



NIGELLA SATIVA: A HERBAL BONE GRAFT

Periodontology

Dr. Ankita Deo	Post graduate student, Department of Periodontics and Implantology Hitkarini Dental College and Hospital,
Dr. K.T Chandrashekar*	Professor and H.O.D, Department of Periodontics and Implantology Hitkarini Dental College and Hospital,*Corresponding Author
Dr. Rohit Mishra	Professor, Department of Periodontics and Implantology Hitkarini Dental College and Hospital,
Dr. Vandana Tripathi	Reader, Department of Periodontics and Implantology Hitkarini Dental College and Hospital,
Dr. Shubhda Malhotra	Post graduate student, Department of Periodontics and Implantology Hitkarini Dental College and Hospital,
Dr. Blessy Shin Sabu	Post graduate student Department of Periodontics and Implantology Hitkarini Dental College and Hospital,

ABSTRACT

Aim- The aim of the study to assess the ability of Nigella sativa seed extract in the treatment of periodontal intrabony defects by clinical and radiographical analysis. **Methodology-** Twenty patients with intrabony defects were divided into two groups. Group I patients received open flap debridement only whereas group II patient underwent open flap debridement along with placement of Nigella sativa seed extract into the intrabony defect. Gingival index, plaque index, probing pocket depth, clinical attachment level and Radiographs were taken preoperatively and six months postoperatively. **Result-** The results indicated that Nigella sativa seed extract produced favourable changes in terms of soft and hard tissue which were assessed both clinically and radiographically. **Conclusion-** This clinical study concluded that the Nigella sativa seed extract can be used as a herbal bone formative material in periodontal intrabony defects.

KEYWORDS

Nigella sativa, intrabony defects, herbal bonegraft.

INTRODUCTION

The aim of periodontal therapy is to maintain health and integrity of dentition for welfare of the patient. Periodontal therapy involves elimination of pathogenic periodontal microflora, leading to favourable clinical changes in periodontium thus causing elimination of infection.¹

Regeneration is defined as the biologic method by which the structural and function performance of lost tissues are fully restored.² An perfect bone graft substitute ought to be osteogenic, osteoinductive, osteoconductive, it should be nontoxic, non-antigenic it doesn't cause infection, root resorption or ankylosis, strong and resilient, easily adaptable, ready and sufficiently available.^{3,4}

However, today's world seeking ways to replace the synthetic drugs with the therapeutic power of natural products to decrease many side effects which result from conventional treatment.⁵

Herbal medications turned out to be a popular form of therapy throughout the world when used in prophylaxis and treatment of various oral diseases, for example Neem twigs can be used as tooth brush and its extract as a mouthwash. Turmeric has anti-microbial, anti-oxidant, anti-inflammatory astringent, anti-septic and analgesic properties due to which it used in treatment of oral ulcers.⁶

Traditional herbal formulas have been developed to promote bone healing in fractures. Many wonderful medicinal plants had been introduced one of which is Nigella sativa. Due to its extensive use, Nigella Sativa became a seed of blessing. Due to its anti-microbial effect, anti-cancer activity, gastroprotective antioxidant activity, immunomodulatory, anti-inflammatory, anti-tumour effects, anti-anxiety effect anti-asthmatic effect, anti-inflammatory effects, it was said that "black seed can cure every disease except death".^{7,8}

The purpose of this prospective, randomized controlled clinical trial is to evaluate the bone forming ability of Nigella sativa seed extract in the treatment of periodontal intrabony defects by clinical and

radiographical analysis.

Materials and methods

Study design- Prospective randomised controlled trial.

Source of data and patient selection- The study population of twenty subjects in 30-55 years of age, with chronic periodontitis and presence of intrabony defects were selected randomly from the outpatient section, Department of Periodontics and Implantology, Hitkarini Dental College and Hospital, Jabalpur, Madhya Pradesh, India. The present study was presented and approved by the ethical committee of Hitkarini Dental College and Hospital, Jabalpur (India). A written consent explaining the character of the study and surgical treatment was signed by all the collaborating patients.

Inclusion criteria:

1. Patients diagnosed as having chronic periodontitis with periodontal pockets of probing depth ≥ 5 mm with radiographic evidence of vertical bone loss.
2. Patients with no history of systemic disease or medication.
3. Patients with good oral hygiene.

Exclusion criteria:

1. Smokers
2. Pregnant women and lactating mothers.

Method of Preparation of Nigella sativa seed extract

Nigella sativa seeds were purchased from local market and supplied to Smyan laboratory (Pansila m'impact), where seeds were washed and dried in shade this procedure known as evaporation to dryness, these seeds were pulverized using pulverizer and sterilized using ethylene vapors in ethylene vapor sterilizer then stored in air tight sterile vials. The resulting Nigella sativa seed extract used as a bone fill material in periodontal intrabony defects.



Figure 1: (a) Nigella Sativa flower, (b) Nigella Sativa fruit, (c) Nigella Sativa seeds, (d) Nigella sativa seed extract

Surgical phase

Pre Surgical Protocol

After an initial examination and treatment planning discussion, all the selected patients underwent Etiotropic phase with oral hygiene instructions. All the patients were subjected to routine blood examination that included hemoglobin, bleeding time, clotting time, total leucocyte count, differential leucocyte count and random blood sugar. ELISA test for HIV and Hepatitis screening test was also done prior to surgical procedure.

Surgical protocol

The selected sites were randomly assigned to either control group or experimental group. Following adequate local anesthesia, crevicular incisions were given, full thickness mucoperiosteal flap reflection and the defect site was exposed. The defects were debrided followed by thorough root planing and irrigation with normal saline. Using an acrylic stent and UNC 15 probe defect depth was measured. Control group site defects were subjected to open flap debridement. The operative site was closed with 3-0 black braided silk sutures and protected with a periodontal dressing.

Experimental group site defect was filled with nigella sativa extract after open flap debridement. The mucoperiosteal flaps were repositioned, black braided (3-0) interrupted silk sutures were placed to obtain primary closure and periodontal dressing was placed.



Figure 1-Experimental group(a,b)Pre-op measurement withUNC-15 probe and stent, (c,d)Defect Depth from CEJ to base of defect, (e)Placement of Nigella sativa seed extract, (f)Suture placed, (g)Periodontal Dressing placed,(h,i)Six months post-op measurement with UNC-15 probe and stent, (j) Radiographic measurement at baseline, (k)Radiographic measurement six month follow up.

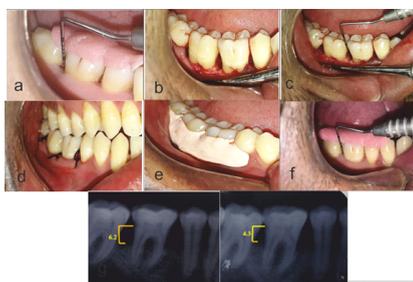


Figure 1-Control group(a)Pre-op measurement withUNC-15 probe and stent, (b) Flap reflection and debridement (c)Defect Depth from

CEJ to base of defect, (d)Suture placed, (e)Periodontal Dressing placed, (f)Six months' post-op measurement with UNC-15 probe and stent, (g) Radiographic measurement at baseline, (h)Radiographic measurement six month follow up.

Maintenance phase

1. After seven days
 - Removal of Periodontal dressing and sutures and irrigation with saline.Reinforcement of oral hygiene instruction.
2. After three months
 - Recording of clinical parameters (plaque index, gingival index, probing depth, relative attachment level).
 - Reinforcement of oral hygiene instructions.
3. After six months
 - Recording of clinical parameters (plaque index, gingival index, probing depth, relative attachment level).
 - Reinforcement of oral hygiene instructions.

Results

Plaque Index

Mean ± SD of plaque index scores at baseline, 3rd month and 6th month in Control and Experimental group were 1.47 ± 0.34 and 1.41 ± 0.24, 0.84 ± 0.30 and 0.80 ± 0.34, 0.74 ± 0.24 and 0.71 ± 0.24 respectively. Unpaired t-test showed no significant difference between Control group and Experimental group for plaque index scores.

Table 1: Comparison of Plaque index scores between Control group and Experimental group at different time intervals.

Time intervals	Groups	Plaque index scores		Unpaired t-test
		Mean ± SD	Min-Max	
Baseline	Control group	1.47 ± 0.34	1.00-2.20	t = 0.451, P = 0.657 (>0.05), Not sig.
	Experimental group	1.41 ± 0.24	1.20-2.00	
At 3rd month	Control group	0.84 ± 0.30	0.60-1.50	t = 0.278, P = 0.784 (>0.05), Not sig.
	Experimental group	0.80 ± 0.34	0.50-1.40	
At 6th month	Control group	0.74 ± 0.24	0.40-1.00	t = 0.283, P = 0.781 (>0.05), Not sig.
	Experimental group	0.71 ± 0.24	0.50-1.00	

Gingival Index

Mean ± SD of gingival index scores at baseline, 3rd month and 6th month in Control and Experimental group were 1.29 ± 0.32 and 1.35 ± 0.20, 0.88 ± 0.24 and 0.81 ± 0.26, 0.77 ± 0.21 and 0.71 ± 0.23 respectively. Unpaired t-test showed no significant difference between Control and Experimental group for gingival index scores.

Table 2: Comparison of gingival index scores between Control group and Experimental group at different time intervals

Time intervals	Groups	Gingival index scores		Unpaired t-test
		Mean ± SD	Min-Max	
Baseline	Control group	1.29 ± 0.32	1.00-1.80	t = -0.497, P = 0.626 (>0.05), Not sig.
	Experimental group	1.35 ± 0.20	1.20-1.70	
At 3rd month	Control group	0.88 ± 0.24	0.60-1.20	t = 0.621, P = 0.543 (>0.05), Not sig.
	Experimental group	0.81 ± 0.26	0.50-1.20	
At 6th month	Control group	0.77 ± 0.21	0.60-1.10	t = 0.610, P = 0.549 (>0.05), Not sig.
	Experimental group	0.71 ± 0.23	0.50-1.00	

Probing Pocket Depth

Mean ± SD of probing pocket depth at baseline, 3rd month, 6th month in Control and Experimental group were 7.90 ± 1.37 mm and 8.70 ± 1.16 mm, 6.20 ± 0.92 mm and 5.40 ± 1.08 mm, 5.70 ± 0.48 mm and 3.60 ± 0.70 mm respectively. Unpaired t-test showed no significant difference between Control and Experimental group at baseline and 3rd month. Whereas 6th month mean probing pocket depth in control group was significantly higher than experimental group.

Table 3: Comparison of Probing pocket depth in Control group and Experimental group at different time intervals.

Time intervals	Groups	Probing pocket depth (mm)		Unpaired t-test
		Mean ± SD	Min-Max	
Baseline	Control group	7.90 ± 1.37	6.00-10.00	t = -1.409, P = 0.176 (>0.05), Not sig.
	Experimental group	8.70 ± 1.16	7.00-10.00	
At 3rd month	Control group	6.20 ± 0.92	5.00-8.00	t = 1.789, P = 0.090 (>0.05), Not sig.
	Experimental group	5.40 ± 1.08	4.00-7.00	
At 6th month	Control group	5.70 ± 0.48	5.00-6.00	t = 7.814, P = 0.000 (<0.001), Very high sig.
	Experimental group	3.60 ± 0.70	3.00-5.00	

Relative Attachment Level

Mean ± SD of relative attachment levels at baseline, 3rd month and 6th month in Control and Experimental group were 12.90 ± 1.79 mm and 13.70 ± 1.16 mm, 11.20 ± 2.25 mm and 10.50 ± 1.18 mm, 10.80 ± 1.14 mm and 8.90 ± 0.88 mm respectively. Unpaired t-test showed no significant difference between Control group and Experimental group for relative attachment levels at baseline and 3rd month. Whereas 6th month mean relative attachment level in Control group was significantly higher than Experimental group

Table 4: Comparison of relative attachment levels in Control group and Experimental group at different time intervals.

Time intervals	Groups	Relative attachment levels (mm)		Unpaired t-test
		Mean ± SD	Min-Max	
Baseline	Control group	12.90 ± 1.79	10.00-15.00	t = -1.185, P = 0.251 (>0.05), Not sig.
	Experimental group	13.70 ± 1.16	12.00-15.00	
At 3rd month	Control group	11.20 ± 2.25	06.00-13.00	t = 0.871, P = 0.395 (>0.05), Not sig.
	Experimental group	10.50 ± 1.18	09.00-12.00	
At 6th month	Control group	10.80 ± 1.14	09.00-12.00	t = 4.191, P = 0.001 (<0.01), Highly sig.
	Experimental group	8.90 ± 0.88	08.00-10.00	

Radiographic assessment depth of defect

Mean ± SD of depth of defect at baseline and 6th month in Control and Experimental group were 6.28 ± 0.64 mm and 6.01 ± 0.77 mm, 5.14 ± 0.78 mm and 3.15 ± 0.57 mm. Unpaired t-test showed no significant difference between Control group and Experimental group for depth of defect at baseline. Whereas at 6th month depth of defect in control group was significantly higher than in experimental group.

Table 5: Comparison of radiographic assessment depth of defect in Control group and Experimental group at different time intervals.

Time intervals	Groups	Depth of defect (mm)		Unpaired t-test
		Mean ± SD	Min-Max	
Baseline	Control group	6.28 ± 0.64	5.26-7.52	t = 0.874, P = 0.393 (>0.05), Not sig.
	Experimental group	6.01 ± 0.77	4.42-6.84	
At 6th month	Control group	5.14 ± 0.78	4.30-6.56	t = 6.506, P = 0.000 (<0.001), Very high significant
	Experimental group	3.15 ± 0.57	2.36-3.78	

Percentage Change In Depth Of Defect

Percentage change in depth of defect from baseline to 6th month between Control and Experimental group. Percentage change in depth of defect in Control and Experimental group were 18% and 47%. Unpaired t-test showed that percentage change in depth of defect in Experimental group was significantly higher than Control group.

Table 6: Comparison of percentage change in depth of defect (from baseline to 6th month) between Control group and Experimental group.

Groups	Percentage change in depth of defect	
	Mean ± SD	Min-Max
Control group	18.12 ± 9.03	10.76-32.18

Experimental group	46.91 ± 10.81	32.47-64.05
Unpaired t-test	t = -6.466, P = 0.000 (<0.001), Very High sig.	

DISCUSSION

The present clinical study indicated that Nigella sativa seed extract produced favourable results in terms of soft and hard tissue changes which were assessed both clinically and radiographically.

The seeds of Nigella sativa showed richness and diversity in its chemical composition. Carbohydrates, proteins, amino acids, volatile and fixed oils are contained in the seeds. Thymoquinone proved to be the main active constituent of the volatile oil.

NgS acts as bioactive and bioinductive materials that enhance bone formation. This could be due to following components: Amino acids which are present in Nigella sativa seed oil contributes in formation of proteins like Osteocalcin, Bone Sialoprotein (Bsp) and Osteopontin.^{9,10}

Osteocalcin: conjointly referred to as bone Gla protein is one among the most plentiful non collagenous proteins (NCPs) in bone. It binds with calcium present in hydroxyapatite (HA) and thus it involves in bone growth & repair.^{11,12,13}

Bone Sialoprotein (Bsp): comprises 15 % of the total non-collagenous protein (NCP) in bone. Bone Sialoprotein helps in initiation of mineral crystal formation.

Osteopontin: One of the important NCPs that induce cell adhesion and binding of mineral.^{14,15}

To the best of our knowledge, no other clinical study has been reported using Nigella sativa seed extract in the treatment of periodontal osseous defects and this is the first clinical study done on Nigella sativa seed extract as a herbal bone regenerative material.

CONCLUSION

In this study, we demonstrated that Nigella sativa seed extract has potential effects on the bone cells. To the best of our knowledge, no other clinical study has been reported using Nigella sativa seed extract in the treatment of periodontal osseous defects and this is the first clinical study. We can conclude that Nigella sativa seed extract can be used as a herbal bone formative material in periodontal intrabony defects.

REFERENCES

- Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., ... & Greenwell, H. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of periodontology*, 89, S173-S182 Yang H, Wen Q, Xue J, Ding Y. Alveolar bone regeneration potential of a traditional Chinese medicine, B u-S hen-G u-C hi-W an, in experimental periodontitis. *Journal of periodontal research*. 2014 Jun;49(3):382-9.
- Janicki, P., & Schmidmaier, G. (2011). What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells. *Injury*, 42, S77-S81.
- Jangid MR, Rakhewar PS, Nayyar AS, Cholepatil A, Chhabra MP (2016). Bone Grafts in Periodontal Regeneration: Factors Impacting Treatment Outcome. *Int. J. Curr. Res. Med. Sci.* 2(7):20-4
- Al-Hijazi, A. Y. (2013). Evaluation of the effect of Nigella Sativa oil and powder on socket healing process. *Evaluation*, 3(11).
- Pandita, V., Patthi, B., Singla, A., Singh, S., Malhi, R., & Vashishtha, V. (2014). Dentistry meets nature-role of herbs in periodontal care: A systematic review. *Journal of Indian Association of Public Health Dentistry*, 12(3), 148.
- jaz, H., Tulain, U. R., Qureshi, J., Danish, Z., Musayab, S., Akhtar, M. F., ... & Khan, I. (2017). Nigella sativa (Phrothetic Medicine): A Review. *Pakistan journal of pharmaceutical sciences*, 30(1).
- Randhawa, M. A., & Al-Ghamdi, M. S. (2002). A review of the pharmaco-therapeutic effects of Nigella sativa. *Pak J Med Res*, 41(2), 77-83.
- Al-Jassir, M. S. (1992). Chemical composition and microflora of black cumim (Nigella sativa L.) seeds growing in Saudi Arabia. *Food Chemistry*, 45(4), 239-242.
- Gilani A, Jabeen Q, Ullakhhan M (2004). A review of medicinal uses and pharmacological activities of Nigella sativa. *Pakistan Journal of Biological Sciences* ,7(4): 441-451.
- Hing KA (2004). Bone repair in the twenty-first century: biology, chemistry or engineering? *Phil Trans R Soc Lond*, 362: 2821 - 2850.
- Stanford CM, Keller JC, Solursh M (1994). Bone cell expression on titanium surfaces is altered by sterilization treatments. *J Dent Res*. 73 (5): 1061 - 1071.
- Nagai M and Ota M. (1994) Pulsating electromagnetic field stimulates mRNA expression of bone morphogenetic protein-2 and -4. *J Dent Res* 73 (10): 1601 - 1605.
- Puleo DA, Nanci A.(1999) Understanding and controlling the bone - implant interface. *Biomaterials* 20, 2311- 2321.
- Alt V, Bitschnau A, Osterling J, Sewing A, Meyer C, Kraus R, Meissners et al.(2006) The effect of combined gentamicin- hydroxyapatite coating for cementless joint prostheses on the reduction of infection rates in a rabbit infection prophylaxis model. *Biomaterials* 27: 4627-4634.
- Bernhardt R, Vanden Dolder J, Bierbaum S, Beutner R, Schamweber D, Jansen J et al.(2005) osteoconductive modifications of Ti - implants in a goat defect model : characterization of bone growth with SR mu CT and histology. *Biomaterials* 26: 2009 - 2019