro Study)

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Abstract: ***Background:*** ***Nigella Sativa or Habbatul Baraka is an important medical plant that has been studied by researchers especially in last decades due to its beneficial medical effects, it has the ability to improve general health, additionally, it has a role in improving oral health through its inorganic and organic constituents and through its antibacterial (ant cariogenic effect). This study was conducted to investigate the effect of Nigella Sativa aqueous extract on microhardness of initial enamel caries (remineralizing effect) in companion with that of sodium fluoride.*** ***Materials and methods:*** ***Fifty maxillary human permanent premolar teeth were used in this study, twenty five teeth were used for pilot study in which the concentration of the dissolved calcium ions was measured and in order to select the concentrations of water extract of Nigella Sativa that used for microhardness test, in pilot study, the teeth were treated with agents (aqueous Nigella Sativa extract and 0.5 sodium fluoride) for two minutes for twenty days the deionized water in this study used as a control negative, then after etching with 2NHCl solution, calcium ions were measured and the concentrations of aqueous Nigella Sativa extract were selected for subsequent microhardness test, the rest number of teeth (25 teeth) were subjected to the pH cycling procedure. The teeth were immerged in twenty ml of the selected agents individually for about four minutes and then rinsed with deionized water. The agents used were 3%, 5%, 7% aqueous Nigella Sativa extract and0.05 sodium fluoride. The samples were subjected to microhardness test using vicker'smicrohardness device before, after the pH cycling and after placing in the selected agents.*** ***Results:*** ***It was found that the Nigella Sativa aqueous extract and sodium fluoride had the ability to increase the microhardness value of demineralized enamel. Statistically, highly significant difference (P > 0.01) was found between 7% Nigella Sativa water extract and 0.05% sodium fluoride, also highly significant difference was found between 7% Nigella extract, 0.05 sodium fluoride and deionized water, while statistical significant difference (P < 0.05) between 3% and 5% of the aqueous extract was recorded.*** ***Conclusion:*** ***The water extract of Nigella Sativa at a concentration of 7% was very effective in initial enamel caries remineralization.***

Keywords: Nigella Sativa, Habbatul Baraka, Microhardness, Calcium ion

1. Introduction

There is an increasing interest regarding the use of plants and herbs in medical fields for various diseases treatment around the world as a result of their less side effects with good and promising results [(1)]. Nigella Sativa also known as Habbatul Baraka is one of the herbal medicines, that has been considered as the miracle herb in the century [(2)]. It (Nigella Sativa) has religious importance and effect among Muslim populations, since the prophet Mohammed said "use the black seed regularly, because it is a cure for every disease, except death" and this might explain its widely used by millions of Muslimpeople [(3)].

Nigella Sativa has many pharmacological effects which include; anti-inflammatory, antiviral, antifungal, anti-parasitic, anti-bacterial and anti-oxidant effect [(4)]. In oral cavity, the use and application of Habbatul Baraka is still under study among different countries around the world. The seeds of Nigella Sativa are composed of Al-Kaloids, fixed oils, proteins, saponin and essential oils, Additionally, several national components are found in Habbatul Baraka seeds, such as essential amino acids, carbohydrates, fatsand vitamins, also, they are considered to be as a good source of potassium, magnesium, zinc and calcium [(5)]. Dental caries is "a dynamic process that occurs when demineralization exceeds remineralization"[(6)]. One of the minerals which plays an important role in the battle that occurs between demineralization and remineralization is calcium, therefore, it can modify the susceptibility of tooth for development and progression of dental caries[(7)]. Through demineralization process, the release of calcium from enamel precedes the release of phosphate, therefore, the use of calcium rather than phosphate would be effective in suppression of demineralization process and hens, it would enhance initial demineralization of initial enamel caries [(8)]. There are few natural products that have been tested in vivo and in vitro and used as remineralizing agents for prevention of dental caries [(9)]. It was found that the hardness of enamel of tooth increases with increased calcium content in enamel, so, for all the above mentioned reasons, and since there was no available previous study concerning the remineralizing effect of Nigella Sativa through testing the microhardness of teeth with initial enamel caries (artificial caries) before and after treating them with water extract of Nigella Saliva in comparison with a remineralizing agent (Sodium Fluoride) and deionized water, thus, this study was designed and conducted.

2. Materials and Methods

The sample consisted of fifty human permanent maxillary first premolar teeth, newly extracted from 11 – 13 years old patients attending Orthodontic Department / College of Dentistry / Baghdad University.

After extraction of teeth, they were cleaned using conventional hand piece with rubber cup and pumice which was not – fluoridated and deionized water, after that, the teeth were stored in 0.1% Solution of thymol at 4C^{o}[10].

Water extract of Nigella Sativa was prepared using the method modified by Ibrahim et al [11] in which "80g powder of Nigella Sativa was placed in an ordinary beaker that contain 100 ml of deionized water, then a magnetic stirrer was used to continuously mix the suspension for 18 hours, then the mixture was filtered using Whitman filter paper number 41, the filter then was left to dry in an open dish for 24 hours and then, freeze dried to obtain a dry powder. A total of 4g of the freeze – dried powder was obtained, of that; 0.5g was dissolved in 100 ml of deionized water to obtain a stock solution of 0.5 w/v of water extract"

It was important to select concentrations of water extract of Nigella Sativa which were used later in microhardness test, this was achieved by doing a pilot study in which different concentrations of water extract of Nigella Saliva were prepared from the stock solution by adding deionized water, concentrations of 3% w/v, 5% w/v and 7% w/v were prepared from the stock solution with addition of deionized water.

Twenty five teeth were randomly divided into five groups, and each group consisted of five teeth, then, they were immersed individually for about 2 minutes once daily for twenty days in 40ml of their assigned test solution that involved, Habbatul Baraka water extract (3%, 5%, 7%) and sodium fluoride (0.05%) which was used for comparison as a control positive that is approved concentration for the daily – home used sodium fluoride [12]. Deionized water teeth group was used as a control negative group.

After immersion of each tooth, they were washed with deionized water individually for five minutes and then the specimens were stored in humid condition which included a deionized water with 0.1% thymol that was added until the next immersion was done, this procedure continued for twenty days, after the twenty days of treatment period, a three mm circular area was selected and made on the buccal surface of the tooth enamel (specimen) and this was done by application of already prepared annular adhesive disc, the rest areas of specimen were covered with a sticky wax, and only the circular window on the enamel was left exposed, etching of circular enamel windows were done for ten seconds using five ml of 2NHCL, this etching was done in separated polyethylene tubes.

Calcium ion concentration was determined and calculated using spectrophotometer device [10].

After this procedure the concentrations of water extracts (3%, 5% , 7%) of Nigella Sativa gave the best results and were used for microhardness test to compare the result of microhardness of the tested solutions with those of NaF and deionized water.

For microhardness test, the rest twenty five teeth were used, they were randomly divided into five groups, each group consisted of five teeth. The groups were divided as followings:
The first group (3% Nigella Sativa water extract group).
The second group (5% Nigella Sativa water extract group).
The third group (7% Nigella Sativa water extract group).
The fourth group (0.05% Sodium fluoride group).
The fifth group (Deionized water group), and as mentioned previously, 5 teeth were used for each agroup.

Tooth surfaces were painted with an acid – resistant nail varnish and only 6 mm diameter was left on buccal surface, the teeth then adapted in a model made of acrylic with the size of the model 30 × 27 mm using red waxes, then the windows of tooth surfaces were ground and polished by using a wet emery paper (400 grit) and the grinding was repeated 10 times for every tooth, then, they were subjected to pH cycling procedure[13].

Initially, microhardness of enamel was measured for normal enamel in each group and then, the microhardness was measured after induction of caries using pH cycling procedure, and the microhardness measurement was done using Vickers microhardness device at a load of 500gm for 30 seconds [14]. Then the teeth were treated by solution of the selected agents and this was done by emerging each specimen (tooth) separately in 20 ml of the solution of the selected agent for 4 minutes, then each tooth was rinsed for two minutes with a deionized water, then the teeth were restored in a deionized water for the next day at a temperature of 37C^{o} (in an incubator), and this procedure was repeated daily for one week.

The difference in the microhardness (Change in the hardness) between the initial and final microhardness reading was calculated for each group which represent the rate of change in hardness [15].

Processing of data was done using SPSS version (18) statistical software. ANOVA (Analysis Of Variance), Dennett's T3 and Tukey Honestly Significant Difference (Tukey HSD) statistical tests were used for evaluation of significant difference among groups. The level of significance was considered to be accepted at 95% ($P < 0.05$).

3. Results

Tables (1), (2) and (3) involve the results concerning pilot study (calcium ion dissolution), in Table (1), the least mean value of calcium dissolution was found in 7% Nigella Sativa water extract group, while the highest value was found in deionized water group.

Table (2) shows that there is a highly significant difference ($P < 0.0.1$) among groups. In Table (3), Dennett's T3 test shows multiple comparison for dissolution of calcium between groups, highly significant difference ($P < 0.01$) was found between 7% Nigella Sativa extract group and NaF group and between 7% Nigella Sativa group with deionized water also highly significant difference was found between NaF and deionized water group, additionally, highly significant difference was found between 5% Nigella Sativa group and deionized water while significant difference was

found between 3% Nigella Sativa group and 5% group of the tested solutions.

Table (4) includes the descriptive (mean and standard deviation values of microhardness (remineralization) and the highest mean value for microhardness is found in 7% Nigella Sativa group while the least mean value is recorded for deionized water group. Table (5) shows the statistical test for microhardness (remineralization), a highly significant difference was found among groups ($P < 0.01$), further statistical test between groups using Tukey HSD test is shown in Table (6) in which highly significant difference was found between each two groups except between 3% Nigella Sativa group and deionized water in which the difference was statistically not significant ($P > 0.05$).

The mean values of microhardness change (remineralization – demineralization) are illustrated in Table (7), the highest mean value was recorded for 7% Nigella Sativa group while the least value was recorded for deionized water group. Statistically, highly significant difference was found among groups as shown in Table (8), further statistical analysis illustrates highly significant difference between each two groups of the tested solutions except for the difference between 3% Nigella Sativa and deionized water groups in which a non-significant difference was found ($P > 0.05$) as shown in Table (9). The mean and standard deviation values of microhardness change (sound enamel – remineralization) for the tested groups are illustrated in Table (10).

From the Table, the least mean value is found in 7% water extract group of Nigella Sativa (this indicates that the remineralizing ability of 7% extract was the best), while the highest mean (changing microhardness between sound and remineralized enamel) was recorded for deionized water group (the least ability for remineralization).

Statistical test for microhardness change (sound – remineralization) is illustrated in Table (11), a statistically, highly significant difference was found among all groups($P < 0.01$).

Further statistical analysis using Tukey Honestly Significant Difference (Tukey HSD) demonstrates highly significant difference between 7% extract and deionized water groups, also highly significant difference is found between NaF and deionized group, furthermore, highly statistical difference was found between 5% Nigella Sativa and deionized groups and between 5% and 7% extract groups, also highly significant difference was found between 3% and 5% Nigella Sativa groups ($P < 0.01$).

Additionally, significant difference was found between 7% Nigella Sativa and NaF, also significant difference was found between 3% extract and deionized water ($P < 0.05$) as shown in Table (12).

Table 1: Descriptive statistics of dissolution of calcium among groups

Groups	Minimum	Maximum	Mean	±SD
3% Nigella sativa water extract	2.020	4.000	2.974	.794
5% Nigella sativa water extract	1.380	2.790	2.152	.593
7% Nigella sativa water extract	.910	1.470	1.170	.224
0.05% Sodium fluoride	1.860	2.370	2.120	.192
Deionized water	3.820	5.210	4.556	.510

Table 2: Statistical test of dissolution of calcium among groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.208	4	8.052	30.270	.000**
Within Groups	5.320	20	.266		
Total	37.528	24			

****Highly Significant (p< 0.01)**

Table 3: Multiple comparison calcium dissolution between groups using Dunnett's T3

Agent (1)	Agent (2)	Mean difference	Sig.
3% Nigella sativa water extract	7% Nigella sativa water extract	.822	.542
	5% Nigella sativa water extract	1.804	.036*
	0.05% Sodium fluoride	.854	.366
	Deionized water	-1.582	.056
5% Nigella sativa water extract	7% Nigella sativa water extract	.982	.109
	0.05% Sodium fluoride	.032	1.000
	Deionized water	-2.404	.001**
7% Nigella sativa water extract	0.05% Sodium fluoride	-.950	.001**
	Deionized water	-3.386	.000**
0.05% Sodium fluoride	Deionized water	-2.436	.001**

***Significant (p< 0.05), **Highly Significant (p< 0.01)**

Table 4: Descriptive of microhardness (remineralization) among groups

Groups	Minimum	Maximum	Mean	±SD
3% Nigella sativa water extract	43.00	50.00	45.4000	2.70185
5% Nigella sativa water extract	55.000	68.000	62.400	5.030
7% Nigella sativa water extract	95.000	99.000	96.800	1.643
0.05% Sodium fluoride	85.000	90.000	87.000	2.000
Deionized water	36.000	46.000	41.400	3.847

Table 5: Statistical test of microhardness (remineralization) among groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12151.600	4	3037.900	280.767	.000**
Within Groups	216.400	20	10.820		
Total	12368.000	24			

****Highly Significant (p< 0.01)**

Table 6: Multiple comparison of microhardness (remineralization) between groups using Tukey Honestly Significant Difference (Tukey HSD)

Agent (1)	Agent (2)	Mean difference	Sig.
3% Nigella sativa water	7% Nigella sativa water	-51.400	.000**
	5% Nigella sativa water	-17.000	.000**
	0.05% Sodium fluoride	-41.600	.000**
	Deionized water	4.000	.338
5% Nigella sativa water	7% Nigella sativa water	-34.400	.000**
	0.05% Sodium fluoride	-24.600	.000**
	Deionized water	21.000	.000**
7% Nigella sativa water	0.05% Sodium fluoride	9.800	.001**
	Deionized water	55.400	.000**
0.05% Sodium fluoride	Deionized water	45.600	.000**

Highly Significant (p< 0.01)

Table 7: Descriptive of microhardness change (remineralization - demineralization) among groups

Groups	Minimum	Maximum	Mean	±SD
3% Nigella sativa water	1.000	10.000	6.200	3.899
5% Nigella sativa water	17.000	30.000	23.600	5.683
7% Nigella sativa water	56.000	68.000	62.800	4.550
0.05% Sodium fluoride	44.000	50.000	47.800	2.683
Deionized water	1.000	1.000	1.000	.000

Table 8: Statistical test of microhardness change (remineralization – demineralization) among groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14131.440	4	3532.860	234.275	.000**
Within Groups	301.600	20	15.080		
Total	14433.040	24			

Highly Significant (p< 0.01)

Table 9: Multiple comparison of microhardness change (remineralization - demineralization) between groups using Dunnett's T3

Agent (1)	Agent (2)	Mean difference	Sig.
3% Nigella sativa water extract	7% Nigella sativa water extract	-17.400	.006**
	5% Nigella sativa water extract	-56.600	.000**
	0.05% Sodium fluoride	-41.600	.000**
	Deionized water	5.200	.213
5% Nigella sativa water extract	7% Nigella sativa water extract	-39.200	.000**
	0.05% Sodium fluoride	-24.200	.001**
	Deionized water	22.600	.005**
7% Nigella sativa water extract	0.05% Sodium fluoride	15.000	.004**
	Deionized water	61.800	.000**
0.05% Sodium fluoride	Deionized water	46.800	.000**

Highly Significant (p< 0.01)

Table 10: Descriptive statistics of microhardness change (sound- remineralization)

Groups	Minimum	Maximum	Mean	Std. Deviation
3% Nigella sativa water extract	198.000	212.000	204.000	5.431
5% Nigella sativa water extract	179.000	225.000	192.400	19.476
7% Nigella sativa water extract	138.000	152.000	144.400	6.107
0.05% Sodium fluoride	159.000	202.000	174.800	17.079
Deionized water	211.000	249.000	232.800	15.156

Table 11: Statistical test of microhardness change (sound-remineralization) among groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21717.440	4	5429.360	28.059	.000**
Within Groups	3870.000	20	193.500		
Total	25587.440	24			

Highly Significant (p< 0.01)

Table 12: Multiple comparison of microhardness change (sound- remineralization) between groups using Tukey Honestly Significant Difference (Tukey HSD)

Agent (1)	Agent (2)	Mean difference	Sig.
3% Nigella sativa water extract	7% Nigella sativa water extract	11.600	.683
	5% Nigella sativa water extract	59.600	.000**
	0.05% Sodium fluoride	29.200	.025*
	Deionized water	-28.800	.028*
5% Nigella sativa water extract	7% Nigella sativa water extract	48.000	.000**
	0.05% Sodium fluoride	17.600	.301
	Deionized water	-40.400	.001**
7% Nigella sativa water extract	0.05% Sodium fluoride	-30.400	.019*
	Deionized water	-88.400	.000**
0.05%Sodium fluoride	Deionized water	-58.000	.000**

*Significant (p< 0.05), **Highly Significant (p< 0.01)

4. Discussion

Nigella Sativa (Habbatul Baraka) is considered as one of an important plants that used for medical purposes [16].

In dental field, it was documented that Nigella Sativa had antimicrobial activity (effect) in oral cavity and this could be related to its content of both organic and inorganic compounds such as minerals (calcium, phosphate …etc.) [1, 17, 18]. These constituents have the antibacterial activity, Additionally, they could interact (react) with outer surface of the enamel [14, 17, 19], thus, nigella sativa extract was used to test its remineralizing ability (microhardness) on teeth and a pilot study was done to select the concentrations of the extract which give the best result (less dissolution ability for calcium ion from etched demineralized teeth), so the concentrations (3% , 5% and 7%) were chosen accordingly. The aqueous extract of Nigella Sativa was used in this study because it resulted in more uniform solution (solvent) of the selected agents, in present study, the pilot study involved determination of the dissolved calcium ions from demineralized teeth using different concentrations of the water extract since calcium is considered as one of the major mineral of the tooth enamel which comprises 33.6% - 39.4% of the hydroxyl – apatite crystal [20, 21].

In current study, samples of teeth enamel were treated with 3% , 5% and 7% water extract of Nigella Sativa and 0.05% NaF, and it was found that the release of calcium ion was the least for the 7% Nigella Sativa extract and the highest release was recorded in deionized water group and this may

indicate an ionic incorporation resulting in decreasing porosity and increasing the microhardness of enamel after application of the extract(7% concentration) against the demineralizing effect of the acid used. Sodium fluoride has the ability to increase the resistance of enamel to dissolution on by effect of acid which results in protection of the teeth against decay [14 , 22], therefore it had been used in this study as one of remineralizing agents (control positive), it is capable to react with the outer surface of enamel resulting in calcium fluoride formation and this results in increasing fluoride concentration on the surface of enamel leading to increase the resistance of the surface to attack by acid[21]. The increase in microhardness of enamel after application of Nigella Sativa extracts could be due to its content of ant cariogenic minerals (calcium, phosphorus and fluoride) [23, 24] that are considered as constituents of a tooth [25], so, these ions will incorporate the outer enamel layer and this also may explain the difference in calcium ion concentration between Nigella Sativa extracts and deionized water. It was apparent from the results of this study that, as the concentration of the extract increases, its remineralizing effect increases (less dissolution of calcium ion and increase in microhardness of enamel) and the explanation for these results is that when the concentration of the extract of black seed increases, the (calcium \ phosphorous) ratio increases and this result in making enamel more hard with increased resistance to attack of acids [26]. Additionally, the concentration of fluoride increases this mean that the water extract of Nigella Sativa at a concentration of 7% gave the best remineralizing action in current study (increase resistance of enamel to acid attack) as compared to the rest tested solution. An Interesting finding in present study was that the remineralizing effect of 7% of Habbatul Baraka extract was better than that of 0.05% sodium fluoride, however, this finding (result) is needed to be confirmed by further investigations and studies prior to recommend the application of the above mentioned concentration (7%) as a remineralizing mouth wash in dental practice for caries prevention.

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