

**EVALUATION OF EFFICACY OF *NIGELLA SATIVA* ON GINGIVAL HEALTH**

Running title: Efficacy of *Nigella sativa* on Gingival Health

**ABSTRACT**

**Background:** Gingivitis is a reversible inflammation of the gingiva. If it is left untreated it will progress to irreversible damage to the tissues like mobility and tooth loss. Previous studies have found that use of an antibacterial agent along with scaling and root planing is ideal for treating periodontal diseases. *Nigella sativa* is a herb with excellent antioxidant, antiinflammatory and antibacterial properties and hence was used in this study.

**Aim:** The aim of the study was to evaluate the efficacy of *Nigella sativa* mouthwash on gingival health in comparison to 0.12% chlorhexidine mouthwash.

**Materials and Methods:** The study was performed as a double-blinded, randomised controlled trial research study. A total of 30 patients were selected randomly, comprising two groups, 15 in each, aged between 18 and 28 years with gingivitis. The first group was advised to oral rinse with 0.12% chlorhexidine mouthwash and the second group with new formulated *Nigella sativa* herbal mouthwash for 1 week. The plaque index and gingival index scores were calculated before administering them the mouthwashes. Then subsequently after one week the plaque and gingival index scores were taken for the 30 patients. The final data was sorted in excel and statistically analysed using IBM SPSS software analysis, unpaired t-test. The results were interpreted as graphs and tabulations.

**Results:** The group 1 patients were found to have a mean plaque index value of  $2.6 \pm 0.49$  before mouthwash usage and post 1 week usage of chlorhexidine the plaque index was  $1.53 \pm 0.51$ . The mean plaque index was  $2.53 \pm 0.51$  prior to usage of mouthwash in group 2 and  $1.27 \pm 0.45$  post usage of *Nigella sativa* mouthwash. The difference in plaque index between both the groups was found to be statistically non-significant ( $p=0.067$ ). The mean gingival index of patients who used chlorhexidine was  $2.53 \pm 0.51$  before the mouthwash usage and  $1.33 \pm 0.45$  after mouthwash usage. In group 2 the gingival index before the mouthwash usage was  $2.47 \pm 0.51$  and after the mouthwash was  $1.47 \pm 0.51$ . However the difference in gingival index between both the groups was not statistically significant ( $p=0.393$ )

**Conclusion:**

The present study reveals that the *Nigella sativa* mouthwash was more effective in plaque control when compared to the standard chlorhexidine mouthwash. Herbal mouthwash shows promising results to be used as an alternative for chlorhexidine mouthwash for management of gingivitis.

Keywords: Chlorhexidine, Gingival Health, Gingivitis, Green Synthesis, Innovative, *Nigella sativa*

## INTRODUCTION:

Periodontal disease affects the gingiva and the alveolar bone and the supporting connective tissues which anchors the tooth in the jaws (1). Periodontal disease is one of the most common chronic diseases which has affected humans for many centuries (2,3). In recent years the occurrence of dental caries has declined and periodontal disease has been the cause of tooth loss, since less teeth are lost due to caries (4). The tooth root, periodontal tooth and the alveolar bone socket are referred to as periodontium (5). Periodontal disease destroys the periodontium, it causes loss of attachment which progresses to alveolar bone loss which results in the loss of the affected teeth (6,7). The severity of the diseases depends upon the host risk factors both modifiable and non modifiable and the environment (8,9).

The two common periodontal diseases are gingivitis and periodontitis (10,11). Gingivitis is the inflammation of the gingiva which harbours the connective tissue attachment that lets the tooth remain in its position (12). This affects only the soft tissue of the gingival epithelium and its connective tissue (13). Gingivitis presents itself by swollen gums, pain and bleeding and if it is left untreated it leads to periodontitis (14–20). Periodontitis is a much more advanced form of periodontal disease and destroys both the soft tissue and the hard tissues of the tooth (21). It affects the supporting tissues and causes attachment loss of the tooth along with bone destruction (22,23). This leads to mobility of the tooth and loss of the tooth (24–28).

*Nigella sativa* is known to be a miraculous herb and it also has a religious and historical background (29). It is also known as black cumin seeds or *Kalonji*. It belongs to the family Ranunculaceae and is a flowering plant. It is native to Southwest Asia and in some southern parts of Europe and North America. It holds an important place in the practice of Unani and Ayurveda (30). The seeds of *Nigella sativa* are used in folk medicine all over the world for prevention and treating several diseases (31). It is a black boat shaped edible seed of size 2 to 4mm length .

In traditional medicine it is used to treat headaches, common colds, nasal congestion, warts, rheumatic diseases and many more (32). Many studies suggest that it helps with allergies, asthma and dyspnea (33–36). It has a very extensive and broad pharmacological use as it is an analgesic

(37), diuretic and antihypertensive (38), antidiabetic (39), antilipemic (40), antimicrobial (41), antifungal (42), antihelminthic (43), anticancer (44), anti-inflammatory (45), bronchodilator, spasmolytic and calcium antagonist (46). *Nigella sativa* is well known for its terpenoids especially thymoquinone and its fatty acids (47). Thymoquinone being an anti-inflammatory and antioxidant helps with the periodontal disease as inflammation and release of reactive oxygen species are the main offenders of periodontal disease pathogenesis (48).

Recently, the use of therapeutic herbs for the treatment of several diseases has been growing due to its promising results and rare side effects and cost effectiveness (49). Periodontal treatment along with adjunctive mouthwashes, irrigations and others improves the patient's hygiene significantly (50). Our team has extensive knowledge and research experience that has translated into high quality publications (51–70). In this context, the aim of the present study was to evaluate the efficacy of *Nigella sativa* on gingival health.

## **MATERIALS AND METHODS**

### **Study population**

The present double-blinded, parallel designed randomized clinical trial was carried out in the Department of Periodontics, Saveetha Dental College and Hospitals, Chennai, India. A total of 30 patients with gingivitis within the age group of 18-28 years were enrolled. The ethical clearance was obtained from the Institutional Ethical Committee and a written informed consent was obtained from all the study participants.

### **Inclusion criteria**

Participants within the age group of 18-28 years who were systemically healthy, presence of at least 20 teeth, probing depth of 1-3 mm, presence of bleeding on probing (BOP) in at least 30% of the sites were included in the study.

### **Exclusion criteria**

Participants allergic to herbal extracts, smokers, pregnant or lactating mothers, participants under long term medications, systemically compromised patients were excluded from the study.

### **Test solutions**

Group 1: Chlorhexidine Mouthwash (Clohex Plus® mouthwash, Dr Reddy's Lab Ltd., Hyderabad, India).

Group 2: *Nigella sativa* mouthwash formulated under laboratory conditions

### **Study design**

A pilot study was conducted using similar herbal mouthwashes to check the feasibility of the study. The prevalence of gingivitis was 80% in the pilot study. Considering the dropouts, the sample size was inflated by 20%, hence the sample size was 30 with 15 participants in each group [Group 1 (CHX mouthwash), Group 2 (*Nigella sativa* mouthwash)]. Participants were assigned to the groups by a person not involved in the study. All the subjects were provided with their assigned mouthrinses and were divided into Group 1 and Group 2 randomly using a simple lottery method with 15 participants in each group. All the mouthrinses were dispensed in identical bottles thereby ensuring subject masking. The examiner and the participants were also blinded with regard to the mouthrinse allocated to them thereby ensuring a double-blinded study. Subjects were instructed to use 10 ml of mouthwash for 1 min twice daily after tooth brushing for a period of 2 weeks.

### **Preparation of the novel herbal mouthwash :**

Dry powder of *Nigella sativa* seeds were suspended in 10 times its quantity of sterile distilled water in a flask and was kept undisturbed for 72 h at 4°C. The aqueous extract thus obtained was decanted and clarified by filtration through double-layered muslin cloth. This solution was then transferred to a porcelain dish and let it evaporate at 40°C.

The dried remnant obtained was stored for making the mouthwash solution. 200 g of the powder was suspended in polyethylene glycol, and distilled water of 800 ml and was allowed to evaporate to get the final concentrate. The final concentrate was then diluted with sterile distilled water to make a mouthwash of 20% (w/v) concentration. Two tablespoons of honey and peppermint extract were added as a natural sweetener and flavoring agent, respectively.

### **Clinical parameters**

- Silness and Loe plaque index (PI)
- Loe and Silness gingival index (GI)

Before recording the clinical parameters at baseline, thorough scaling and root planing was carried out using ultrasonic scaler, patients were advised not to use any oral hygiene aids for the next three days. After three days patients were recalled and gingival and plaque indices were recorded which was the baseline data of the study. Patients were asked to use the mouthwash for two weeks and were recalled after 14 days and both indices were recorded.

### **Statistical analysis**

The data was analyzed using Statistical Package for Social Sciences (SPSS Software, Version 23.0). Descriptive and inferential statistics were done for data summarization and presentation. Unpaired t-test was used to compare the mean values of plaque index and gingival index between the groups. The data were analyzed and interpreted as graphs and tabulations.

### **RESULTS:**

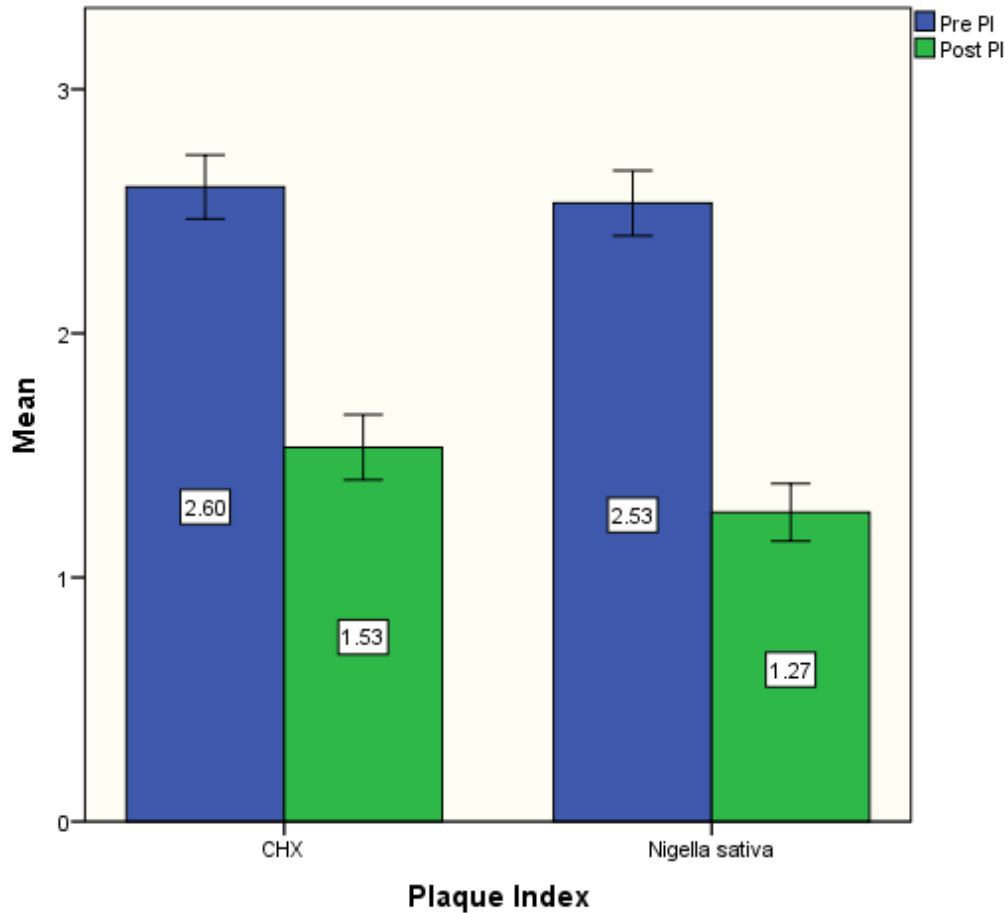
In this present study, a total of 30 gingivitis patients were enrolled for the study and were divided into two groups in which 15 were enrolled in group A (chlorhexidine mouthwash) and 15 were enrolled in group B (*Nigella sativa* mouthwash). Plaque index and gingival index of both the groups were compared at baseline and after 14 days.

In this present study we found that the plaque index had reduced in both standard chlorhexidine and *Nigella sativa* mouthwash. The mean plaque index present before mouthwash in the chlorhexidine group was  $2.6 \pm 0.49$  and in the *Nigella sativa* group was  $2.53 \pm 0.51$  before treatment. The mean plaque index which was recorded after the treatment of mouthwash was  $1.53 \pm 0.51$  in the chlorhexidine group and  $1.27 \pm 0.45$  in the *Nigella sativa* group. The plaque index values were found to be statistically non-significant ( $p=0.067$ ), unpaired t- test [Figure 1, Table 1]

Similarly we also found the gingival index of the study sample, which also had a reduction in both the groups. The gingival index which was present before the treatment was  $2.53 \pm 0.51$  in chlorhexidine group and  $2.47 \pm 0.51$  in the *Nigella sativa* group. The gingival index post treatment was noted to be  $1.33 \pm 0.45$  in chlorhexidine group and  $1.47 \pm 0.51$  in *Nigella sativa* group. The gingival index values were found to be statistically non-significant ( $p=0.393$ ), unpaired t-test [Figure 2, Table 1].

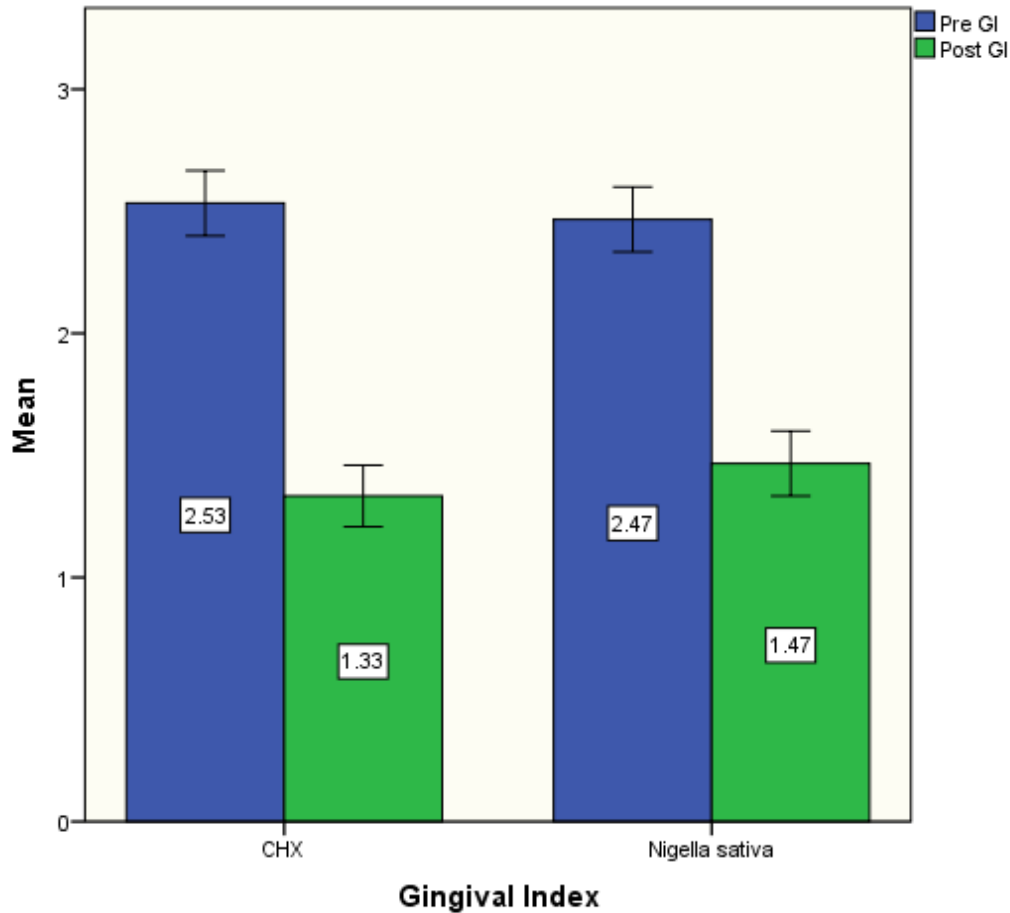
Table 1 - Comparison of mean plaque index and gingival index scores between the study groups using unpaired t test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig	t	df	Sig(2-tailed)	Mean difference	Std. Error difference	95% Confidence Interval of the Difference	
								Lower	Upper
Mean gingival index Equal variances assumed Equal variances not assumed	3.646	.067	4.49	28	.000	-.800	.178	-1.165	-.435
			4.49	27.603	.000	-.800	.178	-1.165	-.435
Mean plaque index Equal variances assumed Equal variances not assumed	.753	.393	1.00	28	.326	.133	.133	-.140	.406
			1.00	27.858	.326	.133	.133	-.140	.407



Error Bars: +/- 1 SE

Figure 1 : Comparison of difference between chlorhexidine and *Nigella sativa* mouthwash in terms of mean plaque index scores. The x-axis depicts the types of mouthwash used in this study, chlorhexidine and *Nigella sativa* mouthwash and the y-axis represents the mean plaque index value. Blue bar depicts the preoperative plaque index scores, green bar depicts the postoperative plaque index scores. The reduction in plaque index scores was higher in the *Nigella sativa* group when compared to the chlorhexidine group. The difference between both the groups were found to be statistically not significant,  $p=0.067$  (unpaired t-test).



Error Bars: +/- 1 SE

Figure 2 : Comparison of difference between chlorhexidine and *Nigella sativa* mouthwash in terms of gingival index scores. The x-axis depicts the types of mouthwash used in this study, chlorhexidine and *Nigella sativa* mouthwash and the y-axis represents the mean gingival index value. Blue bar depicts the preoperative gingival index scores and the green bar depicts the postoperative gingival index scores. The reduction in gingival index scores was higher in the chlorhexidine group when compared to the *Nigella sativa* group. The difference between both the groups were found to be statistically not significant,  $p=0.393$  (unpaired t-test).

## DISCUSSION:

The present study was done to assess the efficacy of *Nigella sativa* on gingival health.

In this present study, we observed that the plaque index decreased in both groups. Khairnar et al found that the herbal mouthwash had a significant plaque control when compared to the standard chlorhexidine (71). A study by Balappanavar found that there was no difference of efficiency between herbal mouthwash and chlorhexidine for plaque control (72). Another study done on green tea also suggests that the herbal mouthwash helps in reducing plaque as effective as chlorhexidine mouthwash (73).

The present study also observed the gingival index reduced in both the groups post usage of the mouthwash. Al-Wafi et al found that the role of thymoquinone present in *Nigella sativa* reduced the gingival inflammation in rat models (74). Mahyari et al found that there was a significant improvement in gingival index from baseline to the end of the trial with herbal mouthwash and chlorhexidine (75). Another study by Cortelli found that there was significant reduction in gingival index post baseline time points in the case of herbal mouthrinse and that it was significantly superior when compared with cetylpyridinium chloride as standard control (76). An in vivo study also found that the herbal mouthrinse group provided the results of striking decrease in both gingival index and plaque index which was similar to our study (77).

It is found that the *Nigella sativa* mouthwash like other herbal mouthwashes are effective in reducing plaque and gingivitis. But there was no difference between the herbal and chlorhexidine mouthwashes. The natural botanical products provide a promising result in treatment of gingivitis. The exact mechanism behind the antiplaque and anti-gingival activities of it are not well known. It could be because of its components like thymoquinone or others like tannins, sterols or oils. Further molecular and clinical studies are needed to investigate the mechanism of action of *Nigella sativa* constituents, especially thymoquinone and to clarify the biomechanics behind its antiplaque and antigingivitis effect.

There are certain limitations to this present study. The duration of the study was only for two weeks and therefore the results of it cannot be generalized for a longer duration of the study.

There was a geographic limitation which had more of the South Indian population. The sample size and the duration of the study can be expanded for better results.

For future scope of the research larger sample size and inclusion of different ethnicity will provide better results. Longitudinal or periodic studies can be done to evaluate the efficiency of Nigella mouthwash on the gingival health.

## **CONCLUSION:**

The present study reveals that the *Nigella sativa* mouthwash was more effective in plaque control when compared to the standard chlorhexidine mouthwash(78) (79) (80) (81) (82) (83) (84) (85) (86) (87) (88) ((88,89) (90) (91). Herbal mouthwash shows promising results to be used as an alternative for chlorhexidine mouthwash for management of gingivitis.

## **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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