

BIRD'S NEST EXTRACT PROMOTES HUMAN STEM CELLS PROLIFERATION

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1. ABSTRACT

The present study aimed to investigate the ability of KaicoLIVE[®]-coded edible bird's nest extract to promote the proliferation of human adipose-derived stem cells (hADSCs). KaicoLIVE[®]-coded edible bird's nest extract was prepared with hot water extraction. MTT assay was used to determine the optimum concentration of KaicoLIVE[®] on cultured hADSCs. Subsequently, scratch assay was performed on hADSCs to assess the wound healing effect of KaicoLIVE[®]. The wound closure rate was measured based on microscopic observation evaluated on a daily basis. RTPCR was used to determine the expression of anti-aging markers (IL-6, Collagen I, and Elastin) and cell cycle-related genes (Cyclin D1, Gadd45, p53, and Rb) of the KaicoLIVE treated group compared to the control group.

The optimum concentration of KaicoLIVE[®] to increase the proliferation of hADSCs is 0.1%, which significantly accelerates the wound closure of hADSCs. KaicoLIVE[®] also increased the anti-aging markers expression in hADSCs whereby the expression of the Cyclin D1 gene was up-regulated as compared to the control group. While Gadd45, a growth arrest and DNA damage inducible gene was down-regulated in KaicoLIVE[®] treated group. These data showed that KaicoLIVE[®] possess the ability to promote cell rejuvenation.

Keywords: KaicoLIVE[®], Edible Bird Nest, Natural product, Human adipose derived stem cell (hADSCs), Proliferative, Rejuvenation.

2. INTRODUCTION

The edible bird's nest (EBN) is a well-known food which has a health enhancing effect and is used to maintain the youthfulness among the Chinese. Investigators have shown that EBN has selective mitogenic effect on human normal cells including human peripheral blood monocytes (co-stimulation with concanavalin A) [1], enhance DNA synthesis in 3T3 fibroblast [2], and adipose-derived stem cells but not the human transformed cell line such as MCF-7 [3]. Hence, EBN has been proposed to have rejuvenating and anti-cancer properties. EBN has been reported to improve immune system, enhance complexion, alleviating asthma, speedy recovery from illness and surgery, improve renal function, increase energy and metabolism [4]. Although EBN has been reported to have many health benefits, scientific evidences on its bioactivity are not well established yet particularly on molecular activity.

3. OBJECTIVE

The objective of the study is to investigate the ability of KaicoLIVE[®]-coded edible bird's nest (EBN) extract to promote the proliferation of human adipose-derived stem cells.

4. METHODOLOGY

Cell Culture

Human adipose-derived stem cell (hADSCs) purchased from ATCC and cultured in Dulbecco's Modified Eagle Medium-Ham's F12 medium (DMEM:F12; 1:1, Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 1% antibiotic-antimycotic (Invitrogen), 1% glutamax (Invitrogen) and 1% vitamin C (Sigma Aldrich). They were then maintained at 37°C and 5% carbon dioxide. The hADSCs were divided into 2 groups: 1) Control (hADSCs cultured in normal medium) and 2) KaicoLIVE[®] (hADSCs cultured in medium supplemented with EBN extract). Each of the group was maintained in similar culture condition for 72 hours.

Scratch Assay

This assay was conducted to investigate the wound healing effect of KaicoLIVE[®] on normal hADSCs and wound closure rate was evaluated through daily microscopic observation. Cells were cultured in 6-well plate at density 5×10⁴ cells/well and once confluence, a defect area was created with a pipette tip.

MTT Assay

MTT (3-[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay was used to determine the optimum concentration of KaicoLIVE[®] on healthy hADSCs.

Quantitative RT-PCR

RT-PCR was performed to determine the expression of anti-aging and cell cycle-related and tumor suppressor-related genes of hADSCs treated with 0.10% KaicoLIVE[®].

Statistical Analysis

Statistical analysis was performed (SPSS, version 20.0) and all the data were presented as mean ± SEM. Statistical significance was calculated using student's t-test, where p < 0.05 was considered significant.

5. RESULTS

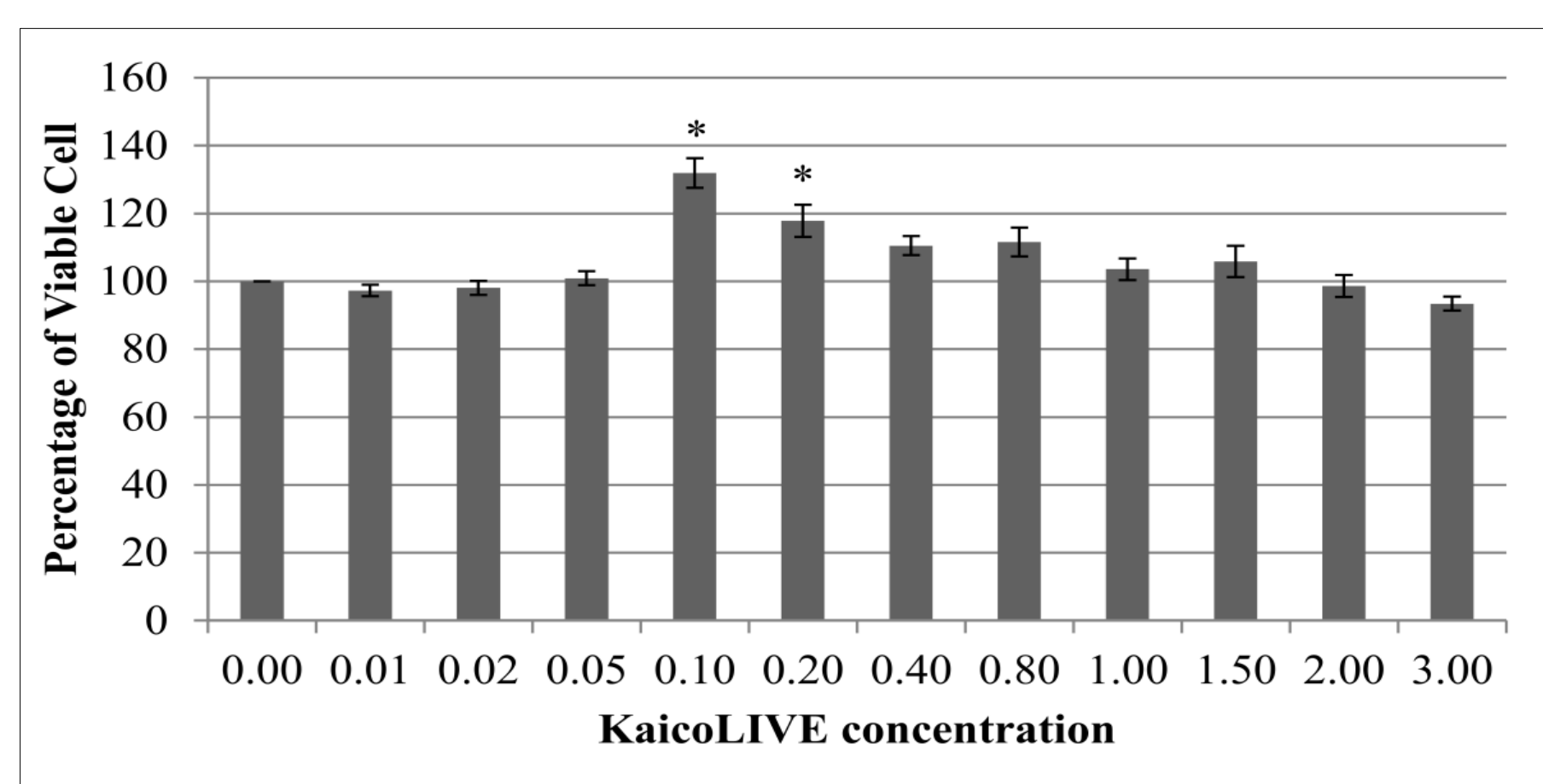
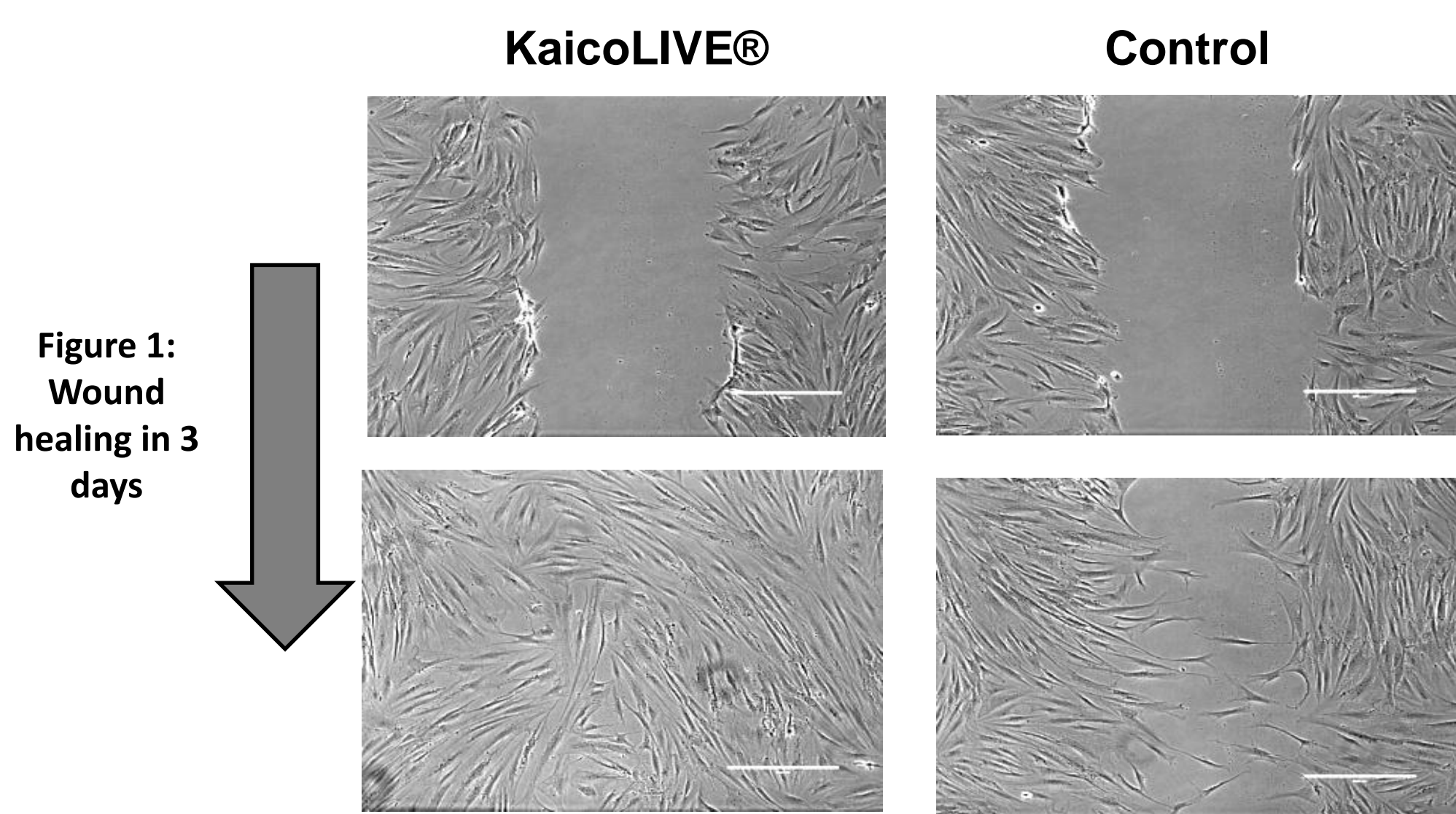


Figure 2. Effects of KaicoLIVE[®] on hADSCs proliferation. The cell proliferation was highest in 0.10% KaicoLIVE[®]. * p < 0.05 relative to control group.

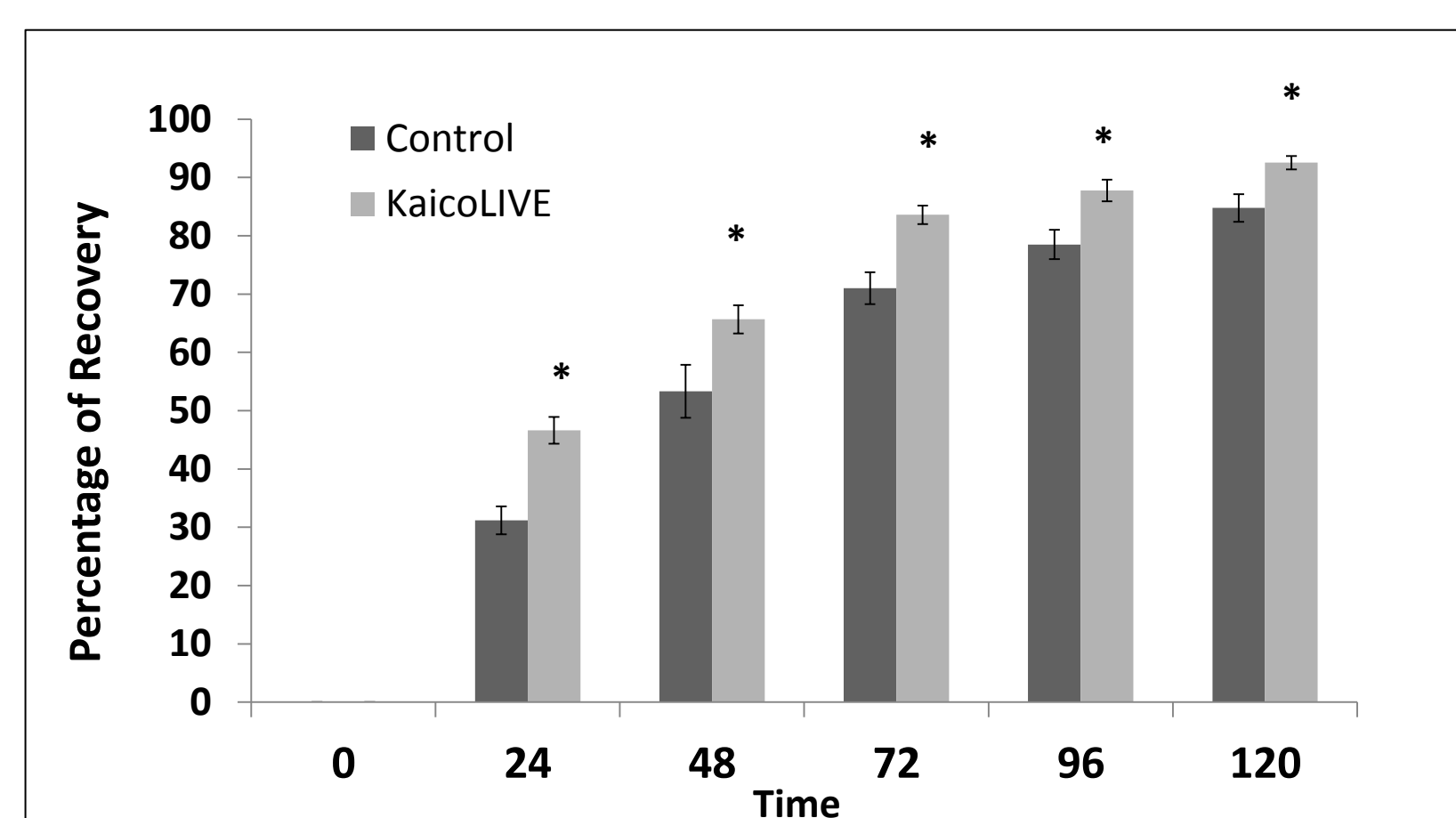


Figure 3. Effects of KaicoLIVE[®] on wound closure. Wound recovery was significantly higher for the KaicoLIVE[®]. * p < 0.05 relative to control group.

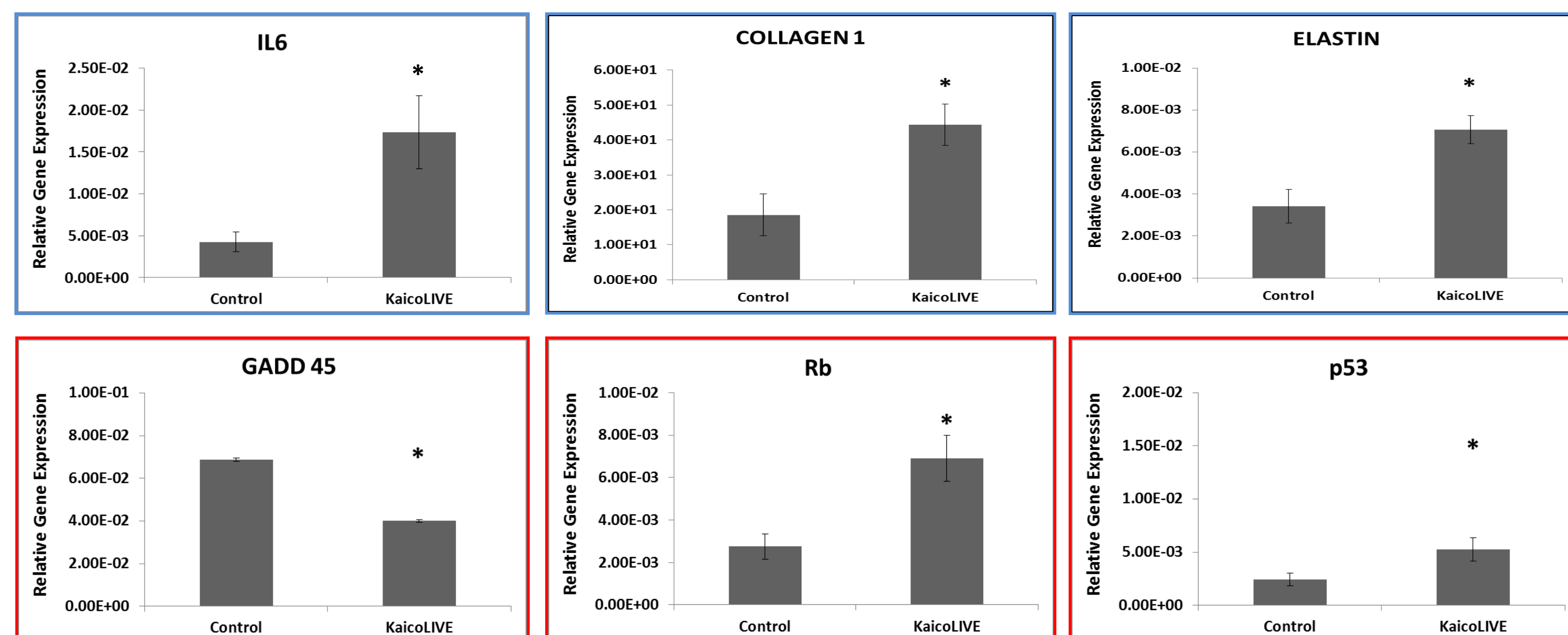


Figure 4. Gene expression analysis of anti-aging-related markers: IL6 (4.07-fold), Collagen 1 (2.39-fold) and Elastin (2.07-fold) showed that they were up-regulated when hADSCs were treated with KaicoLIVE[®] after 72 hours. Data was normalised to GAPDH (housekeeping gene). The values were expressed as mean ± SEM (n=4). * p < 0.05 relative to control group.

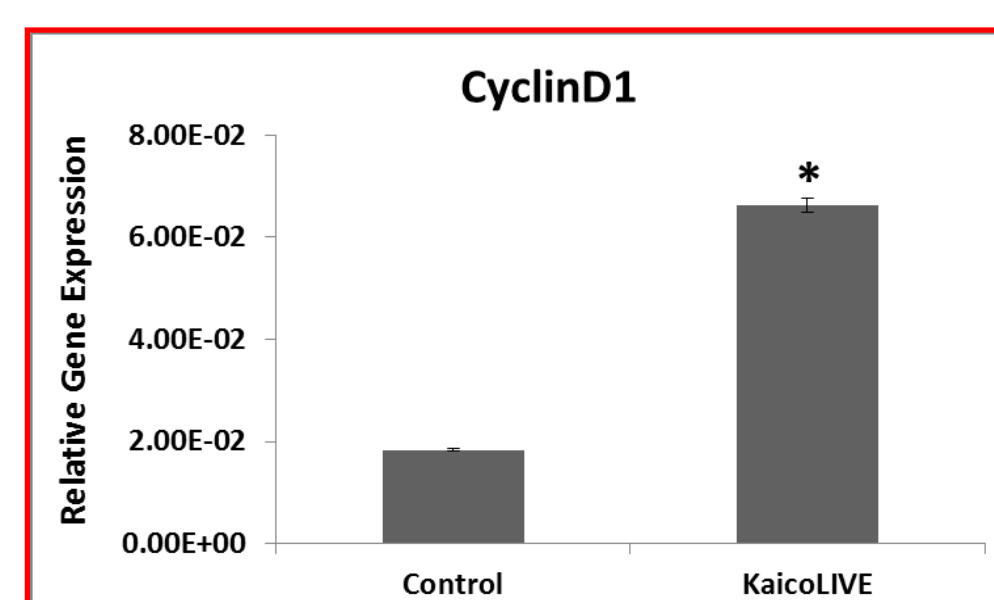


Figure 5. Gene expression analysis of cell cycle-related genes and tumour suppressor-related genes: GADD45 (1.7-fold), Rb (2.50-fold), p53 (2.16-fold) and CyclinD1 (3.6-fold) of non-treated hADSCs (Control) and treated hADSCs (KaicoLIVE[®]) after 72 hours. All genes were up-regulated except for GADD45. The values were expressed as mean ± SEM (n=4). * p < 0.05 relative to control group.

6. DISCUSSION

- ✓ The increase in the anti-aging-related genes expression level of IL6, Collagen 1 and Elastin showed that KaicoLIVE[®] may be able to slow down the progression of aging process.
- ✓ IL6, also an anti-inflammatory marker may be activated by the NFκB and AP-1 pathways [3] when hADSCs were treated with KaicoLIVE[®].
- ✓ All of the cell cycle-related genes and tumour suppressor-related genes used in this study was up-regulated except GADD45 which was down-regulated. GADD45 is a gene that was expressed in response to stress signaling which caused cell cycle arrest [5].
- ✓ While increase in CyclinD1, which play an important role in cell progress, coincides with the increase in Rb as CyclinD1 is known to neutralise the inhibitory action of retinoblastoma protein (Rb). This action allows the transcription of genes required for cell cycle progression [6].
- ✓ Based on the results, KaicoLIVE[®] may be able to promote hADSCs proliferation and protect against inflammatory mechanism during culture, which may potentially slow down the aging process.

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7. CONCLUSION

- This study showed that KaicoLIVE[®] possess the ability to promote cell rejuvenation.
- Based on the data obtained, KaicoLIVE[®] may also be able to promote tissue regeneration but this would require further investigation.

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