EVALUATION OF ANTIBACTERIAL SUBSTANTIVITY OF NIGELLA SATIVA (0.1%) MOUTHWASH – AN IN-VITRO STUDY.

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ABSTRACT:

Title: Evaluation of antibacterial substantivity of Nigella sativa (0.1%) mouthwash.

Introduction: Nigella sativa (Black seed) is a miraculous herb with a rich historical and religious background. It is known to have medicinal properties and the key ingredient being Thymoquinone which is known to have anti-inflammatory and anti-oxidative properties that has been found to have an important role in prevention of periodontal disease.

Objective: To assess and compare the antibacterial substantivity of Nigella sativa mouthwash (0.1%) with chlorhexidine gluconate mouthwash (0.2%).

Methodology: Twenty human extracted teeth were sectioned to obtain only crowns. These sectioned crowns were randomly divided into two groups to evaluate the substantivity by immersing in Nigella sativa mouthwash (0.1%) and Chlorhexidine gluconate mouthwash (0.2%) for one minute, followed by immersion in 10 ml of artificial saliva. After an interval of eight and twelve

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hours, an aliquot was taken from the tubes and analysed by ultraviolet spectrophotometer at 260nm.

Results: The mean substantivity value of Nigella sativa (0.1%) group was relatively higher as compared to chlorhexidine gluconate (0.2%) group at eight hours (P=0.08) and twelve hours time interval (P=0.19).

Conclusion: The mean substantivity value of NS was found to be relatively higher as compared to chlorhexidine gluconate at eight and twelve hours time interval. Therefore NS mouthwash (0.1%) can be used twice daily for its maximum antibacterial effect.

Keywords: Nigella sativa, Substantivity, Gingivitis, Spectrophotometer.

INTRODUCTION

Oral diseases comprises major health problems with dental caries and periodontal diseases being the most important preventable global infectious diseases.[1] Gingivitis is the most prevalent form of periodontal disease. It is an inflammatory response of the gingival tissues resulting from bacterial plaque accumulation located at or beneath the gingival margin.[2] Gingivitis is an reversible process through plaque control, if not intervened, it will further progress to periodontitis.[3] Therefore, elimination of plaque and its control plays a vital role in preventing and/or arresting gingival inflammation and maintenance of good periodontal health.

Plaque control can be achieved by mechanical and chemical measures.[4] Mechanical plaque control aids in prevention of plaque accumulation comprises of toothbrushing and interdental aids such as interproximal brush ,dental floss, wooden toothpicks.[5] Among these, toothbrushing is self-performed, practised each day to mechanically remove dental plaque. However mechanical plaque control in most of the individuals is often in-effective due to time consumption, dexterity required and injury to the adjacent tissues.[6] Thus adjunct use of chemotherapeutic agent with mechanical plaque removal is recommended as they inhibit bacterial colonization, growth, and metabolism, and consequently interrupt the formation of mature biofilm.[7] The use of antimicrobial mouthwashes as an adjunct to mechanical plaque control has become well established in dental practice for the control of Periodontal diseases.[8]

Among various chemical plaque control agents, chlorhexidine gluconate (CHX) is considered as gold standard mouthwash for prevention and treatment of periodontal diseases because of its potent antimicrobial and substantivity properties, but its deleterious effects on the oral mucosa, staining properties,

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taste alterations and calculus formation discourage its long term usage.[8] Hence there is a need and quest for a naturally occurring indigenous and cost-effective oral hygiene aid with similar positive effects and decreased adverse effects.[9]

Medicinal plants have evolved as emerging substances worldwide for the treatment of a wide array of inflammatory conditions and beneficial effects such as anti-oxidant, anti-inflammatory and immunomodulatory.[10]

Nigella sativa is one such miraculous herb with a rich historical and religious background belonging to the family Ranunculaceae. The seeds of N. sativa and their oil have been widely used since centuries in the treatment of various ailments throughout the world.[11]

Nigella sativa (NS) is known to have antihypertensive, antidiabetic, anticancer, diuretic, analgesic, antimalarial effects.[10] The active ingredient Thymoquinone is known for its anti-inflammatory and anti-oxidative properties and has been reported to have an important role in prevention of periodontal disease.[10]

The physical properties of NS was determined and it was found that, the Minimum inhibitory concentration (MIC) of NS extract performed against four microorganisms namely, Streptococcus mitis (S.mitis), Streptococcus mutans (S.mutans), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi) using Thioglycollate broth and it was found to be 0.1%. The NS mouthwash (0.1%) was prepared and pH was adjusted to 7. The antibacterial substantivity of mouthwash is its intrinsic ability to be retained by oral surfaces, and gradually released into oral fluids over many hours.[12] The anti-bacterial substantivity of NS (0.1%) mouthwash has not been reported to the best of our knowledge in published literature. Hence, the aim of the present study was to determine the duration of its antibacterial substantivity and to compare it with the substantivity of chlorhexidine gluconate mouthwash (0.2%).

OBJECTIVES

To assess and compare the antibacterial substantivity of Nigella sativa mouthwash (0.1%) with chlorhexidine gluconate (0.2%) mouthwash.

MATERIALS AND METHODS

Twenty human extracted teeth were sectioned with a water-cooled diamond saw to obtain the crowns. These 20 sectioned crowns were randomly divided into two groups to evaluate the substantivity by immersing in, Group 1 - Nigella sativa (0.1%) mouthwash and Group 2 - Chlorhexidine gluconate (0.2%) mouthwash for one-minute.

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The samples were then immersed in 10ml of artificial saliva and kept in glass tubes. After a time interval of eight and twelve hours, an aliquot of artificial saliva was taken from the glass tubes and the same volume was immediately replaced and analysed by ultraviolet spectrophotometer at 260nm.[12]

RESULTS

The present study was conducted to assess and compare the antibacterial substantivity of Nigella sativa mouthwash (0.1%) with chlorhexidine gluconate (0.2%) mouthwash. The Intergroup comparison of mean Substantivity values at 8 hours and 12 hours time interval was done using Mann Whitney Test and Intragroup comparison of mean Substantivity values between 8 & 12 hrs time intervals was done using Wilcoxon Signed Rank Test.

The present study results observed that, On Intragroup comparison, the mean substantivity value of NS group at 8 hrs time interval was relatively higher (0.9370 ± 0.1722) as compared to 12 hrs time interval period (0.8882 ± 0.0911) with mean score of 0.8882 ± 0.0911. However, this mean difference between 8 hrs and 12 hrs time interval in NS group was not statistically significant [P=0.33] [Table no. 1, Graph no. 1].
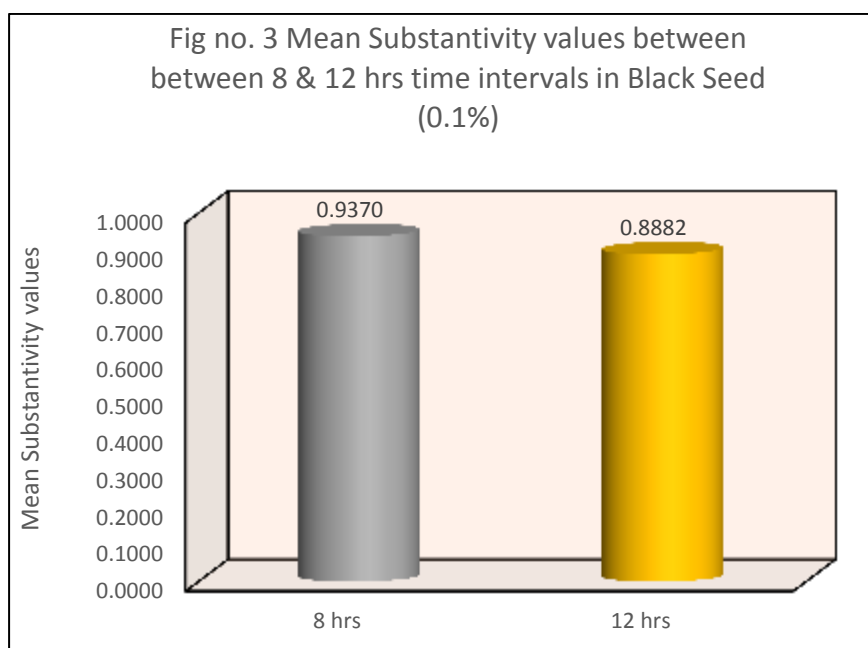
On Intragroup comparison, the mean substantivity value of CHX group at 8 hrs time interval was relatively higher (0.8019 ± 0.1300) as compared 12 hrs time interval period (0.7903 ± 0.1487) with mean score of 0.7903 ± 0.1487. However, this mean difference between 8 hrs and 12 hrs time interval in CHX group was not statistically significant [P=0.72] [Table no. 2, Graph no. 2].

On Intergroup comparison of the mean substantivity value at 8 hrs time interval was relatively higher in NS group (0.9370 ± 0.1722) when compared to CHX group (0.8019 ± 0.1300) with mean score of 0.8019 ± 0.1300. However, this mean difference between 2 groups at 8 hrs time interval was not statistically significant [P=0.08]. [Table no. 3, Graph no.3]

Whereas on Intergroup comparison of the mean substantivity value at 12 hrs time was relatively higher in NS group (0.8882 ± 0.0911) as compared to CHX group (0.7903 ± 0.1487) with mean score of 0.7903 ± 0.1487. However, this mean difference between 2 groups at 12 hrs time interval was not statistically significant [P=0.19]. [Table no. 4, Graph no. 4]
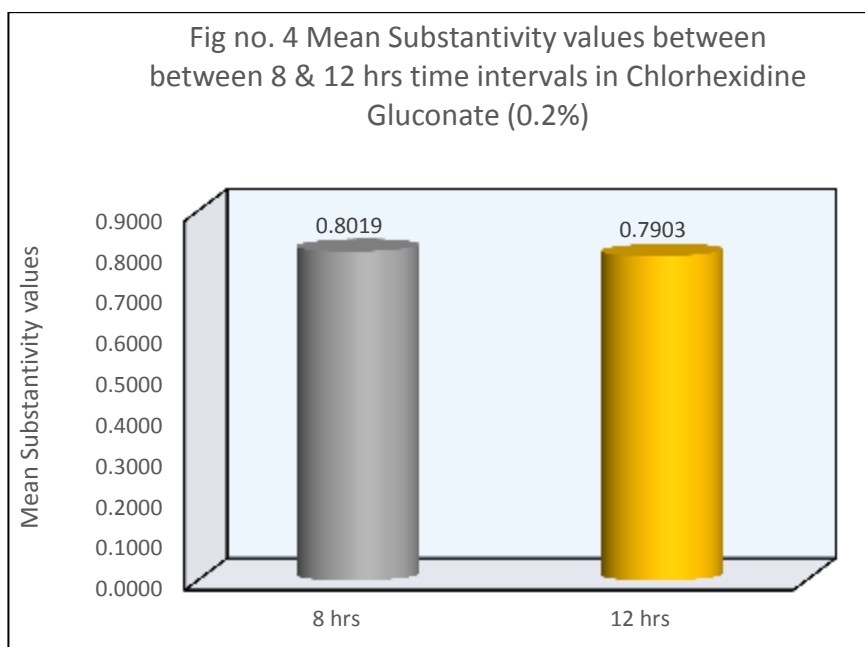
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Table no. 1

Comparison of mean Substantivity values between 8 & 12 hrs time intervals in Nigella sativa (0.1%) using Wilcoxon Signed Rank Test						
Group	Time	N	Mean	SD	Mean Diff	P-Value
Nigella sativa (0.1%)	8 hrs	10	0.9370	0.1722	0.0488	0.33
	12 hrs	10	0.8882	0.0911		

Fig no. 3 Mean Substantivity values between between 8 & 12 hrs time intervals in Black Seed (0.1%)

Graph no. 1 - Mean Substantivity values between 8 & 12 hrs time intervals in NS group

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Table no – 2

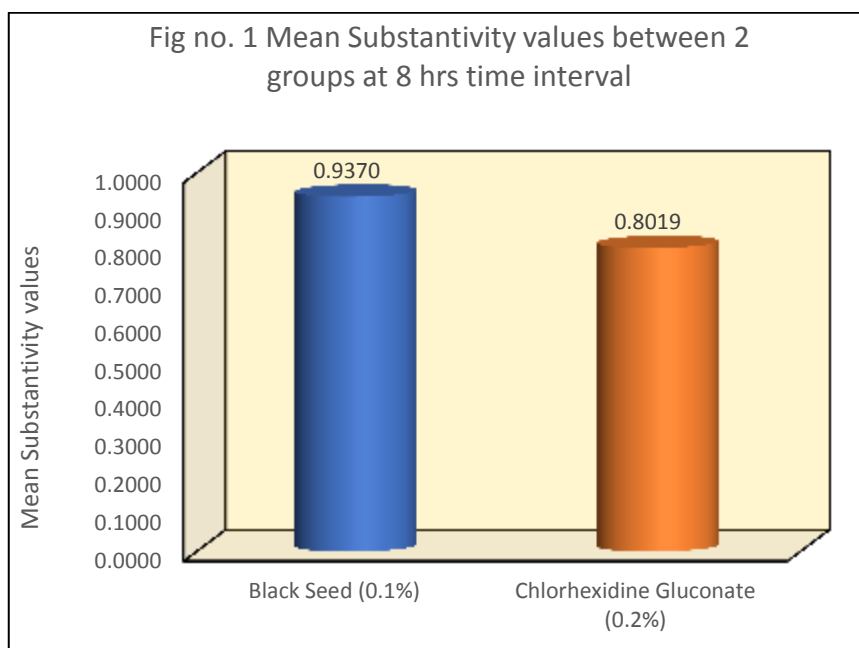| Comparison of mean Substantivity values between 8 & 12 hrs time intervals in Chlorhexidine Gluconate (0.2%) using Wilcoxon Signed Rank Test						
Group	Time	N	Mean	SD	Mean Diff	P-Value
Chlorhexidine Gluconate (0.2%)	8 hrs	10	0.8019	0.1300	0.0116	0.72
	12 hrs	10	0.7903	0.1487		

Fig no. 4 Mean Substantivity values between between 8 & 12 hrs time intervals in Chlorhexidine Gluconate (0.2%)

Graph no. 2 - Mean Substantivity values between between 8 & 12 hrs time intervals in Chlorhexidine Gluconate (0.2%)

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Table no. 3

	Comparison of mean Substantivity values between 2 groups at 8 hrs time interval using Mann Whitney Test					
Time	Groups	N	Mean	SD	Mean Diff	P-Value
8 hrs	Nigella sativa (0.1%)	10	0.9370	0.1722	0.1351	0.08
	Chlorhexidine Gluconate (0.2%)	10	0.8019	0.1300		

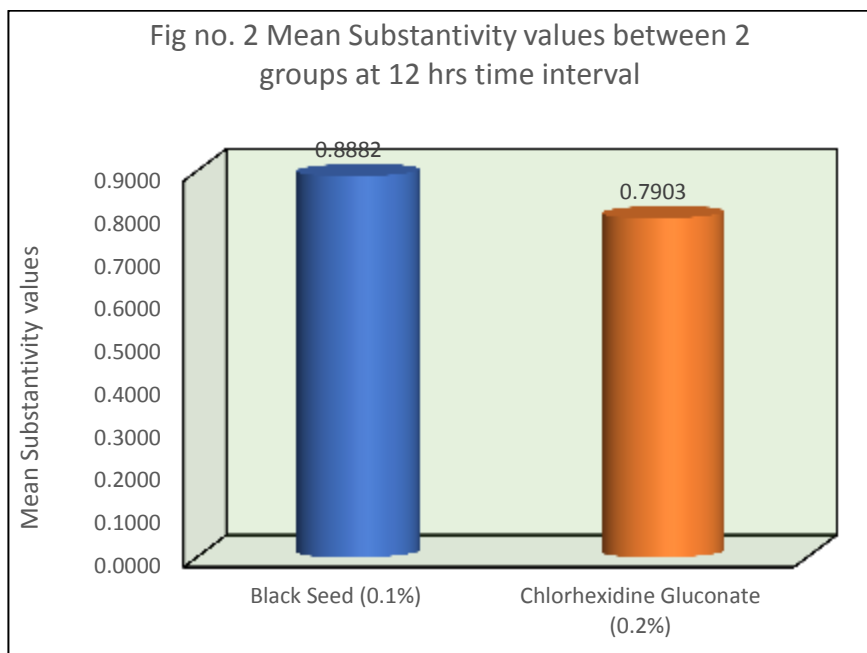Fig no. 1 Mean Substantivity values between 2 groups at 8 hrs time interval

Graph no. 3 - Mean Substantivity values between 2 groups at 8 hrs time interval

Table no. 4

	Comparison of mean Substantivity values between 2 groups at 12 hrs time interval using Mann Whitney Test					
Time	Groups	N	Mean	SD	Mean Diff	P-Value

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12 hrs	Nigella sativa (0.1%)	10	0.8882	0.0911	0.0979	0.19
	Chlorhexidine Gluconate (0.2%)	10	0.7903	0.1487		

Graph no. 4 - Mean Substantivity values between 2 groups at 12 hrs time interval

DISCUSSION

Chlorhexidine gluconate mouthwash is considered as gold standard mouthwash for prevention and treatment of periodontal diseases.[8] It has greater antibacterial activity and substantivity than other bactericidal agents which are used for maintenance of oral health.[13] The greater antibacterial effect is by the fact that the positively charged bis-biguanide molecule gets rapidly attracted by the negatively charged bacterial tooth surfaces and oral mucosal cell surfaces thereby increasing the substantivity through controlled release of the agent. Various studies have been demonstrated the antibacterial substantivity of CHX to last for twelve hours based on the persistence of CHX on the oral surfaces and ability to suppress salivary bacterial counts,[13] but its limitation on long duration may results in staining of teeth, taste alterations and calculus formation.[8] Therefore, the application of natural and herbal products for the treatment of periodontal disease has increased attention recently and provide great benefits.

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Herbal medicines, derived from botanical sources, have been used for various therapeutic benefits in periodontal therapy.[3] Among researches on various herbal components, Nigella sativa is found to possess antibacterial activity against different Gram-positive and Gram-negative organisms.

This in-vitro—pilot study was undertaken to determine the duration of antibacterial substantivity of NS mouthwash (0.1%) and to compare it with the substantivity of chlorhexidine gluconate mouthwash (0.2%).

In the present study, On Intergroup comparison, the mean substantivity values for NS at 8 hrs and 12 hrs time interval was relatively higher as compared to CHX group, however this mean difference was not statistically significant [At 8 hrs: $P = 0.08$, At 12 hrs: $P = 0.19$]. On intragroup comparison, it was found that the mean substantivity value for NS group and CHX group at 8 hrs time interval was relatively higher as compared 12 hrs time interval period and this difference was not statistically significant [NS:$P=0.33$, CHX: $P=0.72$].

There are no studies in published literature regarding evaluating the substantivity of NS mouthwash (0.1%), hence could not compare our results to highlight the importance of determination of antibacterial substantivity. However the possible mechanism of anti-inflammatory and anti-oxidant property of NS is attributed to the presence of main component, Thymoquinone (TQ). TQ is found to have antibacterial activity against most gram-positive and gram-negative micro-organisms. An in-vitro study conducted by Mohammed N A (2012) demonstrated the anti-bacterial effect of ethanol and ether extracts of NS against Streptococcus mutans and Streptococcus mitis.[14] Senthilnathan K et al. (2020) reported the anti-bacterial activity of Nigella sativa Seed Extract against Porphyromonas gingivalis and Prevotella intermedia.[15] These results were in accordance with our study where in the MIC of the Nigella sativa showed antibacterial activity against (S.mitis), Streptococcus mutans (S.mutans), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi).

 Chaieb K et al (2011) observed that the Thymoquinone has antibacterial effect against Gram positive cocci (Staphylococcus aureus and Staphylococcus epidermidis) which exhibited potency to prevent bacterial biofilm formation.[16]

The limitations of the present study are smaller sample size, no sufficient literature to support and compare our results for antibacterial substantivity of Nigella sativa mouthwash. Therefore, further research is warranted with a large sample size to establish the findings of this study.

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CONCLUSION

The present study was performed to determine the antibacterial substantivity of Nigella sativa mouthwash (0.1%) thereby assessing its frequency of usage for the maximum efficiency. The study observed that the mean substantivity value of NS was relatively higher as compared to chlorhexidine gluconate at eight and twelve hours time interval. Therefore NS mouthwash (0.1%) can be used twice daily for its maximum antibacterial effect.

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