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Research Article

Hypoxia Mesenchymal Stem Cells-Derived Exosomes Suppress IL-5 and STAT3 Gene Expression in a Calcipotriol-Induced Atopic Dermatitis Mouse Model

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Abstract

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by Th2-mediated immune dysregulation and activation of pro-inflammatory signaling pathways. Hypoxia mesenchymal stem cells-derived exosomes (EH-MSCs) have emerged as a promising cell-free therapeutic strategy because of their potent immunomodulatory properties. This study investigated the effects of EH-MSCs on IL-5 and STAT3 gene expression in a calcipotriol-induced AD mouse model. Thirty female C57BL/6 mice were randomly assigned to five groups (n = 6/group): healthy control, AD-like + NaCl, AD-like + dexamethasone, AD-like + EH-MSCs (100 µg/kg body weight), and AD-like + EH-MSCs (200 µg/kg body weight). AD-like lesions were induced by topical calcipotriol, and treatments were administered via subcutaneous injection on days 15 and 19. Relative IL-5 and STAT3 gene expression in dorsal skin tissue was quantified using quantitative real-time polymerase chain reaction (qRT-PCR). Calcipotriol induction significantly increased IL-5 and STAT3 gene expression compared with healthy controls. Treatment with EH-MSCs significantly downregulated the expression of both genes ($p < 0.05$). These findings demonstrate that EH-MSCs attenuate Th2-mediated inflammation and STAT3-associated inflammatory signaling, supporting their potential as a novel cell-free therapeutic strategy for atopic dermatitis.

Keywords: **A**topic dermatitis, EH-MSCs, IL-5, STAT3, Calcipotriol.

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1 Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by impaired skin barrier function, persistent pruritus, and immune dysregulation predominantly driven by T helper 2 (Th2)-mediated responses. The pathogenesis of AD is influenced not only by genetic and environmental factors but also by complex interactions between keratinocytes and immune cells, resulting in the overproduction of pro-inflammatory cytokines through the activation of transcription factors, including signal transducer and activator of transcription 3 (STAT-3).[1] Current standard management of AD relies primarily on topical pharmacological agents, such as corticosteroids, calcineurin inhibitors, Janus kinase (JAK) inhibitors, and phosphodiesterase-4 (PDE4) inhibitors.[2] However, these therapies provide only temporary symptomatic relief, and their long-term use is associated with adverse effects, including cutaneous atrophy, telangiectasia, and impaired epidermal barrier function.[3], [4] Therefore, the development of alternative therapeutic strategies that target immune modulation while offering a favorable long-term safety profile remains a clinical priority. Cell-free therapies, particularly exosomes derived from mesenchymal stem cells (MSCs), have emerged as promising immunomodulatory agents. Exosomes obtained from hypoxia-preconditioned MSCs (EH-MSCs) exhibit an enriched cargo of bioactive molecules, including specific microRNAs (miRNAs) and paracrine factors, which enhance their anti-inflammatory and regenerative properties.[5]

Hyperactivation of the Th2 immune axis and dysregulation of intracellular signaling pathways play central roles in the pathogenesis and chronicity of AD. IL-5 is a key pro-inflammatory cytokine that mediates the recruitment, activation, and survival of eosinophils within AD skin lesions, thereby contributing to persistent tissue inflammation and damage. Concurrently, STAT3 functions as a pivotal transcription factor that orchestrates inflammatory signaling cascades, promotes the production of Th2- and Th22-associated cytokines, and induces keratinocyte hyperproliferation.[6] Previous preclinical studies have demonstrated the therapeutic efficacy of MSCs-derived exosomes in alleviating cutaneous inflammation. For example, adipose-derived stem cell (ADSC)-derived exosomes have been shown to partially suppress the mRNA expression of inflammatory cytokines in experimental models of AD.[7] However, the specific potential of EH-MSCs to modulate upstream signaling pathways, particularly STAT3, and suppress downstream effector cytokines such as IL-5 has not been comprehensively investigated. Mechanistically, the bioactive cargo contained within EH-MSCs, including regulatory microRNAs and anti-inflammatory mediators, may inhibit STAT3 activation and phosphorylation, thereby reducing IL-5 expression, interrupting the inflammatory cascade, and promoting skin repair.[8]

Given these considerations, further elucidation of the molecular mechanisms underlying the anti-inflammatory effects of EH-MSCs is warranted. Accordingly, this study aimed to investigate the effects of EH-MSCs administration on IL-5 and STAT3 gene expression in a calcipotriol-induced atopic dermatitis-like C57BL/6 mouse model.

2 Method

2.1 Mouse UC-MSCs Isolation and Characterization

Umbilical cords were collected from a 19-day pregnant female C57BL/6 mice. After removal of the blood vessels, the umbilical cord tissue was minced into small explants and cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco™, NY, USA) and 1% penicillin–streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced every 3 days, and cells were passaged upon reaching approximately 80% confluence. Umbilical cord-derived mesenchymal stem cells (UC-MSCs) at passages 4–5 was used for subsequent experiments.

The immunophenotype of passage 4 UC-MSCs was characterized by flow cytometry to determine the expression of the mesenchymal markers CD90 and CD29 and the hematopoietic and endothelial markers CD45 and CD31, respectively. The multipotent differentiation potential of UC-MSCs was further evaluated by culturing the cells in osteogenic and chondrogenic induction media for 21 days.

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Osteogenic and chondrogenic differentiation was confirmed by Alizarin Red and Alcian Blue staining, respectively, followed by microscopic examination.[9]

2.2 EH-MSCs Preparation and Characterization

Passage 5 UC-MSCs were cultured under standard conditions until approximately 80% confluence. Cells were then subjected to hypoxic preconditioning (5% O_2) in a hypoxic chamber and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. A hypoxic level of 5% O_2 was selected because it closely mimics the physiological oxygen tension of the mesenchymal stem cell niche and has been shown to enhance MSCs survival, paracrine activity, and extracellular vesicle secretion while minimizing hypoxia-induced cellular stress associated with more severe oxygen deprivation (1–2% O_2).[10] The conditioned medium (CM) was collected and sequentially centrifuged at 300 × g for 5 min to remove cells and at 2,000 × g for 30 min to eliminate cellular debris. The resulting supernatant was concentrated using tangential flow filtration (TFF) with a sterile 300-kDa hollow-fiber polyethersulfone membrane to obtain the secretome.

The concentrated secretome was further processed for exosome isolation by centrifugation at 200 × *g* for 5 min at 4°C, followed by centrifugation of the supernatant at 14,000 × *g* for 10 min at 4°C. All EH-MSCs used in this study were prepared from a single UC-MSCs donor, and pooled donor samples were not used, thereby minimizing donor-related variability. The final supernatant containing exosomes was characterized for the exosomal surface marker CD9 using the Exosome Isolation and Analysis Kit (Abcam, Cambridge, UK according to the manufacturer's instructions.[11] Exosome particle size and concentration were determined using a NanoSight Pro (Malvern Panalytical, Malvern, UK). Nanoparticle Tracking Analysis (NTA) software was used to generate particle size distribution profiles and concentration measurements.[12]

2.3 Animals and Study Design

This in vivo experimental study employed a randomized post-test-only control group design using female C57BL/6 mice aged 6–8 weeks and weighing 20–25 g, obtained from Stem Cell and Cancer Research (SCCR) Indonesia. The animals were housed under standard laboratory conditions, including a 12 h light/dark cycle, ambient temperature of 20–24°C, relative humidity of approximately 60%, and adequate ventilation, with free access to standard chow and water throughout the 22-day experimental period. All experimental procedures were approved by the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang (Approval No. 68/II/2026/Komisi Bioetik), and were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

2.4 Atopic Dermatitis (AD)-Like Mouse Model

Thirty-six female C57BL/6 mice were included in this study, comprising 30 mice assigned to the experimental groups and 6 mice (3 healthy and 3 calcipotriol-induced) used for AD-like model validation. Animals were maintained under standard laboratory conditions (24°C, 12 h light/dark cycle) with free access to food and water. Atopic dermatitis (AD)-like lesions were induced in 27 mice by topical application of 20 mg calcipotriol (MC903) to the right ear and dorsal skin once daily for 14 consecutive days. Model validation was performed by comparing ear and dorsal skin thickness between healthy and calcipotriol-induced mice. On day 15, ear and skin thickness were measured using a digital vernier caliper. The validation mice were subsequently euthanized, and dorsal skin tissues were harvested, fixed in 4% paraformaldehyde, and processed for hematoxylin and eosin (H&E) staining. Histopathological evaluation was performed by assessing the thickness of the stratum corneum, epidermis, and dermis.

2.5 EH-MSCs Treatment and Skin Tissue Collection

Following successful induction of AD-like lesions, six healthy mice and twenty-four calcipotriol-induced AD-like mice were randomly assigned to five groups (n = 6 per group): healthy control, AD-like + NaCl, AD-like + dexamethasone, AD-like + EH-MSCs (100 µg/kg body weight), and AD-like + EH-MSCs (200 µg/kg body weight). The EH-MSCs doses were selected based on previously reported effective exosome protein doses in murine inflammatory disease models and were adapted according to body weight to standardize dosing across experimental animals.[13] Treatments were administered by

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subcutaneous injection on days 15 and 19. The subcutaneous route was selected to provide localized and sustained delivery of EH-MSCs to the inflamed skin while minimizing systemic distribution. The dosing interval was based on the reported biological half-life of exosomes (approximately 72 h),[14] allowing maintenance of therapeutic exposure throughout the treatment period. On day 22, all animals were euthanized, and dorsal skin tissues were harvested for subsequent molecular analysis.

2.6 IL-5 and STAT3 Gene Expression

Total RNA was extracted from dorsal skin tissues using RNA Iso Plus reagent, followed by sonication according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from the extracted RNA using a reverse transcription kit following the manufacturer's protocol. Relative gene expression of IL-5 and STAT3 was quantified by quantitative real-time polymerase chain reaction (qRT-PCR) using gene-specific primers: IL-5 (forward: 5′-ATGGAGATTCCCATGAGCAC-3′; reverse: 5′-AGCCCCTGAAAGATTTCTCC-3′), STAT3 (forward: 5′-CATATGCGGCCAGCAAAGAA-3′; reverse: 5′-ATACCTGCTCTGAAGAAACT-3′), and β-actin (forward: 5′-GGCTGTATTCCCCTCCATCG-3′; reverse: 5′-CCAGTTGGTAACAATGCCATGT-3′), which served as the housekeeping gene. PCR amplification was performed under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with β-actin as the internal reference gene.

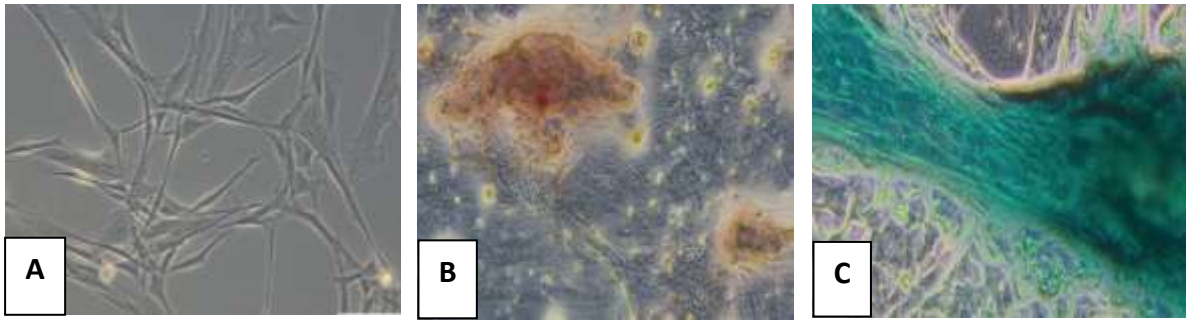
2.7 Data Analysis

Data are presented as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Differences among multiple groups were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey's Honestly Significant Difference (HSD) post hoc test for multiple comparisons. Comparisons between two groups (ear thickness and skin thickness) were performed using an unpaired Student's *t*-test. A two-tailed p value < 0.05 was considered statistically significant.

3 Results and Discussion

3.1 MSCs Characterization

Cultured MSCs exhibited a spindle-shaped, fibroblast-like morphology and adhered firmly to the culture flask surface (Figure 1A). Flow cytometric immunophenotyping demonstrated high expression of the mesenchymal markers CD90 (98.3%) and CD29 (98.2%), with minimal expression of the hematopoietic marker CD45 (0.27%) and the endothelial marker CD31 (4.03%) (Figure 1D). The multipotent differentiation capacity of MSCs was further confirmed by culturing the cells in osteogenic and chondrogenic induction media for 21 days. Osteogenic differentiation was evidenced by calcium deposition, as demonstrated by positive Alizarin Red staining (Figure 1B). Chondrogenic differentiation was confirmed by the accumulation of glycosaminoglycans (GAGs), visualized by Alcian Blue staining (Figure 1C). Collectively, these findings demonstrate that the isolated MSCs fulfilled the minimal criteria established by the International Society for Cellular Therapy (ISCT) for the characterization of mesenchymal stem cells.[15]



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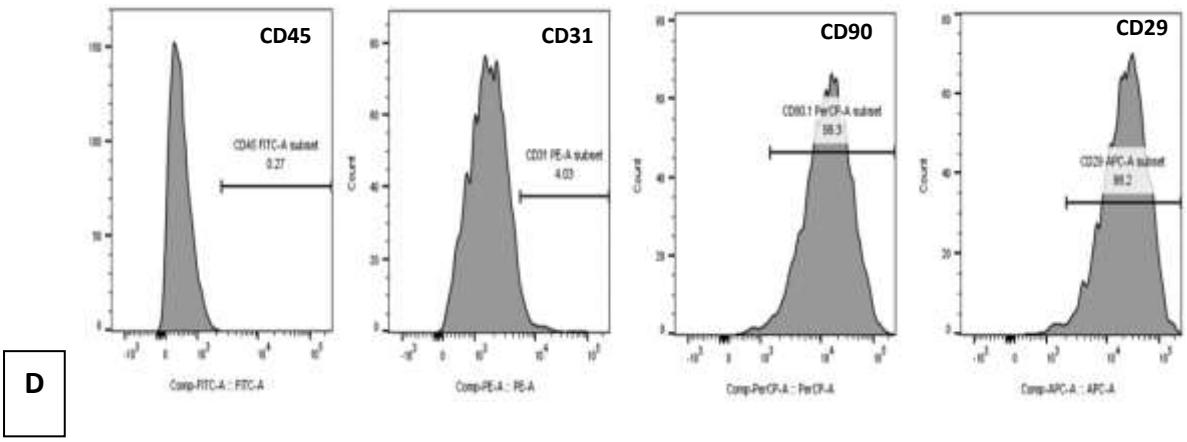

Figure 1 Morphological, immunophenotypic, and differentiation characterization of mesenchymal stem cells (MSCs). (A) Cultured MSCs exhibited a typical spindle-shaped, fibroblast-like morphology. (B) Osteogenic differentiation was confirmed by calcium deposition following Alizarin Red staining. (C) Chondrogenic differentiation was demonstrated by glycosaminoglycan (GAG) deposition following Alcian Blue staining. (D) Flow cytometric analysis showed high expression of the mesenchymal markers CD90 and CD29, with minimal expression of the hematopoietic marker CD45 and the endothelial marker CD31.

3.2 EH-MSCs Characterization

Flow cytometric analysis demonstrated positive expression of the exosomal marker CD9 in the isolated EH-MSCs, with 79.41% CD9-positive particles (Figure 2A). Nanoparticle characterization revealed an average particle size of 133 nm, a modal size of 82.5 nm, and a concentration of 1.41×10^9 particles/mL (Figure 2B), confirming successful isolation of EH-MSCs [16], [17], [18].

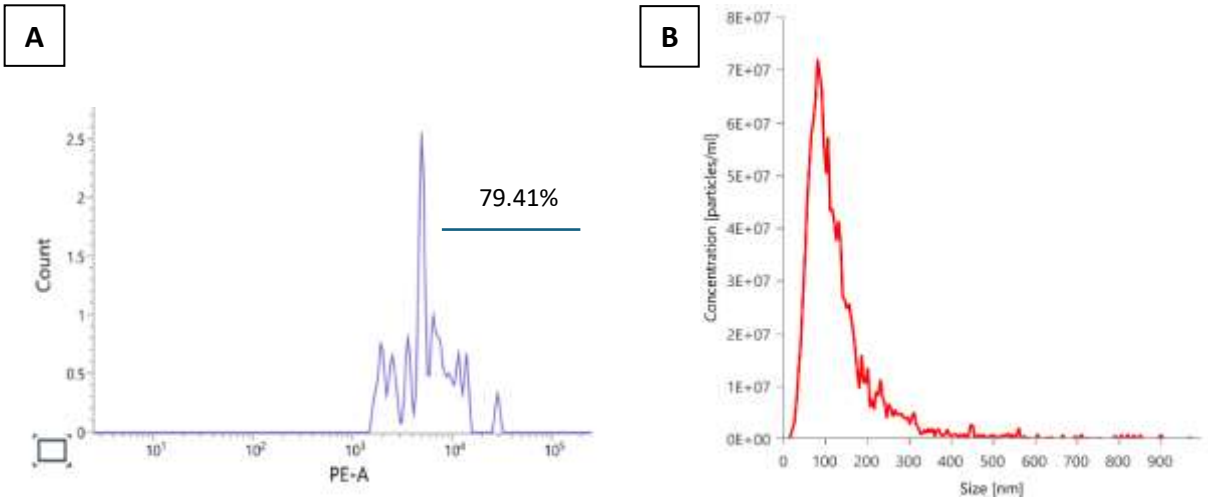

Figure 2. Characterization of EH-MSCs. (A) Flow cytometric analysis demonstrating positive expression of the exosomal marker CD9, confirming successful exosome isolation. (B) Nanoparticle size distribution analysis showing an average particle size of 133 nm, a modal size of 82.5 nm, and a particle concentration of 1.41×10^9 particles/mL.

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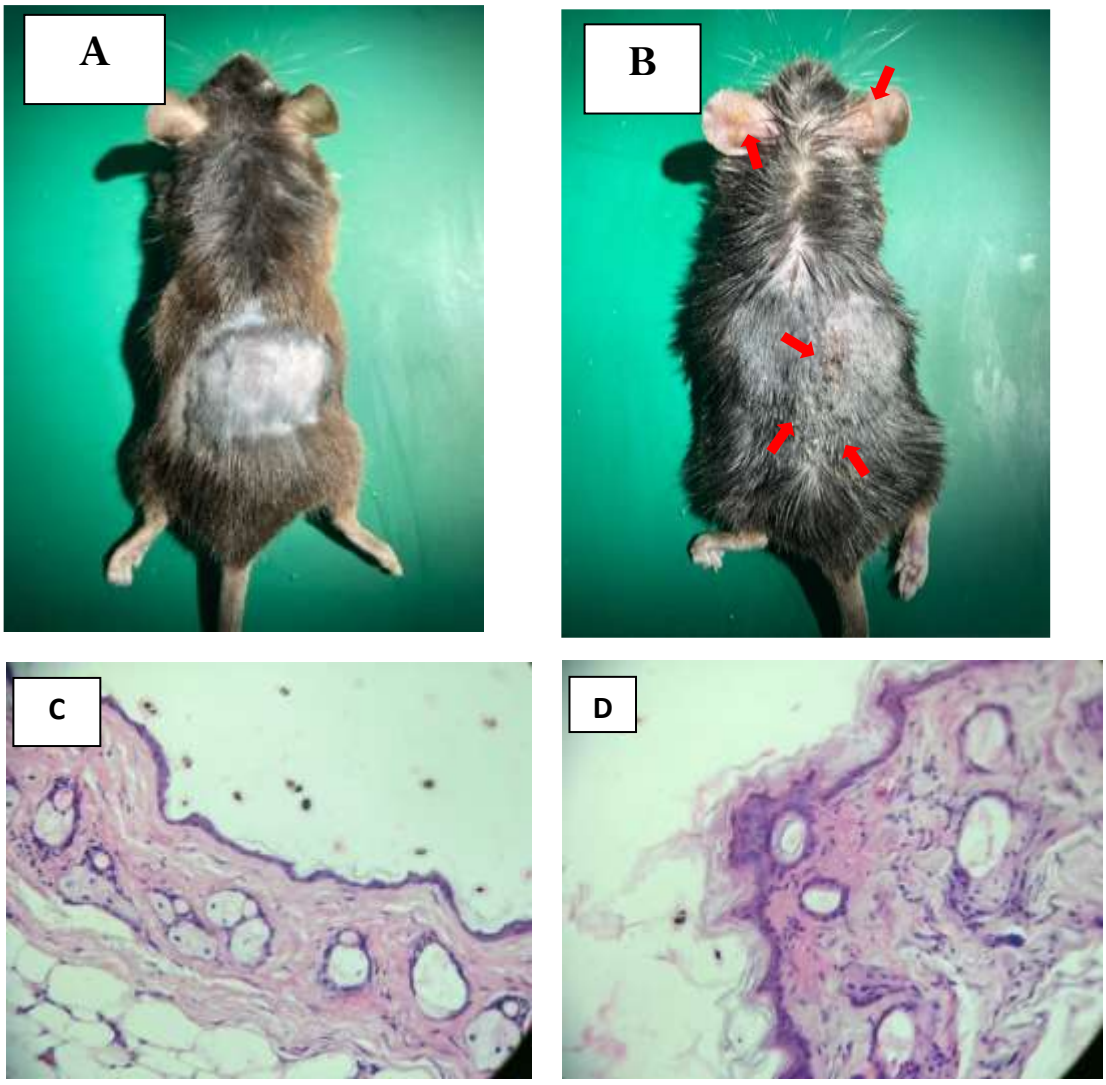
3.3 AD-Like Mouse Model Validation

Validation of the atopic dermatitis (AD)-like mouse model was performed by evaluating clinical and histopathological features in healthy and calcipotriol-induced mice. Macroscopically, healthy mice exhibited intact, elastic skin without erythema, edema, scaling, or crust formation (Figure 3A). In contrast, repeated topical calcipotriol application induced characteristic AD-like skin lesions, including erythema, edema, dry skin, and scaling (Figure 3B). These clinical observations were supported by quantitative measurements of ear and dorsal skin thickness, which served as indicators of cutaneous inflammation. Compared with healthy controls, AD-like mice showed significantly greater ear thickness ($p = 0.0001$) and dorsal skin thickness ($p < 0.0001$) (Figures 3E and 3F).Histopathological examination of hematoxylin and eosin (H&E)-stained dorsal skin sections further confirmed successful AD induction. Skin from healthy mice displayed normal epidermal architecture, a thin stratum corneum without parakeratosis, and intact dermal structure (Figure 3C). In contrast, AD-like mice exhibited marked hyperkeratosis, epidermal hyperplasia (acanthosis), dermal thickening, prominent mononuclear inflammatory cell infiltration, and epidermal edema (spongiosis), all of which are characteristic histopathological features of AD (Figure 3D). Collectively, these clinical, morphometric, and histopathological findings confirmed that repeated topical calcipotriol application successfully established an AD-like mouse model that closely recapitulated the key features of human atopic dermatitis.[19]



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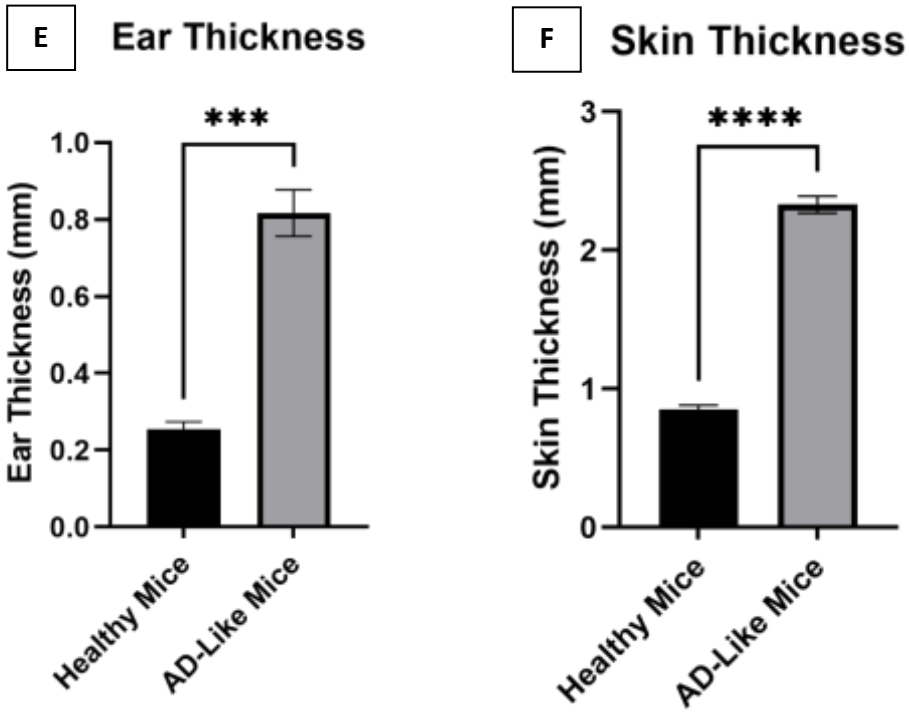

Figure 3 Validation of the calcipotriol-induced atopic dermatitis (AD)-like mouse model. Representative macroscopic images of (A) healthy mice showing normal skin and (B) calcipotriol-induced AD-like mice exhibiting erythema, edema, dry skin, and scaling. Representative hematoxylin and eosin (H&E)-stained dorsal skin sections of (C) healthy mice, displaying normal epidermal architecture and an intact dermis, and (D) AD-like mice, showing hyperkeratosis, epidermal hyperplasia (acanthosis), spongiosis, dermal thickening, and mononuclear inflammatory cell infiltration. Quantitative analysis of (E) ear thickness and (F) dorsal skin thickness as indicators of cutaneous inflammation. Data are presented as the mean ± SD (n = 3 per group). Statistical analysis was performed using an unpaired Student's *t*-test. *** $p = 0.0001$; **** $p < 0.0001$.

3.4 IL-5 dan STAT3 Gene Expression

Relative IL-5 gene expression was significantly reduced in AD-like mice treated with EH-MSCs at doses of 100 µg/kg body weight ($p < 0.05$) and 200 µg/kg body weight ($p < 0.01$) compared with the AD-like mice receiving NaCl (Figure 4A). Similarly, STAT3 gene expression was significantly lower in the dexamethasone-treated group ($p < 0.05$) and in both EH-MSCs-treated groups ($p < 0.001$ and $p < 0.0001$) than in the NaCl-treated AD-like group (Figure 4B). However, no significant difference in IL-5 or STAT3 gene expression was observed between the two EH-MSCs dose groups ($p > 0.05$) (Figure 4).

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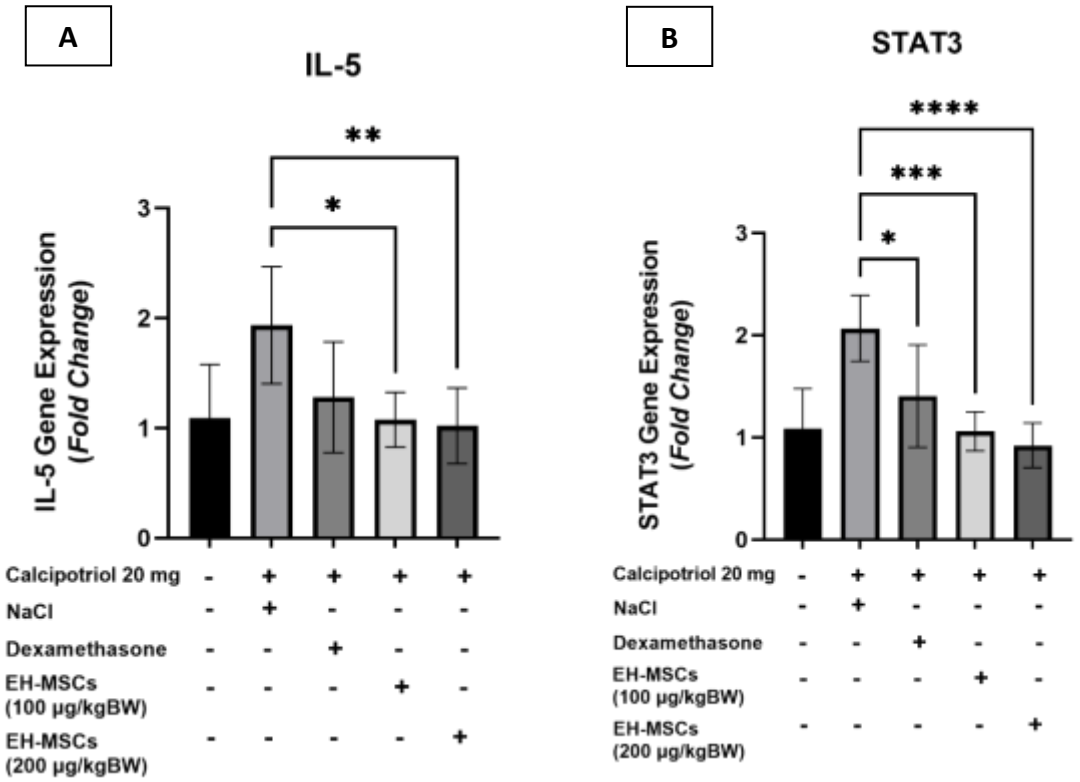

Figure 4 Effects of hypoxia-derived mesenchymal stem cell exosomes (EH-MSCs) on IL-5 and STAT3 gene expression in a calcipotriol-induced atopic dermatitis (AD)-like mouse model. Relative mRNA expression of (A) IL-5 and (B) STAT3 in dorsal skin tissue was quantified by quantitative real-time PCR (qRT-PCR) and normalized to β-actin using the $2^{-\Delta\Delta Ct}$ method. Experimental groups included healthy control, AD-like + NaCl, AD-like + dexamethasone, AD-like + EH-MSCs (100 µg/kg body weight), and AD-like + EH-MSCs (200 µg/kg body weight). Data are presented as mean ± SD (n = 6 per group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by dysregulated immune responses, particularly the predominance of Th2-mediated inflammation.[20] Consistent with this pathophysiology, calcipotriol-induced AD-like mice demonstrated marked upregulation of IL-5 and STAT3 gene expression, reflecting activation of inflammatory signaling pathways. Administration of hypoxia mesenchymal stem cells-derived exosomes (EH-MSCs) significantly suppressed the expression of both genes, suggesting that EH-MSCs alleviate AD-associated inflammation through modulation of Th2 immune responses and STAT3-mediated signaling.[21]

The AD-like mice treated with NaCl exhibited the highest IL-5 gene expression, reflecting the pronounced Th2-mediated inflammatory response induced by calcipotriol. Topical calcipotriol stimulates keratinocytes to release thymic stromal lymphopoietin (TSLP), which activates dendritic cells and type 2 innate lymphoid cells (ILC2s), thereby promoting Th2 cell polarization and enhancing IL-5 production.[8], [22] IL-5 subsequently activates the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, stimulating B-cell maturation and differentiation, which promotes immunoglobulin E (IgE) production. IgE binds to FcεRI receptors on mast cells, triggering degranulation and histamine release upon allergen exposure. In addition, IL-5 is a key regulator of eosinophil proliferation, differentiation, recruitment, and survival through JAK/STAT signaling, resulting in

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eosinophilic infiltration and the sustained release of pro-inflammatory mediators that exacerbate skin inflammation and contribute to the progression of atopic dermatitis.[2], [8], [23]

Administration of EH-MSCs at doses of 100 and 200 μg/kg body weight significantly reduced IL-5 gene expression compared with the NaCl-treated AD-like group. The anti-inflammatory effects of EH-MSCs are attributed to their rich cargo of immunoregulatory bioactive molecules, including anti-inflammatory cytokines, growth factors, messenger RNAs (mRNAs), and regulatory microRNAs (miRNAs), which collectively modulate immune cell function and inflammatory signaling.[24] Previous studies have demonstrated that exosome-derived miR-22-3p suppresses Th2 polarization, thereby attenuating type 2 immune responses.[25] In addition, miR-146a and miR-21-5p inhibit NF-κB activation, leading to reduced activation of macrophages and dendritic cells and subsequent suppression of pro-inflammatory cytokine production.[26], [27] Collectively, these mechanisms likely contribute to the observed downregulation of IL-5 expression following EH-MSCs treatment, thereby mitigating eosinophilic inflammation and Th2-mediated immune responses in atopic dermatitis.

The favorable therapeutic effects observed in the present study may also be influenced by the route of administration. Subcutaneous injection was selected to facilitate localized delivery and prolonged retention of EH-MSCs within the inflamed skin while minimizing systemic distribution. Previous studies have reported that subcutaneously administered extracellular vesicles preferentially accumulate at skin lesions, whereas intravenously administered vesicles undergo rapid systemic biodistribution and sequestration in organs such as the liver and spleen, thereby reducing the fraction reaching the target tissue. Although topical administration represents a non-invasive approach, its therapeutic efficacy may be limited by the barrier function of the stratum corneum and restricted penetration of extracellular vesicles into deeper skin layers. Therefore, subcutaneous administration may provide a more effective strategy for delivering EH-MSCs to inflamed skin in experimental atopic dermatitis. Nevertheless, comparative studies evaluating subcutaneous, topical, and intravenous administration are warranted to determine the optimal delivery route for EH-MSCs-based therapy.[28], [29], [30], [31]

The present study demonstrated that STAT3 gene expression was markedly elevated in the AD-like mice, indicating activation of inflammatory signaling associated with chronic skin inflammation. STAT3 is a key transcription factor activated downstream of cytokine receptors through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway. Persistent STAT3 activation promotes the expression of multiple pro-inflammatory mediators that contribute to keratinocyte hyperproliferation, immune cell activation, and epidermal barrier dysfunction, all of which are hallmarks of atopic dermatitis.[32] Furthermore, sustained STAT3 signaling facilitates the differentiation of T helper 17 (Th17) cells and amplifies chronic inflammatory responses within the skin, thereby exacerbating disease progression and tissue damage.[32], [33]

Treatment with EH-MSCs at doses of 100 and 200 μg/kg body weight significantly reduced STAT3 gene expression compared with the NaCl-treated AD-like group. STAT3 is a central regulator of inflammatory signaling in atopic dermatitis, promoting the production of pro-inflammatory cytokines, including IL-6 and IL-1β, as well as enhancing reactive oxygen species (ROS) generation in keratinocytes, thereby amplifying cutaneous inflammation and epidermal barrier dysfunction.[32], [33] The therapeutic effects of EH-MSCs are likely mediated by their exosomal cargo, particularly regulatory microRNAs. Previous studies have demonstrated that exosome-derived miR-1294 suppresses the STAT3/NF-κB signaling pathway, resulting in reduced STAT3 activation, attenuation of downstream inflammatory cytokine production, and alleviation of skin inflammation in experimental atopic dermatitis.[34] These findings suggest that EH-MSCs mitigate AD-associated inflammation, at least in part, through modulation of the STAT3/NF-κB signaling axis.

Although both EH-MSCs doses significantly suppressed IL-5 and STAT3 expression, no statistically significant differences were observed between the 100 and 200 μg/kg body weight groups. This finding suggests that the lower dose may have already achieved a near-maximal biological response. Similar observations have been reported in previous extracellular vesicle studies, in which increasing the exosome

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dose beyond an effective threshold did not proportionally enhance therapeutic efficacy. This phenomenon may reflect saturation of extracellular vesicle uptake by target cells or saturation of downstream intracellular signaling pathways once sufficient bioactive cargo has been delivered. Consequently, increasing the administered dose may not necessarily translate into greater biological effects.[35] Further dose-escalation studies incorporating pharmacokinetic and biodistribution analyses are warranted to determine the optimal therapeutic dose for EH-MSCs in atopic dermatitis.

Overall, EH-MSCs attenuated inflammation in atopic dermatitis by suppressing IL-5 and STAT3 gene expression, supporting their anti-inflammatory and immunomodulatory potential as a novel cell-free therapeutic approach. However, the present study was limited to the evaluation of IL-5 and STAT3 gene expression, whereas the pathogenesis of atopic dermatitis involves a complex network of inflammatory cytokines, epidermal barrier proteins, and intracellular signaling pathways. Future studies should therefore include additional Th2- and pro-inflammatory cytokines (IL-4, IL-13, IL-31, TNF-α, and IL-6), barrier-related proteins (filaggrin and loricrin), and other signaling pathways, such as NF-κB and MAPK, together with protein expression, histopathological, and skin barrier function analysis. Long-term efficacy and safety studies are also warranted to further elucidate the therapeutic mechanisms and support the clinical translation of EH-MSCs for atopic dermatitis.

4 Conclusion

Administration of EH-MSCs at doses of 100 and 200 μg/kg body weight significantly suppressed IL-5 and STAT3 gene expression in the AD-like mouse model, highlighting their potent anti-inflammatory and immunomodulatory effects. These findings establish a scientific foundation for future preclinical investigations to validate the therapeutic efficacy, elucidate the molecular mechanisms, and explore the clinical translation of EH-MSCs as a promising cell-free treatment for atopic dermatitis.

5 Declaration

5.1 Acknowledgement

The authors would like to express their sincere gratitude to PT Stem Cell and Cancer Research (SCCR) Indonesia for providing research materials, technical support, and laboratory facilities that made this study possible.

5.2 Author Contribution

SP: Conceptualization, Investigation, Methodology, Data Curation, Formal Analysis, Visualization, Writing – Original Draft, Corresponding Author. **FNS:** Methodology, Validation, Formal Analysis, Writing – Review & Editing. **RA:** Project Administration, Validation, Writing – Review & Editing. **RS:** Investigation, Laboratory Work, Data Curation, Resources. **RF:** Conceptualization, Methodology, Resources, Writing – Review & Editing. All authors have read and approved the published version of the manuscript.

5.3 Ethical Clearance

All animal experiments were conducted in accordance with institutional guidelines and ethical standards for the care and use of laboratory animals. The study protocol was reviewed and approved by the Medical/Health Research Bioethics Committee, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia (Approval No. 68/II/2026/Komisi Bioetik).

5.4 Conflict of Interest

The authors declare no conflict of interest.

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