DOI Number: 10.37506/v11/i1/2020/ijphrd/194025

Effect of Black Seed (Nigella Sativa) Extract on Release of Some Minerals from Human Enamel: An in Vitro Study

Nibal Mohammed Hoobi[1], Raya R. Al-Dafaai[2], Baydaa Hussain[1]

[1]Assist. Prof. College of Dentistry, University of Baghdad, [2]Assist. Prof. College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Sciences, Iraq

Abstract

Background: Black seed of *Nigella sativa* has been used for centuries to promote health due to its anti-microbial, anti-oxidant and anti-inflammatory effects. This study was conducted to evaluate the ability of black seed water extract to reduce the release of potassium and zinc ions from enamel after acidic attack.

Materials and Method: Twenty five maxillary human premolars were prepared and divided into five groups. The test solutions were included, black seed water extract in three concentrations (3%, 5% % and 7%), sodium fluoride (0.05%) as control positive and de-ionized water as control negative. The teeth in each group were immersed separately in 40 ml of the test solution for two minutes, once daily and for twenty days. The teeth then prepared for subsequent etching and analysis by atomic flame spectrometer to measure the concentration of the released potassium and zinc ions.

Result: The highest mean value of dissolved potassium ion was found in de-ionized water group followed by 3% water extract of black seed while the lowest one was recorded in 7% black seed water extract. The statistical difference between the five groups was highly significant ($p<0.01$) by using ANOVA test. The highest mean value of dissolved zinc ion was found in de-ionized water group followed by 0.05% sodium fluoride and 3% black seed extract while the lowest one was recorded in 7% water extract of black seed. The statistical difference between the five groups was also highly significant ($p<0.01$).

Conclusion: Water extract of black seed has the ability to reduce the dissolution of potassium and zinc ions from the tooth and might increase tooth resistance against acid attack.

Keywords: *Nigella Sativa, potassium ion, zinc ion.*

Introduction

There is a worldwide increasing interest on the use of herbs or plants in the treatment of various diseases especially in developing countries. Black seed of *Nigella sativa*is an annual flowering plant that widely used in nutrition and medicine. This seedis rich in phenolic compounds, essential fatty acids, proteinsand bioactive compounds[(1)]. Previous studies had searched for its anti-oxidant, anti-cancer, anti-inflammatory and anti-microbial activities of this miracle seed[(2,3,4)].

Enamel is the hardest tissue in the human body. Dental enamel is 95% mineral, 4% water and 1% organic matter by weight percentage. The minerals are composed mainly from calcium and phosphate in addition to the presence of small quantities of other elements[(5)]. Zinc is essential trace element. It accumulates in the surface structures of teeth and occurs in low concentrations in subsurface material. Zinc can reduce enamel demineralization and increase enamel resistance to acid dissolution[(6)]. Regarding potassium ion, enamel content of this ion is very low in comparison with calcium and phosphors contents [(7)]. However, little data are available about the relation between tooth resistant to acid attack and potassium content. A possible relationship between enamel minerals content and caries susceptibility has been advised[(8)]. No previous study was conducted for the effect of water extract of *Nigella sativa* on the release of zinc and potassium ions from human enamel, so this study was conducted.

Materials and Method

Extracted maxillary first premolar teeth from patients aged 10-13 years old were selected from orthodontic department (college of dentistry, university of Baghdad). The total number of teeth was 25. The teeth were cleaned by using conventional hand piece and rubber cup with non-fluoridated pumice and deionized water. After cleaning, the teeth were stored in 0.1% thymol solution at 4º C until use tominimize microbial growth. This step also was done to prevent the dryness and brittleness of teeth. The water extract of black seed was prepared by the modified method of Ibraheem et al[9]. The teeth were divided into five equal groups; each group consisted of five teeth. The test solutions were included, water extract of black seed in three concentrations (3%, 5% % and 7%),sodium fluoride (0.05%) as control positive and de-ionized water as control negative. The teeth in each group were immersed separately for two minutes, once daily,for twenty days in 40 ml of the test solution. After each immersion, the specimens were washed in de-ionized water for 5 minutes and then stored in de-ionized water with thymol (0.1%)at room temperature till the next immersion.

After the treatment period (20 days), a rounded area (3 mm in diameter) were prepared on buccal surface of each tooth by applying adhesive disc and avoiding hypo plastic areas ormicroscopic cracks. The rest of enamel for each tooth was covered by a sticky wax, leaving only the rounded area (window) exposed for subsequent etching. The windows were etched for ten seconds in separated polyethylene tubes. Eachtube is containing five ml of 2NHCL[10]. The concentrations of released potassium and zinc ions were determined calorimetrically by using flame atomic absorption spectrometer.

Statistical analysis: The data was processed using SPSS version 20 statistical software. Means and standard deviation were calculated for each group. One way ANOVA and Dunnett T3 (post hoc test) were used to evaluate the significance of difference between the five groups. Probability values less than 0.05 were considered statistically significant (P<0.05). Values less than 0.01 were considered highly significant (P<0.01).

Results

Concentration of potassium ion release (mean,standard deviation and statistical analysis of ANOVA are illustrated in Table 1.The highest mean value of dissolved potassium ions was found in de-ionized water group followed by 3% water extract of black seed while the lowest mean was recorded in 7% concentration of black seed. The statistical difference between the five groups was highly significant (p<0.01). Table 2 is showing the mean differences of dissolved potassium ions concentrations between each two agents. No statistical significant differences were found between the de-ionized water and black seed extract in concentrations: 3%, 5% and 0.05% of sodium fluoride. No significant difference was found between 5% black seed extract and 0.05% sodium fluoride.

Concentration of zinc ion release (mean, standard deviation and statistical analysis of ANOVA are illustrated in Table 3. The highest mean value of dissolved zinc ion was found in de-ionized water group followed by 0.05% sodium fluoride and black seed extract 3%, while the lowest mean was recorded in water extract of black seed 7%. The statistical difference between the five groups was highly significant (p<0.01).Table 4 is showing statistical mean differences of dissolved zinc ions concentrations between each two agents. No statistical significant difference was found between the black seed extract in 5% and 7%. No significant difference was found between 3% black seed extract and 0.05% sodium fluoride.

Table 1: The released potassium ion concentrations among the selected agents

Selected Agents	No	Mean ± SD	F value	P value
Water black seed extract 3%	5	0.910±0.07	19.44	0.00**
Water black seed extract 5%	5	0.660±0.07		
Water black seed extract 7%	5	0.484±0.06		
Sodium Fluoride 0.05%	5	0.730±0.05		
Deionized water	5	1.634±0.48		

** Highly significant (p<0.01), df (Between Groups=4, Within Groups=20, Total=24)

Table 2: Post hoc test between each two agents (potassium ion concentrations)

Agent 1	Agent 2	Mean Difference	Significant
Water black seedextract 3%	Water black seed extract 5%	0.250**	0.005
	Water black seed extract 7%	0.426**	0.000
	Sodium Fluoride 0.05%	0.180*	0.022
	Deionized water	-0.724	0.159
Water blackseed extract5%	Water black seed extract 7%	0.176*	0.027
	Sodium Fluoride 0.05	-0.070	0.573
	Deionized water	-0.974	0.063
Water blackseed extract 7%	Sodium Fluoride 0.05	-0.246**	0.002
	Deionized water	-1.150*	0.035
SodiumFluoride0.05%	Deionized water	-0.904	0.081

*The mean difference is significant at the 0.05 level., ** The mean difference is highly significant at the 0.01 level.

Table 3: The released zinc ion concentrations among the selected agents

Selected agents	No	Mean ± SD	F value	P value
Water black seed extract 3%	5	4.6260±0.30	168.547	0.00**
Water black seed extract 5%	5	3.3240±0.28		
Water black seed extract 7%	5	2.7760±0.29		
Sodium Fluoride 0.05%	5	4.7260±0.45		
Deionized water	5	8.6140±0.54		

**Highly Significant (p<0.01), df (Between Groups=4, Within Groups=20, Total=24)

Table 4: Post hoc test between each two agents (zinc ionconcentrations)

Agent 1	Agent 2	Mean Difference	Significant
Water black seedextract3%	Water black seed extract 5%	1.302**	0.001
	Water black seed extract 7%	1.850**	0.000
	Sodium Fluoride 0.05	-0.100	1.000
	Deionized water	-3.988**	0.000
Water blackseed extract5%	Water black seed extract 7%	0.548	0.129
	Sodium Fluoride 0.05	-1.402**	0.006
	Deionized water	-5.290**	0.000
Water blackseed extract7%	Sodium Fluoride 0.05%	-1.950**	0.001
	Deionized water	-5.838**	0.000
SodiumFluoride0.05%	Deionized water	-3.888**	0.000

*The mean difference is significant at the 0.05 level., ** The mean difference is highly significant at the 0.01 level.

Discussion

In dentistry, the extract of black seed was tested in many studies to verify its oral effects due to the interested organic and inorganic constituents. The black seed showed its antimicrobial action against *Streptococcus mutans*, *Streptococcus mitis* and other types of bacteria isolated from the oral cavity [11,12]. In addition to that, *Nigella Sativa* extract had an obvious effect on the healing process of oral ulcer[13]. In previous Iraqi study, water extract of black seed was able to decrease the dissolution of inorganic phosphorous ions from teeth [14].In the current study, the water extract of black seed was tested in three concentrations to approve its ability to decrease the dissolution of zinc and potassium ions from the tooth surface and thus increase the hardness of the tooth and the resistant to acid attack. Sodium fluoride was used as control positive due to its effect in inhibiting

demineralization and enhancing remineralization of tooth surface. It is able to react with the outer enamel surface resulting in the formation of calcium fluoride[15].

The study showedthat the release of potassium ions was the least for the 7% extract and the highest release was recorded in deionized water group. The effect of sodium fluoride 0.05% in reducing the release of potassium ion was better than that reported for 3% extract.

The study also reported that the release of zinc ions was theleast for the 7% extract and the highestrelease was recorded in deionized water group. The effect of 3% extract was almost equal to the effect of sodium fluoride 0.05% in reducing the release of zinc ions. This may indicate that the application of black seed water extract can decrease the demineralizing effect of the acid used. It was alsoobvious from this study that, if theconcentration of the extract increases, theremineralizing effect of the water extract will increase. These results approve the findings of other studies regarding the effect of black seed water extract to improve the hardness of the tooth [14,16]. This effect could be attributed to thechemical composition of black seed and its mineralcontents (calcium, phosphorous, potassium, sodium, zinc and iron)which was reported by previous studies[17,18]. However, the finding of this study is needed to be confirmed byfurther investigations and larger sample sizebefore the applicationof this extract in the above mentioned concentration as mouth wash in preventive dentistry.

Conclusion

Water extract of black seed or *Nigella Sativa*has the ability to reduce the dissolution of potassium and zinc ions from the tooth.This effect might increase tooth hardness and its resistanceto acid attack.

Conflict of Interest: None

Funding: self

Ethical Clearance: Not required.

Acknowledgment: the authors are grateful to the teaching staff in the orthodontic department and preventive department in the college of dentistry, university of Baghdad to their help in conducting the current study. Authors thank the medical staff in poisons center of medical city in Baghdad to their efforts in data readings.

References

1. Dinagaran S, Sridhar S,Eganathan P: Chemical composition and antioxidant activities of black seed oil (Nigella sativa L.). Int J Pharm Sci Res 2016; 7(11): 4473-4479.
2. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytotherapy Research 2000; 14(5):323–328.
3. Randhawa MA, Alghamdi MS. Anticancer activity of Nigella sativa (Black Seed) -a review. AJCM 2011; 39(6):1075–1091.
4. Abd-Awn BH, Al-Dhaher ZA, Al-Dafaai RR. The effectofblackseedoilextractsonmutansstreptococci in comparison to chlorhexidinegluconate (in vitro).J BaghColl Dentistry 2012; 24(4):126-131.
5. Baldassarri M, Margolis HC, Beniash E. Compositional determinants of mechanical properties of enamel. J Dent Res 2008; 87:645–649.
6. BrudevoldF,SteadmanLT,Spinelli MA, Amdur BH, Grøn P. A study of zinc in human teeth. Archives of Oral Biology 1963; 8(2): 135-144.
7. Kunin AA, Evdokimova AY, Moiseeva NS. Age-related differencesof tooth enamel morphochemistry in health and dental caries. EPMA J 2015; 6:3.
8. Gutiérrez-Salazar MP,Reyes-Gasga J. Enamel hardness and caries susceptibility in human teeth. Rev Latin Am Met Mat 2001;21(2): 36-40.
9. Ibraheem NK, Ahmed JH, Hassan MK.The effect of fixed oil and water extracts of Nigella sativa on sickle cells: an in vitro study.
10. Barbakow F, Sener B, Snr Lab Tech, Lutz F. Dissolution of phosphorus from human enamel pretreated in vitro using SnF2 stabilized with amine fluoride 297. ClinPrev Dent 1987; 9(5): 3-6.
11. Mohammed NA. Effect of Nigella Sativa L. extractsagainst Streptococcus mutans and Streptococcus mitis inVitro. J Bagh College Dentistry 2012, 24(3):154-7.
12. Nader MI, Al-Thwaini AN, Abdul-Hassan IA, Ali WA. Effect of Nigella Sativa (Black Seed), SalvadoraPersica (Siwak) and Aluminum PotassiumSulphate (Alum) Aqueous Extracts On Isolated Bacteria From Teeth Root Canal. Iraqi J Biotech 2010; 9(1): 99-104.
13. Al-Douri AS, Al-KazazSGhA. The Effect of Nigella Sativa Oil (Black Seed) on the Healing

of Chemically Induced Oral Ulcer in Rabbit (Experimental Study). Al–Rafidain Dent J 2010; 10(1):151-157.

14. Hoobi NM, Rzoqi M G. 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. IJSR 2017;6(2): 1962-1965.
15. Brar GS, Arora AS, Khinda VI, Kallar S, Arora K. Topographic assessment of human enamel surface treated with different topical sodium fluoride agents: Scanning electron microscope consideration. Indian J Dent Res 2017;28:617-22.
16. Hussein B. Effect of Nigella Sativa (Habbatul Baraka) WaterExtract on Micro-hardness of Initial Carious Lesionof Permanent Teeth Enamel Compared to SodiumFluoride (An in Vitro Study). IJSR 2018; 7(1):215-220.
17. Al-Naqeep GN, Ismail MM, Al-Zubairi AS,Esa NM, 2009. Nutrients Composition and Minerals Content of Three Different Samples of Nigella sativa L. Cultivated in Yemen. Asian Journal of Biological Sciences 2009; 2: 43-48.
18. JasimNA, Abid FM. Determination ofmineral composition of Iraqi Nigella Sativa L. seed byAtomic absorption spectrophotometer.Irq Nat J Chem 2011; 42:178-84.