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Research Article

Regenerative Effects of Scrophularia Striata and Adipose Tissue–Derived Stem Cells on Dental Pulp Tissue: An Animal Model

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Abstract

Introduction: Regeneration of pulp tissue after infection is complicated and has frequently failed because current methods cannot control inflammation or eliminate bacterial infection. Numerous studies have demonstrated the effects of flavonoids on regeneration. Scrophularia is an exceptional plant source rich in flavonoids, and many flavonoid compounds have been extracted from it. This study aimed to determine the effects of Scrophularia striata and adipose tissue–derived stem cells on the expression of Kruppel-like factor 4 (KLF4), monocyte chemoattractant protein-1 (MCP-1), and brain-derived neurotrophic factor (BDNF) genes during pulp tissue regeneration in an animal model.

Method: This experimental–laboratory study was conducted on 16 male Wistar rats. The methanolic extract of Scrophularia striata was prepared by maceration (Scro). Adipose tissue was harvested from the bilateral inguinal region of rats that did not undergo surgery, and adipose-derived stem cells (SC) were isolated and characterized. Pulp inflammation was induced in the rats, and pulpectomy was performed at the end of the second week. After placement of a triple-antibiotic paste for 2 weeks, regenerative treatment with drugs and materials was performed. Three weeks after tooth filling, the teeth were randomly divided into four groups (n = 4/group): (1) control (blood clot only), (2) Scrophularia alone (Scro), (3) stem cells alone (SC), and (4) Scrophularia + stem cells (SC+Scro). One to four weeks after tooth filling, the dental pulp was harvested from the animals. Real-time PCR was used to measure the expression levels of KLF4, MCP-1, and BDNF genes. Data were analyzed using one-way ANOVA and Tukey's test ($\alpha = 0.05$).

Result: Injection of Scro, SC, and SC+Scro significantly increased KLF4 gene expression compared with the control group ($P = 0.001$). The highest level of KLF4 expression was observed in the SC group ($P = 0.001$), which was significantly reduced in the SC+Scro group compared with the SC group ($P = 0.001$). Injection of Scro significantly decreased MCP-1 gene expression compared with the control group ($P = 0.001$). Injection of SC did not significantly alter MCP-1 expression compared with the control group; however, injection of SC+Scro significantly increased MCP-1 expression compared with all other groups ($P = 0.001$). Injection of Scro, SC, and SC+Scro significantly increased BDNF gene expression compared with the control group ($P = 0.001$). The highest BDNF expression was observed in the Scro group ($P = 0.001$), which was significantly reduced in the SC+Scro group compared with the Scro and SC groups ($P = 0.001$).

Conclusion : The results of this study showed that application of the Scro extract increased the expression of KLF4 and BDNF genes and decreased MCP-1 expression. Application of the Scro+SC combination increased the expression of KLF4, BDNF, and MCP-1 genes. Application of adipose-derived stem cells increased the expression of KLF4 and BDNF genes, with no significant effect on MCP-1. According to this study, the plant extract enhanced the expression of dentinogenesis-related factors during pulp regeneration.

Keywords: Scrophularia striata, adipose tissue–derived stem cells, pulp tissue regeneration, KLF4, MCP-1, BDNF.

Introduction

Pulp necrosis is also caused by dental caries and most commonly affects first molars in children[1-2]. The most common cause of pulp necrosis in children's teeth with incomplete apices is dental trauma [3]. In immature teeth,

pulp necrosis leads to incomplete root development, thin root canal walls, and open apices, which makes root canal treatment difficult. It has been well documented that, in a tooth with an incomplete apex, when the pulp is destroyed by necrosis, no further dentin is produced to complete apical formation, resulting in a short, weak root that is prone to fracture [3]. The technique of pulp tissue regeneration (REP) is a biological process aimed at replacing damaged structures, including dentin and the dentin–pulp complex, in teeth with immature apices [4]. Creating a favorable environment within the root canal system for regeneration of dental pulp tissue ultimately eliminates the need for conventional root canal treatment. It potentially reduces the risk of tooth fracture in these fragile and immature teeth [4]. The primary goal of REP is the elimination of clinical symptoms and resolution of apical periodontitis. The objective of REP treatment is to fill infected and contaminated canals with living tissue [5].

It is believed that an open root apex is a critical component for REP. Current limitations suggest that REP may be applicable in teeth with apical diameters as small as 0.24 mm or greater. However, no definitive conclusion has yet been reached regarding the correlation between root morphology and REP outcomes [5].

In addition to managing traumatic injuries, REP may be an ideal treatment for immature permanent teeth with necrosis caused by dental anomalies, such as dentinogenesis imperfecta, leading to resolution of root lesions and root maturation [5]. Recently, REP has also been proposed as a treatment option for resorptive dental lesions; however, sufficient evidence is currently lacking, and further studies are required [5].

Regeneration of pulp tissue after infection is complex and has frequently failed because current methods are unable to control inflammation or eliminate bacterial infection adequately. Tissue engineering involves interactions among stem cells, growth factors, and scaffolds. It is well established that deliberate manipulation of these three components can result in functional tissue regeneration that would not occur if repair occurred without intervention[6].

Traditionally, three elements—stem cells, scaffolds, and signaling molecules such as growth factors—are used to achieve dental pulp regeneration and regenerative treatment [5].

All tissues originate from stem cells. These cells are undifferentiated, capable of continuous division and, under appropriate environmental conditions, differentiating into specialized cells or tissues [7]. Adipose-derived stem cells (ADSCs) are a type of mesenchymal stem cell (MSC) that are enzymatically isolated from adipose tissue. These cells express specific surface markers and can differentiate into multiple MSC lineages [8–10]. ADSCs secrete large amounts of important proteins, including growth factors and cytokines, as well as extracellular vesicles and RNAs, which support cellular regeneration, proliferation, differentiation, and migration[11]. In regenerative dentistry, adipose-derived stem cells promote bone formation, periodontal ligament regeneration, cementum formation, reduced inflammatory resorption, and decreased ankylosis following avulsion. Pulp regeneration using pulp stem cells offers advantages such as less invasive extraction procedures, high differentiation potential and proliferation rate, greater resistance to cellular aging, and increased formation of pulp-like tissue compared with bone marrow stem cells [12-14].

Another important factor in pulp tissue reconstruction is signaling molecules such as growth factors. Numerous growth factors have been used to accelerate stem cell differentiation into various lineages, including neurogenic, myogenic, osteogenic, and angiogenic lineages [5].

Scrophularia striata is a plant belonging to the genus Scrophularia. It possesses numerous therapeutic properties, including anti-inflammatory effects and inhibition of oxidative stress. It has also been reported to promote healing of burn wounds infected with Pseudomonas, exhibit therapeutic effects in leishmaniasis, provide neuroprotective effects, protect against neurotoxicity, and act as a growth factor. Traditionally, this plant has been used as an antibacterial agent for treating gastrointestinal inflammation, eye and ear infections, skin burns, hemorrhoids, candidiasis, inflammatory diseases, colds, respiratory conditions, and gingival and oral inflammation. S. striata has anticancer, antibacterial, antifungal, and antidepressant properties [15]. Scrophularia is an exceptional source of flavonoids, and numerous flavonoid compounds have been extracted from it [16]. Many studies have demonstrated the role of flavonoids in regeneration [17]. Some flavonoids increase the proliferation of stem cells

from the apical papilla (SCAP) [16]. Flavonoids can also induce differentiation of stem cells into neurons [18] and exhibit osteogenic and angiogenic properties in bone regeneration [17-18].

Kruppel-like factor 4 (KLF4) is a transcription factor that can directly increase the expression of odontoblast-related genes such as Dmp1, Dspp, and Sp7 by binding to their promoters during odontoblastic differentiation [19]. In addition, the nuclear factor I-C (NFIC) can directly bind to the KLF4 promoter and stimulate its transcriptional activity, thereby regulating Dmp1/Dspp signaling during odontoblast differentiation [20]. MCP-1 is an important biomarker involved in angiogenesis, while BDNF is a key neurotrophic factor. These factors integrate various signaling pathways by responding to inductive signals and modulating the expression of other factors in a DNA sequence–dependent manner. Specific transcription factors, including Dlx3, Runx2, and Sp7, regulate odontoblast differentiation and development. Nevertheless, the precise molecular mechanisms underlying odontoblast differentiation and modulation of signaling pathways and odontoblast marker genes remain unclear [16].

Given that the success rates of pulp regeneration reported in various studies have been highly variable, with maximum success rates of approximately 71–72% [21], and considering the favorable regenerative properties of adipose-derived mesenchymal stem cells and the methanolic extract of Scrophularia striata, the present study aimed to investigate the effects of simultaneous intracanal injection of Scrophularia striata and adipose tissue–derived stem cells on pulp tissue regeneration in rats. To evaluate the effects of these treatments on regeneration, the expression levels of three transcription-related factors in pulp tissue—Kruppel-like factor 4 (KLF4), monocyte chemoattractant protein-1 (MCP-1), and brain-derived neurotrophic factor (BDNF), which are associated with odontoblast differentiation—were measured. In addition, the effects of simultaneous intracanal injection of Scrophularia striata and stem cells on neutrophil migration in pulp tissue were assessed using hematoxylin and eosin staining.

Methods

Study Design and Sample

This experimental-laboratory study was conducted on the maxillary first molars of adult male Wistar rats. The study was performed in the Department of Endodontics, Faculty of Dentistry, Baqiyatallah University of Medical Sciences, from 2024 to 2025. A total of 16 rats were used, with four rats allocated per group. Rats were housed under standard laboratory conditions (22 ± 2 °C, 55 ± 5% humidity, 12-hour light-dark cycle) with free access to water and standard chow (Nuvilab CR-1, PR, Brazil).

Inclusion criteria: Male Wistar rats, 6 weeks old, weighing 180–200 g, Maxillary first molars without caries or anomalies. **Exclusion criteria:** Animals with systemic illness or impaired mobility, Teeth presenting developmental or structural anomalies.

Preparation of Scrophularia striata Extract

Aerial parts of *Scrophularia striata* (flowers, leaves, and stems) were collected during the flowering season from the outskirts of Karaj and authenticated by Dr. Yousef Ajani, a botanist at the National Botanical Garden. Plant material was shade-dried at room temperature and ground into powder.

The methanolic extract was prepared via maceration. Briefly, 1000 g of powdered plant material was soaked in 4000 mL of methanol for 3 days, stirred every 24 hours, and filtered. The residue was re-extracted twice with 3000 mL of methanol for 24 hours each. The combined filtrates were concentrated using a rotary evaporator at 50 °C and further dried in an oven at 30 °C for 24 hours. The final methanolic extract was stored at 4 °C until use.

For this study, a concentration of 100 mg/mL, known for its antioxidant properties, was used. Based on previous reports of molar tooth weight (~1.88 g) and extract concentration, an injection volume of 2.5 µL per tooth was administered [22-24].

Adipose Tissue-Derived Stem Cell (ADSC) Isolation

Adipose tissue was harvested from the bilateral inguinal region of rats under sterile conditions. A 6-cm incision was made along the inguinal fold, the area was disinfected, and fat tissue was excised using a No. 15 scalpel. The tissue was weighed and placed in sterile fetal bovine serum (FBS).

Harvested tissue was minced and washed with phosphate-buffered saline (PBS) containing 5% penicillin/streptomycin. Samples were treated with 0.25% trypsin and incubated in a shaker for 30 minutes. Cells were then centrifuged, and the precipitate was

cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C, 5% CO_2, and 95% humidity in T25 flasks until confluency. Cells were subsequently transferred to T75 flasks for expansion and prepared for flow cytometry analysis. ADSCs were monitored under a microscope (Table 1).

Induction of Pulp Inflammation

Sixteen rats were anesthetized with ketamine/xylazine (80/10 mg/kg). Under a microscope, the maxillary first molars were perforated using a 0.4 mm bur (EndoTracer, Komet, Germany) (Figure 1). Two weeks post-exposure, pulp tissue was left open to induce bacterial contamination. Pulpectomy was performed using small K-files (Mani, Japan). A triple antibiotic paste was applied for 2 weeks prior to regenerative treatment with the experimental materials[25-30].

Animal Grouping and Treatment

Teeth were randomly assigned to four groups (n = 4 per group):

.1 Control: Blood clot only, no injection.

.2 Scro: 100 mg/mL Scrophularia striata extract.

.3 SC: Stem cells only.

.4 SC + Scro: Combination of stem cells and extract.

In the control group, teeth were isolated with a rubber dam (Sanctuary), ISO FUNCTION COMPOUND (GC, USA), and PermaFlo Pink composite (Ultradent, USA). Intracanal bleeding was induced, followed by placement of cold ceramic and restoration with dual-cure glass ionomer cement (Figure 3).

In treatment groups, the designated material(s) were injected into the canals using a Hamilton syringe, followed by cold ceramic placement and sealing with glass ionomer. Occlusal surfaces were reduced to minimize biting forces. Three weeks post-treatment, teeth were extracted, and pulp tissue was harvested and stored at −70 °C for subsequent gene expression analysis.

Real-Time PCR

RNA was extracted from pulp tissue using TRIzol (Gibco-BRL, USA). NanoDrop measured concentration and purity. cDNA synthesis was performed using a cDNA kit. Primers (stock 100 pmol/µL) were diluted to 3 pmol/µL and mixed with Master Mix, water, and cDNA. Reactions were run in triplicate using an ABI Step One plus Real-Time PCR System. Primer specificity was confirmed by melting curve analysis and agarose gel electrophoresis. CT values were exported to Excel, with Actb as the reference gene. Expression of KLF4, MCP-1, and BDNF was quantified according to the manufacturer's protocol.

Histological Analysis

Hematoxylin & Eosin (H&E) Staining: Animals were anesthetized, perfused transcardially with saline and 4% paraformaldehyde (PFA), and teeth with surrounding bone were collected. Samples were fixed overnight, decalcified in 10% EDTA for 14 days, dehydrated through graded ethanol, embedded in paraffin, and coronally sectioned. Sections were mounted on gelatin-coated slides, stained with H&E, and analyzed under a light microscope [31].

Immunohistochemistry (IHC): Paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer. Endogenous peroxidase was blocked with 3% H_2O_2, and non-specific binding was blocked with 10% goat serum. Sections were incubated overnight at 4 °C with primary antibodies against KLF4, MCP-1, and BDNF, followed by biotin-conjugated secondary antibody and streptavidin-peroxidase. Sections were counterstained with Mayer's hematoxylin. Photomicrographs were captured at 100× magnification, and optical density was analyzed using ImageJ[32].

Data analysis

The results were analyzed using GraphPad Prism software at a significance level of 0.05 ($\alpha = 0.05$). One-way ANOVA and Tukey test were used to compare gene expression with the control group.

Table 1. Materials Used for Stem Cell Isolation

Material	Manufacturer	Lot
DMEM	Gibco	2485291x
FBS	Gibco	2378406
L-Glutamine	Bio-IDEA	BI-1202 (100 mL)
PBS	Gibco	1937139
Trypsin/EDTA 0.25%	Bio-IDEA	BI-1602 (100 mL)
Penicillin/Streptomycin (100X)	Bio-IDEA	BI-1203 (100 mL)

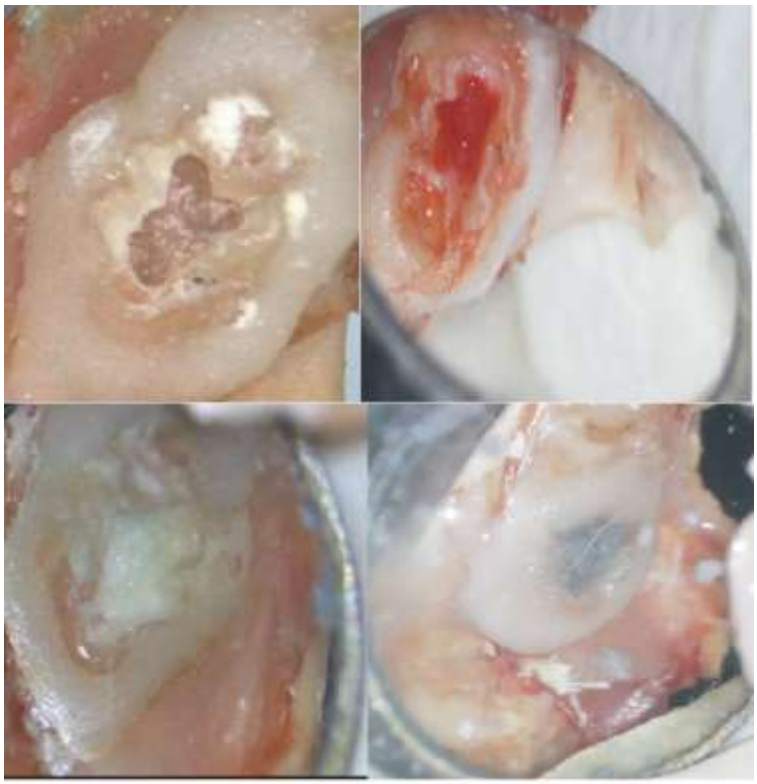

Figure 1: Preparation of the access cavity in the maxillary first molar of the rat and Induction of intracanal bleeding from the periapical area at ×12.5 magnification.

Results

Real-Time PCR Analysis

KLF4 Gene Expression

The fold-change analysis showed that injection of Scrophularia striata alone, stem cells alone, or the combination of Scrophularia striata and stem cells significantly increased KLF4 gene expression compared with the control group (P = 0.001). The highest expression was observed in the stem cell (SC) group (P = 0.001).

Interestingly, the group receiving the combination of Scrophularia striata and stem cells (SC+Scro) exhibited significantly lower KLF4 expression than the SC group alone (P = 0.001). Moreover, SC group expression was significantly higher than the Scro group alone. No other comparisons reached statistical significance.

MCP-1 Gene Expression

Fold-change analysis indicated that injection of Scro alone and SC+Scro significantly increased MCP-1 expression compared to control (P = 0.001). Injection of SC alone did not significantly alter MCP-1 expression relative to control. The SC+Scro group showed a significant increase in MCP-1 expression compared to all other groups (P = 0.001).

BDNF Gene Expression

Fold-change analysis revealed that Scro alone, SC alone, and SC+Scro significantly increased BDNF expression compared to control (P = 0.001). The highest BDNF expression was observed in the Scro group (P = 0.001). The SC+Scro group showed significantly lower expression than the groups receiving Scro or SC alone (P = 0.001). No other differences were statistically significant.

Immunohistochemistry Analysis

KLF4 Protein Expression

ANOVA demonstrated significant differences in mean KLF4 protein expression among groups ($F = 87.01$, $P < 0.0001$). Tukey's post hoc test indicated that the control group had lower expression compared to all experimental groups ($P < 0.001$). No significant difference was observed between Scro and SC groups

(P = 0.09). The SC+Scro group had lower expression than either Scro or SC alone (P < 0.01).

BDNF Protein Expression

ANOVA revealed significant differences among groups (F = 69.82, P < 0.0001). Tukey's test showed that the control group had lower BDNF expression compared to all experimental groups (P < 0.01). Among the three experimental groups, the SC+Scro group exhibited higher expression than either Scro or SC alone (P < 0.001).

MCP-1 Protein Expression

ANOVA showed significant differences among groups (F = 59.66, P < 0.0001). Tukey's test indicated that the control group had higher MCP-1 expression compared to experimental groups (P < 0.001). No significant difference was observed between Scro and SC groups (P = 0.06). The SC+Scro group exhibited lower MCP-1 expression than either treatment alone (P < 0.05).

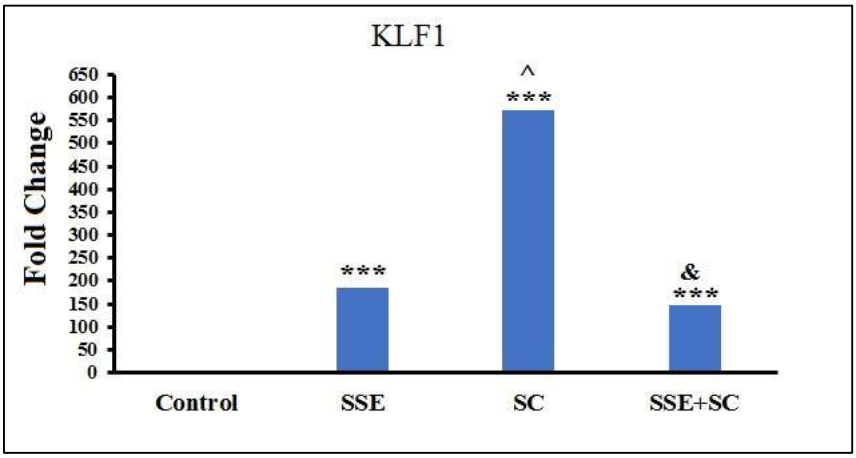

Figure 2: The fold-change of KLF4 in dental pulp tissue across the groups. Data are expressed as mean ± standard error. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Symbols: * indicates significant difference versus control; ^ versus Scro; & versus SC (P = 0.001).

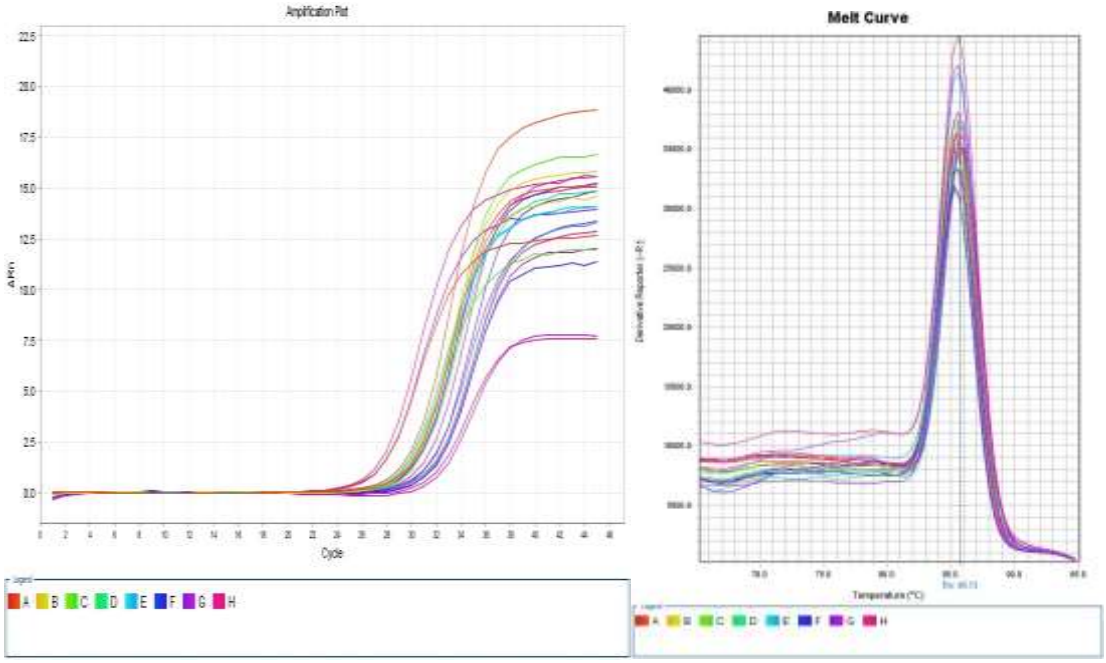

Figure 3: The amplification plot and melting curve of KLF4, respectively.

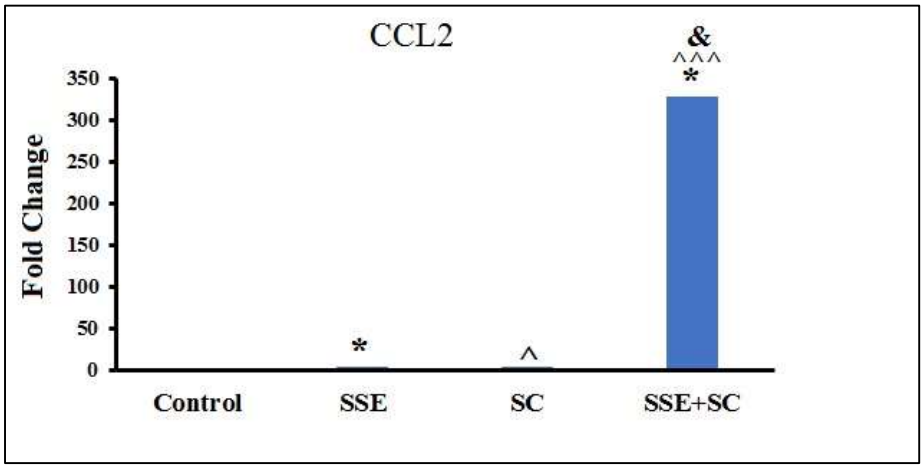

Figure 4: Fold-change of MCP-1 in dental pulp tissue. Data are expressed as mean ± standard error. One-way ANOVA with Tukey's post hoc test was used. Symbols: * vs control; ^ vs Scro; & vs SC (P = 0.001).

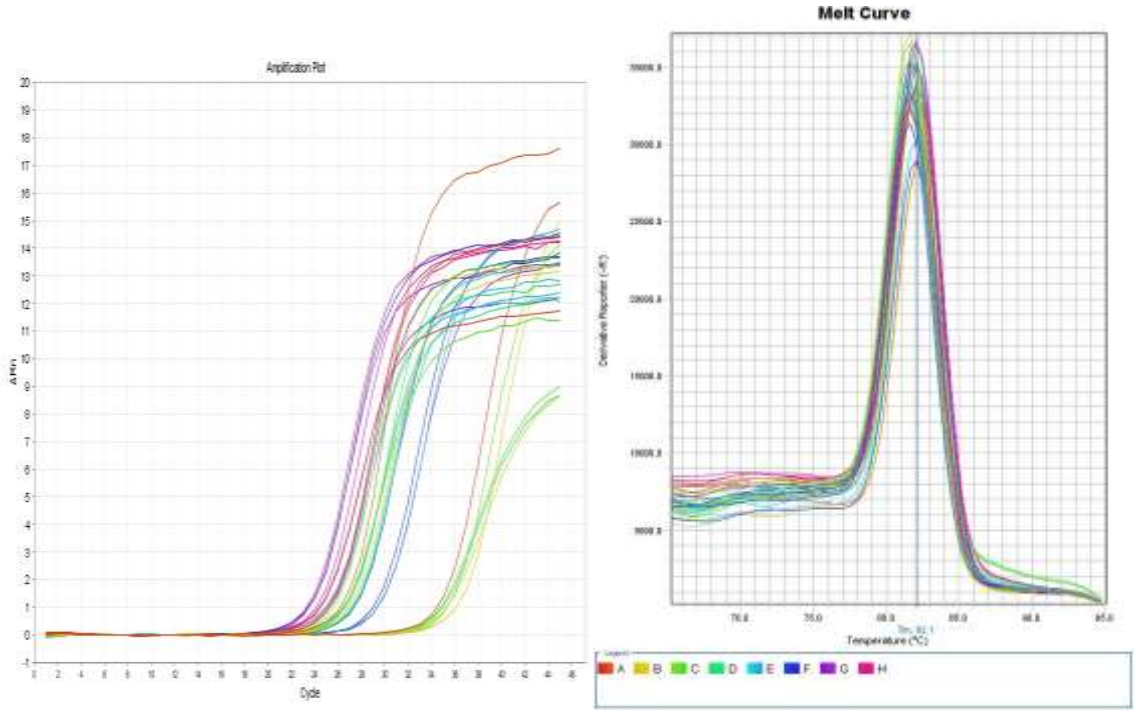

Figure 5: The amplification plot and melting curve for MCP-1.

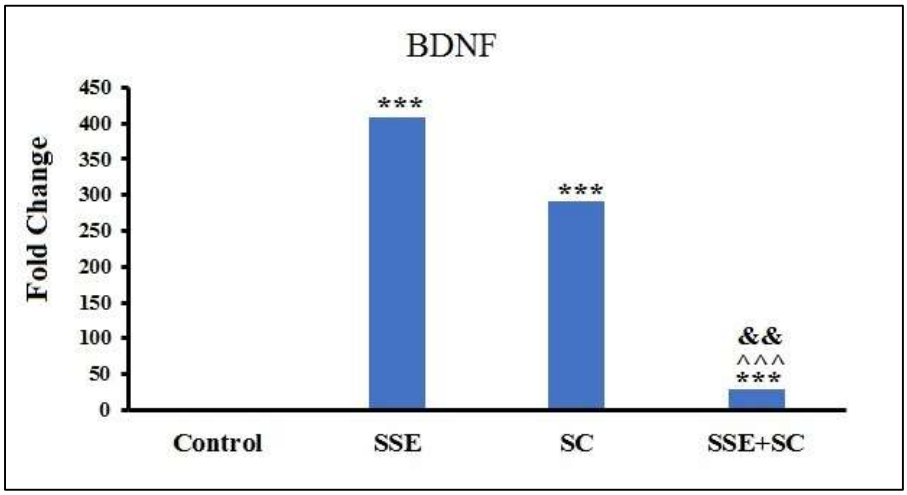

Figure 6: Fold-change of BDNF in dental pulp tissue (mean ± SE). Symbols: * vs control; ^ vs Scro; & vs SC (P = 0.001).

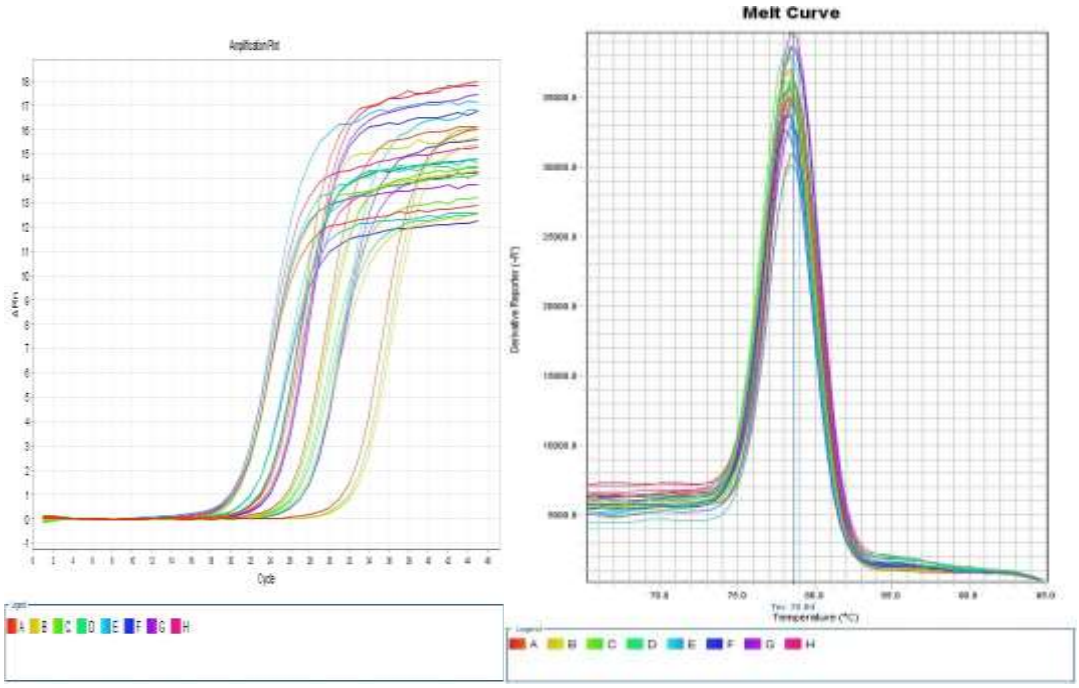

Figure 7: The amplification and melting curves for BDNF.

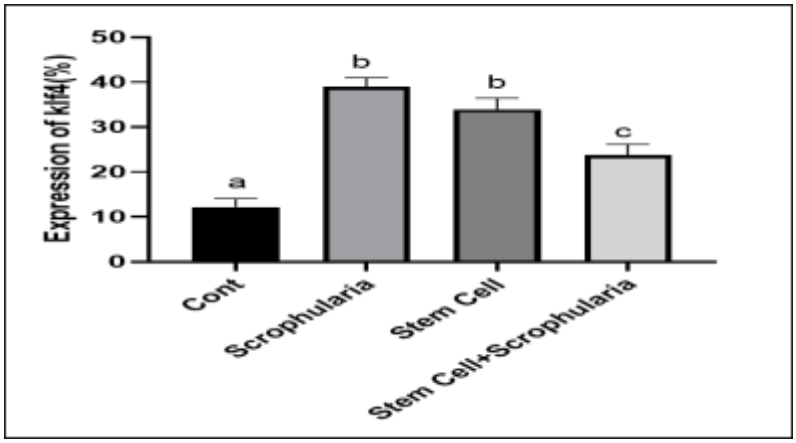

Figure 8: Study of the effects of drug injection on the average expression of the KLF4 protein marker in rat dental pulp tissue.

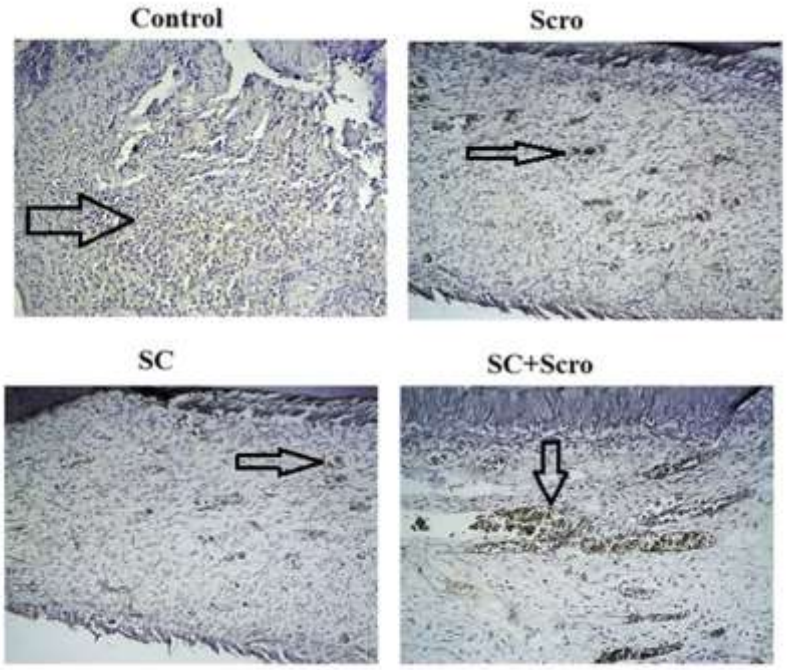

Figure 9: KLF4 immunohistochemical staining across the groups. Data are mean ± SE. Groups with similar letters are not significantly different; different letters indicate significant differences ($P < 0.05$).

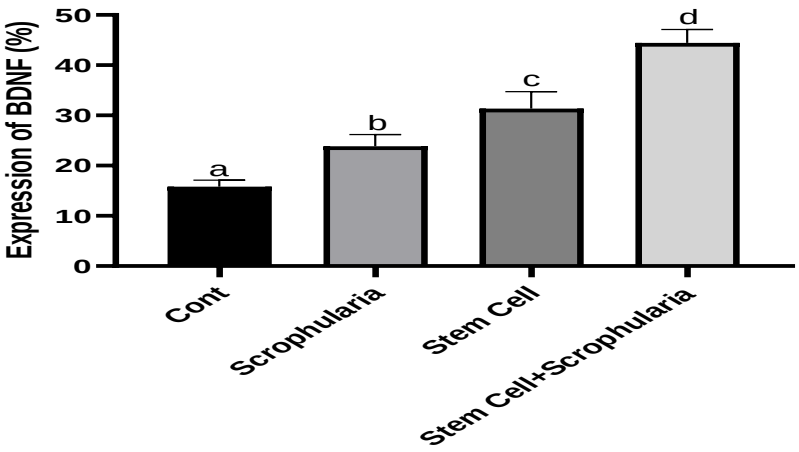

Figure 10: BDNF immunostaining in dental pulp tissue. Data are mean ± SE. Letters indicate statistical differences as above ($P < 0.05$).

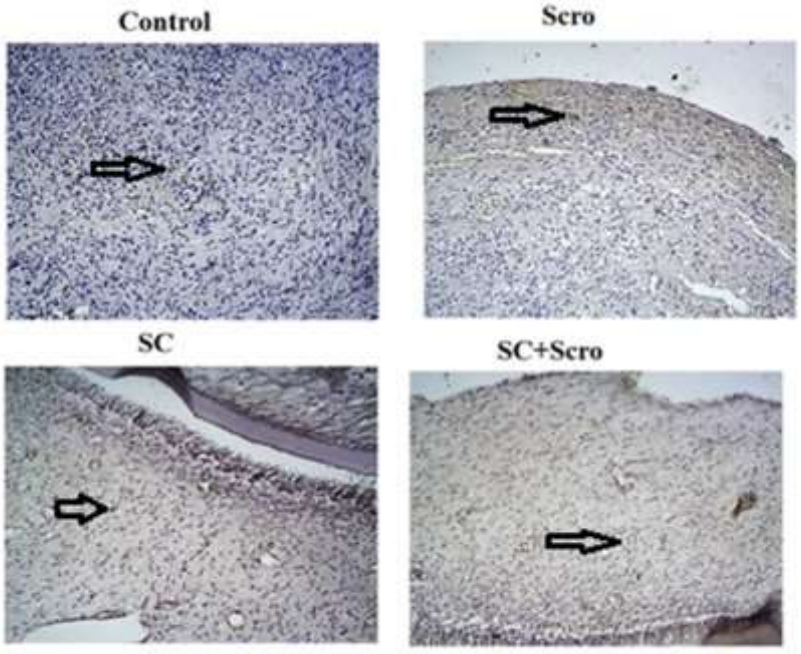

Figure 11: Immunohistochemical images of the BDNF protein marker in rat dental pulp in the four study groups

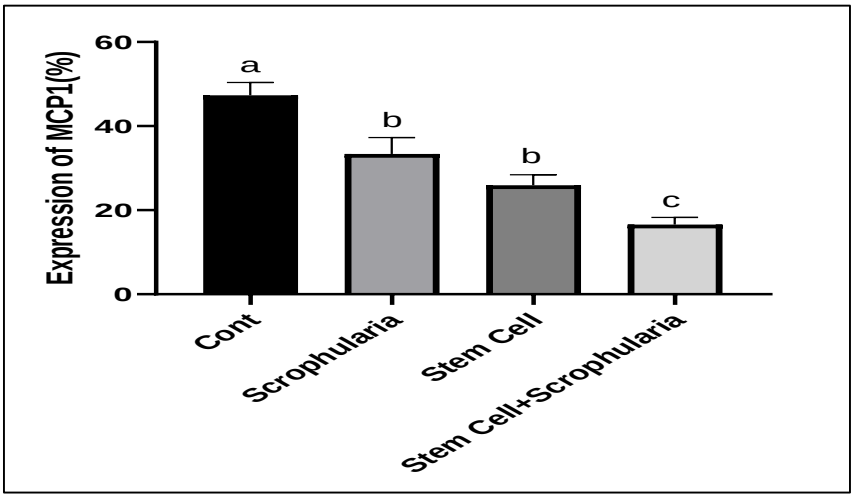

Figure 12: MCP-1 immunostaining in dental pulp tissue. Data are mean ± SE; letters indicate statistical differences ($P < 0.05$).

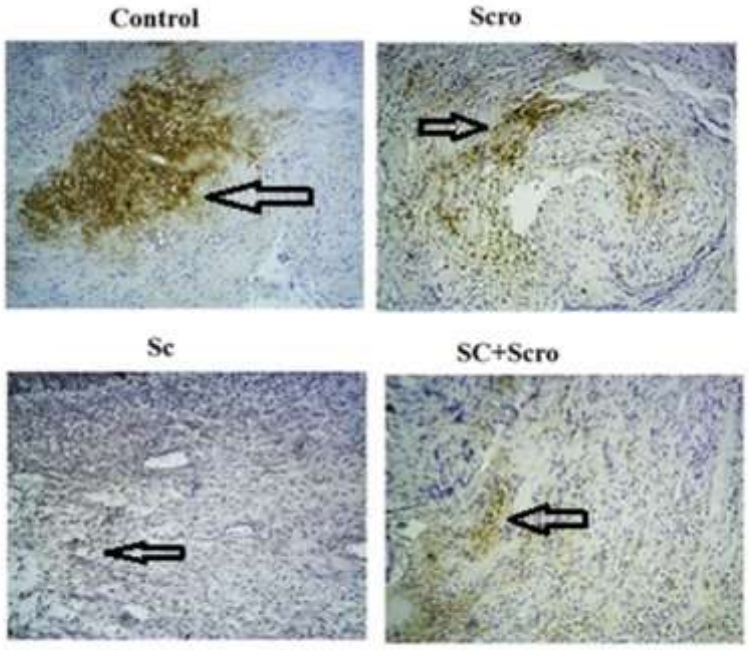

Figure 13: Immunohistochemical images of the protein marker MCP1 in rat dental pulp in the four study groups.

Discussion

Pulp regeneration is a biological strategy for restoring the function of damaged structures of the pulp-dentin complex. It can serve as a practical therapeutic approach to increase the retention rate of teeth affected by pulp infection [34]. Key features of pulp regeneration that should be considered include the formation of new odontoblasts on the existing dentin, as well as neovascularization and neurogenesis (nerve formation) [34, 35]. The latter two are particularly essential for maintaining the long-term vitality of the pulp. One of the main requirements for successful endogenous tissue regeneration is the recruitment of a sufficient number of stem/progenitor cells to the site of injury [35]. This study was conducted to engineer dental

pulp tissue using Scrophularia striata and adipose-derived stem cells (ADSCs).

Mesenchymal stem cells (MSCs) can be isolated from various tissues, including bone, fetal tissue, adipose tissue, muscle, and bone marrow. Previous studies have confirmed that MSCs play crucial roles in tissue repair and regeneration. Adipose-derived stem cells (ADSCs), a population of multipotent stem cells isolated from adipose tissue, are widely distributed in the body and can be easily harvested [36]. ADSCs can be specifically applied in bone tissue engineering to manage alveolar bone defects, particularly in dental implants, periodontal diseases, and the regeneration of the pulp-dentin complex[13, 37].

The concentration and volume of stem cells and plant extract injected were as follows: in the present study, a 100 mg/mL methanolic extract of Scrophularia striata was used, which possesses antioxidant properties [22]. Additionally, previous studies indicate that the weight of each molar tooth is approximately 1.88 g [23]. Regarding the plant extract, based on a concentration of 100 mg/mL per kilogram of tissue weight and the weight of the tooth mentioned, 2.5 µL was injected [22, 24]. In the present study, regarding the dose and number of stem cells injected into the pulp, previous studies indicated that the effective range for adipose-derived MSCs in mice is 1 × 10^7 to 4 × 10^7 cells per kilogram of body weight. Within this range, 2 × 10^7 cells are optimal and more effective (38). Considering that each milliliter of the stem cell suspension contained approximately 1.2 million cells and taking into account the weight of the tooth, 2.5 µL of the suspension was injected using a Hamilton syringe.

In the study by Bagheri-Hosseinabadi et al. (2024), the effect of the hydroalcoholic extract of Scrophularia striata on the differentiation of human adipose-derived stem cells (hADSCs) into chondrogenic and osteogenic lineages was assessed using the MTT assay. The extract reduced hADSC viability at concentrations of 800 and 1000 µg/mL after 24 and 48 hours. In addition to its anti-inflammatory effects, S. striata extract can induce the differentiation of hADSCs toward osteogenic and chondrogenic lineages [39]. In the study by Nam et al. (2020), investigating the effect of Scrophularia species on the differentiation of osteoclasts derived from mouse bone marrow in vitro, it was observed that Scrophularia species can also reduce cell survival [40]. Although in the study by Bigdeli et al. the cytotoxic effect of Iranian Scrophularia extract on colorectal tumor cells (HT29) was observed at concentrations ranging from 0.1 to 10 mg/mL, no cytotoxicity was observed in healthy cells [41]. In the study by Zohiri et al., the toxic effect of ethanolic Scrophularia striata extract was observed at 20% and 50% concentrations on macrophages[42].

KLF4 is expressed explicitly in both polarizing odontoblasts and ameloblasts and is closely associated with the cessation of proliferation and differentiation of these cells, with increased expression [43]. According to IHC findings in the present study, Scro extract increased KLF4 protein expression, promoting odontoblastic differentiation and cellular proliferation. Similarly, stem cells (SC) alone also promoted odontoblastic differentiation and proliferation. However, when used in combination as Scro+SC, although odontoblastic differentiation was observed compared to the control, the level was lower than when Scro or SC was used alone. The absence of synergistic effects may be due to cytotoxicity of the Scro extract on SC, or to SC releasing growth factors or compounds that influence the extract. PCR results were consistent with IHC findings: SC and Scro individually promoted odontoblastic differentiation. Regarding SC+Scro, despite observing odontoblastic differentiation, synergistic effects were still not evident, which may be due to the extract releasing factors that inhibit SC effects, or vice versa.

Lin et al. (2011) found that in dental pulp stem cells cultured in an odontoblastic environment, KLF4 expression increased [44]. This finding aligns with the present study.

Brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB),

form a critical signaling pathway that regulates neuronal survival, growth, and function (45). Emerging evidence suggests that the BDNF-TrkB pathway also plays a key role in inflammatory responses and tissue repair processes (46, 47). BDNF has been shown to contribute to angiogenesis and the stability of cardiac cells in mice and rat-derived endothelial cells. BDNF appears to function via two mechanisms: (1) directly regulating endothelial cell survival and vascular stability, and (2) indirectly regulating capillary network formation via VEGF production[48-50].

The IHC study in the present research showed that intracanal injection of Scro extract and SC led to neurogenic effects. Synergistic effects were observed when Scro+SC was injected, suggesting that the extract likely did not have toxic effects on SC and may have stimulated the expression of secondary mediators or growth factors that specifically reduced SC effects on KLF4. These effects were not observed with BDNF, possibly because they were not released. However, PCR results showed neurogenic effects in the Scro and SC groups, but no synergistic effects in Scro+SC. Not all mRNAs necessarily translate into proteins, so an increase in mRNA does not always indicate an increase in protein levels.

Multiple flavonoid compounds have been extracted from Scrophularia striata [16]. Numerous studies have shown the effects of flavonoids on regeneration. Some flavonoids can increase SCAP proliferation [16] and induce the differentiation of stem cells into neurons [18]. Flavonoids exhibit osteogenic and angiogenic properties in bone regeneration [17]. These effects may explain the increased BDNF gene expression in the Scro group.

Studies by Kim et al. demonstrated that increased BDNF expression in dental pulp stem cells (DPSCs) enhances their regenerative capacity. BDNF/TrkB has a vital role in odontoblastic differentiation of DPSCs under inflammatory conditions [51, 52]. Irfan et al. (2022) reported that BDNF and NGF expression in DPSCs is mediated by inflammation and the C5aR receptor, increasing under inflammatory conditions and potentially promoting neuronal regeneration [53]. In another study, Irfan et al. (2023) found that BDNF expression in DPSCs is regulated by inflammation via the p38MAPKα pathway and C5l2 receptor and is upregulated [54]. Li and Wang (2016) reported that BDNF and NGF not only significantly enhanced the proliferation of bone marrow MSCs (BMSCs) but also promoted osteogenic and neuronal differentiation. In vivo, BDNF was shown to regulate MSC differentiation, stimulate peripheral nerve fiber formation, and induce angiogenesis, thereby improving pulp-like tissue regeneration. Successful revascularization and innervation were observed in pulp tissue regeneration [35]. These findings are consistent with the present study in stem cells.

Monocyte chemoattractant protein-1 (MCP-1), also known as CC chemokine ligand 2 (CCL-2), is a potent chemoattractant for monocytes. It is secreted by various cells in response to inflammatory signals such as TNF-α, IL-1β, and IFN-γ. High MCP-1 expression has been observed in the gingival tissue of adults with periodontitis, indicating its central role in monocyte recruitment in the gingival crevicular fluid (GCF) [55]. MCP-1 contributes to angiogenesis and enhances capillary-like structure formation in human umbilical vein endothelial cells (HUVECs) in vitro [56, 57]. MCP-1 mediates angiogenesis by upregulating VEGF and HIF-α and activating the transcription factor Ets-1, suggesting that its angiogenic properties may be mediated via secondary angiogenic factors [58, 59]. During MSC-mediated tissue regeneration and repair, stem cells can also recruit macrophages by secreting chemokines such as CCL2[60].

According to IHC findings, Scro, SC, and Scro+SC reduced the MCP-1 protein marker. MCP-1 is activated early in inflammation and has dual effects: at high concentrations, it excessively recruits macrophages, and its angiogenic effects are primarily observed in tumors such as melanoma. At lower concentrations, it mainly exhibits anti-inflammatory effects. In the present study, anti-inflammatory effects were observed at low

concentration, which is why angiogenic effects were not evident. PCR results align with this, showing a reduction by the extract. At the same time, SC and Scro+SC increases do not contradict IHC results, as an increase in mRNA does not necessarily indicate an increase in protein.

Hayashi et al. (2015) compared the regenerative potential of cultures derived from dental pulp cells, bone marrow, and SP cells isolated from CD31-negative adipose tissue in an ectopic tooth transplantation model. Increased expression of CXCL14 and MCP-1 was observed in pulp SP cells, suggesting that the MCP-1/CCR2 axis may regulate both endothelial cell migration and new angiogenesis [56]. Ishikawa et al. (2019) studied the osteogenic activity of MCP-1/sSiglec-9 in vitro and in a rat calvarial defect model, showing that MCP-1/sSiglec-9 induced M2 macrophages that expressed higher levels of osteogenic mRNAs, including Igf-1, Tgf-β, Hgf, Bmp2, and Fgf2. MCP-1/sSiglec-9 accelerated bone formation and recruited anti-inflammatory M2 macrophages to the calvarial defect, promoting bone regeneration [61].

Masson's trichrome and H&E staining in line with IHC studies showed that Scro and SC enhanced cellular differentiation and proliferation, increased cell numbers, and reduced edema and inflammation. However, they had little effect on angiogenesis.

In the present study, mouse molars were used, which are a reliable model for testing the biocompatibility of dental materials and instruments in pulp and dentin studies [62]. Additionally, root growth in the first molar of Wistar mice is complete by 8 weeks of age [63].

Conclusion

The present study demonstrated that Scrophularia striata (Scro) extract significantly increased KLF4 and BDNF expression while reducing MCP-1 expression in rat dental pulp. The combination of Scro and adipose-derived stem cells (SC) enhanced the expression of KLF4, BDNF, and MCP-1, thereby improving pulp regeneration. SC alone increased KLF4 and BDNF expression, had no significant effect on MCP-1, and promoted tissue regeneration. Overall, the findings indicate that Scro extract exerts neurogenic, anti-inflammatory, and odontoblastic differentiation effects, while SC shows similar effects. The combined treatment primarily promoted neurogenic effects, as evidenced by increased BDNF expression. These results suggest that S. striata extract can enhance the expression of dentinogenesis-related factors and contribute to effective pulp tissue regeneration.

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Conflict of Interest Disclosures

The authors have no relevant financial or non-financial interests to disclose.

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Authors' Contributions

Conceptualization; Methodology; Validation, Writing & editing: Arezoo Liaghat, Behnam Bolhari, Samira Hajisadeghi, Masood Ghorbani Mahmood Salesi, Narges Marefati, Zahra Bahari.

Ethical Statement

This study was registered with the ethical code (IR.BMSU.AEC.1403.061) and in the history of Baqiyatullah University of Medical Sciences. The protocol of this study was designed in accordance with the ethical principles approved by the international committees for the protection of the rights of laboratory animals. Compliance with the Helsinki rules regarding animal studies, including the use of the minimum possible number of research animals without causing problems in terms of sample size. Emphasis on compliance with hygiene principles and care by those caring for the animals during the study period.

Declaration of Generative AI and AI-assisted

technologies

Not cleared.

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