Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Understanding the effect of ginger on dental pulp stem cells differentiation into chondrocyte

Payal PAWAR[1], Ajay KALE[1], Ramesh BHONDE[2], Pranjali POTDAR[2], Mayuri CHAVAN[1], Avinash KHARAT[2]*

[1] Navsahyadri Institute of Pharmacy, Pune , India

[2] Regenerative Medicine Laboratory, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth, Pimpri , Pune

* Corresponding Author. Avinash Kharat ; E-mail: avinashkharat25@gmail.com (A.K.).

Received: 21 August 2024 / Revised: 30 December 2024/ Accepted: 4 January 2025

ABSTRACT: Ginger is utilised as a plant-based anti-inflammatory and anti-arthritic medication in traditional medicine. Human dental pulp stem cells (hDPSCs) are an ideal in vitro model for screening drugs and biological agents. This study evaluated the effect of ginger extract on the chondrogenic differentiation of hDPSCs. Dental pulp stem cells were isolated using explant culture method. Stem cells surface marker expression and tri-lineage differentiation of hDPSCs were studied. The cytotoxic impact of the ginger extract on hDPSCs was measured using the MTT assay. The impact of ginger on the differentiation of hDPSCs into chondrocytes was examined using gene expression and biochemical staining. The surface markers CD34, CD45, and HLA-DR were negative in the hDPSCs, while CD73, CD90, and CD105 were positive. The hDPSCs exhibited osteocyte, chondrocyte and adipocyte differentiation. The viability of hDPSC was significantly increased at a concentration of 10 µg/ml of ginger. Ginger administration increased the amount of glycosaminoglycan containing extracellular matrix in hDPSCs and demonstrated by Safranin O staining. Ginger extract is a suitable candidate for cartilage repair because it promotes chondrogenic differentiation in hDPSC. Therefore, this could be explored further in the treatment of cartilage disorders. However, further investigation to be required.

KEYWORDS: Dental pulp; drugs screening; chondrocyte; mesenchymal stem cell; traditional medicine.

1. INTRODUCTION

Gingered is a widely dispersed family with medicinal benefits that is well known across the tropics, especially in Southeast Asia. Gingered is a valuable natural resources used for the Spices, food, medications, colours, perfume and other goods [1]. There are around 1200 species in the 53 genera that make up the ginger family [2]. India is one of the *Zingiberaceae* family's richest and most diversified locations. For millennia, people have utilised ginger a well-known herbaceous plant, as a flavouring and herbal remedy [3]. In addition, eating the rhizome of ginger is a common traditional cure for relieving common health issues like pain, nausea and vomiting.It has been stated that more than 100 chemicals have been extracted from ginger. In particular, zingiberene, zingerone, shogaols, and gingerol are the main families of chemicals found in ginger. Terpenes, vitamins, and minerals are also found in ginger [4]. Gingerols are thought to be the main constituents among them and are known to have a number of bioactivities [3]. Researching whether or not bone and cartilage-specific extracellular matrix (ECM) synthesis and cellular development are stimulated is crucial for full tissue renewal and regeneration.

In spite of factors like cost, accessibility, effectiveness, safety, novelty, and non-toxicity, scientists are looking into natural herbal sources for cartilage regeneration. Mesnahcymasl stem cells ideal for various drugs screening owing their differentiation potential and help with tissue regeneration. Several plant extracts and their phytochemicals have been suggested to increase adult stem cell proliferation and differentiation, which has helped in the regeneration of injured and disordered tissues [5]. Similar to bone marrow MSCs, DPSCs exhibit MSC-like characteristic [6]. Because DPSCs are affordable and readily available sources of MSCs. Dental pulp offer promise as a source of stem cells for tissue engineering and regeneration [7]. Osteoporosis and osteoarthritis is one of the most prevalent and widespread disorders that affect the human population [5, 8]. Osteoporosis and osteoarthritis are becoming more common in India due

How to cite this article: Pawar P, Kale A, Bhonde R, Potdar P, Chavan M, Kharat A. Understanding the effect of ginger on dental pulp stem cells differentiation into chondrocyte. J Res Pharm. 2025; 29(5): 1896-1903.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1763604

to a number of factors, including increased life expectancy and urbanisation [9, 10]. Changes in lifestyle, such as diet, exercise, and weight loss are part of the management of various skeletal illnesses [9].

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a range of cell types including osteoblasts (bone cells), neurones (nerve cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells) [11]. The cells that create the cartilage that lines our joints and shields our bones are the subject of our attention. Specific culture conditions and specified media containing transforming growth factor cause differentiation. During chondrogenesis, two different cell types are created such as chondrocytes and chondroblasts. Diffuse nutrition and matrix repair are the functions of chondrocytes, whereas chondroblasts are progenitor cells that secrete the extracellular matrix (ECM). For cartilage to develop, both cell types are necessary [12].

Even though studies on ginger's anti-inflammatory properties in the treatment of arthritis have been scant, not much is known about how ginger affects the regeneration of bone or cartilage tissue in the treatment of bone disorders, nor about how ginger influences stem cell proliferation and differentiation into bone cells. Furthermore, ginger's potential for treating bone tissue directly related to cartilage has not been investigated. Therefore, the goal of the current work was to comprehend how ginger affected the in vitro chondrogenic differentiation of hDPSCs.

2. RESULTS

2.1. Solubility of Ginger Extract

The pure extract of ginger 10 mg, (Figure 1) which was dissolved in sterile distilled water, DMSO, and ethanol at the concentration of 10 mg/ml each. Highest solubility was seen in DMSO. The mixture was filter sterilized using a 0.22-micron filter and 10 ml syringe. This DMSO ginger extract was stored at 4◦C for further use.

Figure 1. Ginger Extract

2.2. Human Dental Pulp Stem Cell Isolation and Cultivation (hDPSCs)

After incubation for seven days, cell outgrowth was observed surrounding the transplant. The morphology of the cells was spindle-shaped and fibroblastic like (Figure 2D). Upon confluence, hDPSCs were trypsinized and separated in a fresh, sterile T-75 flask. The cells had a fibroblastic shape, were adherent, and multiplied in a monolayer. The cells were trypsinized on the fifth or seventh day of incubation, when they had reached confluence (Figure 2E). The cells were further used for the current investigation after being subcultured in T-75 flasks up to passage 5.

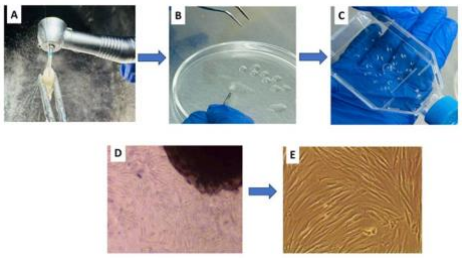

Figure 2. Isolation and culturing of human Dental Pulp Stem Cells (hDPSCs). (A) Extraction of pulp, (B) Dental pulp cut into small sections, (D) Explant culture and, (E) Outgrowth of cells from explant culture

2.3. Surface marker analysis employing FACS to characterise hDPSCs

The percentage of cells that were stained favourably relative to the unstained sample is used to illustrate the results. The isolated and cultivated hDPSCs expressed CD73 (46%), CD90 (91.5%), and CD105 (92.7%), but not CD34 (6.61%), CD45 (4.50%), or HLA-DR (4.41%), according to the FACS histograms Figure 3).This suggested that the cells under cultivation were a uniform population of stem cells with mesenchymal characteristics.

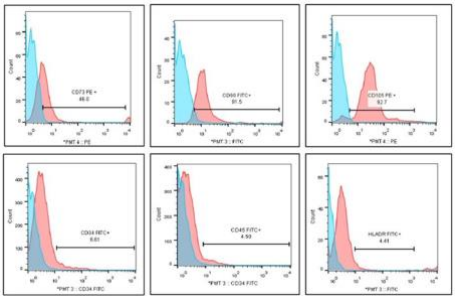

Figure 3. The FACS histograms show that the homogenous population of hDPSCs is positive for surface markers CD73, CD90, and CD105, while they are negative for HLA-DR, CD34, and CD45.

2.4. Tri-lineage differentiation method for characterising hDPSCs

To visualise the tri-lineage differentiation such as osteogenic, chondrogenic, and adipogenic induction medium, the cells were stained with 2% Alizarin Red S, 0.1% Safranin O, and 0.3% Oil Red O respectively. DPSCs developed into calcium-producing osteocytes when stimulated with osteogenic medium; these cells were stained red with 2% Alizarin Red S (Figure 4A).Chondrogenic medium induction encouraged the production of glycosaminoglycan-containing extracellular matrix (ECM), which was stained with 0.1% Safranin O (Figure 4B). The lipid droplet that had collected at the cell periphery of hDPSCs was stained with 0.3% Oil Red O to show that adipogenic conditions encouraged the cells to develop into adipocytes (Figure 4C).

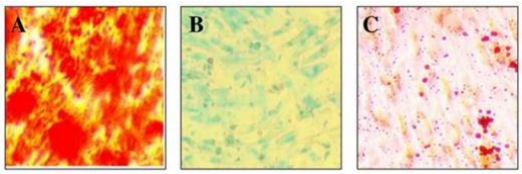

Figure 4. (A) Osteogenic differentiation, scale bar = 100 µm, (B) Chondrogenic differentiation, and scale bar = 100 µm and, (C) Adipogenic differentiation, scale bar = 10 µm

2.5. Ginger extract's cytotoxicity on hDPSCs

MTT assay was used to determine whether ginger extract was cytotoxic to hDPSCs after 24 hrs and 48 hrs of treatment. Complete medium was used as the control, and the concentrations that were examined are 1, 2, 5, 10, 15, 20, and 25 µg/ml. At 560 nm, absorbance was measured, and the results are displayed as mean (n = 3) ± standard error of mean. There was a statistically significant absorbance difference between the values of 5 µg/ml and the control (Figure 5) The absorbance showed that all concentrations of the herbal extract were not cytotoxic and instead increased the viability of the hDPSCs in comparison to the control cells.

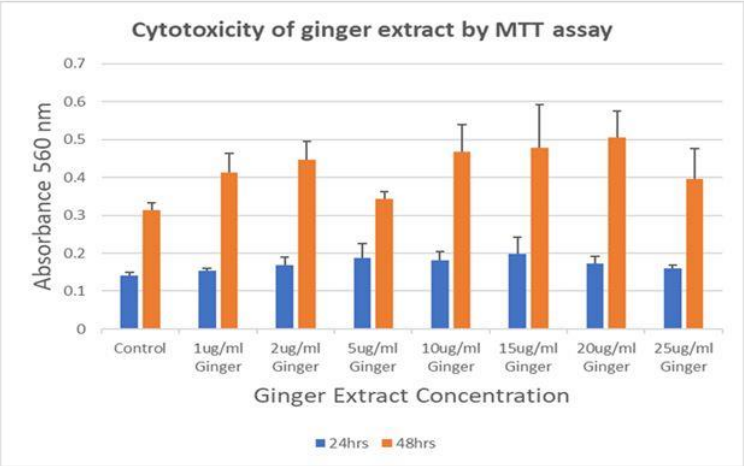

Figure 5. Cytotoxic assessment of ginger extract towards hDPSCs proliferation by MTT assay.

2.6. Ginger extract's impact on hDPSCs' chondrogenic differentiation

The induced-treated chondrogenic ginger test hDPSCs developed into the chondrogenic lineage and were comparable to the corresponding induced-untreated positive control cells, according to biochemical staining performed after 21 days of ginger treatment (Figure 6A - 6B).Therefore; the ginger extract did not prevent differentiating in the presence of induction media. When the stain was quantified, it was shown that, in comparison to the corresponding positive control sample, hDPSC induction and ginger treatment increased the formation of chondrogenic matrix (Figure 6).

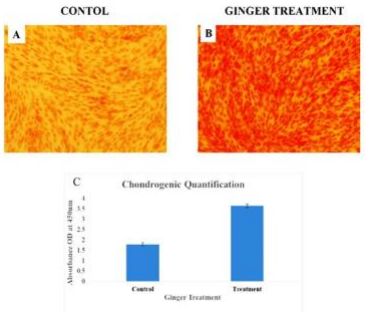

Figure 6. Effect of Ginger extract (5 µg/ml) on chondrogenic differentiation of hDPSCs. (A) Control for chondrogenic differentiation, (B) Ginger treated chondrogenic differentiation and, (C) Chondrogenic quantification.

3. DISCUSSION

Herbal remedies have been routinely used to treat cartilage diseases. In preclinical or clinical research, herbal remedies, or their combination have been popular tools in complementary and alternative medicine for cartilage problems in recent years. Ginger is effective in relieving pain and it can used as a safe product to improve pain in patients with knee osteoarthritis [13]. Ginger possesses anti-inflammatory and antioxidant properties [14]. Various studies reported that ginger consists of active components majorly gingerol, that have anti-inflammatory properties [3].

In vitro cytotoxicity and antioxidant testing involve the testing of herbal extracts against the homogenous culture of human cells. Mesenchymal stem cell (MSCs) is an ideal platform for herbal drug screening [15, 16]. MSCs are plastic adherence; have shown differentiation into various cell types, including bone and cartilage [17].

Comparative studies between conventional medicine and numerous other allopathic medications are best conducted on MSCs [18,19]. This straightforward in vitro approach allows for the differentiation of hMSCs into the lineage of concern and the investigation of the bioactivity of conventional medications. It is well known that ginger extract has anti-inflammatory properties and a distinct phytochemical composition. Ginger extract is reported to include phytochemicals gingerol, flavones, flavonoids, anthocyanin, catechins and isocatechins [13]. One of the important phytochemicals that is known to encourage chondrogenic differentiation of cartilage tissue is gingerol. hDPSCs from dental extracts offer an alternative to mesenchymal stem cells because of their relative abundance, ease of availability, and lack of ethical considerations[20].

The capacity of MSCs to develop into adipocytes, chondrocytes, and osteocytes in particular differentiation cocktails [20, 21]. Osteocytes mineralize the matrix and create calcium aggregates; chondrocytes are encased in a highly structured extracellular matrix (ECM) network made up of high molecular mass proteoglycans; and the maturation of adipocytes produces lipid-rich intracellular vacuoles [22, 23]. After hDPSCs were induced for 21 days, the cells were favourably stained with the appropriate stains, indicating that the cultivated hDPSCs had mesenchymal-like characteristics. All concentrations of ginger had more live cells than the negative control, according to the results of the MTT cytotoxicity study. This was statistically significant at 5 µg/ml. Numerous investigations on ginger have demonstrated the apoptotic and anti-proliferative properties [24]. This is the first study to examine the effect of the plant extract on hDPSCs at lower, clinically relevant concentrations; however, the earlier experiments were conducted on cancer cell lines at considerably greater concentrations. For 21 days, hDPSCS-induced cells received a 5 µg/ml ginger therapy. A recent study examined the differentiation of DPSCs on days 7, 14, and 21, and the results were positive at all times [25]. Similar research done on days 14, 17, and 21 similarly revealed that differentiation started on day 14 and grew until day 21 of induction [26]. In the current investigation, DPSCs were produced for 21 days, and upon termination with the appropriate dye, a notable degree of differentiation was observed. The results of the biochemical test with Safranin showed that ginger improved the matrix mineralization and glycosaminoglycan of hDPSCs that differentiated into chondrocytes. Our data showed that DPSCS could be used as platform for drug screening. In these in-vitro studies, we have screen the ginger and it enhance the chondrogenic differentiation of stem cells. It concluded the ginger could be effective osteoarthritis. In addition, we can use ginger pre-treated dental pulp stem cells for clinical application in osteoarthritis to regenerate the cartilage. Data incorporated in thesis section. However, we would need further investigation in animal model.

4. CONCLUSION

Based on the previously described description, we discovered that ginger is beneficial in enhancing chondrogenesis. It has the potential to enhance cartilage regeneration, which is crucial for enhancing the recovery of inflammatory rheumatic disorders.

5.MATERİAL AND METHODS

5.1. Sample collection

The pure extract of ginger was used for the study. The powder were transported to the Regenerative Medicine Laboratory at Dr. D. Y. Patil Dental College & Hospital, Pune, Maharashtra, and stored at room temperature until further processing.

5.2. Solubility of Ginger Extract

Solubility of ginger extract was checked using different DMSO, PBS, distilled water, and ethanol. The pure extract of ginger 10 mg /ml was dissolved in sterile distilled water, DMSO, and ethanol respectively.

5.3 Human Dental Pulp Stem Cell (hDPSCs) Isolation and Cultivation

The study has approved by the institutional committee for stem cell research (IEC/394/209-20) at Dr. D. Y. Dental College and Hospital in Pune, India. The third molar tooth was removed from healthy donor (n=5) 30 to 45 years age group during orthodontic surgery as biological waste. Using an air-rotor handpiece

in the style of a bur chuck, the dental pulp was extracted (Figure 1A). Using a sterile surgical blade, the tissue was cut into sections of approximately 1 mm (Figure 1B). The pieces were put in a culture flask that had been preconditioned with FBS (Figure 1C). For 24 hours, the flask was kept at 37 °C in an incubator with 5% CO2 and 95% humidity. Culture media media (DMEM basal media, 10% FBS, and 1% antibiotic-antimycotic cocktail) were added after a day, and the flask was then incubated under the same conditions promote cell outgrowth. The explant culture was routinely checked for the growth of hDPSCs using an inverted phase-contrast microscope (Olympus CKX53). The medium was substituted every two to three days until the hDPSC outgrowth achieved 80–90% confluence. After the adhering hDPSCs reached confluence, they were grown in a T-25 flask (passage 1), supplied with full medium that was changed out every two to three days, and trypsinized (0.25% Trypsin-EDTA solution at 37 ◦C for 30 s) without disturbing the explant [16].

5.4. Surface marker analysis employing FACS to characterise hDPSCs

hDPSCs that were confluent and in passage 4 were subjected to surface markers analysis using flow cytometry. After harvesting, the cells were suspended in 1X PBS containing 1% FBS. Anti-human monoclonal antibodies conjugated to PE (phycoerythrin) for surface markers CD73, CD90, and CD105 and FITC (fluorescein isothiocyanate) for surface markers CD34, CD45, and HLA-DR were added to the cell suspension and incubated for 30 minutes at 4 ◦C under dark aseptic conditions. Negative controls consisted of unstained cells and matching isotype controls. Post incubation, the samples were passed through a flow cytometer (Attune NxT, Thermo Scientific) and cleaned with 1X PBS. A total of 10,000 events were recorded for every sample.

5.5. Tri-lineage differentiation method for characterising hDPSCs

In order to evaluate hDPSCs' ability for tri-lineage differentiation, such as osteogenic, chondrogenic, and adipogenic lineages, the appropriate induction medium was used to stimulate the cells. Osteogenic induction media containing DMEM with 1 µm dexamethasone, 1 mM β-glycerophosphate, and 2 mM ascorbate-2-phosphate were used to treat hDPSCs in osteogenic differentiation. In order to induce chondrogenesis, the induction medium comprised of dexamethasone (1 µm), ascorbate-2-phosphate (1 mM), sodium pyruvate (10 µg/ml), L-proline (40 µg/ml), 1X ITS, and TGF-β3 (10 ng/ml). The adipogenic induction medium was composed of DMEM with 1.743 µM insulin, 200 µM indomethacin, 0.5 mM 3-isobutyl-5-methylxanthine, and 1 µM dexamethasone. For 21 days, the cells were induced, and the medium was changed every 2-3 days. In order to verify that hDPSCs had differentiated into osteocytes, chondrocytes, and adipocytes, the cells were stained with 0.1% Alizarin Red S, 0.1% Safranin O, and 0.3% Oil Red O respectively.

5.6. Ginger extract's cytotoxicity on hDPSC

To test the ginger extract's cytotoxicity on hDPSCs, the MTT assay was employed. DPSCs were treated with 1,2, 5, 10, 15 , 20 and 25 µg/ml of ginger extract. After 48 hours of treatment, 50 µl of MTT solution (5 mg/ml) was added and it was incubated for an additional three hours under the same parameters. After a 3 hours duration, the MTT solution was removed and 100 microliters of dimethyl sulfoxide (DMSO) were introduced into every well. Absorbance was measure using microplate spectrophotometer at 560 nm. The SkanIt Software 5.0 software was used for reading.

5.7 Effect of Ginger extract on differentiation of hDPSCs

hDPSCs that were treated with ginger for chondrogenic differentiation. For chondrogenic differentiation, hDPSCs were incubated in differentiation medium with 5 µg/ml of ginger. In the absence of ginger, hDPSCs were treated with the appropriate induction media as a positive control. The cells were stained with Safranin to confirm chondrogenesis after 21 days of stimulation. After that, the cells were examined using an inverted phase-contrast microscopy. The quantitative analysis was performed using ELISA reader absorbance at 405 nm.

5.8 Statistical analysis

One-way ANNOVA used for statistical analysis. The mean ± standard error of mean (SEM) was used to represent the data. The threshold for statistical significance was set at $P<0.05$.

An assertion of generative AI in scholarly literature: The authors state that the current work was not composed using artificial intelligence.

Acknowledgment: The authors would like to thank Dr. D. Y. Patil Vidyapeeth for providing the lab equipment needed to conduct research.

Author's contributions: Payal Pawar: Experimental work- collected the data and manuscript writing. Avinash Kharat and Ajay Kale: Experiment designed and manuscript writing, Pranjali Potdar and Mayuri Chavan: Graphical abstract, Ramesh Bhonde: Data analysis and interpretation of data for the article.

Conflict of interest: The authors state that none of the work described in this publication appears to have been influenced by any known competing financial interests or personal ties.

Data availability: The data that support the findings of this study are available from the corresponding author, [A.K.], on special request.

REFERENCES

[1] Bin Jantan I, Yassin MSM, Chin CB, Chen LL, Sim NL. Antifungal activity of the essential oils of nine Zingiberaceae Species. Pharm Biol. 2008; 41(5): 392–397. https://doi.org/10.1076/phbi.41.5.392.15941

[2] Kress WJ, Prince LM, Williams KJ. The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. Am J Bot. 2002;89(10):1682-1696. https://doi.org/10.3732/ajb.89.10.1682

[3] Kubra IR, Rao LJ. An impression on current developments in the technology, chemistry, and biological activities of ginger (*Zingiber officinale* Roscoe). Crit Rev Food Sci Nutr. 2012;52(8):651-688. https://doi.org/10.1080/10408398.2010.505689

[4] Mahomoodally MF, Aumeeruddy MZ, Rengasamy KRR, Roshan S, Hammad S, Pandohee J, Hu X, Zengin G. Ginger and its active compounds in cancer therapy: From folk uses to nano-therapeutic applications. Semin Cancer Biol. 2021;69:140-149. https://doi.org/10.1016/j.semcancer.2019.08.009

[5] Buhrmann C, Honarvar A, Setayeshmehr M, Karbasi S, Shakibaei M, Valiani A. Herbal remedies as potential in cartilage tissue engineering: An overview of new therapeutic approaches and strategies. Molecules. 2020;25(13):3075. https://doi.org/10.3390/molecules25133075

[6] Anitua E, Troya M, Zalduendo M. Progress in the use of dental pulp stem cells in regenerative medicine. Cytotherapy. 2018;20(4):479-498. https://doi.org/10.1016/j.jcyt.2017.12.011

[7] Ledesma-Martínez E, Mendoza-Núñez VM, Santiago-Osorio E. Mesenchymal stem cells derived from dental pulp: A Review. Stem Cells Int. 2016;2016:4709572. https://doi.org/10.1155/2016/4709572

[8] Salari N, Darvishi N, Bartina Y, Larti M, Kiaei A, Hemmati M, Shohaimi S, Mohammadi M. Global prevalence of osteoporosis among the world older adults: a comprehensive systematic review and meta-analysis. J Orthop Surg Res. 2021; 16(1):669. https://doi.org/10.1186/s13018-021-02772-0

[9] Swärdh E, Jethliya G, Khatri S, Kindblom K, Opava CH. Approaches to osteoarthritis - A qualitative study among patients in a rural setting in Central Western India. Physiother Theory Pract. 2022;38(11):1683-1692. https://doi.org/10.1080/09593985.2021.1872126

[10] Bhadada SK, Chadha M, Sriram U, Pal R, Paul TV, Khadgawat R, Joshi A, Bansal B, Kapoor N, Aggarwal A, Garg MK, Tandon N, Gupta S, Kotwal N, Mahadevan S, Mukhopadhyay S, Mukherjee S, Kukreja SC, Rao SD, Mithal A. The Indian Society for Bone and Mineral Research (ISBMR) position statement for the diagnosis and treatment of osteoporosis in adults. Arch Osteoporos. 2021;16(1):102. https://doi.org/10.1007/s11657-021-00954-1

[11] Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen Med. 2019;4:22. https://doi.org/10.1038/s41536-019-0083-6

[12] Longoni A, Utomo L, van Hooijdonk IE, Bittermann GK, Vetter VC, Kruijt Spanjer EC, Ross J, Rosenberg AJ, Gawlitta D. The chondrogenic differentiation potential of dental pulp stem cells. Eur Cell Mater. 2020;39:121-135. https://doi.org/10.22203/eCM.v039a08

[13] Maryam H, Azhar S, Akhtar MN, Asgar A, Saeed F, Ateeq H, Afzaal M, Akram N, Munir H, Anjum W, Shah MA. Role of bioactive components of ginger in management of osteoarthritis : a review. Int J Food Prop. 2023; 26(1): 1903–1913. https://doi.org/10.1080/10942912.2023.2236811

[14] Li H, Liu Y, Luo D, Ma Y, Zhang J, Li M, Yao L, Shi X, Liu X, Yang K. Ginger for health care: An overview of systematic reviews. Complement Ther Med. 2019;45:114-123. https://doi.org/10.1016/j.ctim.2019.06.002

[15] Kitambi SS, Chandrasekar G. Stem cells: a model for screening, discovery and development of drugs. Stem Cells Cloning. 2011;4:51-9. https://doi.org/10.2147/SCCAA.S16417

[16] Joshi KS, Bhonde R. Insights from Ayurveda for translational stem cell research. J Ayurveda Integr Med. 2014;5(1):4-10. https://doi.org/10.4103/0975-9476.128846.

[17] Patil VR, Kharat AH, Kulkarni DG, Kheur SM, Bhonde RR. Long term explant culture for harvesting homogeneous population of human dental pulp stem cells. Cell Biol Int. 2018;42(12):1602-1610. https://doi.org/10.1002/cbin.11065
[18] Saud B, Malla R, Shrestha K. A review on the effect of plant extract on mesenchymal stem cell proliferation and differentiation. Stem Cells Int. 2019;2019:7513404. https://doi.org/10.1155/2019/7513404
[19] Bhonde R, Sanap A, Joshi K. Mesenchymal stem cells as a platform for research on traditional medicine. J Ayurveda Integr Med. 2021;12(4):722-728. https://doi.org/10.1016/j.jaim.2021.08.012
[20] Li B, Ouchi T, Cao Y, Zhao Z, Men Y. Dental-derived mesenchymal stem cells: State of the art. Front Cell Dev Biol. 2021;9:654559. https://doi.org/10.3389/fcell.2021.654559.
[21] Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. Acta Biomater. 2011;7(2):463-477. https://doi.org/10.1016/j.actbio.2010.07.037.
[22] Sudo K, Kanno M, Miharada K, Ogawa S, Hiroyama T, Saijo K, Nakamura Y. Mesenchymal progenitors able to differentiate into osteogenic, chondrogenic, and/or adipogenic cells in vitro are present in most primary fibroblast-like cell populations. Stem Cells. 2007 ;25(7):1610-1617. https://doi.org/10.1634/stemcells.2006-0504
[23] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739-2749. https://doi.org/10.1634/stemcells.2007-0197.
[24] Chen CY, Chen YN, Shiau JP, Tang JY, Hou MF, Chang HW. Ginger-Derived 3HDT exerts antiproliferative effects on breast cancer cells by apoptosis and DNA Damage. Int J Mol Sci. 2023;24(6):5741. https://doi.org/10.3390/ijms24065741.
[25] Labedz-Maslowska A, Bryniarska N, Kubiak A, Kaczmarzyk T, Sekula-Stryjewska M, Noga S, Boruczkowski D, Madeja Z, Zuba-Surma E. Multilineage differentiation potential of human dental pulp stem cells-ımpact of 3D and hypoxic environment on osteogenesis ın vitro. Int J Mol Sci. 2020;21(17):6172. https://doi.org/10.3390/ijms21176172.
[26] Eggerschwiler B, Canepa DD, Pape HC, Casanova EA, Cinelli P. Automated digital image quantification of histological staining for the analysis of the trilineage differentiation potential of mesenchymal stem cells. Stem Cell Res Ther. 2019;10(1):69. https://doi.org/10.1186/s13287-019-1170-8