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The effects of *Scrophularia striata* extract and adipose-derived stem cells on the expression of VEGF, CGRP, and TNF-α genes in dental pulp regeneration in an animal model

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ABSTRACT

Introduction: *Scrophularia striata* possesses wound-healing, anti-inflammatory, and antioxidant properties. The present study aimed to assess the angiogenic and anti-inflammatory effects of *Scrophularia striata* extract (SSE) and adipose-derived stem cells (SCs) on the expression of vascular endothelial growth factor (VEGF), calcitonin gene-related peptide (CGRP), and tumor necrosis factor-alpha (TNF-α) genes during pulp tissue regeneration in rat.

Methods: The experiment involved 16 male Wistar rats. Teeth were randomly allocated into four experimental groups (n = 4 per group): 1. Control, 2. SSE extract alone, 3. SCs alone, and 4. SCs+SSE. Two weeks following the induction of pulpal infection, intracanal injections of SSE or SCs were administered. After an additional 3 weeks, the treated teeth were extracted to collect dental pulp tissue. Quantitative real-time polymerase chain reaction (qRT-PCR) was applied to measure the VEGF, CGRP, and TNF-α gene expression levels.

Results: The mean VEGF expression in SCs+SSE group was significantly lower than in the control ($P<0.01$), whereas its level in the SSE group was significantly higher ($P<0.01$). The lowest TNF-α expression was observed in the SCs+SSE group, with the SSE group showing a significant increase compared with the control group ($P<0.001$). The lowest CGRP expression was observed in the SCs+SSE group, but mean CGRP gene expression in the studied groups was similar to that of the control group ($P>0.05$).

Conclusion: The results of this study suggest that adding SCs to SSE may provide a protective modulatory effect by suppressing inflammation and angiogenesis without significantly altering CGRP expression.

Implication for health policy/practice/research/medical education:
This experimental study shows that *Scrophularia striata* extract (SSE) increases vascular endothelial growth factor (VEGF) expression, and when used with stem cells (SCs), lowers proinflammatory markers (CGRP and TNF-α). These results suggest a possible treatment that may reduce the need for traditional root canal therapy after pulpal injury. Clinically, this supports the exploration of less invasive methods to preserve pulp tissue. In health policy, creating clear, evidence-based guidelines for these biological treatments could make tissue-preserving care more widely available and reduce long-term dental problems. Future studies should focus on how to apply these findings in practice, including the optimal doses, delivery methods, and safety.

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Introduction

The preservation of dental pulp vitality remains a primary goal in modern endodontic therapy, as maintaining a functional pulp-dentin complex contributes to increased tooth longevity and minimizes the necessity for invasive procedures. Vital pulp therapy helps inhibit apical periodontitis from developing and supports normal root growth. This keeps the tooth in place and working as it should (1). While conventional treatments for pulpal disease are effective at eliminating infection, they often involve removing vital tissue, compromising the tooth's biological and biomechanical properties. Conversely, regenerative endodontic strategies aim to restore the physiological and structural integrity of the dental pulp tissue by utilizing the reparative capacities of stem cells (SCs), growth factors, biodegradable polymers, and polymer-based scaffolds (2,3).

Adipose-derived SCs are promising candidates for regenerative applications because of their accessibility, multipotency, and demonstrated capacity to promote angiogenesis and modulate immune responses (4). The interaction between SCs and bioactive plant-derived compounds represents a potential strategy to improve regenerative outcomes and promote mesenchymal stem cells (MSCs) differentiation, proliferation, and migration (5). The main idea behind these combination therapies is that plant-based bioactive compounds may help create a microenvironment that supports pro-regeneration by reducing oxidative stress and inflammation. This can improve SC survival, SC transplantation, paracrine signaling, differentiation, and maintenance of long-term function (6). For example, in previous studies, *Scrophularia striata* extract (SSE) potentially improved human adipose-derived stem cells (hADSCs) proliferation toward osteogenic and chondrogenic phenotypes, and differentiation of mouse bone marrow-derived osteoclasts (7,8). Taken together, these approaches are expected to have a more substantial effect than using either alone.

Medicinal plants and their derivatives are known for their significant amounts of tannins, flavonoids, saponins, alkaloids, naphthoquinones, and triterpenes; thus, they have been employed for many years to manage various diseases due to their anti-inflammatory, antioxidant, and safety profiles (9-11). In the western region of Iran, *Scrophularia striata* (SS) is a widely used medicinal plant. This species belongs to the genus *Scrophularia* (12,13). It is recognized for various pharmacological properties, such as notable anti-inflammatory and antioxidant effects, neuroprotective, analgesic, antimicrobial, antifungal, and antidepressant activities (14,15).

Angiogenesis and the modulation of inflammatory responses are essential biological processes for successful pulp regeneration, as both vascular supply and regulated immune activity determine the microenvironment necessary for tissue repair and cellular differentiation (16). Growth factors control many cellular mechanisms involved in the repair and regeneration of dental tissue. Vascular endothelial growth factor (VEGF) is an important regulator of both normal and abnormal blood vessel formation, promoting endothelial cell division and encouraging angiogenesis (17). VEGF is known as a vascular permeability factor due to its role in regulating vascular permeability, an essential process in angiogenesis (18). Calcitonin gene-related peptide (CGRP) is a key mediator of infection and is also increased in inflammatory conditions in the pulp. It is a proinflammatory peptide. In the tooth, the CGRP receptor is expressed in ameloblasts and odontoblasts, and is involved in tooth crown and root growth (19). Similarly, tumor necrosis factor-alpha (TNF-α) is a central pro-inflammatory cytokine in the pathophysiology of dental pulp inflammation, synthesized by multiple resident and infiltrating cell populations in response to bacterial infection (20). This cytokine causes tissue damage in the inflamed pulp by attracting immune cells and amplifying the inflammatory response. Inflammation in this area can disrupt repair processes and lead to its necrosis (21).

Although SCs and medicinal plant extracts are known for their roles in regenerative medicine, there is still limited experimental evidence on how they interact to modulate angiogenic and inflammatory markers in dental pulp tissue. This study investigated the effects of SSE and adipose-derived SCs on the expression of VEGF, CGRP, and TNF-α genes during pulp tissue regeneration in an animal model.

Materials and Methods

Animals

Sixteen male Wistar rats, aged six weeks and weighing between 180 and 200 g, were procured from the Laboratory Animal Center of Baqiyatallah University of Medical Sciences. The animals were maintained in plastic cages under controlled environmental conditions, including a 12-hour light/dark cycle and a stable temperature of 22 ± 3 °C. Throughout the study, they were provided ad libitum access to standard laboratory chow and water.

Experimental design

We conducted this study on 16 male Wistar rats. The teeth were randomly divided into four groups (n = 4 animals per group, 8 teeth per group) as follows: 1. Control, 2. SSE alone, 3. SCs alone, and 4. SSE+SCs. Initially, an infection model was induced in all animals. In a way that the right and left maxillary first molars were drilled on the occlusal surface (access cavity preparation) using a contra-angle low-speed dental handpiece equipped with the EndoTracer bur with size number 004# (Komet Dental, Germany), and pulp was exposed to the oral cavity for 2 weeks (Figure 1A) to induce infection (22).

Regenerative endodontic procedure in rats

The regeneration procedure was based on the American

Association of Endodontists Clinical Considerations for a Regenerative Procedure in 2021 (23), as illustrated in Figure 1. General anesthesia was achieved through intraperitoneal injection of a ketamine-xylazine anesthetic regimen (80 mg/kg and 10 mg/kg, respectively). In all groups, teeth were isolated using a rubber dam (Sanctuary dental dam latex, Malaysia), ISO-FUNCTIONAL STICKS (GC International AG), and Permaflo Pink composite (Ultradent, United States). Following the regeneration protocol, after two weeks, pulpectomy and root canal disinfection were done, and a triple antibiotic was placed in the root canals for two weeks. After that, bleeding was induced in all 4 groups' root canals by a small-sized K-file (Mani, Japan) (Figure 1B). In treatment groups 2, 3, and 4, SSE and/or SCs (either individually or in combination [SSE + SCs]) were injected into the canals using a Hamilton syringe. Cold Ceramic (SAMIN, Yazd, Iran) was subsequently placed in all teeth (Figure 1C), and the teeth were restored with dual-cure glass ionomer (Iono Cid_B, FSDS, Iran) (Figure 1D). The occlusal surfaces of all filled teeth were reduced to minimise occlusal force on the teeth. Three weeks after the dental procedure, animals were re-anaesthetised with a ketamine-xylazine mixture (80/10 mg/kg), the teeth were extracted, and the dental pulp tissue was harvested. Subsequently, the expression levels of VEGF, CGRP, and TNF-α in the pulp were quantitatively assessed real-time polymerase chain reaction (RT-qPCR)

All regenerative endodontic procedures on the rat maxillary first molar were performed in accordance with the American Association of Endodontists Clinical Considerations for a Regenerative Procedure (23) under a dental operating microscope magnification. The experimental protocol consisted of the following steps:

A. Preparation of the access cavity and induction of root canal infection by exposing the canal system to the oral environment for two weeks (5× magnification);
B. Following the disinfection phase, induction of intracanal bleeding from the periapical region to establish a blood clot scaffold (12.5× magnification);
C. Placement of cold ceramic material over the formed blood clot (12.5× magnification);
D. Final coronal sealing with glass ionomer cement (12.5× magnification).

This sequence was performed under standardized microscopy to ensure procedural consistency and reproducibility.

Isolation of adipose-derived SCs

Isolation of adipose-derived SCs was based on previous studies (24). Male Wistar rats (4-6 weeks) were sacrificed after an intraperitoneal injection of ketamine/xylazine chlorhydrate. Adipose tissue was meticulously dissected and placed in individual Petri dishes, with one tissue sample per dish. The tissue was finely minced using sterile surgical scissors and subsequently homogenized in 1 mL of sterile phosphate-buffered saline (PBS). The processed adipose tissue was transferred to a sterile 50 mL Falcon tube and washed twice with PBS. Thereafter, 20 mL of collagenase solution (0.5 U/mL) was added, and the tubes were incubated on a shaker at 37 °C for 45 minutes. Enzymatic activity was terminated by adding 20 mL of 10% fetal bovine serum (FBS), followed by centrifugation at 1200 rpm for 5 minutes. The adipose layer and supernatant were discarded, and the resulting cell pellet was washed with 40 mL of PBS and centrifuged

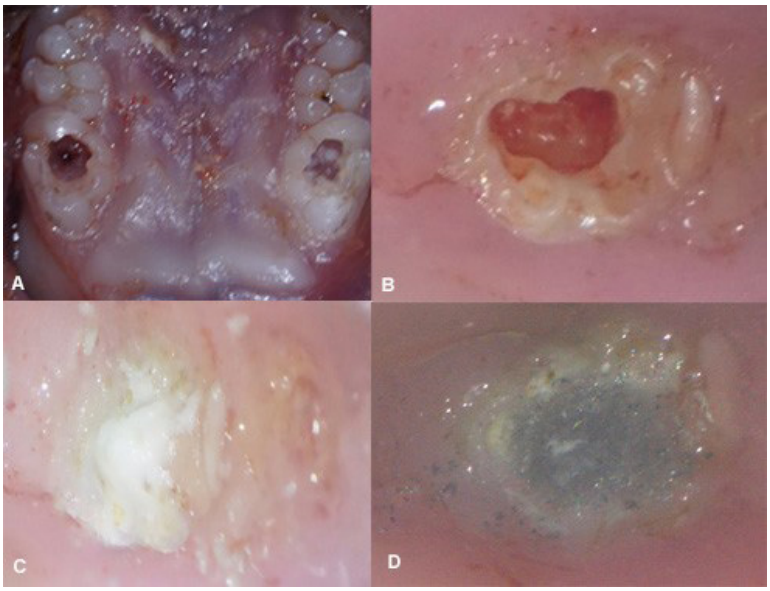

Figure 1. Dental pulp regeneration model in rat maxillary first molars. A) Access cavity preparation and induction of experimental root canal infection by pulp exposure to the oral environment for two weeks (5× magnification). (B) Induction of intracanal bleeding after canal disinfection by mechanical irritation of periapical tissues to form a blood clot scaffold (12.5× magnification). (C) Placement of cold ceramic biomaterial over the formed blood clot (12.5× magnification). (D) Final coronal sealing with dual-cure glass ionomer cement (12.5× magnification).

again under the same conditions. The harvested cell pellet was thoroughly rehydrated in 2 mL of enriched culture medium, which consisted of Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1% penicillin-streptomycin-fungizone (PSF) to ensure optimal cell viability and growth. One milliliter of this cell suspension was then seeded into a T150 flask containing 25 mL of growth medium for subsequent culture. The culture was maintained for 10-14 days until 80-90% confluency was achieved. The culture medium was replenished every 48 hours, and cellular subculturing was performed using 1× trypsin-EDTA to facilitate detachment and propagation.

Flow cytometric analysis of surface markers of adipose-derived SCs

The phenotype of adipose-derived SCs was characterized by flow cytometric analysis. Positive markers (CD44/CD90-PE) and negative markers (CD34-PE, CD45-FITC) were detected using fluorochrome-conjugated monoclonal antibodies. Isotype controls included IgG1-FITC and IgG1-PE. Third-passage adipose-derived SCs were resuspended in PBS at a density of 5×10^5 cells/mL, incubated with the appropriate antibodies for 30 minutes at 4 °C, washed with PBS, and then resuspended in FACS buffer for analysis using a MOFlo™ high-performance cell sorter.

Real-time RT-PCR

The procedure was executed in full compliance with the manufacturer's standardized protocol accompanying the kit. To evaluate the expression of VEGF, CGRP, and TNFα genes in dental pulp by quantitative PCR, RNA was first extracted from tissue samples using TRIzol. To ensure the accuracy of the extraction, the samples were measured using the Nanodrop device. After that, complementary DNA (cDNA) was synthesized from the isolated single-stranded RNA using a commercial cDNA synthesis kit. Subsequently, the primer sequences were diluted to a concentration of 3 pmol/µL with a stock concentration of 100 pmol/µL and combined with other materials such as Master Mix, water, and sample (cDNA) and tested at the appropriate annealing temperature (temperature obtained from the Suntap gel) in triplicate using a Real-Time PCR device (ABI StepOnePlus). The accuracy of each primer was determined after examining the melting curve and the formation of a specific band on the gel. Finally, the CT quantity was output from the Real-Time device to Excel. The Actin beta gene was applied as a reference gene. The expression levels of VEGF, CGRP, and TNFα genes in pulp tissue were analyzed (19,25,26). The PCR primers were synthesized by Invitrogen, and their corresponding sequences are listed in Table 1.

Table 1. Primers for the genes used in the real-time quantitative polymerase chain reaction (PCR) assay

Gene	Primer type	Primer sequence
Actin beta	Forward	GCCTTCCTTCCTGGGTATGG
	Reverse	GAGGTCTTTACGGATGTCAACG
VEGFα	Forward	GCAGAAAGCCTATGAAGTGGTG
	Reverse	CGGGGTACTCGTGGAAGATG
CGRP (Calca)	Forward	AGTCATCGCTCACCAGGGA
	Reverse	GCCAGTAGGCGAGCTTCTTC
TNF-α	Forward	ATGGGCTCCCTCTCATCAGT
	Reverse	GCTTGGTGGTTTGCTACGAC

CGRP: Calcitonin gene-related peptide; TNF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor.

Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS software version 26. Differences in gene expression between control and experimental groups were evaluated using the nonparametric Mann–Whitney U test. Graphical representations were generated with GraphPad Prism version 6.0. A P value of <0.05 was considered indicative of statistical significance.

Results

Flow cytometry

A flow cytometry test was used to assess marker expression and confirm the presence of SCs.CD45 and CD34, used as negative markers, had low expression (Figure 2A).CD44 and CD90, which were used as positive markers, had high expression (98.8% expression for the CD44 marker and 98% expression for the CD90 marker) (Figure 2B).

Adipose SCs were also observed under an inverted microscope. At x10 magnification, individual cells appeared as adherent, elongated, spindle-shaped structures characteristic of mesenchymal SCs. These cells were distributed across the culture surface and exhibited a fibroblast-like morphology, with tapering ends and organization into small bundles or whorled patterns (Figure 3).

Real-time PCR results for CGRP gene expression in rat dental pulp

CGRP gene expression levels in rat dental pulp were the highest in the SCs, SSE, control, and SCs+SSE groups, respectively. The Mann-Whitney U test indicated no statistically significant differences were found between the experimental groups and the control group ($P > 0.05$). Fold change analysis indicated that CGRP gene expression in the SCs group was 2.08 times higher than in the control group. In the SSE group, expression was 1.97 times higher than in the control group. In the SCs+SSE group, expression was reduced to 0.28-fold relative to the control group (Table 2 and Figure 4A).

Additionally, according to the pairwise Man-Whitney U test, no significant differences were observed between

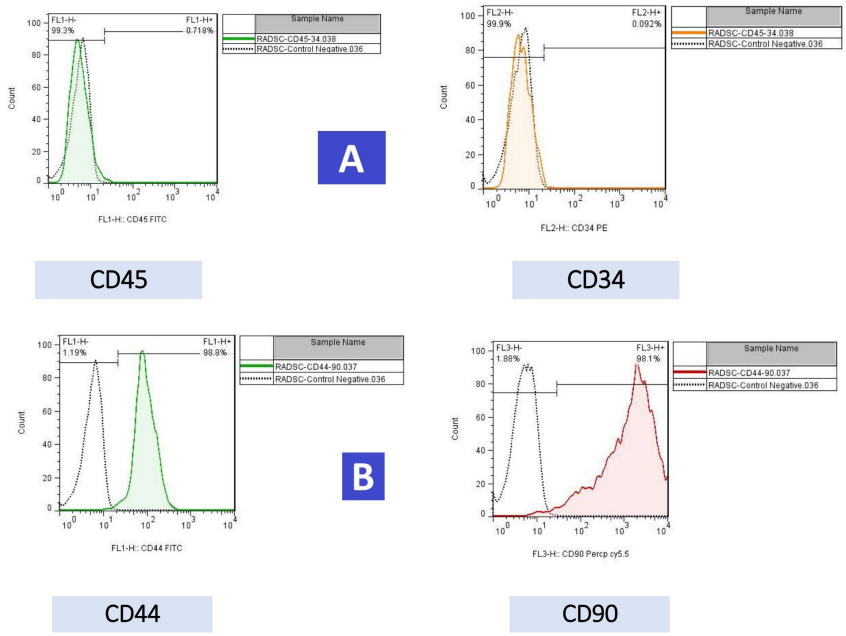

Figure 2. Representative flow cytometry data demonstrating the expression patterns of CD markers in adipose-derived stem cells (SCs). (A) Negative CD markers; (B) Positive CD markers; CD44: Cluster of differentiation 44; CD90: Cluster of differentiation 90 (Thy-1); FITC: Fluorescein isothiocyanate; PerCP-Cy5.5: Peridinin-chlorophyll protein-cyanine 5.5; RADSCs: Rat adipose-derived SCs; FL1-H: Fluorescence channel 1 (FITC); FL3-H: Fluorescence channel 3 (PerCP-Cy5.5).

the SCs and SSE or SCs+SSE groups ($P>0.05$). Likewise, CGRP expression in the SSE group did not differ significantly from the SCs+SSE group ($P>0.05$) (Table 3).

Real-time PCR results for TNF-α gene expression in rat dental pulp

The highest TNF-α gene expression level in rat dental pulp was observed in the SSE, control, SCs, and SCs+SSE groups, respectively. The Mann–Whitney U test indicated that the mean TNF-α expression level in the SSE group was significantly higher than in the control group ($P<0.001$). According to the fold change results, TNF-α expression was reduced in the SCs and SCs+SSE groups relative to the control group, although the differences did not reach statistical significance. TNF-α expression was elevated in the SSE group, which was 10.43-fold higher than in the control group (Table 4 and Figure 4B).

Additionally, TNF-α gene expression in the SSE group was significantly more than in both the SCs and SCs+SSE groups ($P>0.05$). There was no statistically significant difference in TNF-α expression was observed between the SCs and SCs+SSE groups ($P>0.05$) (Table 3).

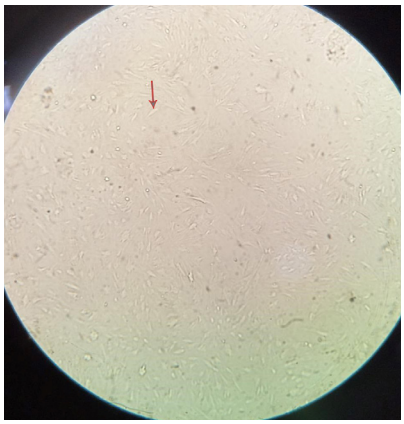

Figure 3. Morphology of adipose mesenchymal stem cells (spindle-shaped) under an inverted microscope at x10 magnification.

Real-time PCR results of VEGF gene expression in rat dental pulp

The highest VEGF gene expression level in rat dental pulp was observed in the SSE, control, SCs, and SCs+SSE groups, respectively. The Mann–Whitney U test indicated that mean VEGF gene expression in the SCs+SSE group was significantly lower than in the control group ($P<0.01$). In contrast, VEGF gene expression was significantly higher

Table 2. Comparison of CGRP gene expression levels in rat dental pulp in various groups relative to the control group

Groups	Mean±SD ΔCt CGRP	Fold Change	*P* value vs control	
SCs	15.64±4.40	2.08	0.32	NS
SSE	15.72±4.13	1.97	0.29	NS
SCs+SSE	18.53±5.07	0.28	0.29	NS
Control	16.70±4.17	1	-	-

SCs: Stem cells; SSE: *Scrophularia striata* extract; SC + SSE: Mix group; Control: Blood clot; CGRP: Calcitonin gene-related peptide; NS: Not significant.

Table 3. Pairwise comparison of ΔCt values for CGRP, TNF-α, and VEGF gene expressions

Groups	SCs- SSE	SCs- SC+SSE	SSE- SC+SSE	SCs- Control	SSE- Control	SC+SSE- Control
ΔCt CGRP	0.91	0.07	0.20	0.32	0.29	0.29
ΔCt TNF-α	0.001	0.95	*P*<0.001	0.77	*P*<0.001	0.72
ΔCt VEGF	0.002	0.35	*P*<0.001	0.32	0.01	0.01

SCs: Stem cells; SSE: *Scrophularia striata* extract; SC + SSE: Mix group; CGRP: Calcitonin gene-related peptide; TNF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor.

Table 4. Comparison of TNF-α gene expression levels in rat dental pulp in various groups relative to the control group

Groups	Mean±SD ΔCt TNF-α	Fold change	*P* value vs control	
SCs	12.54±1.98	0.90	0.77	NS
SSE	9.01±1.76	10.43	<0.001	S
SCs+SSE	12.72±1.53	0.79	0.72	NS
Control	12.39±1.51	1	-	-

SCs: Stem cells; SSE: *Scrophularia striata* extract; SC + SSE: Mix group; Control: Blood clot; TNF-α: Tumor necrosis factor alpha; NS: Not significant; S: significant.

Table 5. Comparison of VEGF gene expression levels in rat dental pulp in various groups relative to the control group

Groups	Mean±SD ΔCt VEGF	Fold change	*P* value vs control	
SCs	9.01±2.33	0.44	0.32	NS
SSE	6.39±0.89	2.72	0.01	S
SCs+SSE	9.49±1.41	0.32	0.01	S
Control	7.84±1.42	1	-	-

SCs: Stem cells; SSE: *Scrophularia striata* extract; SC + SSE: Mix group; Control: Blood clot; VEGF: Vascular endothelial growth factor; NS: Not significant; S: significant.

in the SSE group compared to the control group ($P<0.01$) (Table 5 and Figure 4C).

Additionally, VEGF gene expression in the SSE group was significantly higher than in both the SCs and SCs + SSE groups ($P<0.05$). There was no significant difference in VEGF expression between the SCs and SCs + SSE groups ($P>0.05$) (Table 3).

Discussion

This study examined the effects of SSE, adipose-derived SCs, and their combination on the expression of genes to angiogenesis (VEGF), inflammation (TNF-α), and neuropeptide signaling (CGRP) in a rat model of dental pulp regeneration. Flow cytometry analysis confirmed the mesenchymal identity of the isolated adipose-derived SCs, as indicated by strong expression of CD44 and CD90 and minimal expression of hematopoietic markers CD34 and CD45, supporting their suitability for regenerative applications. In the study by Bagheri-Hosseinabadi et al, the effects of hydroalcoholic SSE on hADSC differentiation into chondrogenic and osteogenic lineages were evaluated using the MTT assay. The SSE reduced hADSC viability at 800 and 1000 µg/mL after 24 and 48 hours. Moreover, SSE was shown to induce hADSC differentiation toward osteogenic and chondrogenic phenotypes (7). A study examined the effects of *Scrophularia* species extract on the differentiation of mouse bone marrow-derived osteoclasts *in vitro*. *Scrophularia* species could similarly reduce cell viability (8). In another study, Iranian species of *Scrophularia* exhibited cytotoxicity against colorectal cancer cells (HT29) at concentrations spanning 0.1 to 10 mg/mL. In contrast, no toxicity was observed in normal cells (27). Zahiri et al also reported toxic effects

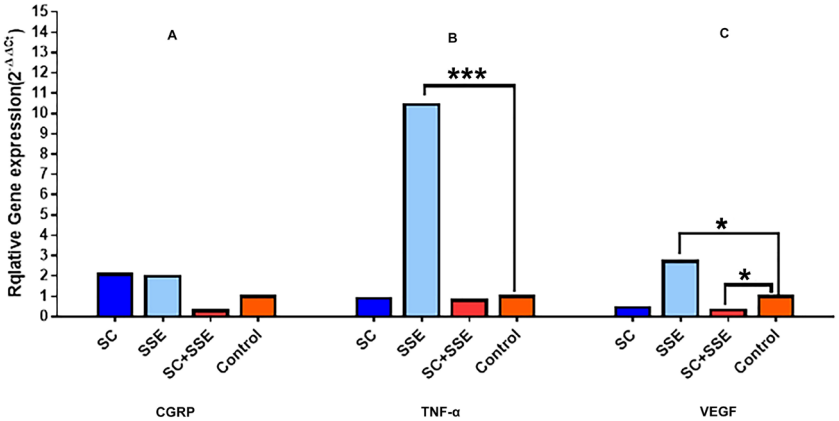

Figure 4. Mean plots of 2-ΔΔCt values for the evaluated genes: (A) CGRP; (B) TNF; (C) VEGF in pulp regeneration in the animal model. *P < 0.05 and *** P < 0.001. SCs: Stem cells; SSE: *Scrophularia striata* extract; SC + SSE: Mix group; Control: Blood clot; CGRP: Calcitonin gene-related peptide; TNF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor.

of the ethanolic SSE at concentrations of 20% and 50% on macrophages (28). The differences observed between the results of the present study and those of previous investigations may be attributed to a combination of methodological and biological factors. Of note, previous investigations have mainly focused on the cytotoxic, anti-inflammatory, and differentiation-inducing effects of SSE in in vitro isolated systems, often using different concentrations of extract and different cell types, such as hADSCs, osteoclast precursors, macrophages, or cancer cell lines. In contrast, the present study evaluated the gene expression profiles of inflammatory and neurovascular markers under conditions that included the combined application of SCs and SSE, which may have altered the biological response through cell-extract interactions and paracrine modulation. Furthermore, differences in SSE preparation (hydroalcoholic or ethanolic extracts), dose, exposure duration, and experimental models may significantly influence cellular outcomes, including cell viability, differentiation, and inflammatory signaling. While previous studies have highlighted SSE-induced cytotoxicity and lineage-specific differentiation at higher concentrations, the results of our study suggest that SSE alone may enhance proinflammatory signaling and angiogenesis, whereas its combination with SCs attenuates these effects. Therefore, discrepancies in results may be due to differences in study design, extract concentration, target cell population, and outcome measures that highlight the context-dependent biological activity of SSE.

The present study found that VEGF expression was significantly improved in the SSE group, whereas it was significantly reduced in the SCs+SSE group, suggesting a reduction in angiogenesis. This decrease may be attributed to potential cell death resulting from the combined treatment. In the current study, the application of adipose-derived SCs to rat pulp reduced VEGF expression, although the decrease was not statistically significant. Fan et al evaluated the effects of ADSC transplantation in a rat model of atherosclerosis. ADSCs were isolated, cultured, and transplanted; the results showed decreased VEGF expression. The authors attributed this decrease to ADSC transplantation's ability to suppress vascular inflammatory responses and endothelial dysfunction in atherosclerotic rats by inhibiting the NF-κB pathway (29). These findings are consistent with the results of our study. Taken together, these findings indicate that SSE and SCs exert distinct biological effects, and the combination of SCs+SSE does not produce a synergistic enhancement of angiogenic or inflammatory gene expression.

The findings also showed that TNF-α expression in the SCs and SCs+SSE groups did not differ significantly from that of the control group. Conversely, the SSE group demonstrated a significant improvement in TNF-α expression relative to the control group. The observed pattern further confirms that SSE and SCs have distinct outcomes, with SCs providing modest immunomodulatory effects that do not amplify the stimulatory effects of SSE. In another study, An et al investigated the impact of TNF-α on endothelial tube formation and vascular network development in a co-culture system of hDPSCs and human umbilical vein endothelial cells (HUVECs). Their findings revealed that TNF-α enhanced angiogenesis and increased VEGF expression (30).

TNF-α plays a dual role, acting as a double-edged sword: at high concentrations, it can suppress cellular differentiation, whereas at lower physiological levels, it can promote cellular differentiation, enhance pulp mineralization, and upregulate the expression of dentin sialoprotein and dentin phosphoprotein (31). The present study showed that adipose-derived SCs reduced TNF-α expression, although this reduction was not statistically significant. Fan et al similarly reported that ADSC transplantation decreased TNF-α levels in rats with atherosclerosis (29). Additionally, a review by Jia et al indicated that SCs therapy was associated with reduced TNF-α levels (32).

According to the present findings, the combined treatment with the extract and SCs did not significantly alter TNF-α expression, although a slight reduction was observed. This mild decrease may be attributable to reduced stem-cell survival, which, in turn, could diminish cytokine production (33).

In the current study, CGRP expression was higher in the SCs group than in the control group, although the difference was not statistically significant. Walker et al reported CGRP expression in adipose tissue and adipose-derived SCs (34), as well as in various other SC types, including those derived from neural tissues (35). CGRP has also been shown to enhance osteoblastic gene expression (36), a finding that aligns with the results of the present study.

In the current study, SSE produced a non-significant increase in CGRP expression. In the study by Lee et al, the efficacy of the ethanolic extract of *Scrophularia buergeriana* (Brainon) was evaluated in a scopolamine-induced mouse model of cognitive impairment. Brainon administration at 10 mL/kg/day dosage for 28 days, induces antioxidant and anti-inflammatory effects through reducing the generation of reactive oxygen species (ROS), IL-1β, IL-6, and TNF-α (37). In a study by Michot et al, which evaluated the effects of CGRP on the viability and proliferation of hDPSCs and their ability to differentiate into mineralizing cells using staining and PCR assays, the authors found that CGRP inhibited hDPSC proliferation, with no stimulatory or inhibitory impact on mineralizing differentiation. They also suggested that CGRP might negatively influence the regenerative or reparative potential of hDPSCs (38).

In the simultaneous use of the extracts and SCs in the present study, a reduction in neurotrophic factor levels was observed, which might be due to an unfavorable interaction between the two components. Overall, the data support the notion that SSE and SCs have independent

effects on gene expression, and their combination does not confer additive or synergistic benefits in dental pulp regeneration.

Conclusion

SSE primarily influenced angiogenic and inflammatory pathways, as indicated by increased VEGF and TNF-α expression, which may suggest a pronounced stimulatory rather than regenerative effect. In contrast, adipose-derived SCs exerted limited influence on angiogenic gene expression but displayed modest immunomodulatory properties. Notably, the combined application of SCs and SSE did not produce regenerative enhancement and, in some instances, reduced angiogenic signaling compared to SSE alone. Collectively, these findings suggest that SSE may provide short-term biological stimulation, whereas SCs exert regulatory effects on inflammation. The lack of combination therapy enhancement underscores the complexity of cell-phytochemical interactions and emphasizes the need for further research to optimize treatment parameters and elucidate their regenerative significance in dental pulp tissue engineering.

Declaration of AI-assisted tools in the writing procedure

Throughout the preparation of this manuscript, the author(s) used Grammarly for language refinement and grammar correction. Afterward, all AI-assisted content was thoroughly reviewed, verified, and edited by the author(s) to ensure accuracy, clarity, and originality. The author(s) take full responsibility for the integrity and final content of the published work.

Conflict of interests

The authors declared no conflict of interest, financial or otherwise.

Ethical considerations

The experimental procedures were reviewed and approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (IR.BMSU.AEC.1403.045) and were conducted in full compliance with institutional guidelines and internationally accepted principles for the humane care and use of laboratory animals (The NIH Guide for the Care and Use of Laboratory Animals).

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