

Exosome Hypoxic-MSCs, Glutathione, and Vitamin C: Effect on IL-10 Levels and CD-163 Expression

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Abstract

Hyperpigmentation of the skin is a result of ultraviolet B (UVB) exposure, which causes oxidative stress due to increased reactive oxygen species (ROS), leading to various skin problems, including melanin accumulation. Exosomes can affect melanocyte activity. Exosomes, as small vesicles released by cells, can affect melanocyte activity and play an important role in various hyperpigmentation processes. The study aims to determine the effect of exosome mesenchymal stem cell hypoxia (EH-MSC) and glutathione with vitamin C on IL-10 levels and CD163 expression. IL-10 gene expression was measured using qRT-PCR, while CD163 expression was analyzed via immunohistochemical staining. A total of 30 male C57BL/6 mice were used and randomly assigned to five different treatment groups. The highest expression of IL-10 was observed in the EH-MSCs-treated group (K4), although the difference was not statistically significant compared to the control ($p = 0.135$). In contrast, the group receiving a combination of EH-MSCs with glutathione and vitamin C (K5) exhibited the highest percentage of CD163 expression, with a statistically significant difference ($p = 0.00$). These findings demonstrate that the administration of EH-MSC and glutathione with vitamin C significantly increased the expression of CD163, but insignificantly increased IL-10 in C57BL/6 mice with a UVB-induced hyperpigmentation model.

Keywords: CD163 expression, exosome mesenchymal stem cell hypoxia (EH-MSC), hyperpigmentation, IL-10 expression.

EH-MSCs, Glutathione, dan Vitamin C: Efeknya pada ekspresi gen IL10 dan CD163.

Abstrak

Hiperpigmentasi kulit merupakan hasil paparan sinar ultraviolet B (UVB) yang menyebabkan stres oksidatif akibat meningkatnya spesies oksigen reaktif (ROS), sehingga menimbulkan sejumlah masalah kulit termasuk penumpukan melanin. Eksosom dapat memengaruhi aktivitas melanosit. Eksosom sebagai vesikel kecil yang dilepaskan oleh sel, dapat memengaruhi aktivitas melanosit dan berperan penting dalam berbagai proses hiperpigmentasi. Penelitian ini bertujuan untuk mengetahui pengaruh hipoksia sel punca mesenkimal eksosom (EH-MSC) dan glutathione dengan vitamin C terhadap kadar IL-10 dan ekspresi CD163. Ekspresi gen IL-10 diukur menggunakan qRT-PCR, sedangkan ekspresi CD163 dianalisis melalui pewarnaan imunohistokimia. Tiga puluh ekor mencit jantan C57BL/6 digunakan, dibagi secara acak ke dalam 5 kelompok perlakuan. Hasil penelitian menunjukkan ekspresi gen IL-10 tertinggi teramati pada kelompok perlakuan EH-MSC saja (K4) ($p = 0,135$) dibandingkan dengan kelompok kontrol. Sementara itu, hasil persentase CD-163 tertinggi terdapat pada kelompok dengan kombinasi EH-MSC dan glutathione dengan perlakuan vitamin C (K5) ($p = 0,00$). Temuan ini menunjukkan bahwa pemberian EH-MSC dan glutathione dengan vitamin C secara signifikan meningkatkan ekspresi CD163, sementara secara statistik tidak meningkatkan IL-10 secara signifikan pada tikus C57BL/6 dengan model hiperpigmentasi yang diinduksi UVB.

Kata Kunci: Ekspresi CD163, ekspresi IL-10, exosome mesenchymal stem cell hypoxia (EH-MSC), hiperpigmentasi.

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1. Introduction

Hyperpigmentation of the skin is the result of exposure to ultraviolet B (UVB) rays which causes oxidative stress due to increased reactive oxygen species (ROS).¹ Increased ROS leads to numerous skin problems, including redness, hardening, melanin accumulation, skin aging, and an increased risk of cancer.² UVB exposure is associated with 8% of skin cancer cases, including metastatic squamous carcinoma. In 2020, hyperpigmentation cases increased significantly, recording more than 100,350 new cases and 6,850 deaths attributed to progression into skin cancer.³ Melanin accumulation and inflammation are key factors contributing to hyperpigmentation.⁴⁻⁶ Current treatments, including topical depigmenting agents and chemical peels, often yield suboptimal results and cause side effects such as skin irritation, dryness, burning, and erythema.⁷ In addition to these side effects, these treatments of hyperpigmentation often require several months to years to achieve noticeable results.⁸ This highlights the urgent need for alternative therapeutic approaches to address hyperpigmentation more effectively.

Recent breakthroughs in regenerative medicine predicted that secretomes derived from mesenchymal stem cells (MSCs), particularly exosomes, offer promising potential for treating hyperpigmentation.⁹ Exosomes can affect melanocyte activity.¹⁰ Exosomes can also affect signaling pathways involved in IL-10 production and CD163 expression in target cells.¹¹ IL-10, as an anti-inflammatory cytokine, can reduce inflammation associated with hyperpigmentation, while CD163, as a marker for macrophages involved in the resolution of inflammation, can also play a role in regulating the inflammatory response associated with skin pigmentation disorders.^{12,13}

Applications using antioxidants, such as vitamin C, that play a role in photoprotection can inhibit the enzyme tyrosinase, thereby reducing melanin formation and eliminating hyperpigmentation spots.¹⁴ Similarly, glutathione exhibits regulatory properties in melanogenesis. Glutathione acts as a skin lightening agent by inhibiting the enzyme tyrosinase.¹⁵ Combining exosomes with antioxidants such as glutathione and vitamin C may enhance their effects in reducing oxidative stress, modulating inflammatory pathways and addressing pigmentation disorders.

Prior reports have highlighted the individual roles of exosomes and antioxidants in skin repair and the regulation of pigmentation. For instance, research in 2023 demonstrated that the uptake of glutathione (GSH) by nanoparticles improved immune responses and reduced oxidative stress.¹⁶ A study by Wang

et al.¹⁷ shows that exosomes influence melanin production through mediation of paracrine, endocrine, and autocrine effects.¹⁷ Various studies of the antioxidant effect of vitamin C have shown that it plays a significant role in collagen synthesis, provides photoprotection, reduces melanin production, and supports immunomodulation.¹⁸

Current study aims to investigate the combined impact of these treatments on the expression of anti-inflammatory marker IL-10 and CD163 in a UVB-induced hyperpigmentation model, potentially offering new insights into more effective therapeutic strategies for managing hyperpigmentation.

2. Methods

2.1. Tools

The establishment of animal models utilized several tools, including UV chambers, exposure and maintenance cages, razors, drinking water containers, and hair clippers. Tools used for data collection included vacutainers, hematocrit tubes, 5 mL pots, 6 mm biopsy punches, centrifuges, micropipettes, a CO₂ incubator, an inverted microscope (Zeiss Primovert, Jena, Germany), and 1.5 mL vial tubes.

2.2. Materials

The materials used in this study were culture medium, Dubbelco's Modified Eagle Medium (DMEM), penicillin (100 U/mL)/streptomycin (100 µg/mL) (Pen-Strep), Fetal Bovine Serum (FBS), and Amphotericin B, which were purchased from Gibco™ Invitrogen.

2.3. Procedures

2.3.1. Ethical Clearance

The experiment protocol has been approved by the ethics commission of the Faculty of Medicine, Sultan Agung Islamic University (No. 414/X/2024/Komisi Bioetik). The study employed a post-test only control group design; the subjects were male mice of the C57BL/6 strain, with a body weight of 20-25 grams. The study was conducted in October-November 2024 at the Animal Model Research Center SCCR Indonesia laboratory.

2.3.2. Mesenchymal Stem Cell Isolated from Umbilical Cord and Validation

The process was conducted in a class 2 biosafety cabinet using sterile equipment and techniques. The mouse umbilical cord was obtained from a single mouse, separated from the blood vessels, washed

with PBS, finely chopped, and evenly distributed in a T75 flask. After a 3-minute incubation for adherence, culture medium (DMEM (Gibco), fungizone (Gibco), Pen-Strep (Gibco), and FBS (Gibco) is added to cover the tissue. The flask is incubated at 37°C with 5% CO₂, with the medium changed every 3 days. Cells began to appear after 3 days and can be harvested after 14 days, once they reach a density of 80%.

The hypoxia process involves by inserting MSCs at 80% density into the hypoxia chamber. Nitrogen gas was introduced until the O₂ level reached 5%, and the flask was incubated for 24 hours at 37°C. After 24 hours, the culture medium was filtered using Tangential Flow Filtration (TFF) (Satorius USA) to obtain the secretome of hypoxic MSC, which was then mixed with a water-based gel at a dose of 5% (P1) and 10% (P2). Secretome injection preparation was done by drawing the secretome fluid into a 1 cc syringe according to the required dose. Validating the ability of EH-MSCs to differentiate into osteogenic and adipogenic lineages using a staining assay. For the adipogenic differentiation assay, oil red O staining was employed. Osteogenic differentiation was confirmed using Alizarin Red staining.¹⁹

To confirm the presence of exosomes derived from EH-MSCs, flow cytometry was used to analyze surface marker expression on umbilical cord MSCs at passage 5. The cells were probed with rat CD29-PE, anti-CD90-FITC, CD45-APC, and CD31-PerCP antibodies (BD Bioscience, CA, USA). The samples were then detected using a BD Accuri C6 Plus flow cytometer (BD Bioscience) and its in-house program.¹⁹

2.3.3. Group Treatment

The subjects used 30 male C57BL/6 mice ranging in 20-25 gr body weight, divided into 5 treatment groups, namely K1: healthy mice, K2: mice exposed to UVB without injection of treatment, K3: mice exposed to UVB with glutathione (100µL) and vitamin C (100µL) injection treatment, K4: mice exposed to UVB with exosome (300µL) treatment, K5: mice exposed to UVB with exosome (300µL) and glutathione (100µL) and vitamin C (100µL) injection treatment. The exosome dosage was determined based on previous research, which used 300 µL of exosomes for subcutaneous injection in Wistar rats.²⁰

The standard therapy considered in this study focuses on the combination of antioxidants and glutathione. Existing standard treatments, as widely reported in various studies,^{21,22} have been shown to have significant negative effects, highlighting the need for alternative approaches.

2.3.4. UV-B exposure.

UV-B exposure began by adapting the mice for 7 days. After the acclimatization period, all mice were assigned to five groups. The mice were then sedated by first disinfecting using an alcohol swab on the lower left abdomen, followed by an intraperitoneal injection of 0.5 cc of a mixture of 90% ketamine and 10% xylazine. The backs of the mice were shaved 2x2 cm wide, then the mice were left until the sedation effect disappeared completely. Furthermore, the mice were exposed to UV-B at a dose of 180 mJ/cm² in a UV chamber for 10 minutes, six times in two weeks. After exposure, all mice were put into cages according to the group. On the 14th day, validation was carried out using Masson Fontana staining.

2.3.5. Masson Fontana Staining Validation for Measured Melanin Level

Skin tissue samples were cut longitudinally to a depth of approximately 3 mm, reaching the subcutaneous layer, for histological examination. The preparation was conducted at the Central Java Animal Health Laboratory. Melanin staining was carried out using the Masson Fontana staining protocol.²³ The procedure began with deparaffinization, followed by incubation in heated Bouin's fluid for 60 minutes, then cooled for 10 minutes. Slides were then incubated in Weigert's iron hematoxylin for 5 minutes, followed by staining with Biebrich Scarlet/Acid Fuchsin for 15 minutes. Next, slides were immersed in a phosphomolybdic acid/phosphotungstic acid solution for 10–15 minutes, then stained with an aniline blue solution for 5–10 minutes. Finally, they were treated with an acetic acid solution for 3–5 minutes before microscopic examination. An increase in melanin content compared to healthy tissue was indicated by the presence of blackish pigmentation in the epidermal layer.²⁴

2.3.6. Tissue Sample Collection and Storage

After 24 hours of the glutathione, vitamin C, and exosome injection treatment, mice were killed using the cervical dislocation method for tissue collection. Skin tissue was taken using a 6 mm punch biopsy from the induced area. Tissue samples were stored in RNA later solution to maintain RNA quality and then stored at -20°C until PCR analysis was performed.

2.3.7. qRT-PCR of IL-10

Total RNA was isolated using TRIzol Reagent (Invitrogen, USA), and complementary DNA (cDNA) was synthesized using SuperScript IV Reverse Transcriptase (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) was then performed with

PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA) on a QuantStudio 3 Real-Time PCR System (Applied Biosystems, USA).²⁰ The primer sequences used for IL-10 were: Forward 5'-GCT CTT ACT GAC TGG CAT GAG-3' and Reverse 5'-CGC AGC TCT AGG AGC ATG TG-3', based on the NCBI reference sequence NM_010548.2. Gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method with efficiency correction, and GAPDH was used as the internal reference gene.

2.3.8. Immunohistochemical of CD163

Paraffin-embedded tissuesamples were deparaffinized using alcohol and xylene. After rehydration, the slides were probed with a CD163 antibody (1:100, Abcam, clone CD3-H52E8), followed by the application of a biotinylated secondary antibody. Observation was carried out using streptavidin peroxidase with ImageJ to quantify the expression levels of CD163.²⁴ One section was used per replication group, with five observation points per section. Observations were conducted using an inverted microscope (Carl Zeiss, Jena, Germany) with 40× magnification and numerical aperture (NA) of 0.65.

2.3.9. Data Analysis

The analysis included descriptive statistics, along with tests for normality and homogeneity. IL-10 expression data were normally distributed but did not meet the homogeneity assumption ($p > 0.05$), so a one-way ANOVA was conducted ($p < 0.05$), followed by a Tamhane post hoc test to identify differences between groups. In contrast, CD163 expression data were both normally distributed and homogeneous ($p > 0.05$), allowing for a one-way ANOVA ($p < 0.05$), accompanied by an LSD post hoc test to examine group differences through the SPSS program (version 22.0).

3. Results

3.1. Validation of EH-MSCs.

The MSCs from the umbilical cords of pregnant mice were collected and cultured in plastic flasks. Upon examination of the cultured MSCs under a microscope, spindle-shaped cells were observed adhering to the bottom of the flask, reaching 80% confluence, as shown in Figure 1A. The results of the isolation process for MSCs were validated by assessing their ability to differentiate into osteogenic and adipogenic lineages. For the adipogenic differentiation assay, Oil Red O staining was employed to visualize the development of red lipid droplets (Figure 1B). The differentiation into osteogenic was validated based on the presence of calcium deposits, which were distinctly visible in red (Figure 1C).

The findings from isolating hypoxic MSCs demonstrated their capability to express a range of specific surface markers, which were confirmed through flow cytometry. This investigation aimed to isolate MSCs to achieve pure MSCs utilizing secretomes derived from MSCs that had been preconditioned under hypoxic conditions with a 5% O₂ concentration, employing Tangential Flow Filtration (TFF); molecules within the 100-500 kDa range were identified as exosomes. The EH-MSCs were examined using flow cytometry to assess the presence of exosome markers, specifically CD63, CD81, and CD9. Ultimately, the analysis revealed a positive identification of exosomes through the markers CD81, CD63, and CD9 (Fig. 2).

3.2. Macroscopic and Histological validation of skin tissue of the C57BL/6 mice hyperpigmentation model.

A macroscopic assessment was performed to verify whether UVB exposure effectively leads to hyperpigmentation in the skin of mice. The

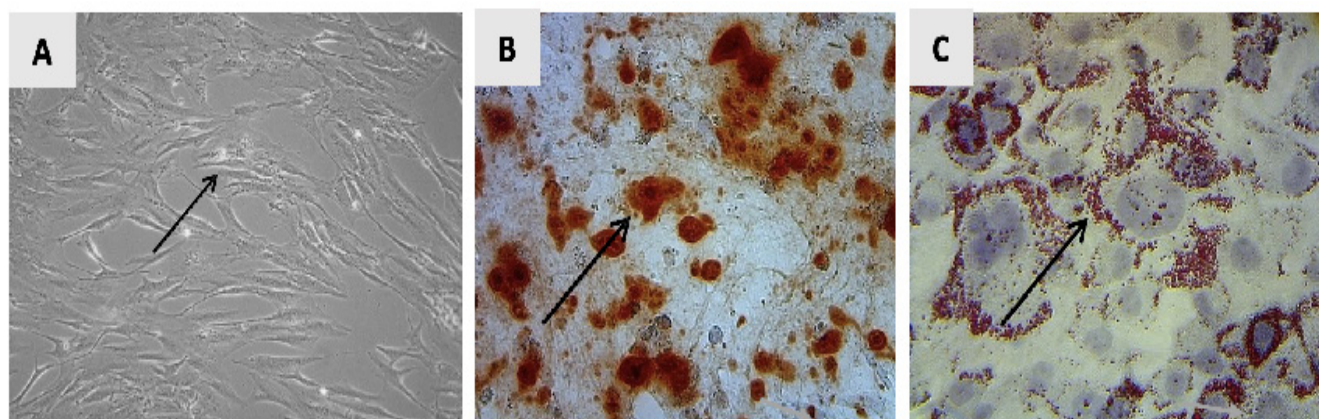


Figure 1. (A) MSCs culture results show spindle-shaped cells (black arrows). (B) Adipogenic differentiation test with oil red O staining. The red color (indicated by the yellow arrow) indicates lipid droplets, and (C) Osteogenic differentiation test with Alizarin red staining. The red color (indicated by the red arrow) indicates calcium deposits.

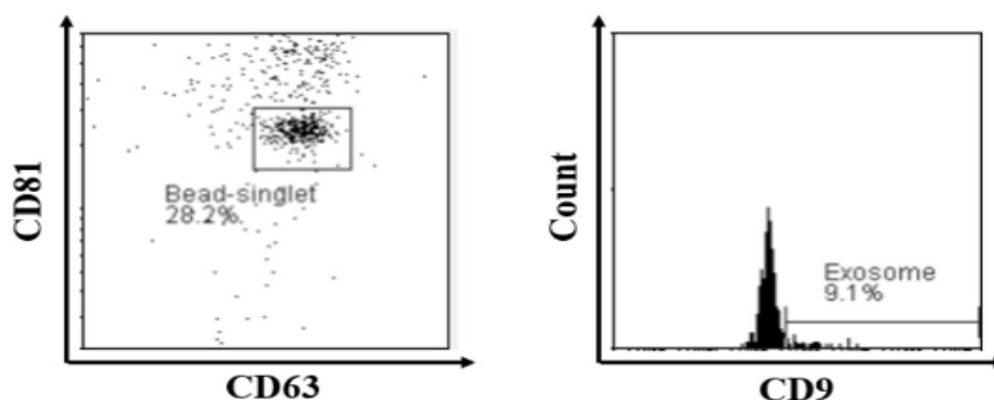


Figure 2. Exosome validation against CD81, CD63, and CD9 marker expression using flow cytometry

macroscopic evaluation involved comparing the condition of mice subjected to UVB exposure with that of the control group. The observations indicated alterations in skin color compared to healthy mouse skin (Figure 3A). The exposed mouse skin shows a darkening of color in the UVB-exposed area (Figure 3B). The validation results from Masson Fontana staining on skin samples from healthy mice (Figure 3C) are compared to those from the treatment group exposed to UVB, showing a black colour in the region corresponding to melanocytes (Figure 3D). An excess of melanin production can cause skin damage and result in hyperpigmentation. Melanin has the ability to absorb UVB radiation, offering photoprotection and interacting with keratinocytes during the melanin synthesis process.²⁵ Microscopic changes observed in the dermis layer of the skin reveal an increase in

melanin content, as evidenced by the findings from the Masson Fontana staining analysis.

3.3. IL-10 gene expression and CD163 percentage in skin tissue of a C57BL/6 mouse hyperpigmentation model

The increase in IL-10 indicates an increase in anti-inflammatory activity, which helps prevent hyperpigmentation.^{26,27} The results of RT-qPCR analysis showed that the highest average expression of IL-10 was obtained in the K4 treatment group, although it did not show a significant difference compared to the negative control group, K2 ($p>0.05$) (Fig. 4). Lower average expression of IL-10 was shown in both K3 and K5 groups (Figure 4).

Macroscopic features of mice skin exposure to UVB.

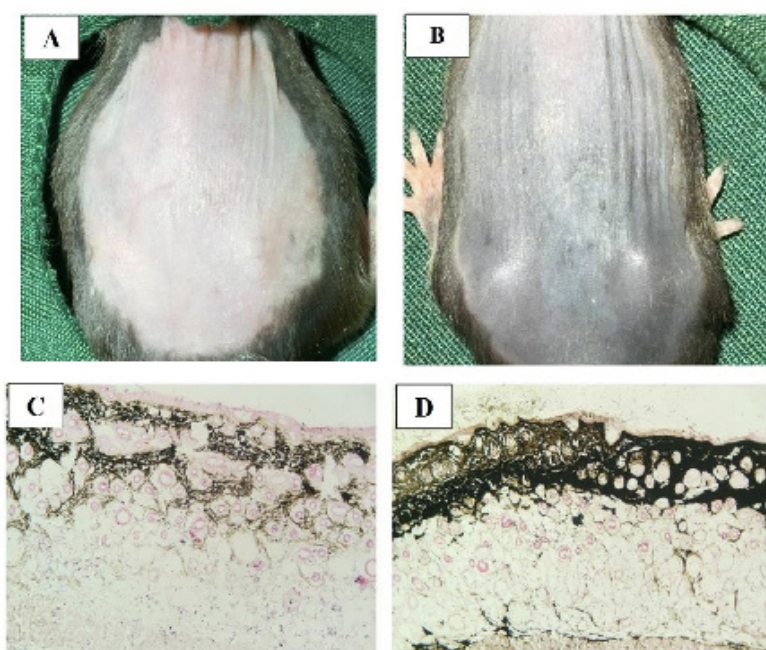


Figure 3. Macroscopic and morphological features of mice skin exposed to UVB for 14 days. (A) Macroscopic features of the skin of mice not exposed to UVB. (B) Macroscopic features of mice skin exposure to UVB. (C) Morphology of the skin of mice not exposed to UVB. (D) Morphology of mouse skin exposed to UVB

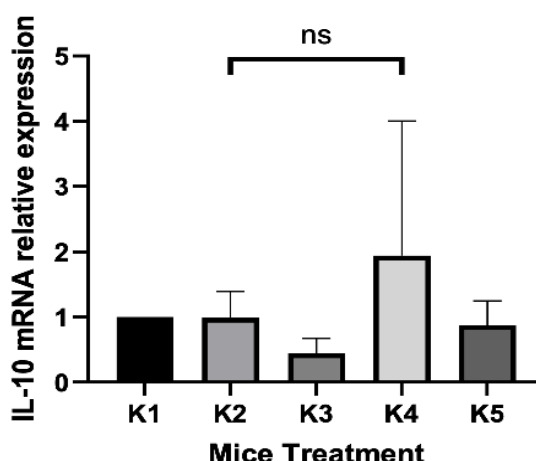


Figure 4. Average expression of IL-10 in C57BL/6 mice hyperpigmentation model. Data are presented as mean $\pm$ SD (n=6). There was no significant difference between the negative control (K2) and the treatment groups (K3, K4, and K5). K1: healthy mice, K2: mice exposed to UVB without injection of treatment, K3: mice exposed to UVB with glutathione and vitamin C injection treatment, K4: mice exposed to UVB with exosome treatment, K5: mice exposed to UVB with exosome and glutathione and vitamin C injection treatment. Relative expression of IL-10 means that the expression is not absolute, but rather relative to the group considered as the control (K1) and also relative to the housekeeping gene.

(C) Morphology of the skin of mice not exposed to UVB. (D) Morphology of mouse skin exposed to UVB. In a large animal model, a single early dose of exosomes is optimal for triggering the desired effect.²⁸ This study demonstrates that a single dose of EH-MSCs is more optimal for inducing an anti-inflammatory response through increased IL-10, whereas combined doses may cause receptor saturation on target cells or activate negative regulatory mechanisms, which reduces exosome saturation efficiency.²⁹

Expression of CD-163 showed a significant difference in both single (K3 and K4) and combined (K5) groups compared to the K2 control group ($p < 0.05$) (Figure 5).

The highest CD-163 average expression result was obtained by the combined treatment of EH-MSCs and glutathione with vitamin C. Lower average expression was shown in the non-combined treatment, K3 and K4 groups (Figure 5). A previous study has shown that MSCs increase CD163 expression.³⁰ This study indicates that EH-MSCs can also increase CD163 expression. Combination of EH-MSCs with glutathione and vitamin C also further increases CD163 expression. It can be concluded that administering a combination injection of EH-MSC and glutathione with vitamin C increases the expression of CD163 in C57BL/6 mice with hyperpigmentation models.

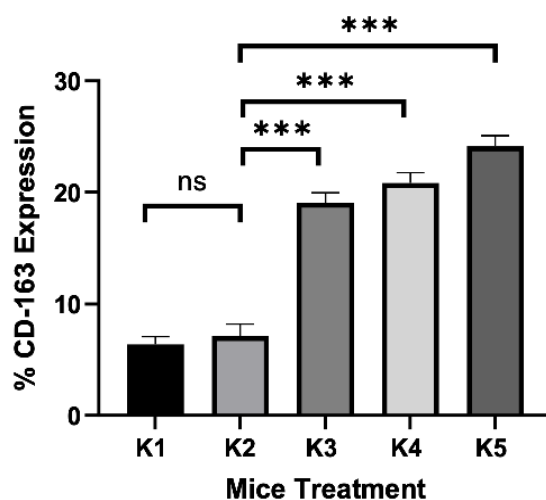


Figure 5. Average expression of CD-163 in the C57BL/6 mice hyperpigmentation model. Data are presented as mean $\pm$ SD (n=6). There is a significant difference between negative control (K2) and treatment groups (K3, K4, and K5). ns: $p > 0.05$, ***: $p \leq 0.00$; K1: healthy mice, K2: mice exposed to UVB without injection of treatment, K3: mice exposed to UVB with glutathione and vitamin C injection treatment, K4: mice exposed to UVB with exosome treatment, K5: mice exposed to UVB with exosome and glutathione and vitamin C injection treatment.

4. Discussion

This study evaluated the effect of exosome-mesenchymal stem cell hypoxia (EH-MSC) administration, combined with glutathione and vitamin C, on IL-10 and CD163 gene expression in C57BL/6 mice, using hyperpigmentation as a model. IL-10 and CD-163 have important roles in inflammation and immunoregulation. IL-10 is an anti-inflammatory cytokine that increases the activity of M2 macrophages³¹, characterized by the expression of CD163. CD-163, a protein scavenger receptor often used as an M2 marker³², is involved in phagocytosis,³³ reducing inflammation,³⁴ and tissue healing.³⁵

A study by Scuteri et al.³⁶ showed that UVB exposure in hyperpigmentation model mice, which were then given exosome injection treatment, significantly increased IL-10 expression. This is in accordance with the results of the EH-MSCs injection group, which showed an increase in IL-10 expression compared to the negative control (Fig. 4). The ability of EH-MSCs to modulate IL-10 expression is likely mediated through the bioactive molecules in exosomes, such as miRNA and anti-inflammatory proteins, which polarize macrophages to the M2 phenotype.³⁷ M2 macrophages produce large amounts of IL-10 to support tissue regeneration.³⁸

The STAT3 signaling pathway plays a crucial role in regulating IL-10.³⁹ Exosomes, including miRNAs, have been shown to activate STAT3 signaling, resulting in increased IL-10 production and the suppression of pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β .^{40,41} This mechanism not only reduces inflammatory damage but also prevents excessive melanogenesis triggered by chronic inflammation. Additionally, exosomal Transforming Growth Factor Beta 1 (TGF- β 1) and Hepatocyte Growth Factor (HGF) further enhance IL-10 production by promoting macrophage polarization to the M2 phenotype.^{42,43} Increased IL-10 production can help reduce inflammatory damage and prevent hyperstimulation of melanogenesis.³⁹ Wu et al⁴⁴ also reported that increased IL-10 can reduce pro-inflammatory cytokines, thereby creating a conducive immune environment that reduces chronic inflammation associated with hyperpigmentation.

Exosomes have the ability to transport microRNAs and proteins that modulate IL-10 expression, helping to reduce inflammation and calm excessive immune responses.⁴⁵ This is very useful in treating hyperpigmentation triggered by inflammation. In addition, IL-10, transmitted through exosomes, can inhibit inflammatory pathways that affect melanogenesis by reducing oxidative stress and local inflammation, thereby preventing excessive activation of MITF, a key regulator of melanin synthesis.⁴⁶

This study also showed that injection of EH-MSCs together with glutathione and vitamin C resulted in the highest CD-163 gene expression in hyperpigmentation model mice. Increased CD-163 expression is associated with macrophage activation to the M2 phenotype that regulates anti-inflammatory phenotype.⁴⁷ CD-163 plays a role in reducing inflammation and facilitating tissue regeneration.⁴⁸ The study by Han et al.⁴⁹ stated that M2 macrophages, which are characterized by CD-163 expression, can promote hyperpigmentation through interaction with melanocytes. Melanocytes cultured with M2 macrophages showed a significant increase in melanin biosynthesis.⁵⁰

UVB exposure produces local oxidative stress and inflammation that stimulate macrophages to adopt an M2 phenotype, increasing CD-163 expression.^{51,52} The STAT3 signaling pathway, triggered by IL-10 or TGF- β , contributes to increased CD-163 expression to address tissue damage and support regeneration.⁵³⁻⁵⁵ Exosomes, especially EH-MSCs, amplify this response by carrying bioactive molecules⁵⁶ such as TGF- β and microRNAs that suppress pro-inflammatory pathways and enhance anti-inflammatory activity. By inducing M2 macrophages and CD-163 expression, the inflammatory process and oxidative stress can be suppressed, reducing the stimulation of melanocytes to produce melanin.

Vitamin C and glutathione work synergistically by increasing the Nrf2 pathway and decreasing the NF- κ B pathway,⁵⁷ which supports CD-163 expression and anti-inflammatory and antioxidant functions.⁵⁸ Thus, CD-163 plays a vital role in reducing hyperpigmentation through modulating the inflammatory response and antioxidant capacity, helping to balance melanin production and maintain healthy skin.

However, this study has limitations because it did not use a comparison group with standard drugs for hyperpigmentation, for example ascorbic acid, arbutin, kojic acid, or hydroquinone, which work as tyrosinase inhibitors.⁵⁹ The addition of a comparison group could increase the validity of the results. Although the combination therapy of exosome injection, glutathione, and vitamin C is promising for treating hyperpigmentation, further development is needed to ensure safety through clinical trials before it can be widely applied.

5. Conclusion

Treatment of single dose exosome mesenchymal stem cell hypoxia (EH-MSCs) increasing IL-10 expression, but combination treatment of EH-MSCs and glutathione with vitamin C not increasing IL-10

expression in C57BL/6 mice with hyperpigmentation model. Administration of EH-MSC and glutathione with vitamin C, both single dose or combination increasing the expression of CD163 gene in C57BL/6 mice with hyperpigmentation model. Thus, show the potential of using combination of EH-MSCs and glutathione with vitamin C to treatment hyperpigmentation.

Conflict of Interest

The authors declare no conflicts of interest.

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