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Therapeutic Potential of Secretome from Hypoxic-Mesenchymal Stem Cell (SH-MSC) in Regulating PDGF and IL-1β Gene Expression in Fluconazole-Related Alopecia

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Abstracts. Fluconazole long therapy causes severe alopecia by increasing interleukin-1ß (IL-1ß) and decreasing platelet-derived growth factor (PDGF). Secretomes from hypoxic mesenchymal stem cells (SH-MSCs) contain anti-inflammatory and growth factors that potentially aid in repairing damaged hair follicles. Therefore, this study aimed to analyze the effect of SH-MSCs on PDGF and IL-1β gene expression to develop a more effective alopecia therapy with minimal side effects. MSCs were extracted from the rat's umbilical cord and cultured under hypoxic conditions for 24 hours before secretome collection. The experiment used six rats per group for a positive control placebo (K1), a negative control group treated with fluconazole + placebo (K2), and topical gel containing 10% and 20% SH-MSCs for K3 and K4, respectively. The rat's PDGF and IL-1β gene expression was measured after 29 days of treatment and validated using histopathological analysis to evaluate hair follicles. The results showed that SH-MSC application significantly increased PDGF and decreased IL-1β gene expression (LSD test result $p < 0.001$). The highest expression of PDGF was observed in K4, which is 1.71 ± 0.31 fold change, followed by K3 with 0.806 ± 0.12 fold change. Conversely, IL-1β mRNA expression was significantly decreased in K4, which is a 1.41 ± 0.20 fold change, followed by K3 with a 2.71 ± 0.23 fold change (Mann-Whitney test result $p < 0.001$). Applying SH-MSC improved hair follicle tissue by increasing anagen cell type than telogen. This study indicates that SH-MSCs have the potential to be developed as a topical gel for the treatment of fluconazole-related alopecia.

Keywords: alopecia-like; IL-1β; PDGF dan SH-MSCs;

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INTRODUCTION

Alopecia or baldness mainly occurs in men aged 30-50 and is considered an unaesthetically disturbing disease. More than 25,000 cases have been recorded in the last five years at Dr. Soetomo Hospital Surabaya, Indonesia (Anindhita et al., 2018). Generally, the skin is very susceptible to radiation or chemical exposure, which potentially damages the cells and causes various diseases (Lisdiana & Ernawati, 2015). In the current case, using certain antifungals, such as fluconazole, for candidiasis therapy triggers alopecia that involves an inflammation response in the skin (Hornik et al. 2021). Long-term fluconazole therapy has been reported to have side effects in increasing alopecia by more than 25% since initial administration (Benitez & Carver, 2019; Thompson et al., 2019). Despite this, the mechanism associated with fluconazole-induced alopecia is still not widely known and may be related to the inhibition of cytochrome P450 enzymes (Alammari et al., 2020; Ogawa et al., 2020). This enzyme inhibitor decreases testosterone levels, reducing follicular cells' liveliness and causing hair loss (Benitez & Carver, 2019).

Recent research has shown that fluconazole induces telogen effluvium, a type of hair loss that occurs when the hair follicle cell cycle enters an untimely resting phase (Yoshihara et al., 2022). In addition, recent studies in mouse samples showed that fluconazole-related alopecia is characterized by decreased expression of genes associated with endogenous retinoid synthesis and retinoid receptors (Foitzik et al. 2005). Endogenous retinoids, particularly all-trans retinoid acid

(ATRA), play an essential role in the cycle and differentiation of hair follicles (Nan et al., 2020). Extended exposure to fluconazole also affects the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), which trigger apoptosis of hair follicle cells (Ashtiani et al. 2019; Liang et al. 2023). In other cases, fluconazole affects the signaling pathway in connective tissue cells, inhibiting platelet-derived growth factor (PDGF) production, which causes hair loss (Steward et al., 2020).

Currently, treatments such as platelet-rich plasma (PRP), a growth factor for hair, minoxidil 2.5–5%, and finasteride are only temporary, with the result that more effective alternative therapies are needed. One potential and relatively safe alternative therapy approach is the administration of secretome hypoxia mesenchymal stem cells (SH-MSCs) (Salhab et al., 2022; Shimizu et al., 2022). SH-MSCs have the advantage of having low immunogenicity, minimal side effects, and ease of absorption through the skin barrier and into the dermis due to their small molecular size (AlSogair, 2019; Rambwawasvika, 2021). Several studies have reported the success of stem cells against alopecia (Egger et al., 2020; Krefft-Trzciniecka et al., 2023; Talebzadeh & Talebzadeh, 2023). However, no studies have used SH-MSCs, especially related to fluconazole-related alopecia inflammatory agents, especially IL-1β and PDGF. Therefore, this study aims to analyze the effect of SH-MSCs on the expression of PDGF and IL-1β genes in vivo to develop therapies against baldness that are more effective and have minimal side effects. Hopefully, this study will be used as fundamental empirical evidence for using SH-MSC to treat alopecia.

METHODS

The study was a post-test-only control group design using homogenous male Wistar rats weighing 200 and 250 grams. Experimental animals were obtained from the Stem Cell and Cancer Research (SCCR) Laboratory, Semarang City, Central Java, Indonesia. The use of experimental animals in this study has received approval from the Ethical Clearance Commission, Faculty of Medicine, Universitas Islam Sultan Agung Semarang, No. 318/VIII/2023/Komisi Bioetika.

A total of 24 rats were randomly divided into four groups with equal numbers. The condition of the cage was set at 60% humidity, 20-24 °C, 12-14 hours of light exposure, and nonstop ventilation. The male Wistar rats were given only standard feed for seven weeks from pretreatment to termination. Each rat in each group was bald on the dorsal and given different treatments per each group. In the positive control group (K1), healthy rats were given 0.9% NaCl; the negative control group (K2) was a fluconazole-related alopecia-like model rat. In the K3 group, the fluconazole-related alopecia rat model was treated with a topical gel of 10% SH-MSCs at a dose of 200 mg/day by smear. Then, the K4 group was fluconazole-related alopecia rats with a topical gel of 20% SH-MSCs at a dose of 200 mg/day.

Mesenchymal Stem Cell (MSC) Isolation Procedure from Umbilical Cord

MSC isolation was carried out in a class 2 biosafety cabinet with sterility level and sterile equipment following procedures from Nugraha & Putra (2018). In this study, MSC was obtained from the umbilical cord of rat fetuses collected aseptically and put into a sterile container given 0.9% NaCl to be cleaned using phosphate buffer solution (PBS) and blood vessel removal. The clean umbilical cord must be finely chopped, placed evenly in the corning 25T flask, and left to stand for three minutes for cell attachment (Nugraha & Putra, 2018).

A mixture of Dulbecco's modified eagle medium (DMEM), 0.25% fungizon, 1% Pen-Strep, and fetal bovine serum (FBS) was added gradually until the tissue was covered. The explants are then stored in an incubator with 5% CO_2 at 37 °C. Cell culture growth was observed for up to 14 days, and a substrate replacement was performed every three days. The medium replacement was conducted by discharging half and adding a new up to cover the entire cell. Culture cell maintenance was continued until cell conciliation reached 80% of the cells and was ready for further treatment (Sazli et al., 2023)

MSC growth was validated through microscopic observation and fluorescently labeled antibodies method using flow cytometry to measure CD90, CD29, CD45, and CD31 expression. The capacity of MSCs to develop into different mature cells is examined based on cell

differentiation towards osteocytes and adipocytes. The examination was done by giving Alizarin red staining and oil red o-dye in osteogenic and adipogenic cultures medium, respectively (Zukhiroh et al., 2022).

MSC hypoxia induction and secretome extraction

MSCs were isolated and then incubated under hypoxic conditions, and they were carried out according to the procedure from Fredianto et al. (2023), with adjustments to cell conditions. Hypoxic conditions are intended to increase the production of SH-MSC. Each flask culture of MSCs with 80% confluence was added 10 mL of DMEM media and placed in a hypoxic chamber with N_2 gas administration until the O_2 content reached 5% and incubated at 37 °C for 24 hours. The culture media was removed after 24 hours and filtered with tangential flow filtration (TFT) to produce SH-MSCs.

Manufacture of topical gel preparations

Treatment for one smear on dorsal rats with an area of 2 cm x 2 cm requires 200 mg of gel containing 10% SH-MSC for K3 and 20% SH-MSC for K4. A topical gel was made aseptically by mixing SH-MSC in NaCl (1:1) until homogeneous, then mixed with water-based gel until reaching 200 mg. Based on the dose adjustment, 20 µL and 40 µL SH-MSC were used for each treatment in each K3 and K4 group.

Treatment, preparation, and histological observations

The rats were adapted for one week before treatment and then injected with a combination of 20 ml/kgBW xylazine and 60 ml/kgBW ketamine for anesthesia. The rats that had lost their response were then shaved off their dorsal hair. As many as 100 mg of fluconazole was applied evenly to the shaved area for 14 days for the K2-K4 groups. Topical gel administration of SH-MSCs at 200 mg/day doses in the K3 and K4 groups was carried out from day 15th to day 28th.

Rats were sacrificed on day 29th by being injected with 10 ml of a lethal-dose cocktail containing 6 ml ketamine and 4 ml xylazine. The skin organs were removed, preserved in an RNAase-free cryotube containing RNA-later, and stored at -80 °C (Restimulia et al. 2021). The remaining obtained skin organs were then used for histologically embedded specimens prepared with hematoxylin-eosin (HE) dye by following Hristu et al. (2021).

RNA extraction, cDNA synthesis, and IL-1ß and PDGF gene expression readings

A total of 50 mg of skin sample was put into a tube containing 1 mL of triazole, then homogenized using an ultrasonicator and incubated at room temperature for 5 minutes. The RNA isolation process was performed using GENEzol™ Reagent (Geneaid: New Taipei City, Taiwan) according to the manufacturer's standard procedure. Isolated RNA was then converted into cDNA using ReverTra Ace® qPCR RT Master Mix (Toyobo: Osaka, Jepang) following the manufacturer's procedures.

Real time-PCR was performed to analyze gene expression through mRNA reading of IL-1β and PDGF genes. A total of 1 µL of cDNA sample was added to 10 µL of SensiFAST SYBR No-ROX Mix (Meridian Bioscience: Ohio, US), 0.8 µL forward-reverse primers of IL-1ß and PDGF genes, and 7.4 uL nuclease-free water. The PDGF primers were F: 5'-TGC CAG AGC CTG CTC TTAC-3' and R: 5'-GATAT GCC ACG GAG ATA AGC GA-3'. The IL-1β primers were F: 5'-CGA CAG TGA GGA GAA TGCC-3' and R: 5'-CAC AGC CAC AAT GAG TGA CAC-3'. The qPCR process goes through the initial denaturation stage at 95 °C for 2 minutes, denaturation at 95 °C for 5 seconds, and annealing at 56 °C for 20 seconds, a total of 40 cycles. The qPCR process was performed by analyzing the hydrolyzed probe at a wavelength of 520 nm and quantified using EcoStudy software.

Data Analysis

The gene expression was then analyzed using the Shapiro-Wilk test and Levene test for data normalization analysis, followed by the One-Way ANOVA test and the Post Hoc Least Significant Difference (LSD) test for PDGF anKruskall-Walli's's test, followed by the Mann-Whitney test for IL-1β gene expression. All methods of testing equality of variance were carried out with a confidence level of 95% and a significance level of $p = 0.050$. All statistical analyses were performed using SPSS 26.0 for Windows desktop applications.

RESULTS AND DISCUSSION

MSC Development

Microscopic observation showed that MSC cells were attached to the bottom of the flask and had a spindle-like cell shape. Furthermore, validation using flow cytometry showed high expression of CD90 (97.6%), CD29 (97.7%), and

low expression of CD45 (1.5%) and CD31 (3.2%), indicating that MSCs had successfully grown (Fang et al. 2019) (Figure 1).

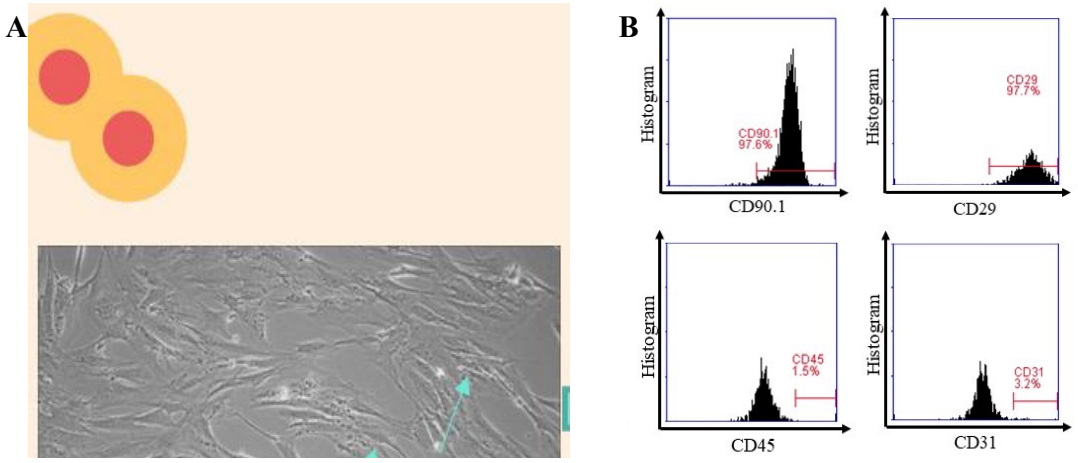

Figure 1. MSC cell culture with 80% confluence obtained a spindle-like form of cells (A) and the results of flow cytometry analysis of CD90, CD29, CD45, and CD31 expression (B). Cell observation was performed using a microscope with 100x magnification.

The capacity of MSCs to differentiate into mature cells and undergo specialization was observed after the application of alizarin red and oil red dye to osteogenic and adipogenic cultures medium. In this study, calcium and fat deposits indicated MSCs that had differentiated into osteocytes and adiposity (Figure 2).

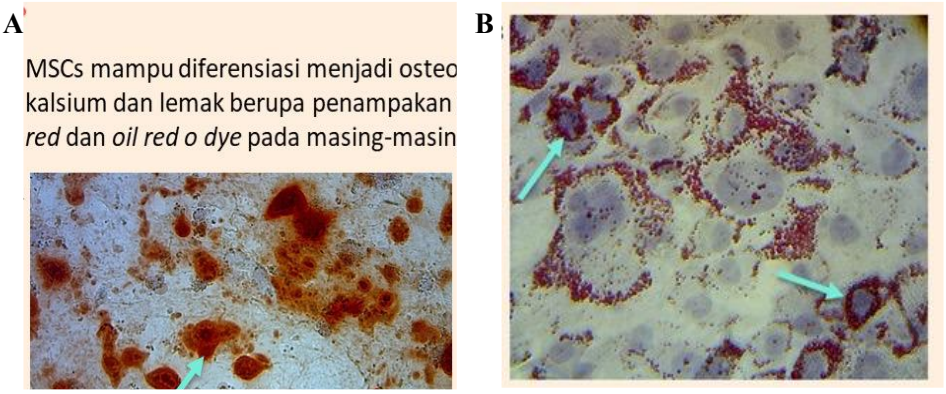

Figure 2. MSCs differentiate into osteocytes indicated by calcium-stained red due to alizarin red (A) staining and adipocytes that store lipids in oil red (B). Blue arrows indicate the location of calcium accumulation in osteocytes and lipids in adiposity. Microscopic observations using 100x magnification.

Effects of SH-MSCs gel administration on IL-1ß and PDGF gene expression

Fluconazole exposure causes inflammation, characterized by changes in alopecia-related cytokine production. These changes include decreased expression of the chemokine PDGF gene by activated platelets and increased expression of the alopecia-related pro-inflammatory cytokine, including IL-1ß gene (Hudgens et al., 2016). This study also showed that the administration of SH-MSC significantly increased ($p < 0.001$) the expression of the PDGF gene while decreasing the expression of the IL-1ß gene, which has potential in the treatment of alopecia (Figure 3).

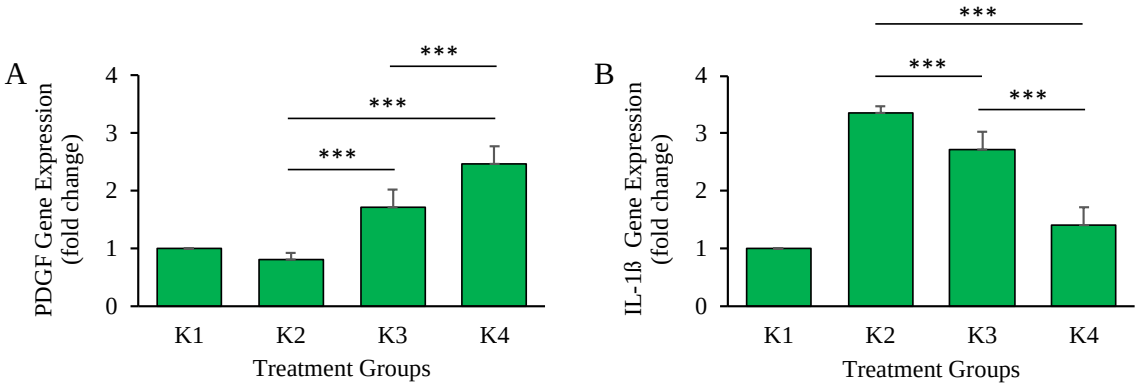

Figure 3. mRNA expression of PDGF (A) and IL-1ß (B) gene in each treatment group changed significantly as the SH-MSC concentration increased. The superscript three stars (***) indicate significant differences based on the LSD test ($p < 0.001$) for PDGF mRNA expression and the Mann-Whitney Test ($p < 0.001$) for IL-1ß mRNA.

In the alopecia condition, PDGF gene expression was decreased compared to normal control (K1), although it is not significantly different ($p > 0.050$). Then, it is significantly different compared to the K3 group, with PDGF expression reaching 1.71 ± 0.31 fold change ($p < 0.001$). Furthermore, increasing SH-MSC concentration in K4 significantly impacted PDGF gene expression up to 2.47 ± 0.30 and better than K3. Several studies have shown that PDGF plays an essential role in follicle stem cell differentiation (González et al. 2017; Zhu et al. 2020), growth and morphogenesis of hair follicle cells (Lin et al. 2022; Rishikaysh et al. 2014; Saxena et al. 2019; Zhu et al. 2020).

This study also showed that prolonged exposure to fluconazole triggers an inflammatory response through significantly increased IL-1ß gene expression, but not significant for PDGF. This was demonstrated by a significant increase of IL-1ß gene expression in fluconazole-related alopecia (K3) up to 2.71 ± 0.23 fold change. Furthermore, SH-MSC therapy also had a significant effect on reducing inflammation in the skin tissue. Effectively, the highest decrease in IL-1ß gene expression was observed in the group with 20% SH-MSC administration (K4) to reach 1.41 ± 0.20 fold change. Recent studies explain that the administration of fluconazole for long-term fungal therapy can potentially trigger oxidative stress, which activates nuclear factor kappa B (NF-κB) signaling (Janganati et al. 2018). Activation of NF-κB leads to phosphorylation of the inflammasome, which increases pre-IL-1ß production. The inflammasome pathway also activates caspase-1, which plays a role in the maturation of IL-1ß in the inflammatory response (Koenen et al. 2011; Nagar et al. 2019). Inflammatory conditions in the skin tissue directly cause damage to hair follicle cells, resulting in alopecia

In detail, fluconazole is a triazole antifungal drug that inhibits the cytochrome P-450 lanosterol 14α-demethylase enzyme (CYP51). This enzyme is important for the production of ergosterol, a component of the fungal cell membrane (Monk et al., 2020). By inhibiting CYP51, fluconazole disrupts the formation of fungal cell membranes, leading to impaired material transport and cell death (Berkow & Lockhart, 2017; Dornelas-Figueira et al., 2023). However, fluconazole also interacts with other cytochrome P-450 enzymes, including CYP2C9, CYP2C19, CYP3A4, and CYP3A5, although to a lesser extent than CYP51 (Biswas et al., 2023).

The relationship between P-450 enzyme inhibition and skin inflammation is complex and poorly understood. However, fluconazole inhibition of CYP enzymes is thought to be associated with a decreased ability of the skin to metabolize xenobiotics (Ogawa et al., 2020), detoxification of drugs, radical oxygen species, and pollutants (Sevrioukova, 2019; Zhao et al., 2021), and leads to the regulation of inflammation (Kuhn et al., 2021). In addition, fluconazole may interfere with the metabolism of arachidonic acid as a precursor of inflammatory mediators such as prostaglandins and leukotrienes. Inhibition of these enzymes may lead to arachidonic acid accumulation and excessive production of inflammatory mediators (Patel et al., 2020).

Inhibition of P-450 enzymes also contributes to increased ROS levels, triggering oxidative stress and inflammation as indicated by increased secretion of pro-inflammatory cytokines. ROS imbalance in the hair follicle causes oxidative

stress and damages cellular components (Liu et al., 2022). Fluconazole and oxidative stress further activate Toll-like Receptors (TLRs) and NOD-like Receptors (NLRs), triggering the activation of danger-associated molecule patterns (DAMP) (Lai et al., 2018). Fluconazole is also known to modulate DAMP receptor synthesis, causing an exaggerated inflammatory response (Pathakumari et al., 2020) and hair follicle dysfunction, which further leads to hair loss (Shin et al., 2019).

Excess ROS in the follicular area recruits immune cells and activates the nuclear factor kappa B (NF-κB) signaling pathway through activation of the inhibitory kappa B (IkB) kinase (IKK) complex (Sun, 2017; Wang et al. 2019). The process of fluconazole-related inflammation activates IKK through a mechanism that remains unclear; however, it may also be triggered by the p-450 inhibition during the alopecia-like process. This cascade leads to phosphorylation of the IkB protein, which affects polyubiquitination and proteolysis, releasing IkB binding to the p50 and p65 heterodimer complex in the cytoplasm (Mustafa et al., 2021). Once released, the p50 and p65 protein complexes move into the nucleus and increase the production of proinflammatory cytokines, such as TNF-α and IL-1β (Wu et al., 2020).

In the murine hair cycle, IL-1α and IL-1β increase sharply with the onset of the spontaneous catagen phase, known as the alopecia process's transition phase (Olayinka & Richmond, 2021). This phase is relatively short and lasts for a few weeks. During the catagen phase, the hair follicle detaches from the blood supply and shrinks. This causes the lower part of the hair follicle (the reed) to move closer to the skin surface. The phase is associated with increased expression of IL-1ß receptor signal transduction in the early stages, which is related to uneven hair loss resembling alopecia areata (Kim & Christiano, 2021; Liu et al., 2021).

The improvement of inflammatory conditions occurred better in the group of rats given SH-MSCs with high concentrations. SH-MSCs gel has anti-inflammatory effects in alopecia-like animal models by upregulating PDGF mRNA expression and downregulating IL-1β mRNA. This suggests that increasing the SH-MSH concentration may synergistically impact follicle repair due to fluconazole exposure. Hair follicle repair is indicated by a decrease in inflammatory conditions that trigger new hair growth in cases of alopecia. This condition is confirmed by the tissue skin specimen observed in histology analysis under the microscope. Comparison of skin tissue from the K1 and K2 rat groups on day 14th showed differences in follicular cell phases, where K1 was dominated by anagen cells (active hair growth). In contrast, K2 was dominated by telogen cells (hair follicle rests, old hair shaft falls out) (Figure 4A-B). In addition, observations on the 29th day showed that SH-MSC administration to the skin tissue restored the condition of telogen cells to anagen. The early-anagen stage indicates this condition in the deeper layers of the skin (Figure 4K1-K4).

At the end of the study, the follicle cell types were observed and showed that the number of anagen cells in the K3 and K4 groups was higher than control group K1 and K2 (Table 1). This result indicates that hair follicle growth is improving in the experimental animals due to SH-MSCs topical gel application. The highest percentage of anagen was performed in the K4 group with the administration of 20 µL SH-MSH per 200 mg topical gel.

Table 1. Phase comparison of anagen and telogen on day 29

Groups	Anagen		Telogen		Total
	∑	%	∑	%	
K1 (healthy control)	55	64.71	30	35.29	85
K2 (alopecia)	40	81.63	9	18.34	49
K3 (alopecia + 10% SH-MSC)	109	54.21	49	45.79	107
K4 (alopecia + 20% SH-MSC)	91	59.87	61	40.13	152

The percentage of telogen follicle cells in the K3 and K4 groups was relatively high but lower than anagen-type follicle cells. This indicates follicular damage that may be caused by oxidative stress due to fluconazole but improved with SH-MSH administration. This study's results align with previous research, which explains that secretome can restore hair follicle damage (Salhab et al., 2022) and skin rejuvenation (Balasubramanian et al., 2017).

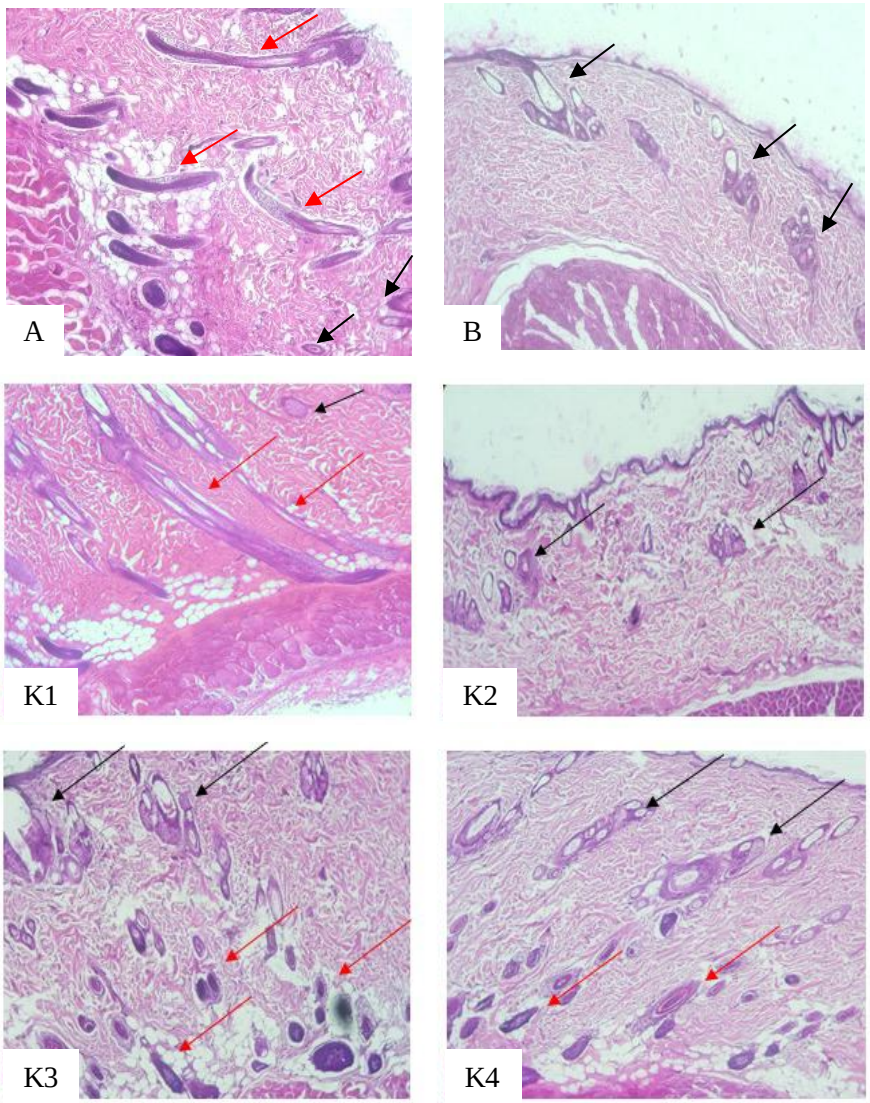

Figure 4. Skin follicle morphology of normal model rats (A), fluconazole-related alopecia (B) on day 14th, and model rats in control positive (K1), fluconazole-related alopecia control group (K2), Fluconazzole-related alopecia treated with 10 µL SH-MSH (K3), and 20 µL of SH-MSH (K4) on day 29th. The black arrow is the telogen follicle, and the red arrow is the anagen follicle. Histology observation was performed at 400X microscope magnification.

Hair follicles are greatly affected by chemical exposures that cause oxidative stress conditions in the scalp. At the same time, follicular cell rejuvenation and repair mechanisms continue to occur by activating follicular growth, proliferation, and maintenance factor PDGF (Ji et al., 2021). PDGF secretion by platelets stimulates stem cell mitosis to activate dermal papillae and inhibits apoptosis during the cell cycle, then forms microcirculation (Deptuła et al., 2020; Pazzaglia et al., 2019). Possible mechanisms in hair growth are PDGF release through platelet activation and wound regeneration mechanisms, follicular stem cell overexpression during wound healing, and angiogenesis (Zhang et al., 2021).

Fluconazole was shown to disrupt the signal balance, interfering with the synthesis and decreasing the levels of PDGF, causing hair loss. In addition, fluconazole alters PDGF receptor expression in hair follicle cells, which inhibits PDGF-induced signaling. PDGF also plays a role in hair follicle differentiation, the process by which hair follicle cells mature and produce hair fibers (Shivakumar et al., 2021). Decreased PDGF levels interfere follicular cell differentiation process, causing abnormalities in hair shaft formation and structure (Guan et al., 2023). This condition was observed in this study, where rats exposed to fluconazole experienced hair follicle damage and were dominated by telogen follicle cells.

PDGF stimulates the proliferation of hair

follicle keratinocytes, the cells that form the hair shaft, through specific receptors (PDGF-R) on hair follicle stem cells, triggering a series of signals that promote cell division (Guan et al., 2023). PDGF-R also activates the Wnt signaling pathway that regulates hair follicle cell differentiation by regulating the expression of genes related to hair follicle morphogenesis and keratinocyte differentiation (Gentile & Garcovich, 2019; Hermans et al. 2021; Hu et al., 2021). This differentiation process is essential for transforming proliferating keratinocytes into mature hair shaft cells. The synergistic action of PDGF and Wnt signaling is evident in their complementary roles in hair follicle development. PDGF initiates the proliferation of hair follicle cells, providing the building blocks for hair shaft formation. The Wnt signaling pathway then takes over, regulating the differentiation of these cells into mature hair shaft components (Gentile & Garcovich, 2019).

SH-MSCs secrete various chemokines cytokines, including IL-10 and growth factors, PDGF, vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β). In addition, the following cytokines and mediators are known as mesenchymal stem cells (H-MSCs): IL-1β, TGFβ, TGFα, bFGF, EGF, VEGF, PDGF, KGF, G-CSF, GM-SCF, TNF, IGF -1, and HGF (Eleuteri & Fierabracci, 2019; Eriani et al., 2018; Rahimi et al. 2021). High concentrations of secretory IL-10 can suppress pro-inflammatory cytokine production and activate tolerogenic macrophage and Treg cell activation (Shi et al., 2021). IL-10 release also decreases ROS concentration and stops systemic inflammation. Previous studies claim IL-10 can reduce inflammation by activating several intracellular proteins, including suppressor of cytokine signaling 3 (SOSC3) via transducer and activator of transcription 3 (STAT-3) (Cevey et al., 2019; Clark et al., 2019). Activation of the STAT3 pathway leads to the synthesis and release of SOSC3 protein, thereby inhibiting pro-inflammatory signaling pathways while increasing PDGF release.

Regarding the study findings, SH-MSCs topical gel dose-dependently significantly increases PDGF gene expression in skin tissues of the fluconazole-related alopecia rat model. The PDGF component in SH-MSCs also plays a role in activating sonic hedgehog (SHH) signaling and lymphoid enhancer-binding factor 1 (LEF-1), which promotes hair growth (Bak et al., 2020; Hermans et al., 2021). Cytokines and chemokines in SH-MSCs transmit signals and growth factors by platelets, promoting cell proliferation to prolong the anagen phase, stimulating hair follicle development, and suppressing apoptosis (Damayanti et al., 2021). Growth factors in SH-MSCs also activate hair follicle stem cells to renew damaged hair follicles and maintain hair morphology so that it is also possible to support hair regeneration (Yuan et al., 2020).

However, this study still lacks elucidating signaling pathways and specific markers that can be used to develop more precise treatments. In addition, this study also did not measure the side effects that may be caused by the administration of SH-MSC topical gel treatment during the study.

CONCLUSION

This study showed that the administration of 10-20% of SH-MSCs in topical gel at a dose of 200 μL was able to reduce IL-1β mRNA expression and increase PDGF mRNA expression compared to the control (K2) in fluconazole-related alopecia model rats. SH-MSCs were able to control the inflammatory process in the model mice and improve the histology structure of tmice'se's skin well. This study proves that SH-MSCs have the potential to be developed as a topical gel for the treatment of alopecia or baldness caused by exposure to fluconazole. Further research must be developed to map the molecular mechanism in explaining inflammation-related fluconazole and system biology signaling pathways, including SOSC3, STAT3, and NF-kB.

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