Chapter 1

Introduction

1.1 Background

Skin aging refers to the decrease in structural and functional feature of the skin, which could be caused by intrinsic or extrinsic factors (Chaudhary et al., 2019). Intrinsic factors that induces skin aging include chronological aging, genetics, and hormonal changes (Ambrose & Chew, 2021). Skin aging caused by extrinsic factors mainly came from environmental exposure such as UV radiation and pollution, or could be caused by lifestyle choices (Krutmann et al., 2021). Both intrinsic and extrinsic skin aging manifests itself through similar attributes which is seen in fine lines, wrinkles, loss of skin elasticity, and dryness of the skin (Zhang & Duan, 2018). In the laboratory setting, aging could be induced with several substances that has been known to damage the cells such as the addition of hydrogen peroxide (H_2O_2) and induction of UVB radiation. DNA damage, mitochondrial malfunction, and oxidative stress could happen when H_2O_2 is introduced to the cell as it converts into a more reactive molecule known as hydroxyl radicals (OH) (Rinnerthaler et al., 2015). This condition leads to cell death and decrease in cellular metabolic activity that is similar to the condition of aging caused by H_2O_2 that is either produced through normal cellular activity or external factors such as pollution or lifestyle choices (Wen et al., 2017). UVB radiation imitates aging process caused by sun exposure where direct physical damage occurs, UVB is absorbed through the epidermis layers in the skin (Hama et al., 2025). The damage of UVB in the cell is seen through direct DNA damage and also leads to increase production of ROS in the cells, creating an oxidative stress condition that would lead to mitochondrial malfunction and eventual cell death (Papaccio et al., 2022)

In recent years, regenerative medicine, namely stem cell therapy, has gained traction in the medical field to address various medical cases that damage tissue cells (Mao & Mooney, 2015). Stem cells have been used for aesthetic cosmetic purposes such as for skin rejuvenation, anti-aging therapies, and hair growth procedures which are commonly advertised by plastic surgeons (Salvatore et al.,

2023). The key between stem cells anti-aging therapy is their ability to increase cellular signalling to Wheimprove cell growth, promotes the production of collagen, and enhancing the structure and function of damage skin (El Assaad et al., 2024). However, stem cell therapy also have its own drawbacks which mainly comes from its ethical sourcing, risk of tumor formation, and high cost of production (Yousuf & Singh, 2022). An alternative and new cell-free treatment have been developed which uses secretome, the secreted biomolecules of cells that was found to have many beneficial properties particularly for skin regeneration and rejuvenation therapy..

Secretomes consist of multiple proteins that includes growth factors, hormones, cytokines, proteases, angiogenic factors, genetic materials, and lipid mediators in varying degrees of concentration (Daneshmandi et al., 2020). Secretome naturally provides cell-to-cell communication in eukaryotic cells, however, recent studies have discovered that secretome also has positive effects on tissue regeneration exhibiting promising potential for novel cell-free therapy (Abbasi-Malati et al., 2018).

Umbilical cord-mesenchymal stem cells secretome (UC-MSCS) has received particular attention due to the cells' non-invasive harvesting methods and strong self-renewal capabilities (Semenova et al., 2021). Zou et al (2022) concluded that hUC-MSCS exhibit a promising and effective anti-photoaging property that can be utilized to treat skin aging due to its inhibition of cell apoptosis, reduction of Reactive oxygen species (ROS) production, and promotion of cell proliferation and motility. Furthermore, Park et al (2021) report on the presence of 18 cytokines that are associated with skin condition improvements, namely Basic Fibroblast Growth Factor (BFGF), Epidermal Growth Factor (EGF), and Glial cell line-derived neurotrophic factor (GDNF), that may play a role in reducing melanin, improving anti-cellular oxidative stress, and anti-wrinkle effects in UC-MSCS. In another study, it was found that UC-MSCS enhances skin barrier and regulates skin cell apoptosis and detoxification, suitable for treating atopic dermatitis and acne (Wang et al, 2022). These findings highlight the potential of secretome-based treatments in advancing regenerative and aesthetic therapies.

There remain unanswered questions regarding the preventative cytoprotective potential of the UC-MSC secretome especially those sourced from local UC-MSC in Indonesia. Therefore, this research

aims to investigate the cytoprotective effects of UC-MSC secretome by subjecting cells to stress inducers, specifically hydrogen peroxide treatment and UVB exposure in Human Keratinocyte (HaCaT) cells. HaCaT cell line was used in this research as they construct 95% of the epidermis layer in the skin, making it suitable for observing the aging related damage. HaCaT has also been used for various skin related research purposes, making them thoroughly characterized and are known to respond to aging related stressors, especially hydrogen peroxide and UVB radiation. The sample for this study came from PT Kalbe Farma, one of the leading pharmaceutical companies in Indonesia, which produces secretome that is derived from UC-MSCs. The product is currently still in development and are available for research use only. This research also evaluates the product's protein characteristics through its total protein content and molecular weight analysis prior to the cytoprotective analysis to better understand the concentration and composition of the secretome sample in regards to their cytoprotective ability as a pre-treatment against aging inducers.

1.2 Objective

The research aims to examine the cytoprotective ability of UC-MSC secretome produced by PT Kalbe Farma as pre-treatment when HaCaT cells are treated with aging inducer through hydrogen peroxide and UVB radiation. The aim is also supported with protein characteristic study in the secretome total protein quantification and molecular weight analysis.

1.3 Hypothesis

H0 : Secretome derived from UC-MSC produced by PT Kalbe Farma does not exhibit any cytoprotective potency when used as pre-treatment in HaCaT cells treated with aging inducers.

H1: Secretome derived from UC-MSC produced by PT Kalbe Farma exhibit significant cytoprotective potency when used as pre-treatment in HaCaT cells treated with aging inducers.