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Therapeutic Potential of Herbal Blend and Essential Nutrients Formulation in Promoting Chondrogenic Differentiation: A Study Using Human Dental Pulp Stem Cells

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ABSTRACT

Background: Osteoarthritis remained a significant healthcare challenge, with current treatments primarily focusing on symptom management rather than chondrocyte regeneration. Human dental pulp stem cells (hDPSCs) emerged as a promising cell source for chondrocyte regeneration. This study investigated the potential of herbal blend and essential nutrients formulation to enhance chondrogenic differentiation of hDPSCs and its implications for osteoarthritis treatment.
Materials and Methods: The researchers evaluated herbal blend and essential nutrients formulation effects on hDPSCs across concentrations ranging from 1-50 µg/ml. The investigators used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for cytotoxicity evaluation, while they assessed chondrogenic differentiation using Safranin O staining to detect glycosaminoglycan production.
Results: The formulation exhibited optimal cell viability at 5 µg/ml and 15 µg/ml. Chondrogenic differentiation showed the most pronounced effects at 5 µg/ml, demonstrating significantly enhanced glycosaminoglycan production compared to both control and 10 µg/ml treatment groups. The 10 µg/ml concentration, while showing less effectiveness than 5 µg/ml, demonstrated marked improvement over control conditions.
Conclusion: This study established the potential of herbal blend and essential nutrients formulation as a promising therapeutic agent for osteoarthritis treatment, demonstrating optimal efficacy at 5 µg/ml for both cell viability and chondrogenic differentiation of hDPSCs. The synergistic effects of formulations herbal components and essential nutrients on hDPSC-mediated cartilage regeneration, offering a novel approach to regenerative therapy for osteoarthritis.

KEYWORDS: Human dental pulp stem cells, Chondrocyte, Osteoarthritis, Shallaki, Curcumin

INTRODUCTION

Osteoarthritis (OA) represents a complex systemic disease affecting multiple joint components, including the synovium, articular cartilage, subchondral bone, tendons, and muscles [1]. The condition can arise idiopathically or develop due to aging, trauma, malformations, or inflammatory diseases [2]. With a prevalence of up to 10% in males and 18% in females over 48 years of

age, OA has emerged as a significant public health concern, with current therapeutic approaches failing to adequately address clinical needs [3]. The loss of articular cartilage in OA is characterized by proteolytic degradation of the chondral matrix, triggering the release of inflammatory cytokines, including interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNF-α). This cascade leads to the secretion of matrix-degrading enzymes from chondrocytes, perpetuating tissue breakdown [4].

While current pharmacological interventions, including cyclooxygenase-2-selective or nonselective nonsteroidal anti-inflammatory drugs and biological agents like TNF-α-binding antibodies or IL-1 inhibitors [5], provide symptomatic relief, their ability to address structural damage remains limited. Non-pharmacological regenerative techniques following the 3R paradigm (reconstruction, repair, and replacement) have been developed, but approaches such as micro fracture and tissue transplantation show poor long-term outcomes [6].

In this context, the exploration of alternative therapeutic approaches, particularly those derived from natural sources, has gained significant attention. A novel herbal formulation combining traditional medicinal plants (Shallaki, Curcumin, Valerian, Guggul, Hadjod, Ashwagandha) with essential nutrients (Vitamin C, Vitamin D3, and Magnesium), represents a promising intervention for OA management. This formulation leverages the established therapeutic properties of its constituents, which have demonstrated anti-inflammatory, antioxidant, and tissue-regenerative properties in various studies.

Recent advances in stem cell therapy, particularly the use of human dental pulp stem cells hDPSCs, have opened new avenues for cartilage regeneration. Human dental pulp stem cells are self-renewing mesenchymal stem cells residing within the dental pulp's perivascular niche [7,8], originate from cranial neural crest and express both MSC and neural stem cell markers [9]. Their accessibility from extracted third molars and capacity to differentiate into various cell types, including chondrocytes, make them an attractive candidate for cartilage regeneration strategies [10,11].

The integration of traditional herbal medicine with modern stem cell therapy represents a novel approach to addressing the limitations of current OA treatments. This study investigates the potential synergistic effects of formulations herbal components on hDPSC-mediated cartilage regeneration, with particular focus on their influence on chondrocyte differentiation and matrix production. Understanding these interactions could provide valuable insights into developing more effective therapeutic strategies for OA management, combining the regenerative potential of stem cells with the established benefits of traditional herbal medicine.

METHODOLOGY

The cytotoxicity and chondrogenic differentiation potential of formulation on hDPSCs were evaluated through the following procedures:

The MTT [3-(4-5 dimethylthiozol-2-yl) 2-5 diphenyl-tetrazolium bromide] assay was employed to evaluate the cytotoxicity of formulation. hDPSCs were seeded in 96-well plates at a density of 7000 cells/well and allowed to attach for 24 hours at 37°C in 5% CO_2 atmosphere. A stock solution of herbal blend and essential nutrients formulation (10 mg/ml) was prepared in dimethyl sulfoxide (DMSO), from which various concentrations (1, 2, 5, 10, 15, 20, 25, and 50 µg/ml) were prepared. After 48 hours of treatment, 50 µl of MTT solution (5 mg/ml) was added to each well. Following 3 hours of incubation, the formed formazan crystals were solubilized using MTT lysis buffer. Absorbance was measured using a plate reader (Thermofisher USA) at 560 nm, with a reference wavelength of 630 nm.

For chondrogenic differentiation, hDPSCs were trypsinized and seeded at a density of 1.5 x 106 cells/cm^2 in Serum Free Medium-1

(SFM-i). After two days of culture, the medium was replaced with osteogenic differentiation media supplemented with herbal blend and essential nutrients formulation at concentrations of 5 µg/ml and 10 µg/ml. Differentiation media without herbal blend and essential nutrients formulation served as a positive control. The chondrogenic differentiation was assessed using Safranin O staining, which specifically stains glycosaminoglycan's present in chondrocytes.

RESULTS

Assessment of cytotoxicity

The MTT assay results demonstrated the effect of herbal blend and essential nutrients formulation on DPSC viability across different concentrations ranging from 1 to 50 µg/ml. The absorbance values measured at 560 nm showed varying cell viability patterns compared to the control. Notably, cells treated with 5 µg/ml of herbal blend and essential nutrients formulation exhibited the highest absorbance, indicating enhanced cell viability at this concentration. A second peak in cell viability was observed at 15 µg/ml. The control group showed a baseline absorbance. As the drug concentration increased beyond 15 µg/ml, there was a gradual decrease in absorbance values, with the lowest readings observed at higher concentrations of 25 and 50 µg/ml. However, even at these higher concentrations, the absorbance values remained comparable to the control group, suggesting that herbal blend and essential nutrients formulation does not exhibit cytotoxicity across the tested concentration range. The cells maintained their viability throughout the treatment period, indicating the safety of formulation for therapeutic applications.

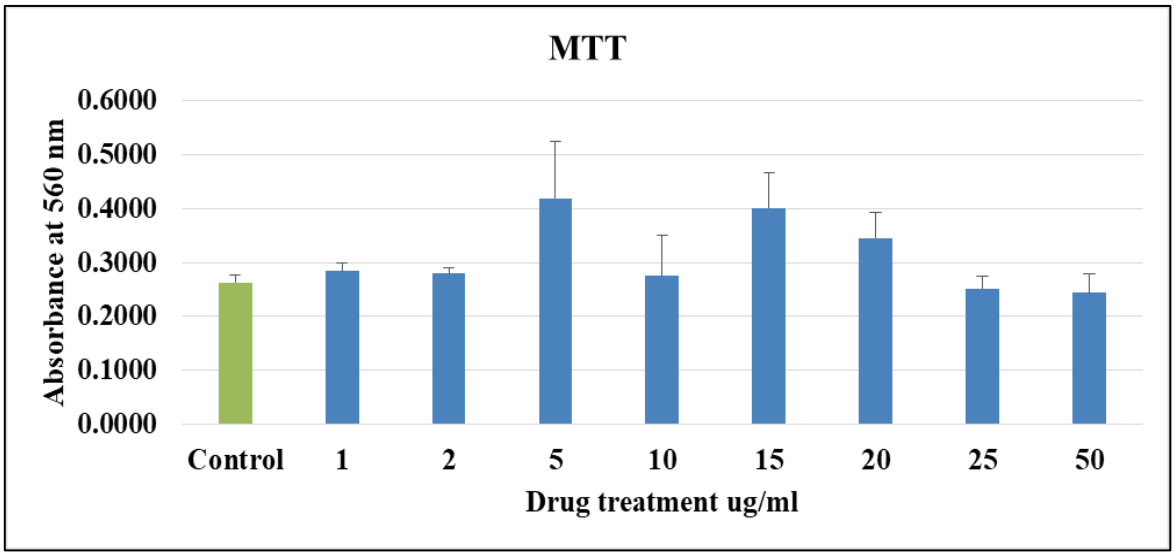

Figure 1: MTT assay showing cell viability of hDPSCs after 48-hour treatment with different concentrations of herbal blend and essential nutrients formulation.

Assessment of chondrogenic differentiation

The figure 2 illustrates the quantitative analysis of chondrocyte differentiation in hDPSCs treated with herbal blend and essential nutrients formulation at different concentrations (5 µg/ml and 10 µg/ml) compared to the control group. The results were measured at 560 nm. Treatment with herbal blend and essential nutrients formulation at 5 µg/ml demonstrated the highest level of chondrogenic differentiation, showing a significant increase in absorbance compared to the control group. The 10 µg/ml concentration also exhibited enhanced chondrogenic differentiation, which was notably higher than the control but slightly lower than the 5 µg/ml treatment.

These findings indicate that herbal blend and essential nutrients formulation effectively

promotes chondrogenic differentiation of hDPSCs, with 5 µg/ml being the optimal concentration for maximizing this effect.

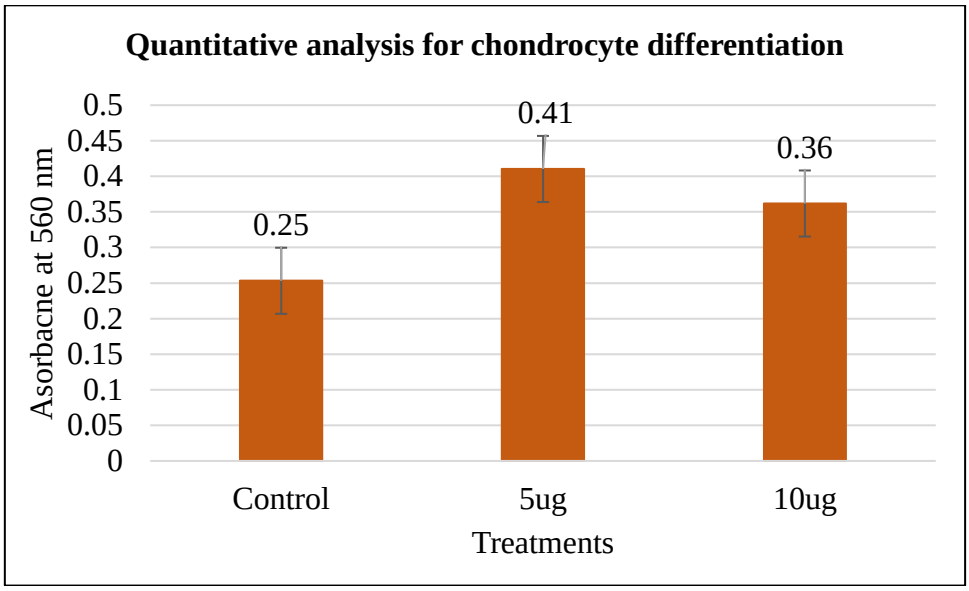

Figure 2: Quantitative analysis of chondrogenic differentiation in hDPSCs treated with different concentrations of herbal blend and essential nutrients formulation (5 and 10 µg/ml).

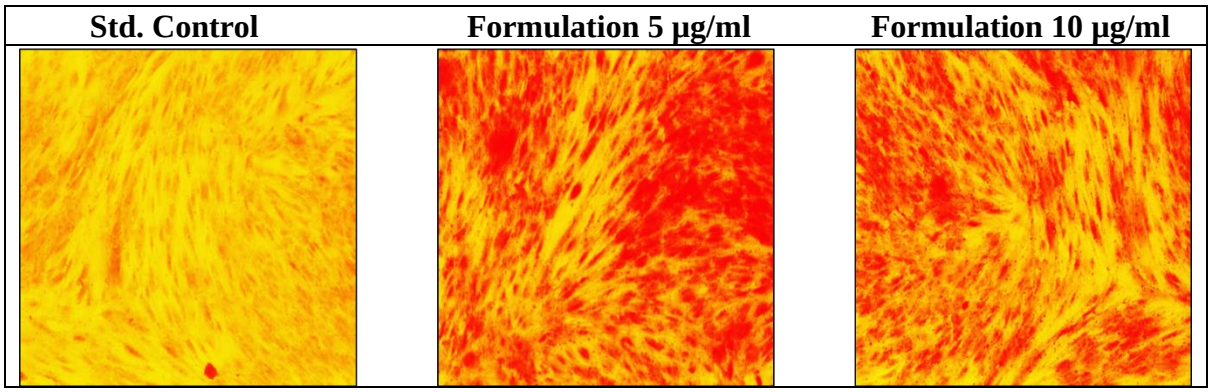

Figure 3: Chondrogenic proliferation with staining of the cells

The figure 3 demonstrate that the hDPSCs proliferated as a monolayer during the differentiation process. Notably, formulation showed enhanced chondrogenic differentiation potential at concentrations of 5 µg/ml and 10 µg/ml. The successful differentiation into chondrocytes was confirmed by positive Safranin O staining, which revealed the presence of glycosaminoglycan's characteristic of chondrocyte formation.

The presence of glycosaminoglycan's in the differentiated cells, as demonstrated by Safranin O staining, provided conclusive evidence of successful chondrogenic differentiation. This confirms the efficacy of herbal blend and essential nutrients formulation in promoting chondrogenic lineage commitment of hDPSCs.

DISCUSSION

The present study demonstrates the significant potential of human dental pulp stem cells (hDPSCs) as a viable source for cartilage regeneration through chondrogenic differentiation, enhanced by herbal blend and essential nutrients formulation preconditioning. The cytotoxicity analysis revealed that herbal blend and essential nutrients formulation exhibits a favourable

safety profile across a wide concentration range (1-50 µg/ml), with particularly enhanced cell viability observed at 5 µg/ml and 15 µg/ml concentrations. This biphasic response pattern suggests a dose-dependent modulatory effect on cellular metabolism, while maintaining cell viability even at higher concentrations. The sustained viability at higher concentrations (25-50 µg/ml) indicates a broad therapeutic window, which is crucial for clinical applications.

The chondrogenic differentiation studies yielded particularly noteworthy results, with herbal blend and essential nutrients formulation demonstrating significant potential in promoting cartilage-specific tissue formation. The optimal concentration for chondrogenic induction was identified at 5 µg/ml, where the highest level of differentiation was observed compared to both the control group and the 10 µg/ml treatment. This finding suggests a precise dose-dependent mechanism of action, where lower concentrations may be more effective in triggering chondrogenic pathways. The successful differentiation was conclusively demonstrated through Safranin O staining, which revealed substantial glycosaminoglycan production - a key indicator of functional chondrocyte formation.

The enhanced chondrogenic differentiation observed at 5 µg/ml, coupled with increased cell viability at this concentration, suggests a potential synergistic mechanism whereby herbal blend and essential nutrients formulation not only promotes cell survival but also actively supports lineage-specific differentiation. This dual effect is particularly significant in the context of regenerative medicine, where both cell survival and appropriate differentiation are crucial for successful tissue regeneration. The slightly reduced effectiveness at 10 µg/ml, while still maintaining significant improvement over control conditions, indicates a possible concentration threshold beyond which the chondrogenic effects may begin to plateau.

This research builds upon previous studies, such as that of Asgari N et al., who demonstrated the effectiveness of hydrogel with curcumin for cartilage regeneration in a cartilage model for micro tissue chondrogenic regeneration [12]. Similarly, another study showed that curcumin provides a microenvironment conducive to chondrogenic proliferation while deactivating IL-1β-induced activation of NF-KB and expression of apoptotic and pro-inflammatory genes in chondrocytes [13].

These findings are particularly relevant when considered alongside the current limitations of osteoarthritis treatments. Traditional pharmacological interventions primarily focus on symptom management rather than tissue regeneration, while existing regenerative approaches often show limited long-term efficacy. The ability of herbal blend and essential nutrients formulation to promote both cell viability and chondrogenic differentiation of hDPSCs represents a promising avenue for developing more effective therapeutic strategies. The natural composition of formulation, combining traditional medicinal plants with essential nutrients, may offer advantages in terms of biocompatibility and reduced side effects compared to synthetic alternatives.

CONCLUSION

The study concludes potential of dental pulp stem cells in cartilage regeneration when stimulated with herbal blend and essential nutrients formulation. The successful chondrogenic differentiation of hDPSCs opens new avenues for cartilage tissue engineering and regenerative medicine. Future research should focus on investigating the long-term stability of the differentiated chondrocytes derived from dental pulp stem cells in both animal models and human clinical trials. Additionally, further studies are needed to elucidate the molecular mechanisms governing hDPSC chondrogenic differentiation and to optimize culture conditions for large-scale therapeutic applications. The accessibility and differentiation capacity of dental pulp stem

cells, combined with their response to herbal blend and essential nutrients formulation, presents a promising approach for developing more effective regenerative therapies for cartilage-related disorders.

Declaration by Authors

Ethical Approval: Approved

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Competing Interests: The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCE

1. Lee WY, Wang B. Cartilage repair by mesenchymal stem cells: clinical trial update and perspectives. Journal of orthopaedic translation. 2017 Apr 1; 9:76-88.
2. Verhaar J. B| Degenerative and Inflammatory Joint Diseases. The EFORT White Book: "Orthopaedics and Traumatology in Europe" [Internet]. 2021.
3. Allen KD, Thoma LM, Golightly YM. Epidemiology of osteoarthritis. Osteoarthritis and cartilage. 2022 Feb 1;30(2):184-95.
4. Liu S, Deng Z, Chen K, et al. Cartilage tissue engineering: From pro-inflammatory and anti-inflammatory cytokines to osteoarthritis treatments. Molecular Medicine Reports. 2022 Mar 1;25(3):1-5.
5. Zhang W, Moskowitz RW, Nuki G, et al. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis and cartilage. 2008 Feb 1;16(2):137-62.
6. Haleem AM, Chu CR. Advances in tissue engineering techniques for articular cartilage repair. Operative Techniques in Orthopaedics. 2010 Jun 1;20(2):76-89.
7. Gulotta LV, Chaudhury S, Wiznia D. Stem cells for augmenting tendon repair. Stem cells international. 2012;2012(1):291431.
8. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences. 2000 Dec 5;97(25):13625-30.
9. Sakai K, Yamamoto A, Matsubara K, et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. The Journal of clinical investigation. 2012 Jan 3;122(1):80-90.
10. Chen K, Xiong H, Xu N, et al. Chondrogenic potential of stem cells from human exfoliated deciduous teeth in vitro and in vivo. Acta Odontologica Scandinavica. 2014 Nov 1;72(8):664-72.
11. Dai J, Wang J, Lu J, et al. The effect of co-culturing costal chondrocytes and dental pulp stem cells combined with exogenous FGF9 protein on chondrogenesis and ossification in engineered cartilage. Biomaterials. 2012 Nov 1;33(31):7699-711.
12. Asgari N, Bagheri F, Eslaminejad MB, et al. Dual functional construct containing kartogenin releasing microtissues and curcumin for cartilage regeneration. Stem cell research & therapy. 2020 Dec; 11:1-5.
13. Buhrmann C, Mobasheri A, Matis U, et al. Curcumin mediated suppression of nuclear factor-κB promotes chondrogenic differentiation of mesenchymal stem cells in a high-density co-culture microenvironment. Arthritis research & therapy. 2010 Aug; 12:1-5.

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