International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

A Histological Evaluation Of Nigella Sativa As A Direct Pulp Capping Material (An In-Vivo Study)

Research Article

Ebrahim Faour[1], Mohannad Laflouf[2], Ahmad Manadili[3], Abdullah Ateek[4], Muaaz Alkhouli[1*], Zuhair Al-Nerabieh[1]

[1] MSc in pediatric dentistry, Faculty of Dentistry, Damascus University, Syria.
[2] Professor in pediatric dentistry, Faculty of Dentistry, Damascus University, Syria.
[3] Professor in oral pathology, Faculty of Dentistry, Damascus University, Syria.
[4] MSc in oral and maxillofacial surgery, Faculty of Dentistry, Damascus University, Syria.

Abstract

Objective: The aim of this study is to evaluate the histological pulp response following direct pulp capping with Nigella Sativa paste (NS)in comparison to calcium hydroxide(CH) in rabbits' teeth.
Methodology: 15-New Zealand rabbits were selected in this study and divided into 3 groups according to pulp capping period (two days, two and four weeks). calcium hydroxide and Nigella Sativa paste were used for capping upper and lower central incisors (4 incisors). The teeth were restored by glass ionomer cement (GIC) as permanent restorations . After that animal were scarificed and teeth were dissected and prepared for histological evaluation using Hematoxylin Eosin (HE) stains.
Results: The results showed that NS caused statistically significantly less severe inflammatory reactions than CH at all-time intervals. Regarding the hard tissue formation, NS showed a statistically significantly thicker formation after a two-week period, while after4 weeks period, all Nigella sativa samples showedhard tissue formation thicker than CH samples but not statistically significant. There were statistically significant differences regarding tissue organization after a period of one and two weeks between NS group and CH group, but after four weeks there were no statistically significant differences.
Conclusions: Nigella Sativa paste can be as a direct pulp capping material as it led to a faster hard tissue formation than calcium hydroxide with less inflammation.

Keywords: Pulp Capping; Nigella Sativa Extract; Calcium Hydroxide; Rabbit's Teeth.

Introduction

Pulpal vitality is critical to the maintenance of the structural integrity and normal physiological function of teeth [1].

Studies demonstrated that exposed pulps possess an inherent capacity for healing through cell reorganization and bridge formation when a proper biologic seal is provided and maintained against leakage of oral contaminants [2, 3].

Direct pulp capping is a conservative therapy frequently performed for preserving pulp vitality when the pulp is exposed during dental treatment. When the pulp tissue is properly protected and preserved with a capping material, the wound may heal uneventfully [4]. An ideal direct pulp-capping material should control infection, preserve the vitality of the pulp tissue, stimulate the repair process, adhere to the dentin tightly without permitting leakage, and promote the formation of a hard tissue barrier and a dentin bridge [5, 6]. Calcium hydroxide has been a material of choice for direct pulp capping for several years because it inhibits bacterial growth and exhibits reparative properties [7, 8]. However, it has several drawbacks, such as the porosity of its induced hard tissue, its inferior adherence to dentin, and the microleakage produced from its decomposition [9, 10].

Many materials have been used to cover the exposed pulp, and have been extensively histologically and clinically studied and have achieved different success rates, such as zinc oxide eugenol (ZoE) [11], glass ionomer cement (GIC)[12], adhesive systems [13], calcium hydroxide [14], mineral trioxide aggregate (MTA)[15], Biodentine [16] and calcium-enriched mixture cement CEM [17].

***Corresponding Author**:
Muaaz Alkhouli,
MSc in pediatric dentistry, Faculty of Dentistry, Damascus University, Syria.
E-mail: Muaaz.alkhouli@outlook.com

Received: June 24, 2021
Accepted: July 06, 2021
Published: July 19, 2021

Citation: Ebrahim Faour, Mohannad Laflouf, Ahmad Manadili, Abdullah Ateek, Muaaz Alkhouli, Zuhair Al-Nerabieh. A Histological Evaluation Of Nigella Sativa As A Direct Pulp Capping Material (An In-Vivo Study) *Int J Dentistry Oral Sci.* 2021;8(7):3393-3401. doi: http://dx.doi.org/10.19070/2377-8075-21000690

Over the past decade, herbal medicine has become a topic of increasing global importance. A larger number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [18].

Nigella sativa (NS) shows very valuable biomedical properties such as antioxidant, antimicrobial, anticancer, anti-inflammatory [19].

Nigella sativa is an annual flowering plant in the family Ranunculaceae also called black cumin, black seed, or Habbatul Barakah is native to the south and southwest Asia, and is cultivated in several countries in the Mediterranean region, South Europe, Syria, Turkey, and Saudi Arabia [20, 21].

Researchers have attributed the health promoting benefits of the black seed to its active components and high nutritional content.3 The seeds are composed of 28-36% fixed oils, proteins, alkaloids and saponins, and 0.4-2.5% essential oils. Many pharmacologically active compounds have been isolated from black seeds, but the most reported active constituents are thymoquinone (TQ), dithymoquinone, thymol, and thymohydroquinone [22, 23].

Abd-Awn et al [24] conducted a research using an agar diffusion test followed by a minimum bactericidal concentration (MBC) determination to test the NS oil extract's sensitivity to S. mutans and its ability to inhibit bacterial adherence to the dental plaque compared with chlorhexidine gluconate. The results showed that the black seed oil extract has 10% MBC against S. mutans [24].

In the rat model proposed by Al-Wafi et al, [25] besides the anticaries assessment, an evaluation of the potential preventive role of TQ on gingival inflammation was conducted. The results revealed that rats treated with TQ in drinking water or an oral gel had statistically significant lower periodontal indices and subgingival bacterial counts in comparison with both the negative and positive control groups. Additionally, their mandibular tissues, which were taken for histological examination to determine the degree of inflammation, demonstrated no signs of inflammation compared with the controls.

Another study [18] searched for a new capping medicament in pediatric dentistry to replace formocresol because of its reported side effects. The study compared the histopathological pulp response to NS oil and formocresol (FC) in dogs. The results showed that NS specimens histologically revealed mild to moderate vasodilatation with few inflammatory cells and a continuous odontoblastic layer. On the other hand, FC specimens showed advanced inflammation with severe vasodilatation and inflammatory cell infiltration and degeneration. Thus, application of NS maintained the vitality of the pulp, which makes it a good pulpotomy agent in clinical practice [18].

Al-Douri and Al-Kazaz(26) carried out an experiment on 12 rabbits. The authors created the ulcers with 0.3 ml of 1% formalin injections in the cheek mucosa of the rabbits followed by topical application of NS twice a day for 3 days. The animals were sacrificed on the fifth day, and their cheek mucosae were histologically examined. The results showed a significant healing process enhancement with NS treatment, and a marked anti-inflammatory activity and differences in the rate of epithelization between the NS and control groups [26].

Therefore, the aim of this study was to evaluate the histological response of a healthy rabbit pulp to direct pulp capping with Nigella sativa compared to calcium hydroxide. The null hypothesis was a lack of difference in the response of the pulp tissue between the two direct pulp capping material.

Materials and Method

Selection Of The Experimental Animals

Fifteen adult males of New Zealand white rabbits weighed about 2 Kg were selected. Females were excluded due to periodic hormonal reasons and the possibility of pregnancy during the study period.

The animals were individually housed in animals incubator and maintained under clean housing conditions and fed it.They were controlled diet and received daily care in the animal house of Faculty of Veterinary Medicine, Hama University.

The necessary medical examinations were carried out by the specialized veterinarian to ensure the integrity of these animals, and that they are free from any current medical condition that may negatively affect the course of the experimental stages.

Ethical Regulation

The research was approved by the research ethics committee, Faculty of Dentistry, Damascus University.

Sample Size

The sample size calculation was calculated using G*Power 3.1.9 (Franz Faul, Universität Kiel), 0.05 significance level, and 80% statistical power and effect size 0.80 . It was estimated that 21 teeth in each group (CH , NS) were required, yet the sample size was raised to 30 teeth in each group.

Distribution of study groups

Fifteen rabbits was randomly subdivided into three subgroups (5 rabbits each), according to the pulp capping period (two days ,two and four weeks). Where teeth capped with calcium hydroxide for two days in the subgroup (C.T1), capped with calcium hydroxide for two weeks in the subgroup (C.T2) and capped with calcium hydroxide for four weeks in the subgroup (C.T3). Meanwhile in (N.T1) they were capped with Nagilla sativa for two days, (N.T2) capped with Nagilla sativa for two weeks and capped with Nagilla sativa for four weeks in the subgroup (N.T3).

Two upper and two lower central incisors were utilized in each rabbit. Split mouth technique was utilized where the two tested materials were used in both sides of the mouth in the same animal. One central incisor was capped with calcium hydroxide and the other with Nagilla sativa.

Capping Materials

1. Calcium Hydroxide (Urbical, ProMedica, Germany).
2. Nigella Sativa Oil (Herb Pharm; Nigella Sativa oil Liquid Ex-

tract; 1 fl oz; 30 ml) is a commercial product obtained from the American iHerb website through online purchase.

Preparing the animal of the experiment

Each rabbit was anaesthetized through administration of 5 mg/kg of xylazine solution (Xyla, Interchemie Holland) intra muscular in the quadriceps femorais muscle using needle with an appropriate gauge and After five minutes, the rabbit is given a muscle injection of ketamine hydrochloride 30 mg/kg (Elsaad pharma, Syria) in the muscle of the other extreme.

Experimental procedures

The working field was disinfected by 0.2% chlorhexidine solutionand dried by cotton rolls.Then Class V cavity preparation was done in the gingival third of the labial surface of each toothof permanent central incisors in a standardized protocol. A class V cavity with the dimension of 1.5 mm × 2 mm wasprepared using a sterilepear-shaped diamond bur (0.10 ISO standards). After cavitypreparation, the pulp horn was mechanically exposed approximately 1 mm in diameter in the middle of the pulpal floor by drilling with a sterilehigh-speed diamond bur (0.10 ISO standards) under copioussterile water irrigation.Proper hemostasis was achieved through controlling of bleeding by pellets of cotton moistured with sterile saline with gentle pressure. For avoidance of cross contamination each cavity was prepared by a sterile bur. The calcium hydroxide was mixed according the manufacturer' instructions and applied on exposure sites in the One central incisor using Liner Placement Instrument (caulk, Dentsply) and the other with nagilla sativa oil that it was mixed with zinc oxide to a thick consistency on a glass pad with the aid of spatula, the mix was placed on the exposure site by means of small ball burnisher. Then cavities were sealed with glass ionomer cement (Medifil, ProMedica, Germany).

Animal Care

After completion of dental procedure, the animals were taken care of according to the protocol of Canadian Council on Animal Care and in coherence with the Three Rs (replacement, reduction, reinforcement) of animal ethics [27].

Animal scarification

The rabbits were scarified after 2 dayes and 2, 4weeks. Once the rabbits were sacrificed, the teeth and the surroundingalveolar bone were dissected en bloc and prepared for histopathologic evaluation using Hematoxylin Eosin (HE) stains.

Histological evaluation & procedures (Passcoe and Gatehouse, 1986)

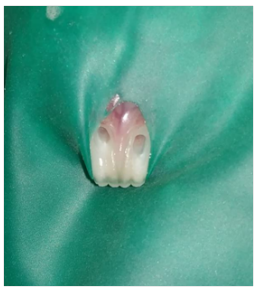
After rabbit scarification teeth were put immediately in fixative 10% formalin for 48- 72 hours. the specimens were washed under running tap water to remove the excess of the fixative. Decalcifications of the specimens were carried out using 10% Nitric acid (HNO3) for 3 dayes. Teeth were cut longitudinally through the pulp in a labial-palatal plane. The samples were then washed with a continuous water stream for 24h. Water was removed from the tissue gradually by putting it in ascending grades of Ethyl alcohol; 50%, 70%, and 90% then in absolute alcohol and finally with xylene . When xylene was completely replaced the alcohol in the tissue, the specimens became clear, they were embedded in dish filled with melted paraffin then removed from the dishes with a warm forceps and placed in the a box of melted hard paraffin, the bottom of which was the surface of cutting. Tissues were cut into sections of 5um thickness, and then stained with Haematoxylin and eosin (H&E) and examined by optical microscopy (Olympus, Tokyo, Japan) at 10x, 40x and 100x magnifications [33]. The sections were blindly evaluated by experienced pathologists and calibrated according to the criteria described in tables . Evaluation criteria for inflammatory cell response are given in Table 1, for tissue disorganization in Table 2 and for Dentine bridge formation in Table 3.

Statistical analysis

The criteria for each specimen were determined and the results were submitted to statistical analysis, using the software Statisti-

Figure 1. Nigella Sativa Oil.

Figure 2. Class V cavity preparation.



cal Packages for Social Sciences (SPSS version 20). Data in the present study assumed non-parametric distribution. Level of significance was set at a P value of (P≤0.05). The inflammatory response, tissue disorganization and hard tissue formation scores were subjected to non-parametric Kruskal-Wallis test to detect the significant differences among the groups and the Mann Whitney U test was used for two-by-two comparisons.

Results

After Pulp Capping For Two Days

70% of specimens of calcium hydroxidesubgroup (C.T1) showed-moderate inflammatory cell infiltration(score 2)(▶Fig. 10), while 30% of specimens showedslight inflammatory cell infiltration(score 1) and demonstrated slight pulp tissue disorganization at the exposure site. As regard to Nigella Sativa group (N.T1) 100% of specimens showed Slight inflammatory cell infiltration (score: 1)(▶Fig. 9) and just 50% of specimens demonstrated slight pulp tissue disorganization at the exposure site.

For this period both of (C.T1) and (N.T1) subgroups in all specimens showed Absence of hard tissue deposition (score 0).

After Pulp Capping For Two Weeks

60% of specimens calcium hydroxide subgroup (C.T2)showed slight inflammatory cell infiltration (score: 1)and 40% of specimensdemonstrated moderate pulp tissue disorganization at the exposure site(score: 1). Meanwhile, 60% of specimens in the Nigella Sativa subgroup (N.T2) showed absence of inflammatory cells(score: 0)(▶Fig. 11) while the 40% of specimens showed Slight inflammatory cell infiltration (score 1)and all specimenss-howednormal tissue morphology.

Table 1. Evaluation criterias for the inflammatory response [28].

Grade	Criteria
0	A few or no scattered inflammatory cells present at the pulp exposure site or below the hard-barrier formation
1	Slight inflammatory cell infiltration with polymorphonuclear or mononuclear leukocytes below the pulp exposure site but limited to the coronal portion of the pulp.
2	moderate inflammatory cell infiltration below the exposure site and extended to the middle of the pulp.
3	severe inflammatory cell infiltration involving the whole pulp
4	Pulp necrosis

Table 2. Evaluation criterias for tissue disorganization [29].

Grade	Criteria
0	Normal or almost normal tissue morphology below the pulp exposure site
1	Slight disorganization immediately below the pulp exposure site or adjacent to the hard-tissue formation(such asodontoblast-like cells, odontoblasts, and pulp tissue pattern disorganization or odontoblast hyperactivity) but central pulp normal
2	Total disorganization of the pulp tissue morphology
3	Pulp necrosis

Table 3. Evaluation criterias for hard tissue formation; dentin bridge [30].

Grade	Criteria
0	Absence of hard tissue deposition
1	Mild hard tissue deposition beneath the exposed area or partially formed hard tissue
2	Moderate hard tissue deposition beneath the exposed area (the exposed area is not completely closed)
3	Heavy hard tissue deposition beneath the exposed area

Table 4. Mean ranks of different subgroups and results of Mann-Whiteny for the comparison between inflammatory response ,Bridge Formation and Tissue Disorganization in the two groups at (2 days, 2 weeks, 4 weeks).

		Inflammation			Tissue Disorganization			hard tissue formation		
		2 Days	2 weeks	4 weeks	2 Days	2 weeks	4 weeks	2 days	2 weeks	4 weeks
Mean ranks	Calcium Hydroxide	14.00a	14.30a	12.00a	13.00a	13.00a	11.50a	10.50a	8.10a	8.75a
	Nigella Sativa	7.00b	6.70b	9.00b	8.00b	8.00b	9.50a	10.50a	12.90b	12.25a
Mann-Whitney U test		15	12	35	25	25	40	50	26	32.5
P-Value		0.001	0.002	0.067	0.012	0.012	0.146	1	0.044	0.15

The values with the same superscript letter (a, b) within the same column are not statistically significantly different.Significant at P ≤ 0.05

60% of calcium hydroxidespecimens (C.T2) showed mild hard tissue deposition (score 1)(▶Fig. 5) and 20% showedmoderate hard tissue deposition (score 2), while 20% showed absence of hard tissue deposition(score 0).

On the other hand, 60% Nigella sativa specimensshowedmoderate hard tissue deposition (score 2)(▶Fig. 4), and the other 40% showedmild hard tissue deposition (score 1)(▶Fig. 3).

After Four Weeks Of Pulp Capping

Just 30% of (C.T3) specimens showed slight inflammatory cell infiltration (score:1)while the 70% of specimens showed absence of inflammatory cell infiltration (score 0)and just 20% of specimensdemonstrated slight pulp tissue disorganization. As regard to Nigella Sativa (N.T3) 100% of specimens showed absence of inflammatory cell infiltration (score 0) and all specimensshowednormal tissue morphology.

For this period 30% of calcium hydroxide specimens (C.T3) showed heavy hard tissue deposition(▶Fig. 8) and 40% of the specimens (C.T3) showed moderate hard tissue deposition (score 2)(▶Fig. 7)while 30% showed mild hard tissue deposition (score 1). As forNigella sativa specimens showed heavy hard tissue deposition(score 3) at 50%(▶Fig. 6), and moderate hard tissue deposition (score 2) at 50%.

On comparing subgroups for interaction between materials and time; Kruskal-Wallis test showed significant differences between subgroups in each group.

The Mann-Whiteny U test for pair wise comparison showed that at 2 days interval the Nigella Sativa statistically significantly less severe inflammatory reactions than CH at all-time intervals. Regarding the hard tissue formation, NS showed a statistically significantly thicker formation after a two-week period, while after 4 weeks period, all Nigella sativa samples showed hard tissue formation thicker than CH samples but not statistically significant. There were statistically significant differences regarding tissue organization after a period of one and two weeks between NS group and CH group, but after four weeks there were no statistically significant differences. pair wise comparisons using Mann-Whiteny U tests are shown in Table (4).

Discussion

Nigella sativa is a promising indigenous plant possessing several medicinal properties have been under extensive research in recent years. Careful scientific evaluation of the safety of essential oils derived from the seeds of plants is mandatory before they can be clinically applied. Experiments on animals for pre-clinical biocompatibility evaluation provide an accurate method of evaluating clinical response to dental materials.[18] Therefore this study

Figure 3. Two weeks post capping with Nagilla sativa showing mild hard tissue deposition (A). H&E stain ×40.

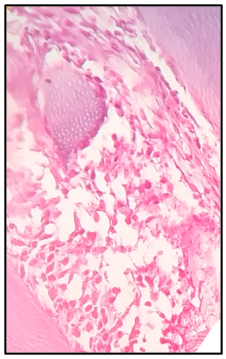


Figure 4. Two weeks post capping with Nagilla sativa showing Moderate hard tissue deposition (A). H&E stain ×10.

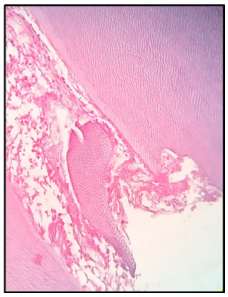


Figure 5. Tow weeks post capping with calcium hydroxide showing mild hard tissue deposition (A). H&E stain ×10.

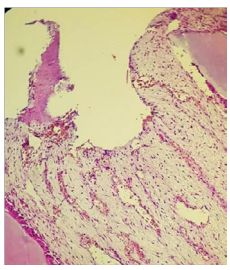


Figure 6. Four weeks post-capping with Nagilla sativa showing heavy hard tissue deposition (A). H&E X 10.

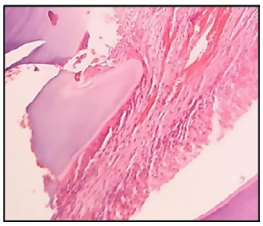


Figure 7. Four weeks post-capping with calcium hydroxide showing moderate hard tissue deposition (A). H&E X 10.

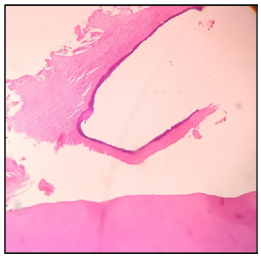


Figure 8. Four weeks post-capping with calcium hydroxide showing heavy hard tissue deposition (A). H&E X 10.

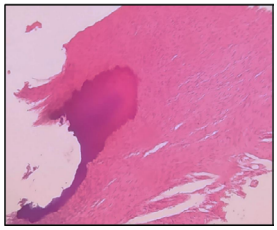


Figure 9. Tow-days post capping with Nagilla sativa,showing mild inflammation and edema of the pulp tissues with dilatation of blood vesselsand PMNS infiltration (B). H&E stain 40X.

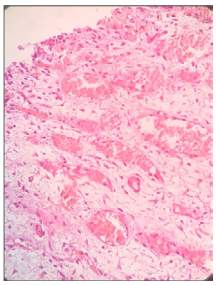


Figure 10. Tow-days post capping with calcium hydroxide, showing moderate inflammation and PMNS infiltration extended to the middle third with dilatation of blood vessels (A). H&E stain 10X.

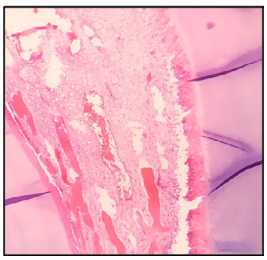
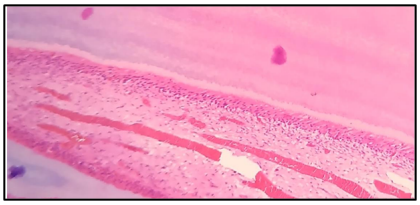


Figure 11. Tow-weeks post capping with Nagilla sativa showing central pulp normal.



was conducted to evaluate histopathologically the effect of NS extract on vital pulp tissue compared to calcium hydroxide in rabbits.

The animal model selected in the present study was rabbits because their pulp tissues are comparable with that of human [31], The reasons that rabbits, particularly a New Zealand white species, were used in this study are because of their short life span,[32] their larger tooth size than that of other rodents' teeth, which is suitable for restorative procedures, and their similar tooth

structure and jaw to human teeth.[33] In addition, several teeth can be selected for any experiment in one rabbit so the number of animals used in one study is reduced . also, using split mouth technique so that both medicaments tested in the same animal in alternate sides of the mouth.

The follow up period was short term extended only 4 weeks this because of rapid formation of secondary and tertiary dentin in rabbits, possibly due to open-apex tooth roots and continuous tooth eruption throughout their life [34]. Because rabbit teeth grow or erupt continuously, these growth or eruption is held in balance by dental abrasion from chewing a diet high in fiber [35]. Several previous studies used comparable follow up periods [30, 35-38].

In this study, demineralization using 10% nitric acid for 3 days was performed based on a protocol derived from 2 previous studies, (39, 40) whose results demonstrate quick and efficient demineralization of calcified tissues, while preserving the integrity of pulp tissue.

In this study, the criteria for histological evaluation included inflammatory response, Tissue Disorganization as well as dentin bridge formation using Hematoxylin Eosin stains.

Calcium hydroxide is considered as the gold standard for direct pulp capping since its use in dentistry in 1921. Moreover, it is able to discharge bioactive molecules in the dentin matrix [12]. However, it does not stimulate dentinogenesis, thus leading to questions over its biochemical suitability for pulp [12, 41].

Histological analyses suggest that calcium hydroxide creates reparative dentin with tunneling defects, hence increasing the susceptibility of the pulp to long-term bacterial infections [41]. In addition, calcium hydroxide provides a poor seal, and its selfcure preparations are soluble. Consequently, the capped zone is exposed over time thus promoting the reinfection of the cavity. Moreover, calcium hydroxide induces inflammation and is thus likely to affect the healing process, a factor that further affects its use [42].

Furthermore, it has a very high pH which is associated with necrosis at the wound site [43]. The drawbacks of calcium hydroxide are that it provides a poor seal, and it's selfcure preparations are soluble and exposed to dissolution over time [12]. Because of its high pH level, a foci of necrosis forms at the wound site [43].

Results this study showed that calcium hydroxide group more inflammatory response and Tissue Disorganization compared with Nigella Sativa group at all follow up periods.

After two days capping pulp with calcium hydroxide showed inflammatory response and PMNs infiltration and Slight tissue disorganization at all specimens . But after two weeks period inflammatory score gradually decreased but stayed Slightly inflammatory response till the fourth week interval. This could be explained by highly alkaline to $Ca(OH)_2$ produces a superficial burn covering a scar at the pulp surface and producing pulpal inflammation closely associated with the presence of necrotic area.

The findings from the calcium hydroxide group are consistent with previousstudies [30, 35, 44, 45].

Nigella sativa group showed Slight inflammatory response and Slight tissue disorganization. but after two weeks period inflammatory score dramatically decreased, and then was observed absence any inflammatory response after four weeks capping period.

This outcome could be credited to the chemical make-up of the Nigella Sativa especially Thymoquinone compound. Thymoquinone is the main active ingredient of nigella sativa seeds has osteogenic, anti-inflammatory, antibacterial, antioxidant, and analgesic effects, while having low impact on normal cells [46]. Moreover, it was found that thymoquinone exerts its anti-inflammatory function through its effect on some mediators of inflammation as leukotriene.[47]

Furthermore,Thymoquinone has an antibacterial activity and antibiotics could potentiate its activity [48] Khattab and Omar study found that NS reduced the microbial flora of the infected root canals significantly [49].

The results of NS agree with the study of Omar and Khattab(18) in pulp response to NS oil in dogs as a pulpotomy agent in Pediatric Dentistry . Few specimens showed scattered inflammatory cell infiltration with mild to moderate vasodilatation and the odontoblastic layer was continuous.

Regarding the results of hard tissue formation, no evidence of hard tissue formation after two days periodwith both calcium hydroxide and Nigella sativa groups.

According to Goldberg [50] cell repair begins after inflammation control, with replacement of the injured or necrotic region by undifferentiated cells.

After transformation, these cells give rise to tissue similar to the previous undamaged tissue, with three successive phases of cell renewal: slight inflammation associated with cell recruitment, cell proliferation filling the lesion site, and cell differentiation in the pulp, creating neo-odontoblasts for the production of reparative dentin.

This study showed that the Nigella sativa significantly faster bridge formation at two and four weeks follow up intervals compared to the $Ca(OH)_2$.

The ability of calcium hydroxide to allow pulp repair and hard tissue formation has been reported in previous histological studies [30, 37, 38]. The formation of the hard tissue after the application of calcium hydroxide is mediated via the alkaline phosphatase enzyme, stimulated by hydroxyl ions at pH 10.2 and calcium-dependent pyrophosphatase [51]. The alkaline environment appears to favor the further differentiation of pulp cells into odontoblast-like cells, which synthesize and deposit the dentin matrix, giving rise to the hard tissue [52, 53].

Formation of reparative dentin due to $Ca(OH)_2$ application is not due to the bio-inductive role of this material, but it is formed as a result of a defense mechanism by the pulp due to the very irritating nature of the material. In such a way, reparative dentin production is much the same as scar tissue formation, during a wound-healing process [8].

we can explaim NS results about hard tissue formation by TQ which has shown promising ability to produce continuous layers of odontoblasts in an animal study [18].

Furthermore TQ has direct stimulating effect on human dental pulp cells as evidenced by its ability to increase the expression level of ALP, which is a marker for differentiation [54].

That ALP activity is a marker for the initial differentiation of odontoblasts [55]. Alkaline phosphatase is a pre-osteoblastic key marker that is abundantly expressed at early stages of osteogenic differentiation and is responsible for bone mineralization [56].

During dentin formation, odontoblast cells synthesize and secrete several noncollagenous proteins into the dentin extracellular matrix [57]. Among these, dentin sialoprotein DSP and alkaline phosphatase ALP are considered to play a regulatory role in the mineralization of reparative dentin [57].

The results of the present study confirmed the favorable outcome of NS paste when compared to H_2O_2 in terms of lack of inflammation , normal tissue morphology and faster hard tissue formation. Furthermore it may be considered a biologically accepted material since it is a natural oil which induced minimal inflammatory response, kept the pulp vital or capable of repair, in addition to being inexpensive and widely available. All These factors highlight the importance of conducting further studies on NS which has encouraging expectations as a direct pulp capping material in dentist. Furthermore, to the best of our knowledge, none of studies have ever tested the enhancing effect of Nigella sativa on pulp wound healing.

Since this animal experiment was designed to histologically test only the biological property of NS oil product in promotion of pulp wound healing. future studies are required to further investigate the sealing ability, solubility of the product, porosity of newly formed dentin bridge, and the quantities of released active chemicals from the product. Moreover, it is necessary to test this product in human teeth because differences in pulp tissue to repair itself between an open-apex root in the rabbit's tooth and a closedapex root in the human tooth are likely to occur.

Conclusion

Within the limitations of this study it could be concluded that NS is a potential material for direct pulp capping with better biological response of pulp tissue and could find many applications in the field of regenerative endodontics.

References

[1]. Silva GA, Lanza LD, Lopes-Júnior N, Moreira A, Alves JB. Direct pulp capping with a dentin bonding system in human teeth: a clinical and histological evaluation. Oper. Dent. 2006 May;31(3):297-307.

[2]. Stanley HR. Pulp capping: conserving the dental pulp--can it be done? Is it worth it? Oral Surg Oral Med Oral Pathol. 1989 Nov;68(5):628-39.Pubmed PMID: 2682429.

[3]. Baume LJ, Holz J. Long term clinical assessment of direct pulp capping. Int. Dent. J. 1981 Dec 1;31(4):251-60.

[4]. Suzuki M, Taira Y, Kato C, Shinkai K, Katoh Y. Histological evaluation of direct pulp capping of rat pulp with experimentally developed low-viscosity adhesives containing reparative dentin-promoting agents. J Dent. 2016 Jan;44:27-36.Pubmed PMID: 26620099.

[5]. Bogen G, Kim JS, Bakland LK. Direct pulp capping with mineral trioxide aggregate: an observational study. J Am Dent Assoc. 2008 Mar;139(3):305-15.Pubmed PMID: 18310735.

[6]. Tziafas D. The future role of a molecular approach to pulp-dentinal regeneration. Caries Res. 2004 May-Jun;38(3):314-20.Pubmed PMID: 15153705.

[7]. Koliniotou-Koumpia E, Tziafas D. Pulpal responses following direct pulp capping of healthy dog teeth with dentine adhesive systems. J Dent. 2005 Sep;33(8):639-47.Pubmed PMID: 16139695.

[8]. Cox CF, Sübay RK, Ostro E, Suzuki S, Suzuki SH. Tunnel defects in dentin bridges: their formation following direct pulp capping. Oper. Dent. 1996 Jan 1;21(1):4-11.

[9]. Willershausen B, Willershausen I, Ross A, Velikonja S, Kasaj A, Blettner M. Retrospective study on direct pulp capping with calcium hydroxide. Quintessence Int. 2011 Feb 1;42(2).

[10]. Hwas M, Sandrik JL. Acid and water solubility and strength of calcium hydroxide bases. J Am Dent Assoc. 1984 Jan;108(1):46-8.Pubmed PMID: 6582113.

[11]. Azevedo LM, Casas-Apayco LC, Villavicencio Espinoza CA, Wang L, Navarro MF, Atta MT. Effect of resin-modified glass-ionomer cement lining and composite layering technique on the adhesive interface of lateral wall. J Appl Oral Sci. 2015 May-Jun;23(3):315-20.Pubmed PMID: 26221927.

[12]. Hilton TJ. Keys to clinical success with pulp capping: a review of the literature. Operative dentistry. 2009;34(5):615.

[13]. Costa CA, Vaerten MA, Edwards CA, Hanks CT. Cytotoxic effects of current dental adhesive systems on immortalized odontoblast cell line MDPC-23. Dent Mater. 1999 Nov;15(6):434-41.Pubmed PMID: 10863445.

[14]. Mente J, Hufnagel S, Leo M, Michel A, Gehrig H, Panagidis D, et al. Treatment outcome of mineral trioxide aggregate or calcium hydroxide direct pulp capping: long-term results. J. Endod. 2014 Nov 1;40(11):1746-51.

[15]. Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J. Endod. 2013 Jun 1;39(6):743-7.

[16]. Brizuela C, Ormeño A, Cabrera C, Cabezas R, Silva CI, Ramírez V, et al. Direct Pulp Capping with Calcium Hydroxide, Mineral Trioxide Aggregate, and Biodentine in Permanent Young Teeth with Caries: A Randomized Clinical Trial. J Endod. 2017 Nov;43(11):1776-1780.Pubmed PMID: 28917577.

[17]. Nosrat A, Peimani A, Asgary S. A preliminary report on histological outcome of pulpotomy with endodontic biomaterials vs calcium hydroxide. Restor Dent Endod. 2013 Nov;38(4):227-33.Pubmed PMID: 24303358.

[18]. Omar OM, Khattab NM, Khater DS. Nigella sativa oil as a pulp medicament for pulpotomized teeth: a histopathological evaluation. J Clin Pediatr Dent. 2012;36(4):335-41.Pubmed PMID: 23019828.

[19]. Mekhemar M, Hassan Y, Dörfer C. Nigella sativa and Thymoquinone: A Natural Blessing for Periodontal Therapy. Antioxidants (Basel). 2020 Dec 11;9(12):1260.Pubmed PMID: 33322636.

[20]. Tariq M. Nigella sativa seeds: folklore treatment in modern day medicine. Saudi J Gastroenterol. 2008 Jul;14(3):105-6.Pubmed PMID: 19568515.

[21]. Shrivastava RM, Agrawal RC, Parveen ZE. A review on therapeutic applications of Nigella sativa. J Chem Chem Sci. 2011;1(4):241-8.

[22]. Ahmad I, Tripathi J, Sharma M, Karchulli MS, Umer L. Nigella sativa–a medicinal herb with immense therapeutic potential (a systematic review). Int J Biol Pharm Res. 2014;5(9):755-62.

[23]. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013 May;3(5):337-52.Pubmed PMID: 23646296.

[24]. Abd-Awn B, Al-Dhaher Z, Al-Dafaai R. The effect of black seed oil extracts on mutans streptococci in comparison to chlorhexidine gluconate (in vitro). J. Baghdad Coll. Dent. 2012;24:126-31.

[25]. Al-Wafi HA. Benefits of Thymoquinone, a Nigella sativa extract in preventing dental caries initiation and improving gingival health (Doctoral dissertation, Tufts University School of Dental Medicine).2014.

[26]. Al-Douri AS, Al-kazaz SG. The Effect of Nigella Sativa Oil (Black Seed) on the Healing of Chemically In-duced Oral Ulcer in Rabbit (Experimen-tal Study). RDENTJ. 2010 Jan 1;10(1):151-7.

[27]. Fenwick N, Danielson P, Griffin G. Survey of Canadian animal-based researchers' views on the Three Rs: replacement, reduction and refinement. PLoS One. 2011;6(8):e22478.Pubmed PMID: 21857928.

[28]. Cobanoglu N, Alptekin T, Kitagawa H, Blatz MB, Imazato S, Ozer F. Evaluation of human pulp tissue response following direct pulp capping with a self-etching adhesive system containing MDPB. Dent Mater J. 2021 May 29;40(3):2020-145.Pubmed PMID: 33563846.

[29]. Shayegan A, Atash R, Petein M, Abbeele AV. Nanohydroxyapatite used as a pulpotomy and direct pulp capping agent in primary pig teeth. J Dent Child (Chic). 2010 May-Aug;77(2):77-83.Pubmed PMID: 20819402.

[30]. Salah M, Kataia MM, Kataia EM, El Din EA, Essa ME. Evaluation of eggshell powder as an experimental direct pulp capping material. Futur. Dent. J.. 2018 Dec 1;4(2):160-4.

[31]. Beldüz N, Yilmaz Y, Özbek E, Kalkan Y, Demirci T. The effect of neodymium-doped yttrium aluminum garnet laser irradiation on rabbit dental pulp tissue. Photomed Laser Surg. 2010 Dec 1;28(6):747-50.
[32]. Stübinger S, Dard M. The rabbit as experimental model for research in implant dentistry and related tissue regeneration. J. Investig. Surg. 2013 Oct 1;26(5):266-82.
[33]. Mapara M, Thomas BS, Bhat KM. Rabbit as an animal model for experimental research. J. Dent. Res. 2012 Jan;9(1):111.
[34]. Aljandan B, AlHassan H, Saghah A, Rasheed M, Ali AA. The effectiveness of using different pulp-capping agents on the healing response of the pulp. Indian J. Dent. Res. 2012 Sep 1;23(5):633.
[35]. Gamal AM, Khattab NM, Fouda TA, Tohamy SM. Histological evaluation of response to direct pulp capping with propolis: experimental study in rabbit. IJAR. 2017;5(2):2325-33.
[36]. Haddad M, Lefranc G, Aftimos G. Local application of IGF1 on dental pulp mechanically exposed; in vivo study on rabbit. Bull Group Int Rech Sci Stomatol Odontol. 2003 Jan-Apr;45(1):12-7.Pubmed PMID: 14535054.
[37]. Safy RK, Ragab MH. Comparative Histological Study of Two Different Pulp Capping Agents in Rabbits Teeth. Egypt. Dent. J. 2019 Jul 1;65(3-July (Fixed Prosthodontics, Dental Materials, Conservative Dentistry & Endodontics)):2699-707.
[38]. Alagha A, Nourallah A, Alhariri S. Dexamethasone- loaded polymeric porous sponge as a direct pulp capping agent. J Biomater Sci Polym Ed. 2020 Sep;31(13):1689-1705.Pubmed PMID: 32402228.
[39]. Gupta S, Jawanda MK, Sm M, Bharti A. Qualitative histological evaluation of hard and soft tissue components of human permanent teeth using various decalcifying agents - a comparative study. J Clin Diagn Res. 2014 Sep;8(9):ZC69-72.Pubmed PMID: 25386527.
[40]. Prasad P, Donoghue M. A comparative study of various decalcification techniques. Indian J. Dent. Res. 2013 May 1;24(3):302.
[41]. Accorinte ML, Loguercio AD, Reis A, Costa CA. Response of human pulps capped with different self-etch adhesive systems. Clin. Oral Investig. 2008 Jun;12(2):119-27.
[42]. Yasuda Y, Ogawa M, Arakawa T, Kadowaki T, Saito T. The effect of mineral trioxide aggregate on the mineralization ability of rat dental pulp cells: an in vitro study. J. Endod. 2008 Sep 1;34(9):1057-60.
[43]. Estrela C, Holland R. Calcium hydroxide: study based on scientific evidences. J. Appl. Oral Sci. 2003;11(4):269-82.
[44]. Chicarelli LP, Webber MB, Amorim JP, Rangel AL, Camilotti V, Sinhoreti MA, et al. Effect of Tricalcium Silicate on Direct Pulp Capping: Experimental Study in Rats. Eur. J. Dent. 2021 Feb;15(01):101-8.
[45]. Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M, et al. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aust Dent J. 2010 Mar;55(1):59-64.Pubmed PMID: 20415913.
[46]. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytotherapy Research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives. 2003 Apr;17(4):299-305.
[47]. Mansour M, Tornhamre S. Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. J Enzyme Inhib Med Chem. 2004 Oct;19(5):431-6.Pubmed PMID: 15648658.
[48]. Halawani E. Antibacterial activity of thymoquinone and thymohydroquinone of Nigella sativa L. and their interaction with some antibiotics. Adv Biol Res. 2009;3(5-6):148-52.
[49]. Khattab NM, Omar OM. An investigation on the relative efficacy of Nigella sativa oil compared to formocresol as pulp medicaments in non-vital primary molar pulpotomies. Egypt Dent J. 2006;52(1):169-77.
[50]. Goldberg M, Farges JC, Lacerda-Pinheiro S, Six N, Jegat N, Decup F, et al. Inflammatory and immunological aspects of dental pulp repair. Pharmacol. Res. 2008 Aug 1;58(2):137-47.
[51]. SCIAKY I, PISANTI S. Localization of calcium placed over amputated pulps in dogs' teeth. J Dent Res. 1960 Nov-Dec;39(6):1128-32.Pubmed PMID: 13749282.
[52]. Schröder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. J Dent Res. 1985 Apr;64(4):541-8.Pubmed PMID: 3857254.
[53]. Stanley HR. Criteria for standardizing and increasing credibility of direct pulp capping studies. Am J Dent. 1998 Jan;11 Spec No:S17-34.Pubmed PMID: 9760878.
[54]. Alwafi HA. Effect of thymoquinone on odontogensis of human dental pulp cells (Doctoral dissertation, Boston University).2018.
[55]. Yokose S, Kadokura H, Tajima Y, Fujieda K, Katayama I, Matsuoka T, et al. Establishment and characterization of a culture system for enzymatically released rat dental pulp cells. Calcif Tissue Int. 2000 Feb;66(2):139-44.Pubmed PMID: 10652962.
[56]. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, et al. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Arch Oral Biol. 2011 Jul;56(7):709-21.Pubmed PMID: 21227403.
[57]. Paranjpe A, Zhang H, Johnson JD. Effects of mineral trioxide aggregate on human dental pulp cells after pulp-capping procedures. J. Endod. 2010 Jun 1;36(6):1042-7.