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Dissolution of Inorganic Phosphorous Ion from Teeth Treated with Different Concentrations of Aqueous Extract of Nigella Sativa (Black Seed) in Comparison with Sodium Fluoride: An in Vitro Study

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Abstract: ***Background: The medicinal plants which were provided by nature and explored by human to disclose its appreciated values in the clinical field. Black cumin seed have important activity on both human's general and oral health. This study had been used to evaluate the ability of Nigella Sativa aqueous extract to increase tooth resistance against acid attack when compared to Sodium Fluoride. Materials and Methods: Twenty five maxillary human premolars were used and treated with the selected solutions as three aqueous extracts of the Nigella Sativa (3%, 5% and 7%) and Sodium Fluoride (0.05% NAF) as control positive, for 2 minutes once daily For 20days time interval, de ionized water was used as control negative.2NHCL etching solution was used and the atomic flame spectrometer was used to measure the concentration of the phosphorous ion release. Result: the least phosphorous ion release from the etched enamel was found in the 7% black seed aqueous extract then 5% aqueous black seed extract followed by 0.05% Sodium Fluoride, statistically highly Significant difference between each two groups except that there was statistically significant result between the two aqueous black seed extracts (3% and 5%) and between 3% and 0.05% NAF (sodium fluoride) and statistically not significant difference between 5% Nigella-sativa aqueous extract and0.05% NAF. Conclusion: Nigella Sativa aqueous extract registered a mark able benefit of increase tooth resistance against acid dissolution thus it should be used not from Islam view but from its welfare of the mankind and its beneficial effects on people***

Keywords: Nigella Sativa aqueous extract, etching solution, phosphorous ion release

1. Introduction

The direction towards herbs and their products is the basis for many unique pharmaceuticals which used for eradicating various dangerous diseases [(1)]. Neigella sativa plant also known as Black seed, black caraway seed, Habbatu Sawda and Habatul Baraka ‘‘the Blessed Seed’’ grows in countries of Mediterranean sea and west of Asia which used as spice, food preservative and herbal medicine for treatment of many diseases and that was approved by Prophet Mohammed who stated that" the black seed can heal every disease except death" , thus it is a apart of 'Tibb-e-Nabavi', or "Medicine of the Prophet (Muhammad)" [(2,3)].So many therapeutic effects of Black seed found in cardiovascular, respiratory, and gastro-intestinal diseases, antidiabetic, anti microbial effect as an antibacterial, antifungal , antiparasitic and antiviral, anti histaminic effect, antitumor and anticancer due to its Immunopotentiator, anti-inflammatory and antioxidant activities [(4)] .

The chemical compostion of Black seed are volatile oil, fixed oil, protein, carbohydrates, alkaloids, saponins, amino and fatty acids , trace elements , heavy metals and minerals [(5,6,7,8)]. *N.Sativa* contain so numerous inorganic elements by range from (1.79%-3.74%) as calcium, phosphorous, potassium, sodium and iron [(9)]. Mohammed et al in 2009[(10)] found that *N.Sativa's* mineral content was 4.20% and that calcium and phosphorous ions concentration means in mg/100gm was 570 and 543 respectively which were followed by potassium and these high contents of calcium and phosphorus are supported by many studies [(11,12,13)].It is found that minerals concentration of Niegella sativa could be attributed to weather, location and genetic differences [(14,15)]. Many studies approved the application of oil extract of N.Sativa in dentistry such as antibacterial [(16,17)], root canal medicaments [(18)], treatment of dry socket [(19)], enhancing the osseous integration of dental implants [(20)] , relief of oral mucositis induced chemotherapeutic drugs [(21)] as well as an anticandidal infection[(22)] .As available, there is no previous Iraqi study was achieved for N.sativa aqueous extract to improve teeth mineralization, so this study was achieved.

2. Materials and Methods

Twenty five randomly extracted human maxillary first premolar teeth from (10-13) years old patients from orthodontic department were selected for this study. Using conventional hand piece and rubber cup with non-fluoridated pumice and deionized water to clean these teeth and then they were stored in 0.1% thymol solution at 4º C until use, to minimize brittleness of enamel and microbial growth [(18)] .Black seed water extract was prepared by the modified method of Ibraheem et al (2010) [(19)].The sample was divided into five equal groups, each group consisted of five teeth and then the teeth were immersed individually for two minutes once daily over twenty days in forty ml of their assigned test solution

which included, black seed water extract (3%, 5%% and 7%) as these concentrations showed the best results by application of pilot study and sodium fluoride (0.05%) which is the approved concentration of daily home- used sodium fluoride [20]. De-ionized water group was used as a control negative. After each immersion, the specimens were water washed in deionized water for 5 minutes, then stored in humid condition of deionized water to which 0.1% thymol was added until the next immersion. After the twenty day treatment period, a circular area (3 mm in diameter) were selected on buccal surface of each enamel specimen by applying prepared annular adhesive disc, avoiding microscopic cracks and hypo plastic areas. The rest of specimen was covered with a sticky wax, leaving only the circular enamel window exposed for subsequent etching, and then the windows were etched for ten seconds in separated polyethylene tubes, each containing five ml of 2NHCL. The concentration of released phosphorus ion was determined calorimetrically by the Molybdenum – Vanadate method. A ready-made Kit (Bio Maghreb, Tunisia) was supplied. Inorganic phosphorus reacts with ammonium molybdate in the presence of sulphuric acid to form a phosphomolybdate complex. The color of molybdenum blue was proportional to the phosphorus concentration [21].The data was processed using SPSS version 18 statistical software. ANOVA (Analysis Of Variance) and Dunnett T3 tests were used to evaluate the significance of difference between different groups. The significance level was accepted at 95% (P<0.05).

3. Results

The descriptive and inferential analysis of release phosphorus ion concentration according to different concentration of Nigella-sativa (black seed) aqueous extract and sodium fluoride were illustrated in Table (1).The highest mean value of dissolved phosphorus ion wad found in deionized water group followed by sodium fluoride treated one, while the lowest one was recorded in aqueous black seed extract (7%) treated one and this was achieved by ANOVA test which was showed a highly significant difference between different groups**.** Results in Table (2) showed statistical difference of dissolved phosphorus ion concentration between each two selective agents using Dunnett T3 post hoc test. All the results which were recorded in Table (2) were found to be statistically highly Significant difference between each two groups except that there was statistically significant result between the two aqueous black seed extracts **(**3% and 5%**)** and between 3% and 0.05% NAF (sodium fluoride) and statistically not significant difference between 5% Nigella-sativa aqueous extract and 0.05% NAF .

Table1: Concentration of phosphorous ion release (mean, standard deviation and statistical analysis ANOVA) dissolved in HCL from enamel treated with selected agents.

Group	No.	Mean	±SD	F-value	P-value
Aqueous black seed extract3%	5	0.86	0.09	87.899	0.000**
Aqueous black seed extract 5%	5	0.67	0.07		
Aqueous black seed extract7%	5	0.34	0.08		
Sodium Flouride0.05	5	0.61	0.07		
Deionized water	5	2.38	0.40		

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

Table 2: Dunnett T3 post hoc test between each two agents.

Agent (1)	Agent (2)	Mean Difference	Sig.
Aqueous black seed extract3%	Aqueous black seed extract 5%	0.19*	0.047
	Aqueous black seed extract7%	0.52**	.000
	Sodium Flouride0.05%	0.25*	0.010
	Deionized water	-1.52**	.005
Aqueous black seed extract 5%	Aqueous black seed extract7%	0.33**	.001
	Sodium Flouride0.05%	0.06	.813
	Deionized water	-1.71**	.004
Aqueous black seed extract7%	Sodium Flouride0.05%	-0.27**	.003
	Deionized water	-2.04**	.002
Sodium Flouride0.05%	Deionized water	-1.77**	.003
*. The mean difference is significant at the 0.05 level.			
** The mean difference is Highly significant at the 0.01 level.			

4. Discussion

Black cumin Seed regarded one of the principal plants in the natural medicine[30]. Niegella sativa had been used for many years as a protective tool against numerous body disorders. In dentistry, It was approved that extract of NS had drastic oral antimicrobial effect due to its organic and inorganic constituents as minerals like calcium and phosphate…etc[16,17,18].These agents could have antibacterial action as well as they might react with the outer enamel surface [16,23,24]. thus an aqueous extract of black seed (Nigella Sativa) (3%,5%,and 7%) was chosen to confirm its ability to harden the outer enamel surfaace .Sodium Fluoride agents was chosen as control positive due to its remineralizing ability, its uniform stability, not cause tooth discoloration and not harmful to gingiva [24, 25], while de-ionized water was used as control negative.)So many types of Nigella Sativa extracts beside aqueous one as oil extract, ethanolic,methanolic, ether and hexane.The aqueous extract was selected in this study to uniform solvent of the used agents. Changes in the calcium/phosphate ratio indicate changes in the inorganic constituents of Hydroxyapatite crystals of tooth structure [26].The concentration of phosphorous ion release after etching with 2NHCL acid following treatment of the samples with the test solutions was higher for the de-ionized water than the test solutions with the highly significant difference this is could be attributed to incorporations of ions which responsible for microhardness of enamel and reduce its porosity and increase its resistance against demineralization .Regarding the black seed extract , this may be related to its chemical composition of as it contains anticariogenic minerals as phosphorous and calcium which was supported by many studies[9,10,11,12,13] which are the so important constituents of the tooth structure[27],thus this could contributing to increase the microhardness of the tooth structure by incorporating of the metals into the outer layer of enamel and this clarify the difference of phosphorous ion concentration between aqueous black seed extracts and de-ionized water. Reaction of fluoride (Sodium fluoride) with the outer enamel layer to form fluoride containing compounds mainly calcium fluoride, thus the increase in the concentration of fluoride in enamel surface made the tooth surface more resistant to dissolution by acid attack and stable crystals (flour apatite crystals)[28], this could explain the lower concentration of phosphorous ion release by sodium fluoride than that of de-ionized water. The result of the present study revealed that the

increase in the concentration of black seeds extract leads to decrease the enamel dissolution, this can be explained by that the increase of the concentration of N.Sativa aqueous extract could increase the concentration of minerals in the extract which lead to increase of calcium\phosphate molar ratio(Ca\P) thus make enamel harder and increase resistance to acid attack[29]. So the finding of this study is that 7% Black cumin Seed aqueous extract is the best to increase tooth against acid attack when compared to other tested solutions. As a result N.Sativa can be used in prevention of oral diseases due to that it is cheap, available and can make so appreciable clinical results but this need further clinical researches and studies on N.Sativa plant to disclose so many uses of this plant on both general and oral health findings of the people.

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