JTL 11 (1) JUNE 2022
Page: 73-83

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Protective effect of Hypoxia Mesenchymal Stem Cell-derived Exosomes on NF-kB (P65) expression and catalase activity in UV-B induced skin aging

Favoury Felie Basuki Rahmawati[1], **Sri Priyantini**[1], **Eko Setiawan**[1*]

[1] Faculty of Medicine Biomedical Sciences Sultan Agung Islamic University Semarang, Indonesia

Abstract: Exposure to ultraviolet-B (UV-B) radiation can elevate free radical levels due to an imbalance between reactive oxygen species (ROS) production and antioxidant enzyme activity, particularly catalase. Elevated free radicals induce oxidative stress, initiating an inflammatory response via the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway and subsequently triggering the release of pro-inflammatory mediators. Recent studies suggest that exosomes derived from mesenchymal stem cells (E-MSCs) contain bioactive molecules with antioxidant and anti-inflammatory properties. Administration of E-MSCs has been reported to suppress pro-inflammatory mediator release, demonstrating potential as a therapeutic agent for photoaging skin. This study aimed to evaluate the effects of subcutaneous E-MSC (E-MSC) injections on NF-kB (P65) expression and catalase enzyme levels in the skin of male Wistar rats subjected to premature aging induced by UV-B exposure. An in vivo experiment employing a randomized post-test-only control group design was utilized, involving five treatment groups. Data were statistically analyzed using the Kruskal-Walls test for NF-kB expression and One-Way ANOVA for catalase enzyme levels. Results indicated significant differences among groups in NF-kB (P65) expression (p=0.005) and catalase enzyme activity (p=0.001). Specifically, subcutaneous administration of EH-MSC at doses of 200 µL and 300 µL significantly reduced NF-kB (P65) expression and enhanced catalase enzyme activity, suggesting a protective role against UV-B-induced skin aging in Wistar rats.

Keywords: *Exosome Mesenchymal Stem Cell* (E-MSC); *NF-kB (P65)* Expression; *Catalase Enzyme Expression*

INTRODUCTION

Ultraviolet B (UV-B) radiation that continuously exposes human skin can cause chronic inflammation that results in a decrease in collagen in the skin.[1] UV-B exposure can cause increased levels of free radicals due to an imbalance between *reactive oxygen species* (ROS) and antioxidant enzymes such as catalase.[2] An increase in free radicals causes oxidative stress that leads to an inflammatory response through the NF-kB pathway and triggers the release of inflammatory mediators.[3] An increase in free radicals causes oxidative stress that leads to an inflammatory response through the NF-kB pathway and triggers the release of inflammatory mediators.[4] Recent research states that Exosome *Mesenchymal Stem Cell* (E-MSC) contains molecules that act as antioxidants and anti-inflammatory as E-MSC injection at a dose of 100µL subcutaneously can prevent the release of pro-inflammatory mediators and become a potential agent for treatment of skin that undergoes photoaging.[5,6] However, the role of Exosome *Hypoxia Mesenchymal Stem Cell* (EH-MSC) injection at doses of 200 µL and 300 µL on *catalase* and NF-kB p65 levels in *collagen loss mice* exposed to UV-B has

Corresponding author*.
E-mail address: drekosetiawan@unissula.ac.id (Eko Setiawan)
DOI: 10.29238/teknolabjournal.v14i1.540
Received 03 September 2024; Received in revised form 24 December 2024; Accepted 07 May 2025

not been studied. This prompted the study to examine the effect of EH-MSC on catalase and NF-kB p65 levels in collagen loss mice exposed to UV-B.

Epidemiological studies have consistently shown that the prevalence of *collagen loss* due to UV-B exposure tends to increase, especially in regions with high levels of sun exposure including Indonesia.[7] Populations that live in tropical areas or that are often exposed to direct sunlight have a greater risk of significant collagen loss. This high prevalence can be found in various age groups, ranging from young to old. Standard therapy for skin conditions that experience *collagen loss* due to UV-B exposure uses *hyaluronic acid* (HA), however the role of HA is limited to hydration and structural integrity of the extra-cellular matrix (ECM) creating a supportive environment for collagen synthesis and maintenance, but does not stop the inflammatory and oxidative stress problems that are the source of *collagen loss problems*.[8] This will cause a relapse in *collagen loss* if you stop using HA.[9]

Exposure to ultraviolet B light triggers the molecular pathway leading to suppression of *catalase capacity* and increased expression of *nuclear factor kappa beta* (NF-kB) p65.[3,10] The NF-kB transcription factor regulates various aspects of innate and adaptive immune function and functions as an important mediator in the inflammatory response, NF-kB p65 is capable of inducing the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines, and also participates in inflammatory regulation.[10] NF-kB also regulates the activation of the antioxidant enzyme Catalase. UV B radiation stimulates the production of ROS in skin cells, creating oxidative cells that affect antioxidants including catalase. In addition, ROS plays a role in the activation of the NF-kB pathway thereby increasing the expression of pro-inflammatory genes as pro-inflammatory cytokines, this contributes to inflammation and immune response. Recent research has found that NF-kB expression can suppress catalase activity so that inflammation that occurs continuously due to UV B exposure will reduce catalase levels so that ROS levels will increase and oxidative stress will decrease.[11,12]

Mecenchymal stem cell *exosomes* contain various types of micro RNA and cytokines that play an important role in relieving inflammation.[13] MiRNAs such as miR-146a, miR-155 and miR-21 have been identified as key regulators in controlling the inflammatory response. miR-146a and mir-155 have a role in suppressing TNFα expression and regulating the NF-kB pathway, whereas miR-21 is involved in reducing oxidative stress.[14,15,16] In addition, MSC exosomes also contain anti-inflammatory substances such as interleukin-10 (IL-10) and pro-inflammatory cytokines that are carefully regulated to manage the balance of immune and inflammatory responses.[17] Meanwhile, in the MSC condition, which goes through the process of *hypoxia* conditions, it can promote better cell proliferation and maturity through the release of several *growth factors* in the medium than in the *normoxic medium* (NM) condition.[18] Existing therapies for UVB-induced skin aging primarily focus on hydration (e.g., hyaluronic acid) and antioxidant supplementation, which do not address underlying molecular pathways such as NF-kB signaling. Limited research has explored the role of hypoxia-preconditioned MSC exosomes in modulating inflammation and oxidative stress markers in vivo. This study is unique in evaluating the dose-dependent effects of EH-MSCs on oxidative stress and inflammation markers, specifically NF-kB (p65) and catalase, in an in vivo UVB-induced skin aging model. These findings could advance the development of regenerative therapies for skin aging, providing a novel, non-invasive alternative to current antioxidant-based treatments.

MATERIAL AND METHOD

Research This study is an in vivo experimental test with mice that experience collagen loss due to exposure to UVB rays as the research subject. The research design used in this study is *Randomized Post Test only Control*

Group Design. This study consisted of 5 treatments. The doses of 200 µL and 300 µL were chosen based on prior preclinical studies indicating optimal anti-inflammatory and antioxidant activity in vivo, without adverse effects.[5,6] NF-kB (p65) was selected as a marker due to its pivotal role in mediating inflammation and oxidative stress pathways. Catalase was chosen for its critical antioxidant function in neutralizing reactive oxygen species (ROS) generated by UVB exposure.[19]

Tools and material

UV *light* (broadband with *peak* emission at 302 nm) with energy of 150 mJ/cm², razor, exposure cage, maintenance cage, rat drinking water bottle, hair clipper, *vacutainer*, hematocrit tube, 6 mm *biopsy punch*, centrifuge, micropipette 1, 1000 µL micropipette tip, *vial tube* 1.5 mL, PCR *machine illumina*, ELISA CAT kit, microscope, *staining jar, coated desk glass, cover glass,* laptop. Ketamine, *xylazine*, ethanol, aquades, oform, *p65 primer,* ELISA CAT kit. Equipment used in MSC culture and exosome isolation includes BSC, CO2 incubator, centrifuge, uPulse tangential flow filtration and TFF filtration membranes measuring 100 kDa and 500 kDa used for exosome isolation from MSC culture medium. The tools used for MSC and EH-MSC validation include Flowcytometer, inverted microscope, incubator. Other equipment used includes micropipettes, centrifuge tubes, cell culture plates, beher bottles, shakers, pH meters, autoclaves and UV lamps.

The materials used in the study included culture materials consisting of DMEM, Fetal Bovine Serum, Phosphate Buffer Saline, Antibiotics and antifungals, culture flasks, centrifuge tubes, pipette tips. Materials used for exosome insulation include NaCl, and Aquabidest. The research materials needed in the process of making animal models and sampling include 70% alcohol and sterile cotton, ketamine, xylazine, vial tubes, RIPA buffers and bisturi knives. Materials required for ELISA analysis include Elisa Kit Rat Catalase and RT – PCR for NF-kB (p65) expression examination.

MSC isolation procedure from umbilical cord

The entire process is carried out in a class 2 biosafety cabinet, using sterile equipment and done with high sterility techniques. *Umbilical cords* are collected in a transport medium. Using tweezers, *the umbilical cord* is placed onto the petri dish, the *umbilical cord* is washed thoroughly using PBS. *The umbilical cord* is cut and the blood vessel is removed. *The umbilical cord* is finely chopped and placed on the 25T flask evenly, let it sit for 3 minutes until the tissue is attached to the surface of the flask. A complete medium consisting of DMEM, fungizone, penstrep, and FBS) is added slowly until it covers the tissue. The explant is incubated in an incubator at a temperature of 37ºC and 5% CO2. Cells will emerge after approximately 14 days from the beginning of the culture process. Replacement of the medium is carried out every 3 days by removing half of the medium and replacing it with a new complete medium. Cell maintenance is carried out until the cell reaches 80% confulness. Then the cells are harvested (*passage*) for propagation, passage 1 of the confluent is then subcultured until passage 4, condition medium is collected after the cells reach 70% confluence or more.

Isolasi exosome MSCS hypoxia

The condition medium that has been collected from passage 4, then in a centrifuge of 300xg for 5 minutes to remove the cells, then in a centrifuge of 200xg for 30 minutes to remove debris. *Supernatant* that has been centrifuged is then filtered by the TFF (*Tangential Flow Filtration*) method using *sterile hollow fiber polyether-sulfone membrane* 10, 50, 100, 500 kD. MSC that has reached 80% confluence is added complete medium up to 10 mL. The flask that has contained the MSC is then placed in the *hypoxic chamber.* Nitrogen gas is delivered through an inlet valve and an oxygen meter is placed in the sensor hole to measure the oxygen concentration in the chamber. Nitrogen is added until the indicator needle shows a concentration of 5% oxygen. *The chamber* that has been filled with flasks is

incubated for 24 hours at a temperature of 37°C. Hypoxia conditioning was achieved by incubating MSC cultures in a chamber with 5% oxygen concentration for 24 hours, enhancing the secretion of exosomes enriched in miRNAs and growth factors.[20] After 24 hours, the culture medium was taken and filtered using TFT with 100-500 kDa to obtain EH-MSC which was then in accordance with the P1 and P2 doses. Wash 2 times with *stain buffer* (PBS) and resuspension with 300-500µL *stain buffer* (PBS) or 1 wash *buffer* (FBS). Read in *flow cytometry* use tubes 1-5 as controls for setting up *cytometry* (as compensation). Validation of EH-MSC content using flowcytometry with surface markers in the form of CD63+, CD81+, CD9+. The validated results containing EH-MSCs were stored in 2.5 ml tubes and stored at 2-8 °C.

Tissue sampling

Tissue sampling for RT-PCR (p65) and CAT ELISA analysis was carried out on the 29th day after the first day of treatment. The entire rat was first killed by means of cervical dislocation before the tissue was taken. The tissue was taken using a 6 mm punch biopsy on the skin exposed to UV-B. The tissue sample was divided into two for deficiencies by soaking in 10% formalin for 24 hours and to be inserted into RNA Later. The tissue is put into formalin for 24 hours and then stored in a tube containing 70% and stored at room temperature until the process of making paraffin preparate. The samples that are put into RNA Later are then stored at a room temperature of -80°C until the RT-PCR and ELISA analysis process.

Data analysis

The Kruskal-Walls test was applied for NF-kB (p65) due to non-normal data distribution, followed by the Mann-Whitney test for pairwise comparisons. One Way ANOVA was used for catalase levels with Post Hoc Tamhane for non-homogeneous data. Data analysis was carried out using SPSS 26.0 software in the Windows desktop platform.

RESULTS AND DISCUSSION

Isolation of MSCs was carried out in the *Stem Cells and Cancer Research* (SCCR) laboratory in Semarang, using *umbilical cord* of pregnant rats that were 21 days old. After isolation, the cells are cultured in petri dishes containing a specific medium. After reaching the fourth passase, an image of the cell attached to the base of the flask with *spindle-like cell morphology* was obtained in microscopic observation (Figure 1).

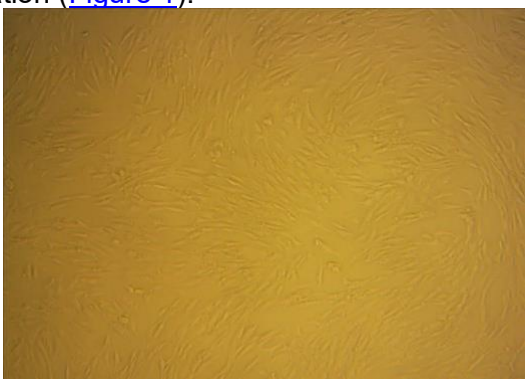

Figure 1. MSCs passage 4 confluence 80% (40x magnification)

This study examined how subcutaneous injection of EH-MSC affected NF-kB p65 and Catalase levels in 36 male rats of the wistar strain who experienced collagen loss due to UV-B exposure at a distance of 20cm with the intensity used was 1xMED (150mj/cm²) for 8 minutes carried out 10 times in 14 days to make the mice a model of collagen loss. Validation of the formation of *a collagen loss* model in mice macroscopic by looking at wrinkles on the skin of mice exposed to UVB compared to mice exposed to UVB rays, Based on visual observations, it was seen

that mice exposed to UVB had more pronounced wrinkles compared to mice that were not exposed. As seen in Figure 2, in addition to macroscopic validation, validation was also carried out microscopically using Masson Trichome staining to evaluate collagen density in UVB-exposed mice. The results of the painting showed that there was a decrease in collagen density after UVB exposure.

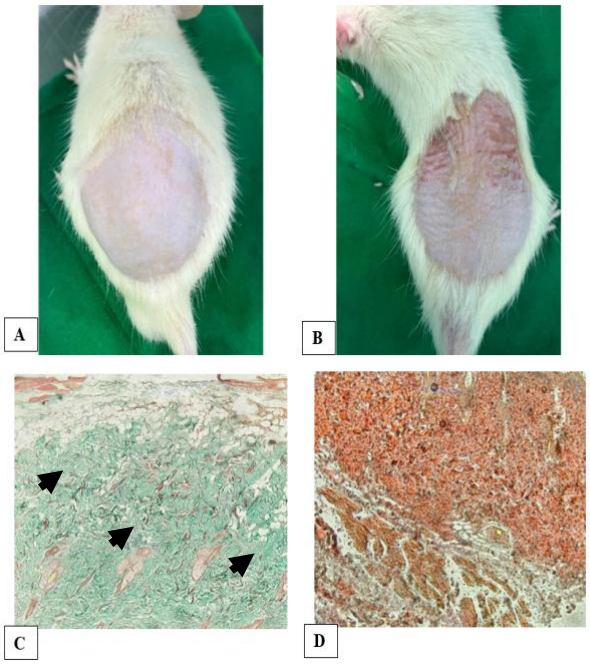

Figure 2. Collagen Loss *Validation*. Wrinkles were more pronounced in mice exposed to UVB (B) compared to those not exposed (A). Collagen shown in blue (black arrow) was less visible in the group exposed to UVB (D), compared to the group without UVB exposure (C)

Rats without UVB exposure were used as healthy controls (K1), while rats that experienced a decrease in collagen density due to UVB exposure were randomly divided into 4 treatment groups as follows: K2 (mice exposed to UVB and given 200µL injection of 0.9% NaCl subcutaneously), K3 (rats exposed to UVB were given Hyaluronic Acid injection at a dose of 200 uL subcutaneously), K4 (rats exposed to UVB were given subcutaneous injection of EH-MSC at a dose of 200 uL subcutaneously), and K5 (UVB-exposed rats were given subcutaneous injection of EH-MSC at a dose of 300 uL subcutaneously). EH-MSC injection was given 1 time on the 21st day and tissue sampling was done on the 29th day. The tissue was then homogenized using RIPA buffer with inhibitor protease inhibition. After the tissue formed the suspension, centrifugation was carried out and supernatants were collected for analysis of NF-kB p65 expression by RT-PCR and Catalase using the ELISA method.

The analysis data of NF-kB p65 expression and catalase enzyme levels listed in table 1 were tested for normality in each NF-kB p65 expression group and catalase enzyme levels. The results of the analysis showed that the expression of NF-kB p65 was abnormal because there were several groups whose results were obtained <0.05, but the levels of catalase enzyme were normally distributed with a value of p>0.05. So that from these results, only the level of the catalase enzyme was tested for

homogeneity. Based on the results of the normality test in table 1, the data on catalase enzyme levels were normal and homogeneous, then using the One Way Anova test, significant results were obtained with a value of ($p<0.05$), meaning that the data on catalase enzyme levels were statistically different and meaningful. This shows that the administration of EH-MSC has an effect on the level of catalase enzyme.

Table 1. Results of Average Analysis, Normality Test, Homogeneity Test on NFkB Expression, and Catalase Enzyme Levels

Variable	Group K1 N=6	K2 N=6	K3 N=6	K4 N=6	K5 N=6	Sig (p)
NF-kB p65 Expression						
Mean	1.00	3.30	1.59	0.92	0.63	
Std.deviasi	0.00	1.19	1.22	0.33	0.29	
Shapiro Wilk	**0.167***	**0.810***	0.012	0.006	0.016	
Lavene Test						0.000
Kruskal Wallis						**0.005*****
Catalase Levels						
Mean	10.84	5.37	12.46	14.16	16.78	
Std.deviasi	1.70	0.39	1.88	0.63	0.77	
Shapiro Wilk	**0.174***	**0.453***	**0.575***	**0.134***	**0.511***	
Levene Test						0.001
One Way Anova						**0.001*****

Table 2. Differences in NF-kB p65 Expression Between 2 Groups

Groups	P Value
K1 vs K2	**0.004***
K1 vs K3	0.747
K1 vs K4	0.330
K1 vs K5	**0.009***
K2 vs K3	0.053
K2 vs K4	**0.004***
K2 vs K5	**0.004***
K3 vs K4	0.746
K3 vs K5	0.053
K4 vs K5	0.104

**Man Whitney Test with a significant value of p<0.05*

Table 3. Differences in Catalase Levels Between 2 Groups

Groups	P Value
K1 vs K2	**0.004***
K1 vs K3	0.805
K1 vs K4	**0.035***
K1 vs K5	**0.001***
K2 vs K3	**0.003***
K2 vs K4	**0.001***
K2 vs K5	**0.001***
K3 vs K4	0.556
K3 vs K5	**0.015***
K4 vs K5	**0.001***

**LSD Post Hoc Test with a significant value of p<0.05*

The NF-kB p65 expression data was abnormal and not homogeneous, so a Non-Parametric test was carried out, namely the Kruskal Wallis test, which obtained significant results (table 3). The significant reduction in NF-kB (p65) expression in the K4 and K5 groups indicates that EH-MSCs effectively suppress

ROS-mediated activation of inflammatory signaling pathways (figure 3). This aligns with the known role of miR-146a and miR-21 in inhibiting NF-kB activation.[21]

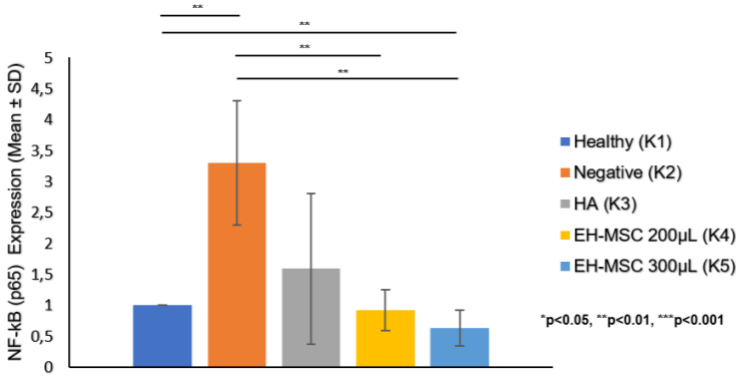

Figure 3. Average NF-kB p65 Expression graph

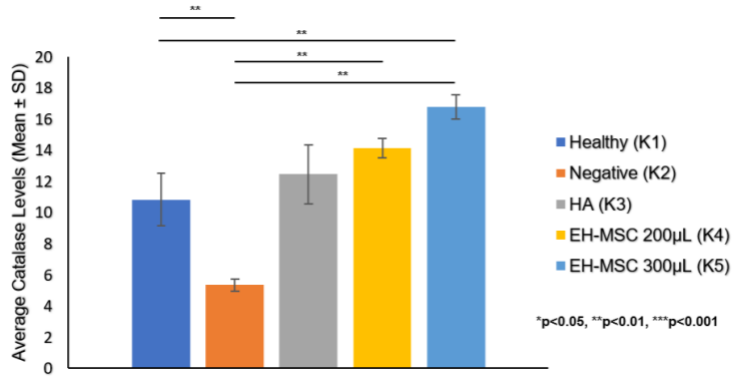

Figure 4. Average Catalase levels

Data on catalase enzyme levels that have significant differences after being tested parametrically *in One Way Anova*. Furthermore, to evaluate the difference between the two treatment groups, a *Post Hoc Tamhane test was carried out* because the data was normal and not homogeneous as shown in table 2. The dose-dependent increase in catalase levels in the EH-MSC-treated groups highlights the exosomes' ability to enhance antioxidant defenses. This is likely mediated by miRNAs such as miR-155 and miR-200c, which regulate catalase gene expression and ROS neutralization.[22]

The Photoaging process involves the skin's complex response to UV-B rays that cause significant structural and molecular changes. UV-B exposure triggers skin DNA damage, increases ROS production, causes oxidative stress and stimulates collagen loss leading to premature skin aging.[6] UV-B exposure causes oxidative sress in the skin, activating the NF-kB p65 signaling pathway.[23]

Exosomes of *mesenchymal stem cells* (MSCs) have been an interesting subject of research in regenerative therapy because of their potential to stimulate tissue regeneration and regulate the immune response.[24] Among the components contained in these exosomes, miRNAs play a key role in mediating the anti-inflammatory and immunomodulatory effects of MSCs.[25] miR-146a, miR-155 and miR-21 were identified as key regulators in controlling the inflammatory response. miR-146a and miR-155 have a role in suppressing the expression of pro-inflammatory cytokines and regulating the NF-kB pathway, whereas miR-21 is involved in reducing oxidative stress.[14] This is important because NF-kB activation is linked to the production of pro-inflammatory cytokines.

Meanwhile, the NF-kB pathway is one of the signals involved in the regulation of expression and pro-inflammatory genes. Exosomes MSCs can

contain miRNAs that inhibit NF-kB activation. In addition, through NF-kB regulation, exosomes can also affect the expression of the CD86 gene and lead to decreased M1 resistance so that M2 increases in macrophages.[23] The suppression of NF-kB (p65) expression observed in EH-MSC-treated groups is consistent with the miRNA-mediated inhibition of pro-inflammatory cytokines. miR-146a and miR-21, found in MSC exosomes, likely downregulate NF-kB transcriptional activity, thereby reducing inflammation.[26] Additionally, the increase in catalase levels suggests that EH-MSCs enhance antioxidant defenses by upregulating catalase gene expression via miR-155 and miR-2.[27]

This study showed that the level of catalase enzyme experienced a very significant increase compared to the negative control group. Then in NF-kB expression, p65 experienced a significant decrease compared to the negative control group. This shows that EH-MSCs have an influence on CAT levels and NF-kB p65 expression. This is because EH-MSCs have great potential in modulating the activity of antioxidant enzymes including catalase through miRNA content.[28] Some of the miRNAs in EH-MSCs are known to have an important role in increasing the activity of the catalase enzyme and regulating cellular antioxidant defenses. For example, miR-155 is involved in the regulation of the response to oxidative stress and can modulate genes associated with anti-oxidative activity, including the enzyme catalase. In addition, miR-21 plays a role in various cellular processes such as proliferation, apoptosis and response to oxidative stress, and can affect the expression of antioxidant enzymes.[29] miR-126 also has an important role in angiogenesis and tissue regeneration, as well as in the regulation of anti-oxidant defense mechanisms. Meanwhile, miR-146a reduces oxidative stress and inflammation, which has an impact on enzymes such as catalase. Finally, miR-200c, which is involved in the regulation of oxidative stress, has a modulating effect on the expression of antioxidant enzymes.[13–15]

Each dose of EH-MSCs injected showed a decrease in NF-kB p65 expression and an increase in different levels of CAT enzyme, indicating that each dose showed a different response to NF-kB p65 expression and CAT enzyme levels, meaning that each dose had a different response to inflammation. When compared between the two doses, at doses of 200 uL and 300 uL, the same can reduce the expression of NF-kB p65 compared to the control group. Previous research has stated that exosome-MSC therapeutics and how they work are relevant for degenerative diseases, as well as the quality control measures necessary for the development of exosome-derived therapies.[30] These findings align with Park *et al*. (2023), who demonstrated that exosome treatments reduce oxidative stress markers in UVB-damaged skin models. However, our study extends this by highlighting the dose-dependent effects of hypoxia-preconditioned exosomes.

Previous studies reported that the overall secretome secreted by MSCs was able to suppress inflammation as seen from the decrease in NF-kB p65 expression, in addition to that MSCs were known to be able to stimulate the production of antioxidant proteins including catalase which can counteract ROS, this is in accordance with this study which found a decrease in NF-kB p65 expression and an increase in catalase enzyme levels after EH-MSC exposure to UV-B.

EH-MSCs offer a promising therapeutic approach for managing oxidative stress and inflammation in skin aging. Future formulations could include topical applications or injectable therapies targeting similar molecular pathways. This study did not evaluate the specific miRNA or protein content of EH-MSCs, limiting the mechanistic understanding of their effects. Future research should focus on detailed profiling of exosomal cargo and its correlation with observed outcomes.

CONCLUSION

This study demonstrates that hypoxia-preconditioned MSC exosomes significantly reduce NF-kB (p65) expression and enhance catalase levels in a UVB-induced skin aging model. These findings highlight the potential of EH-MSCs as a novel, non-invasive therapeutic option for oxidative stress-related skin conditions. Future studies should aim to optimize dosing regimens and investigate the translational potential of EH-MSC therapies in clinical settings.

AUTHORS' CONTRIBUTIONS

Favoury Felie Basuki Rahmawati: Supervision, Conceptualization, Data curation, Reviewing. Sri Priyantini: Data curation, Investigation, Writing-Original draft preparation. Eko Setiawan: Supervision, Methodology, Validation.

ACKNOWLEDGEMENT

We would like to thank the Head of the Semarang Stem Cell Cancer and Research (SCCR) Laboratory for all his support for this research.

FUNDING INFORMATION

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

There is no conflict of interest.

REFERENCE

1. Rittié L, Fisher GJ. İnsan cildinin doğal ve güneş kaynaklı yaşlanması Natural and sun-induced aging of human skin. *Cold Spring Harbor Perspectives in Medicine*. 2015:1-14.
2. Nakai K, Tsuruta D. What are reactive oxygen species, free radicals, and oxidative stress in skin diseases? *Int J Mol Sci*. 2021;22(19). doi:10.3390/ijms221910799
3. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Res*. 2011;21(1):103-115. doi:10.1038/cr.2010.178
4. Anti-inflammatories N. Role of PGE-2 and Other Inflammatory Mediators in Skin Aging and Their Inhibition by Topical. Published online 2019. doi:10.3390/cosmetics6010006
5. Park AY, Lee JOK, Jang Y, et al. Exosomes derived from human dermal fibroblasts protect against UVB-induced skin photoaging. *Int J Mol Med*. 2023;52(6):1-15. doi:10.3892/ijmm.2023.5323
6. Qiu S, Sun J, Zhou T, Gao G, He Z, Liang T. Spiculation Sign Recognition in a Pulmonary Nodule Based on Spiking Neural P Systems. *Biomed Res Int*. 2020;2020. doi:10.1155/2020/6406395
7. Ahmad Z, Damayanti. Penuaan Kulit : Patofisiologi dan Manifestasi Klinis. *Berkala Ilmu Kesehatan Kulit dan Kelamin – Periodical of Dermatology and Venereology*. 2018;30(03):208-215.
8. Chircov C, Grumezescu AM. Hyaluronic acid-based scaffolds for tissue engineering. 2019;(January 2018).
9. Nguyen Trong H, Thi Phuong TV, Nguyen Van T, et al. The efficacy and safety of hyaluronic acid microinjection for skin rejuvenation in Vietnam. *Open Access Maced J Med Sci*. 2019;7(2):234-236. doi:10.3889/oamjms.2019.059

10. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2(March). doi:10.1038/sigtrans.2017.23
11. Chin T, Lee XE, Ng PY, Lee Y, Dreesen O. The role of cellular senescence in skin aging and age-related skin pathologies. *Front Physiol*. 2023;14(November):1-15. doi:10.3389/fphys.2023.1297637
12. Ngo V, Duennwald ML. Nrf2 and Oxidative Stress: A General Overview of Mechanisms and Implications in Human Disease. *Antioxidants*. 2022;11(12). doi:10.3390/antiox11122345
13. Xiong M, Zhang Q, Hu W, et al. The novel mechanisms and applications of exosomes in dermatology and cutaneous medical aesthetics. *Pharmacol Res*. 2021;166. doi:10.1016/j.phrs.2021.105490
14. Xiong Y, Xiong Y, Zhang H, et al. hPMSCs-Derived Exosomal miRNA-21 Protects Against Aging-Related Oxidative Damage of CD4+ T Cells by Targeting the PTEN/PI3K-Nrf2 Axis. *Front Immunol*. 2021;12(November):1-13. doi:10.3389/fimmu.2021.780897
15. Gilyazova I, Asadullina D, Kagirova E, et al. MiRNA-146a—A Key Player in Immunity and Diseases. *Int J Mol Sci*. 2023;24(16). doi:10.3390/ijms241612767
16. Alexander M, Hu R, Runtsch MC, et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nat Commun*. 2015;6. doi:10.1038/ncomms8321
17. Topis JB, Amelia V, Suliansyah I. Systematic Literature Review: Identification of Interleukin-10 in Mesenchymal Stem Cells for Inflammation. *Jurnal Biologi Tropis*. 2023;(2016).
18. Yustianingsih V, Sumarawati T, Putra A. Hypoxia enhances self-renewal properties and markers of mesenchymal stem cells. *Universa Medicina*. 2019;38(3):164-171. doi:10.18051/univmed.2019.v38.164-171
19. Lingappan K. NF-κB in oxidative stress. *Curr Opin Toxicol*. 2018;7:81-86. doi:10.1016/j.cotox.2017.11.002
20. Qu Q, Liu L, Wang L, et al. Exosomes derived from hypoxic mesenchymal stem cells restore ovarian function by enhancing angiogenesis. *Stem Cell Research and Therapy* . 2024;15(1). doi:10.1186/s13287-024-04111-6
21. Markopoulos GS, Roupakia E, Tokamani M, et al. Roles of NF-κB signaling in the regulation of miRNAs impacting on inflammation in cancer. *Biomedicines*. 2018;6(2). doi:10.3390/biomedicines6020040
22. Zheng B, Yin W na, Suzuki T, et al. Exosome-Mediated miR-155 Transfer from Smooth Muscle Cells to Endothelial Cells Induces Endothelial Injury and Promotes Atherosclerosis. *Molecular Therapy*. 2017;25(6):1279-1294. doi:10.1016/j.ymthe.2017.03.031
23. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2(March). doi:10.1038/sigtrans.2017.23
24. Hu JC, Zheng CX, Sui BD, Liu WJ, Jin Y. Mesenchymal stem cell-derived exosomes: A novel and potential remedy for cutaneous wound healing and regeneration. *World J Stem Cells*. 2022;14(5):318-329. doi:10.4252/wjsc.v14.i5.318
25. Cai J, Wu J, Wang J, et al. Extracellular vesicles derived from different sources of mesenchymal stem cells: Therapeutic effects and translational potential. *Cell Biosci*. 2020;10(1):1-14. doi:10.1186/s13578-020-00427-x
26. Zhongfei Zhang, Xiaoxiong Zou, Run Zhang, Yu Xie, Zhiming Feng, Feng li. Human umbilical cord mesenchymal stem cell-derived exosomal miR-146a-5p reduces microglial-mediated neuroinflammation via suppression of the IRAK1/TRAF6 signaling pathway after ischemic stroke. *Aging*. 2021;13.
27. Chang WL, Ko CH. The Role of Oxidative Stress in Vitiligo: An Update on Its Pathogenesis and Therapeutic Implications. *Cells*. 2023;12(6). doi:10.3390/cells12060936

28. Rafieerad A, Saleth LR, Khanahmadi S, Amiri A, Alagarsamy KN, Dhingra S. Periodic Table of Immunomodulatory Elements and Derived Two-Dimensional Biomaterials. *Advanced Science*. Published online February 10, 2025. doi:10.1002/advs.202406324
29. Lin YH. MicroRNA networks modulate oxidative stress in cancer. *Int J Mol Sci*. 2019;20(18). doi:10.3390/ijms20184497
30. Ha DH, Kim H keun, Lee J, et al. 皮肤修复综述Skin Regeneration. *Cells*. 2020;9(1157):4-6.