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Effect of Nigella Sativa Oil on the Stemness Properties of Human Dental Pulp Stem Cells: An invitro study

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ABSTRACT

Purpose: This research to evaluate and compare the effect of Nigella Sativa Oil (NSO) on the stemness properties of human dental pulp stem cells (DPSCs). **Materials and methods:** (DPSCs) were isolated from the pulp of three permanent teeth indicated for extraction for therapeutic purposes. DPSCs were cultured with NS oil at concentration 10μM. and with CaOH and MTA extracts as positive controls. Cells cultured without any extract were used as negative controls. Stemness properties of DPSCs were assessed terms of proliferation, migration, and the ability to enhance wound healing. Moreover, the osteogenic differentiation potential of DPSCs cultured with NSO was evaluated. **Results:** No significant statistical difference between groups was detected groups regarding cell proliferation rate. Also, no significant difference between the numbers of migrated DPSCs in the NSO group when compared to those in the –ve control group ($p>0.05$) while there was a significant difference between the number of migrated DPSCs in the NSO and +ve control groups. The ability to enhance the healing of the wound was higher in the MTA group rather than in the NSO group. Finally, successful osteogenic differentiation of DPSCs manifested as calcified nodules occurred in all groups. **Conclusions:** Because of the limitation of the current research it concluded that NSO may have the potentials for pulp capping and regenerative applications. **Conclusions:**Despite the limitations of this study, NSO proved to have no adverse effect on DPSCs proliferation rate and can induce osteogenic differentiation of DPSCs. On the other hand, NSO showed little effect on cell migration and wound healing in DPSCs.

Keywords: Dental stem cells, Cytotoxicity, Osteogenic differentiation, Calcium hydroxide, MTA, Nigella Sativa.

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INTRODUCTION

Human dental pulp plays an important role in the formation and nutrition of dentin as well as in the innervation and defense of the teeth[(1)]. Controlling the invasion of bacteria and preserving pulp tissue vitality, which led to dentine-like tissue generation after pulp exposure, therefore offers great endodontics[(2)].

Teeth are a natural and easily accessible source for stem cells capable of self-regeneration and differentiation of multi-lineage into odontoblasts, neurons, osteoblasts, and endothelial cells[(3)]. Dental stem cells can be derived from the periodontal ligament, dental pulp, and apical papilla[(4)].

Vital pulp treatment, keeps dental pulp vital and retains teeth, includes procedures such as indirect or direct capping of the pulp, and partial or complete pulpotomy that induces the formation of reparative dentine[(5)]. Pulpotomy varies from pulp-capping in that a small portion of the coronal pulp is amputated before the capping material is applied. The materials used in the vital pulp procedures should be sufficiently biocompatible and bioactive to stimulate the activity of DPSCs and to repair pulp in primary and permanent teeth[(6)]. Dental pulp inflammation may be caused by carious lesions and/or physical injury. Mild or moderate inflammation leads to pulp regeneration while pulp death is induced by severe and/or chronic inflammation. The capping material can influence the equilibrium between the inflammation of the tissue and regeneration [(6)].

Studying the properties of various capping materials is very important in the context of cell viability of regenerating cells, transcriptional profile, and their Capability to affect the differentiation process. CaOH is widely applied for direct pulp dressing with sufficient biological responses. Unfortunately, CaOH has a weak cohesive strength, marginal leakage, and insufficient antibacterial effect[(7)]. MTA is a well-known pulp capping agent due to its high biocompatibility, antimicrobial properties, and sealing performance. In vitro, MTA can promote the proliferation and migration of mesenchymal stem cells derived from human bone marrow[(8)].

NS is an annual plant in the family Ranunculaceae. NS and its active ingredient, Thymoquinone, have been extensively researched and found to have a variety of pharmacological properties, including antimicrobial (antibacterial, anthelmintic, antifungal, and antiviral), anti-inflammatory, analgesic, histamine release inhibitor, antihypertensive, hypoglycemic, anticarcinogenic, antioxidant, and hepatoprotective activities[(9)]. Since NS and Thymoquinone have antiplaque activity, they can help prevent caries and periodontal disease[(10)].

The pulp reaction is thought to vary with the use of various available materials, depending on their biocompatibility, which may cause serious damage to this tissue[(11)]. Cell culture methods are beneficial in evaluating the biocompatibility of materials used in dental practice. In fact, in vitro assays with cell cultures are frequently used to explain the

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methods involved in various biological reactions and to assess cell behavior in certain circumstances[12].

Therefore, this work was performed to compare the effect of the tested materials on the viability of DPSCs and emphasize their influence in stimulating the reparative potential of dental pulp stem cells.

MATERIALS AND METHODS

The study included four five groups:

- Untreated cells as the negative control group.
- Nigella Sativa Oil.
- Dimethyl sulfoxide (DMSO) (vehicle) group.
- Mineral trioxide aggregate as a positive control group.
- Calcium hydroxide as a positive control group.

1- Isolation and culture of DPSCs:

This study was approved by the Research Ethics Committee (REC) of the Faculty of Dental Medicine for Boys, Al-Azhar University (**Ref No.78/82/03-19**). Teeth were obtained from children according to ethical guidelines of the Faculty of Dental Medicine for Boys, Al-Azhar University. Signed informed consent was obtained from the parents/guardians (n= 3 teeth from 7 to 9 years).

Extirpated pulp was cut into small fragments then digested using 0.2% collagenase type II (Serva Electrophores, Germany) for 1 hour at 37^{o}C. Isolated cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza Yerviers SPRL, Belgium) supplemented with penicillin/streptomycin (Invitrogen Co., USA), and 15% fetal bovine serum (FBS) (Life Science, United Kingdom), and then incubated at 37ºC and 5% CO2. When the adherent cells became 70%-80% confluent they were passaged. The cells at the fifth passage were used in the subsequent experiments.

2- Preparation of materials condition media:

The Nigella Sativa oil (CAP pharma, Egypt) 5µl was dissolved in 250 µl DMSO (Samosata, Germany) by vigorous shaking using a vortex (Cole-parmer, Germany).The solution was then centrifuged at 1300 rpm for 5 mins. The clear supernatant was re-diluted in DMSO so the final concentration becomes 10µM [13].

According to the manufacturer's instructions, the powder of MTA (ANGELUS, Brazil)/ CaOH (Prevest dentpro, India) was blended with distilled water. The mixture was sterilized by its exposure to ultraviolet light for 24 hours inside a biological safety cabinet. 5 ml of DMEM without FBS was applied to the prepared mixture after 24 hours and incubated for another 24 hours at 37°C. a 0.22 m filter was used to filter the solution [8].

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3- Cell proliferation assay:

Cell counting kit 8 (CCK-8) (Sigma, Germany) was used to assess cell proliferation. DPSCs (1x104/well) were cultured for 24 hours in DMEM supplemented with 15% FBS in a 96-well plate. The media were then replaced with conditioned media of NSO, MTA, calcium hydroxide, and DEMSO for 24 and 48 hours.. 10 l/well of CCK-8 was added at each time interval and incubated for 4 hours. At 450 nm, the absorbance was measured using a microplate reader (BMG Lab-Tech, Germany). The experiments were carried out in triplicate[14, 15].

4- Cell migration assay:

A. Transwell migration assay: A two-chamber Transwell system (Greiner bio-one, Switzerland) was used to test cell migration (8mm pore size and 6.5 mm diameter). In the lower wells of a 24-well plate, the material extract was added, and supplemented with 15% FBS. Migration Chambers were put in place and 0.5 x 10^5 cells were suspended in 100 ul serum-free DMEM and seeded on the migration chambers. The upper chambers were moved to the lower wells and incubated for 24 hours at 37^{o}C. The cells were then fixed for 2 minutes in 4 % formaldehyde and stained for 15 minutes with Giemsa stain (Biodiagmostic, Egypt). Cells that failed to migrate were gently wiped off with a cotton swab from inside the chamber. The numbers of migrating cells in each well were counted in random fields after the wells were examined under an inverted phase-contrast light microscope (DMi1-Leica, Germany). The tests were carried out in triplicate independently[16-18].

B. Wound healing assay: In a 6 well plate, DPSCs were cultured for 24 hours until they reached 70-80% confluency. With a sterile 1-ml pipette tip, a scratch was made. The material extracts were added and supplemented with 15% FBS. photographs were taken under the microscope to measure the wound width using image J software. all cells were incubated for 24 hours. The cells were then stained with Gemisa dye, and photographs were taken with Image J software to examine cell migration and wound healing. The tests were carried out in triplicate independently[19-21].

5- Osteogenic differentiation assay:

As a positive monitor, an osteogenic differentiation medium (Gibco®, StemPro®, USA) supplemented with 15% FBS was used. Different material extracts supplemented with 15% FBS were also used to culture DPSCs. All cells were incubated for 21 days and the media was changed every 3 days. the cells were fixed in 4% formaldehyde solution after 21 days and then stained with 2% alizarin red (Loba chemic, India). The experiments were carried out three times [7, 22, 23].

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6- Statistical analysis:

SPSS 23 (Statistical Package for Scientific Studies) for Windows was used to conduct the data analysis. The description of variables was presented in the form of mean, and standard deviation (SD). The Shapiro-Wilk test was used to search for normality in the results. The one-way analysis of variance (ANOVA) test was used to compare quantitative variables between classes, followed by Tukey's Post hoc test. Results were expressed in the form of P-values. The significance level was set at $P \leq 0.05$.

RESULTS

1- Cell proliferation:

Statistically, there was no significant difference between the proliferation rates of DPSCs in the NSO group, and DMSO group after 24 hours and 48 hours when compared to those in -ve Control group and MTA and CaOH as +ve Control groups ($p>0.05$). Moreover, DMSO, when used as a vehicle for NSO, showed little effect on the proliferation rate of the cultured cells(Table 1 and Fig. 1, a).

Table (1): The mean absorbance rate of all groups after 24h & 48h

Time	**Control**	**DMSO**	**MTA**	**CaOH**	**NSO**	**P-value**
	Mean ±SD	**Mean±SD**	**Mean ±SD**	**Mean±SD**	**Mean±SD**	
24h	10.49±6.66	10.07±8.15	10.29±8.18	11.62±11.56	10.88±8.08	0.984
48h	14.54±3.3	15.4±6.5	17.21±10.8	13.1±8.87	13.3±9.8	0.122
SD: Standard deviation						

2- Cell migration:

A. Transwell migration assay:

Image J analysis showed there were no detectable variations in the count of migrated cells in NSO, DEMSO, and negative control groups ($p>0.05$) while there was a significant difference between the number of migrated DPSCs in the NSO and +ve control groups ($p<0.05$). Moreover, there was no significant difference between the numbers of migrated DPSCs in the DMSO group (52.33±5.07) and -ve control group and NSO group ($p>0.05$) (Table 2 and Fig. 1,b).

Table (2): The mean numbers of migrated cells in all groups

	Control	**DMSO**	**MTA**	**CaOH**	**NSO**	**P-value**
Mean	55.66	52.33	213.44	83.11	52.44	**<0.0001***
SD±	±5.95	±5.07	±17.38	±4.62	±5.07	

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B. Wound healing assay:

The area of the induced wound was analyzed using Image J immediately after the scratch (day zero) and on days one and two.

The mean area of wound in the untreated control cultures on day zero was 125.77±8.82 and on day one was 96.11±4.98 indicating the decrease of wound area by 24% while the mean area on day two was 31.33±5.93 indicating the decrease of wound area by 75%.

The mean area of wound in the CaOH group on day zero was 124.22±5.91and on day one was 91±6.24indicating the decrease of wound area by 27% while the mean area on day two was 39.55±3.90 indicating the decrease of wound area by 68%.

The mean area of wound in the MTA group on day zero was 122.77±6.13and on day one was 88.77±6.62 indicating the decrease of wound area by 28% while the mean area on day two was 38.33±6.18 indicating the decrease of wound area by 69%.

The mean area of wound in the NSO group on day zero was 128.55±4.87 and on day one was 104.55±4.55 indicating the decrease of wound area by 19% while the mean area on day two was 61.33±5.22 indicating that the area of the wound increased 52%.

The mean area of wound in the DMSO group on day zero was 124.11±6.84 and on day one was 94.22±3.19 indicating the decrease of wound area by 24% while the mean area on day two was 32±6.48 indicating the decrease of wound area by 94% (Figure. 1,c).

3- Osteogenic differentiation assay:

Successful osteogenic differentiation of DPSCs manifested as calcified nodules occurred in all groups. However, the number and size of the calcified nodules were higher and bigger in the osteogenic media and MTA groups followed by the CaOH group and finally the NSO. These results indicate that although NSO can induce osteogenic differentiation of DPSCs, it fails to have the same osteoinductive effect as MTA and CaOH. (Figure 2)

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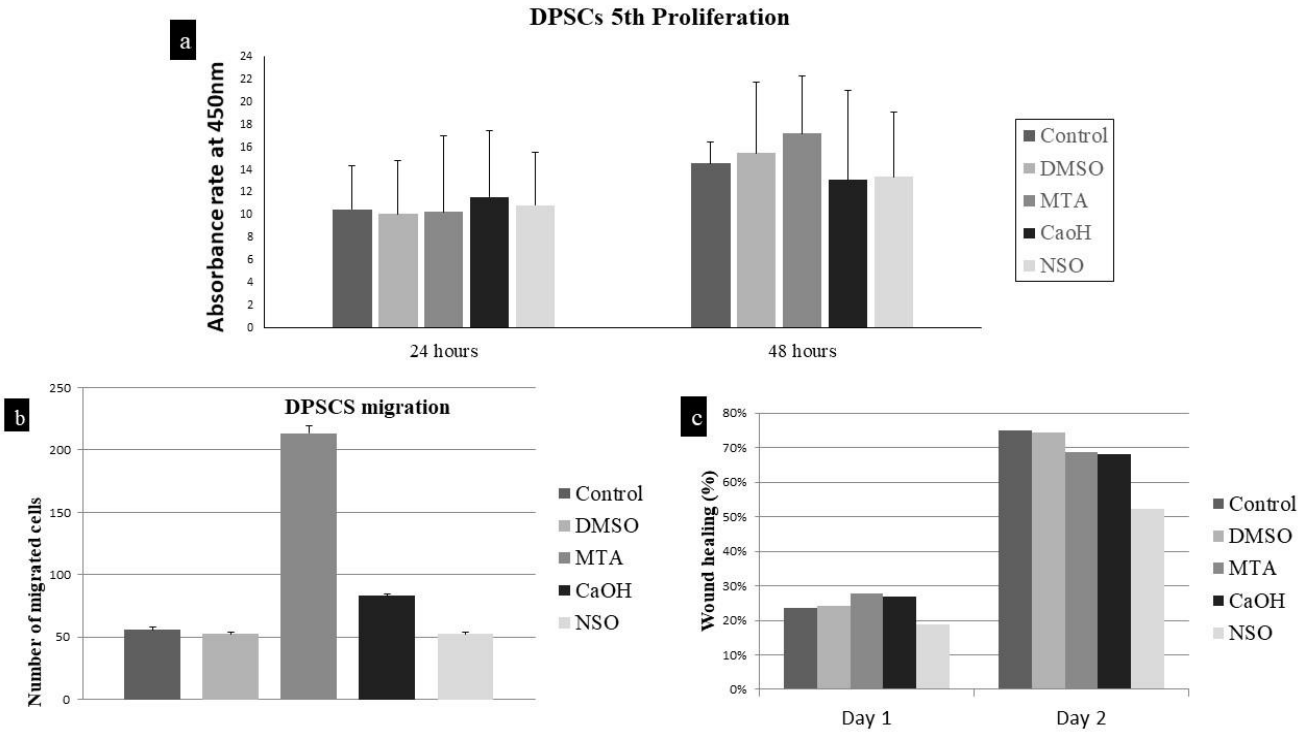

Figure 1: Bar graph revealing the mean absorbance rate of all groups after 24h & 48h (a), Bar chart showing the mean numbers of migrated cells within all groups (b), and Bar chart showing the percentage of wound healing on day one and two in all groups (c).

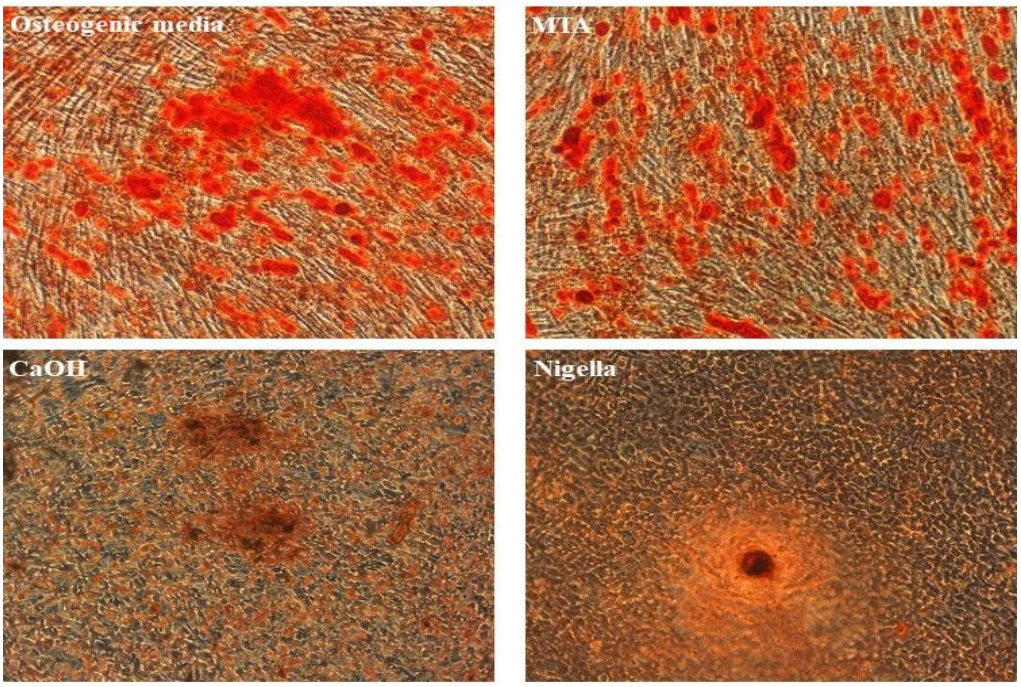

Figure (2): Showing osteogenic differentiation of DPSCs on day 30 (Alizarine red stain). Arrows show the calcified nodules (original magnification x10)

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DISCUSSION

for dental restorative materials to be ideal and allow DPSCs proliferation, migration, and differentiation on the scaffolds, they should be bioactive and biocompatible[24].

Umbilical cord blood, fat tissue, and bone marrow are the most common sources of MSCs. However, to isolate MSCs from these sources, aspiration is used, which is an invasive and painful procedure for donors. Umbilical cord cells have limited availability because they are only available for a short period of time after delivery [25]. Marrow MSCs also have a variety of drawbacks, including the difficulty of harvesting marrow, the small number of MSCs in marrow tissue, and the cells' in vitro aging issues[26].

On the other hand, primary and permanent teeth are considered an easily accessible source for isolation of stem cells and subsequent expansion for tissue engineering. Also, the dental pulp collection is a better and simpler procedure than that of bone marrow. furthermore, studies have revealed that human pulp derived stem cells have great multi-lineage differentiation and high proliferation rates compared to other types of stem cells. The advanced plasticity and abundance of the human DPSCs make them suitable for tissue engineering which is the foundation of pulp capping [27, 28].

The current study evaluated the effect of NSO on cells proliferation. The time taken for the cells to proliferate when NSO was used, was also determined, with the analyses of the cultures being reviewed at 24 hours and 48 hours exposure. Our results show that, there was no significant difference between the proliferation rates of DPSCs in NSO group after 24 hours and 48 hours when compared to those in the -ve control group and +ve control groups (MTA and CaOH). In other words, neither NSO possess proliferative nor cytotoxic effects on DPSCs.

Other studies, investigated the effect of NSO on cells proliferation, and found that NSO could induce proliferation of fibroblasts, and collagen synthesis during wound healing in rabbit[29]. Another study found that, by securing a supportive condition for BM-MSCs, NSO application improved the therapeutic potential of BM-MSCs transplantation in response to the liver injury induced by radiation.[13].

On the other hand, NSO has been shown in several studies to have anti-proliferative properties, the ability to cause cell cycle arrest, and the ability to induce pro-apoptotic effects in cancer cells[30, 31]. Interestingly, NSO induces and up regulates cell proliferation in other cell types and normal cells with certain concentration ranges and time considerations[32].

On the contrary, MO had a marked cytotoxic effect on both human breast adenocarcinoma and breast epithelial cell lines[33]. These observations afford yet another evidence for the safety of NSO for human consumption and consideration of their use in cell stimulatory drugs.

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Upon examining the migration potential properties of NSO on DPSCs, it was found that NSO had no positive effect on DPSCs migration. Moreover, MTA and CaOH showed a better migration induction effect than that with NSO test group.

The report that examined the effect of NSO on cells migration demonstrated that NSO could enhance the migratory potential of BM-MSCs [13]. In vitro, NSO increases the total antioxidant efficiency and migration potential of mouse MSCs, according to another study [34].

A decrease in the wound areas occurred in all groups and became more with time. A study observed the effect of NSO on human gingival fibroblasts. It was found that NSO could accelerate wound healing activities[34]. Another study had a similar observation in that wound healing could be hastened by NSO application[35].

Several studies examined the association of NSO and skin wound healing. The reports include different kinds of wounds, namely burn, excisional, and diabetic wounds. seven studies showed positive results for skin wound healing associated with NSO. The process of wound healing might be because of the anti-inflammatory, antioxidant, and antibacterial properties of NSO [29].

DPSCs differentiation into odontoblasts and osteoblasts is critical for early odontogenic differentiation and late dentine mineralization. Bioactive materials used for pulp tissue healing should enhance the dentinogenic potential of pulp stem cells[8]. NSO treated cells showed increased osteogenic differentiation. Calcium granules were clear and compact 21 days post NSO treatment, which was a sign of good differentiation. Our findings were agreed with a study was carried out to investigate the osteogenic inductive potential of NSO on dental pulp MSCs. This study revealed that NSO could induce dental pulp MSCs osteogenic differentiation[36].

Our results disagree with other studies which showed that NSO can improve the proliferation and migration of MSCs. This could be attributed to the fact that only one concentration and one type of NSO was used, which was available at the time of the study. Moreover, other extraction techniques should have been considered.

CONCLUSIONS

Under the limitations of that research, it concluded that:

NSO neither cytotoxic nor can induce the proliferation of stem cells isolated from human premolar permanent teeth. Also, it was found that it has the lowest effect on migration of DPSCs and wound healing. Finally, NSO can induce osteogenic differentiation of DPSCs but not the same osteoinductive effect as MTA and CaOH.

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Future implementation of NSO in dental pulp capping materials may help to improve direct pulp capping, pulpotomy procedures, decrease its failure rate, and may be a useful therapeutic agent for pulpal repair.

REFERENCES

1. Aguilar P, Linsuwanont P. Vital pulp therapy in vital permanent teeth with cariously exposed pulp: a systematic review. Journal of endodontics. 2011;37(5):581-7.
2. Schmalz G, Smith AJ. Pulp development, repair, and regeneration: challenges of the transition from traditional dentistry to biologically based therapies. Journal of endodontics. 2014;40(4):S2-S5.
3. Fischbach GD, Fischbach RL. Stem cells: science, policy, and ethics. The Journal of clinical investigation. 2004;114(10):1364-70.
4. Zhai Q, Dong Z, Wang W, Li B, Jin Y. Dental stem cell and dental tissue regeneration. Frontiers of medicine. 2019;13(2):152-9.
5. Collado-González M, García-Bernal D, Oñate-Sánchez R, Ortolani-Seltenerich P, Álvarez-Muro T, Lozano A, et al. Cytotoxicity and bioactivity of various pulpotomy materials on stem cells from human exfoliated primary teeth. International endodontic journal. 2017;50:e19-e30.
6. Araújo LB, Cosme-Silva L, Fernandes AP, Oliveira TMd, Cavalcanti BdN, Gomes Filho JE, et al. Effects of mineral trioxide aggregate, BiodentineTM and calcium hydroxide on viability, proliferation, migration and differentiation of stem cells from human exfoliated deciduous teeth. Journal of Applied Oral Science. 2018;26.
7. Ajlan SA, Ashri NY, Aldahmash AM, Alnbaheen MS. Osteogenic differentiation of dental pulp stem cells under the influence of three different materials. BMC oral health. 2015;15(1):1-10.
8. Youssef A-R, Emara R, Taher MM, Al-Allaf FA, Almalki M, Almasri MA, et al. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. BMC Oral Health. 2019;19(1):1-9.
9. Ahmad I, Tripathi J, Sharma M, Karchulli M, Umer L. Nigella sativa–a medicinal herb with immense therapeutic potential (a systematic review). Int J Biol Pharm Res. 2014;5(9):755-62.
10. AlAttas SA, Fat'heya MZ, Turkistany SA. Nigella sativa and its active constituent thymoquinone in oral health. Saudi medical journal. 2016;37(3):235.
11. Chandrasekhar H, Sharma S. Efficient Agents for Pulp Protection: A Review. Journal of Pharmaceutical Sciences and Research. 2016;8(11):1308.
12. Pires C, Botton G, Cadoná F, Machado A, Azzolin V, da Cruz I, et al. Induction of cytotoxicity, oxidative stress and genotoxicity by root filling pastes used in primary teeth. International endodontic journal. 2016;49(8):737-45.
13. Radwan RR, Mohamed HA. Nigella sativa oil modulates the therapeutic efficacy of mesenchymal stem cells against liver injury in irradiated rats. Journal of Photochemistry and Photobiology B: Biology. 2018;178:447-56.
14. Tsai C-L, Ke M-C, Chen Y-H, Kuo H-K, Yu H-J, Chen C-T, et al. Mineral trioxide aggregate affects cell viability and induces apoptosis of stem cells from human exfoliated deciduous teeth. BMC Pharmacology and Toxicology. 2018;19(1):1-10.

15. Chen I, Salhab I, Setzer FC, Kim S, Nah H-D. A new calcium silicate–based bioceramic material promotes human osteo-and odontogenic stem cell proliferation and survival via the extracellular signal-regulated kinase signaling pathway. Journal of endodontics. 2016;42(3):480-6.
16. Lymperi S, Taraslia V, Tsatsoulis IN, Samara A, Velentzas AD, Agrafioti A, et al. Dental stem cell migration on pulp ceiling cavities filled with MTA, dentin chips, or bio-oss. BioMed research international. 2015;2015.
17. Zhu L, Yang J, Zhang J, Peng B. A comparative study of BioAggregate and ProRoot MTA on adhesion, migration, and attachment of human dental pulp cells. Journal of endodontics. 2014;40(8):1118-23.
18. Pijuan J, Barceló C, Moreno DF, Maiques O, Sisó P, Marti RM, et al. In vitro cell migration, invasion, and adhesion assays: from cell imaging to data analysis. Frontiers in cell and developmental biology. 2019;7:107.
19. Tomás-Catalá CJ, Collado-González M, García-Bernal D, Oñate-Sánchez RE, Forner L, Llena C, et al. Biocompatibility of new pulp-capping materials NeoMTA plus, MTA repair HP, and biodentine on human dental pulp stem cells. Journal of endodontics. 2018;44(1):126-32.
20. Zhang J, Zhu L, Cheng X, Lin Y, Yan P, Peng B. Promotion of dental pulp cell migration and pulp repair by a bioceramic putty involving FGFR-mediated signaling pathways. Journal of dental research. 2015;94(6):853-62.
21. Yue C, Guo Z, Luo Y, Yuan J, Wan X, Mo Z. c-Jun overexpression accelerates wound healing in diabetic rats by human umbilical cord-derived mesenchymal stem cells. Stem cells international. 2020;2020.
22. El-Edel RH, Hassouna MM, Abd El-Gayed AM, Noreldin RI, Turky AZ. Isolation and osteogenic differentiation of umbilical cord mesenchymal stem cells. Menoufia Medical Journal. 2020;33(1):231.
23. Liu G, Xu G, Gao Z, Liu Z, Xu J, Wang J, et al. Demineralized dentin matrix induces odontoblastic differentiation of dental pulp stem cells. Cells Tissues Organs. 2016;201(1):65-76.
24. An S, Gong Q, Huang Y. Promotive effect of zinc ions on the vitality, migration, and osteogenic differentiation of human dental pulp cells. Biological trace element research. 2017;175(1):112-21.
25. Alkhalil M, Smajilagic A, Redzic A. Human dental pulp mesenchymal stem cells isolation and osteoblast differentiation. Med Glas (Zenica). 2015;12(1):27-32.
26. Samiei M, Aghazadeh M, Movassaghpour AA, Fallah A, Aminabadi NA, Pakdel SMV, et al. Isolation and characterization of dental pulp stem cells from primary and permanent teeth. J Am Sci. 2013;9(12):153-7.
27. Naz S, Khan FR, Zohra RR, Lakhundi SS, Khan MS, Mohammed N, et al. Isolation and culture of dental pulp stem cells from permanent and deciduous teeth. Pakistan journal of medical sciences. 2019;35(4):997.
28. Karamzadeh R, Eslaminejad MB, Aflatoonian R. Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. Journal of visualized experiments: JoVE. 2012(69).
29. Sallehuddin N, Nordin A, Bt Hj Idrus R, Fauzi MB. Nigella sativa and Its Active Compound, Thymoquinone, Accelerate Wound Healing in an In Vivo Animal Model: A Comprehensive Review. International journal of environmental research and public health. 2020;17(11):4160.
30. Arslan BA, Isik FB, Gur H, Ozen F, Catal T. Apoptotic effect of Nigella sativa on human lymphoma U937 cells. Pharmacognosy magazine. 2017;13(Suppl 3):S628.

31. Al-Sheddi ES, Farshori NN, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Cytotoxicity of Nigella sativa seed oil and extract against human lung cancer cell line. Asian Pacific Journal of Cancer Prevention. 2014;15(2):983-7.
32. Sung B, Prasad S, Yadav VR, Aggarwal BB. Cancer cell signaling pathways targeted by spice-derived nutraceuticals. Nutrition and cancer. 2012;64(2):173-97.
33. Fernandes EE, Pulwale AV, Patil GA, Moghe AS. Probing regenerative potential of Moringa oleifera aqueous extracts using in vitro cellular assays. Pharmacognosy research. 2016;8(4):231.
34. Rezaei N, Sardarzadeh T, Sisakhtnezhad S. Thymoquinone promotes mouse mesenchymal stem cells migration in vitro and induces their immunogenicity in vivo. Toxicology and applied pharmacology. 2020;387:114851.
35. Elgohary HM, Al Jaouni SK, Selim SA. Effect of ultrasound-enhanced Nigella sativa seeds oil on wound healing: An animal model. Journal of Taibah University medical sciences. 2018;13(5):438-43.
36. Mendi A. Nigella sativa oil could induce osteogenic differentiation of dental pulp mesenchymal stem cells: clinical nutrition for dentistry. Food and health. 2018;4(1):19-24.