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Effect of secretome of adipose stem cell (ASC) in photoaging skin

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Effect of secretome of adipose stem cell (ASC) in photoaging skin

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ABSTRACT

Introduction: UV light is the principal cause of photoaging, a kind of aging. The problem arises because it is less enjoyable. Utilizing the secretome of adipose stem cells (ASC), which is rich in growth factors, could be a novel anti-photoaging therapy. The secretome of ASCs has been extensively examined for their role in wound healing, but their function in photoaging has just lately been investigated. This study looked at changes in epidermal and dermal thickness, expression of MMP-1 and TIMP-1, as well as dermal collagen density to assess the influence of the secretome of ASC in photoaging treatment.
Methods: 28 male Wistar rats, 10-12 weeks old, were randomly assigned to one week of acclimation in each of four groups: UV-irradiated group with topical secretome of ASC (Group P1); UV-irradiated group with topical vehicle only (Group P2); only topical secretome of ASC, non-UV-irradiated (Group P3); and non-UV-irradiated and no topical treatment (Group P4) (Group P0). Prior to treating the rats, the UV exposure was administered for six weeks. Then, for a period of four weeks, the secretome ASC was administered as a treatment.
Results: The epidermal and dermal thickness were significantly improved after four weeks treatment compared to UV irradiated with topical vehicle only group ($p<0.05$). The expression of TIMP-1 and dermal collagen density were significantly increased ($p<0.05$).
Conclusion: Secretome of ASC may be a solution for photoaging skin.

Keywords: Photoaging, secretome, adipose stem cell, epidermal thickness, dermal thickness, MMP-1, TIMP-1, dermal collagen density.
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INTRODUCTION

The largest organ in the human body, the skin, is exposed to a variety of environmental elements, including ultraviolet (UV), smoking, and pollution. Those factors together with intrinsic factor may accelerated skin aging.[1–3] There are two types of skin aging, namely extrinsic aging (photoaging) and intrinsic aging (chronological aging). Chronic UV exposure is major causative factor for photoaging.[4,5] It may cause reduced regenerative capacity of epidermal and dermal cells, varied epidermal thickness, disruption of dermal and epidermal junction and degeneration of collagen and elastin.[5–8] UV causes reactive oxygen species (ROS), which trigger several downstream signal transduction pathways such as mitogen activated proteinkinase (MAPK), nuclear factor-κB (NF-κB) and nuclear-factor-erythroid 2-related factor 2 (Nrf2).[9] Activation of the MAPK pathway causes an increase in activator protein (AP-1) thereby increasing the production of matrix metalloproteinase (MMP) which will reduce collagen and cause extracellular matrix (ECM) damage.[10] Under normal conditions, there is a balance between tissue metalloproteinase inhibitor (TIMP) and MMP. Exposure to UV will produce ROS which can inactivate TIMP and simultaneously MMP synthesis and activation occurs. Numerous studies demonstrate that three forms of MMP, MMP-1, MMP-3, and MMP-9, are increased by UV light on human skin in vivo.[11]

Previously treatments for photoaging are less satisfying so that people find better treatment to be more satisfied.[12] In our study, we used secretome of adipose stem cell (ASC) as the main active ingredient of our treatment for photoaged skin. Adipose stem cell (ASC) is an adult stem cell which is easily to obtain in large quantities and relatively free from ethical issues. Studies had shown secreted factor of ASC called secretome, contains a lot of extracellular proteins, cytokines and growth factor (GF). Previous study had shown that secretome of ASC had capability to reduce collagen degradation in wound healing. We investigated the effect of secretome of ASC on photoaged rats skin. The result of this study was expected to be the basic research of the next study about topical secretome of ASC on improvement of photoaging in human subjects.

MATERIAL AND METHODS

Animals

Male Wistar rats (*Rattus norwegicus*), 10–12 weeks old, weighing an average of 150–200 grams, served as the research subjects for this study, which was conducted in a two-week acclimation period at the Laboratory of the Institute of Tropical Disease at Airlangga University

in Surabaya.

The Faculty of Veterinary at Universitas Airlangga in Surabaya, Indonesia, has an ethics committee that has approved the standard animal care and experimental protocol. Four groups of rats were formed at random, and each was given its own cage. These groups included the UV irradiated group with topical secretome of ASC (Group P1), the UV irradiated group with topical vehicle alone (Group P2), the only topical secretome of ASC, non-UV irradiated (Group P3), and the non-UV irradiated group with no topical therapy (Group P0).

Ultraviolet irradiation procedure

The backs of the rats were roughly 4x4 cm2 in size before UV irradiation. The 14 rats (groups P1 and P2) were photoaged for 6 weeks using UV lamps (Philips TL 20W/01 RS, Ultraviolet B Broadband TL lamps). Emission wavelengths range from 290 to 315 nm. To gauge UV irradiance, a UV meter (UV light meter, UV 340B) was employed. 30 cm separated the lamp from the animal's back. In the first three weeks, radiation treatments were carried out three times per week at progressively higher dosages. The irradiation was only done twice a week for the following three weeks. The total dose of radiation for six weeks was 4,2 J/cm^2.

Preparation and treatment of secretome of ASC

We employed the topical secretome of ASC that was received from the Dr. Soetomo General Hospital in Surabaya's Stem Cell and Tissue Bank Department. In compliance with international norms for tissue donation and human stem cell culture, the donor's adipose tissue was lawfully harvested, and the culture process and secretome separation were also carried out. After six weeks of UV exposure, rats' previously-shaved rear parts received topical ASC secretome twice daily for four weeks.

Histopathology analysis

On the same day, by the same technician, slices of the histopathology examination that were taken from the rat's back skin tissue and fixed in 10 percent buffered formalin for 24 hours were cut into 3-5 mm thick slices, embedded in paraffin, and stained with masson trichome and haematoxylin and eosin (H&E). While dermal collagen density was assessed using Masson Trichrome staining, epidermal and dermal thickness were assessed using haematoxylin and eosin staining.

Immunohistochemistry examination

Skin samples were incubated with primary MMP-1 and TIMP-1 polyclonal antibodies (BIOSS, USA) in antibody dilution buffer and then rinsed with phosphate buffer saline to analyze MMP-1 and TIMP-1 (PBS). The Immuno Reactive Score (IRS) approach was used to assess the semi-quantitative expression of MMP-1 and TIMP-1.

Statistical analysis

Data from the research status were gathered, after which cleaning, editing, and coding were done. The Statistical Package for the Social Sciences (SPSS) data format version 20.0 was used to enter the obtained data (SPSS, Inc., Chicago, Illinois). Given that there were fewer than 30 samples in each group and that the data were normally distributed ($p > 0.05$), the normality test combined with the Shapiro-Wilk test was the statistical test that was employed. Data that were homogeneous and normally distributed were analyzed for comparison using parametric statistical tests and the One Way Anova test.

RESULTS

In this study, male Wistar rats aged 10-12 weeks were irradiated with UVB lamp before treated with topical treatment for six weeks. Subjects were divided into four groups, UV irradiated group with topical secretome of ASC (Group P1); UV irradiated group with topical vehicle alone (Group P2); non-UV irradiated with topical secretome of ASC (Group P3) and non-UV irradiated and no treatment (Group P0). Both topical treatments, secretome of ASC or vehicle alone, were given twice a day for four weeks.

Effect of topical secretome of ASC on epidermal and dermal thickness in UVB irradiated rats

In this study, we measured epidermal and dermal thickness used H&E staining to assess the effect of topical secretome of ASC on responses to UV irradiated rats. The haematoxylin and eosin-stained skin images of each group are shown in Fig. 1. Our result showed that epidermal thickness in UV irradiated group with topical secretome of ASC (Group P1) was significantly thinning than UV irradiated with topical vehicle alone group (Group P2) and non-UV irradiated with topical secretome of ASC group (Group P3) ($p < 0.05$).

The dermal thickness in UV irradiated group with topical secretome of ASC (Group P1) was significantly thicker than UV irradiated group with topical vehicle alone (Group P2) and non-UV irradiated and no treatment group (Group P0) by 18.84% and 23.17%, respectively ($p<0.05$). The dermal thickness in non-UV irradiated with topical secretome of ASC group (Group P3) was thicker than non-UV irradiated and no treatment group (Group P0), but it was not significant ($p>0.05$). These results demonstrate that topical treatment with secretome of ASC, could attenuate the UV-induced dermal thinning and also epidermal thickening in photoaged rats.

Effect of topical secretome of ASC on MMP-1 and TIMP-1 expression in UVB irradiated rats

Data analysis using *one-sample Shapiro wilk test* showed that MMP-1 and TIMP-1 expression data were not normally distributed ($p>0.05$). Our study showed in Fig. 2 that MMP-1 expression in UV irradiated group with topical secretome of ASC (Group P1) was higher than UV irradiated group with topical vehicle alone (Group P2), non-UV irradiated with topical secretome of ASC (Group P3) non-UV irradiated and no treatment (Group P0) but it was not significant difference.

Our study showed in Fig. 3 that TIMP-1 expression UV irradiated group with topical secretome of ASC (Group P1) was significantly higher than UV irradiated group with topical vehicle alone (Group P2), non-UV irradiated with topical secretome of ASC (Group P3) and non-UV irradiated and no treatment (Group P0) ($p<0.05$) .

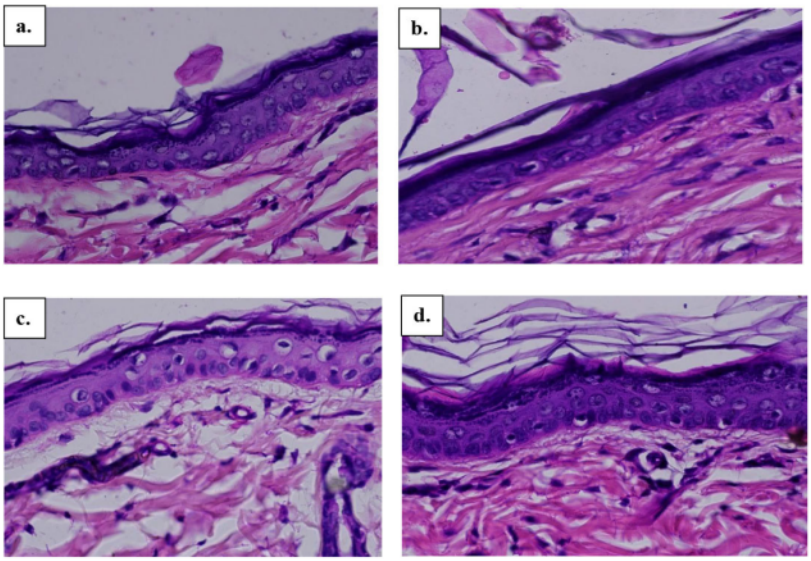

Figure 1. Changes in epidermal and dermal thickness post-experimental (a) Group P1; (b) Group P2, (c) Group P3; (d) Group P0 (p value<0.05).

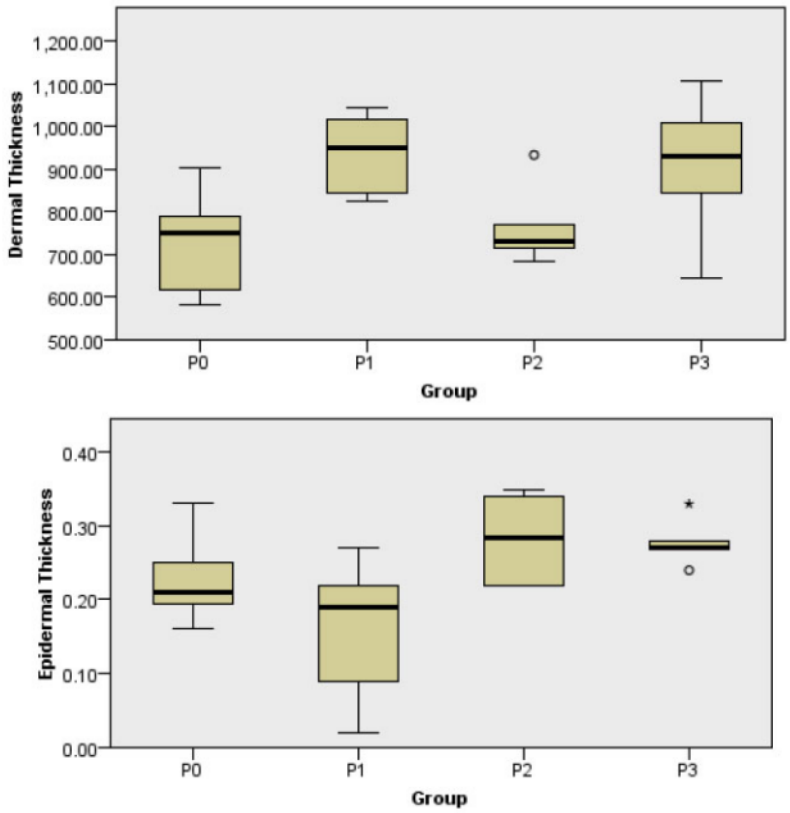

Figure 2. Effect of topical secretome of ASC on epidermal and dermal thickness in UVB irradiated rats.

Effect of topical secretome of ASC on dermal collagen density in UVB irradiated rats

The dermal collagen density measurement, used Masson Trichome staining, was measured after 4 weeks treatment with secretome of ASC. The highest value for dermal collagen density was found in UV irradiated with topical secretome of ASC group (Group P1), while the lowest value was found in non-UV irradiated with topical secretome of ASC (Group P3). Dermal collagen density in UV irradiated group with topical secretome of ASC (Group P1) was significantly higher than UV irradiated group with topical vehicle alone (Group P2) and non-UV irradiated with topical secretome of ASC (Group P3) ($p<0.05$).

DISCUSSION

Intrinsic and extrinsic aging are aging processes which occur simultaneously. Extrinsic aging or photoaging is mediated mainly by UV irradiation and more frequent than skin cancer. It is characterized by fine or deep wrinkles, dryness, roughness, skin atrophy, telangiectasia, and also dyspigmentation. Increased stratum compaction, thickened granular cell layer in the epidermis, and thinning epidermis are the histologic characteristics of photoaged skin, which causes the skin to become dry and rough.[4,13,14] Mechanism of photoaging is activation of cell surface receptors which can activate intracellular signaling and synthesis of transcription factors like activation protein-1 (AP-1). This protein will inhibit collagen synthesis also increase collagen degradation.[13,14] Comparing photoaged skin to skin that has been protected from the sun, there is a statistically significant drop in total collagen of 20%.[15] Study of Amirthalingam et al reported that animals in UVB-irradiated group showed an increase in epidermal and dermal thickness by 14.25±1.19 pixels, 99.21±8.73 pixels, respectively.[16]

Secretome of ASC can be used to repair damaged skin such as reduce wrinkle, improve skin barrier, and augment skin thickness.[16] In vitro study by Moon and colleagues (2012) and also Kim and colleagues (2020) reported an increase of production of extracellular matrix

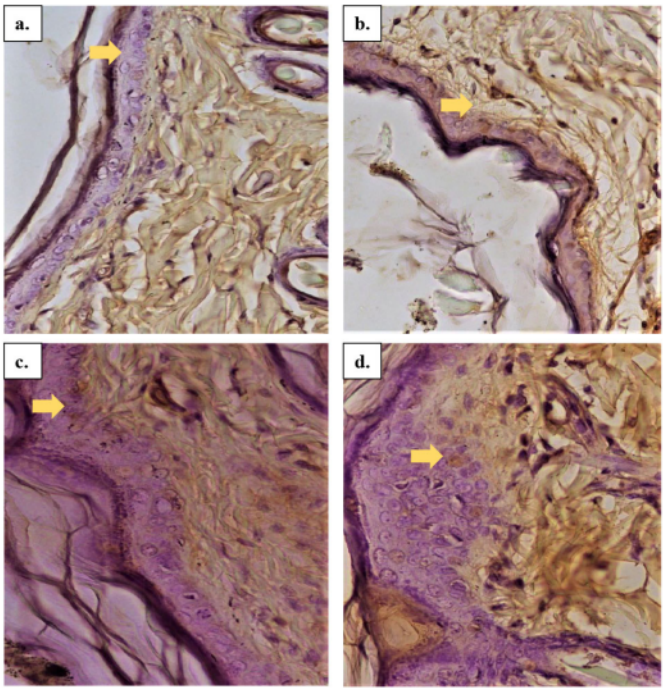

Figure 3. Immunohistochemistry result of MMP-1 expression (a) Group P1; (b) Group P2, (c) Group P3; (d) Group P0. (400x magnification).

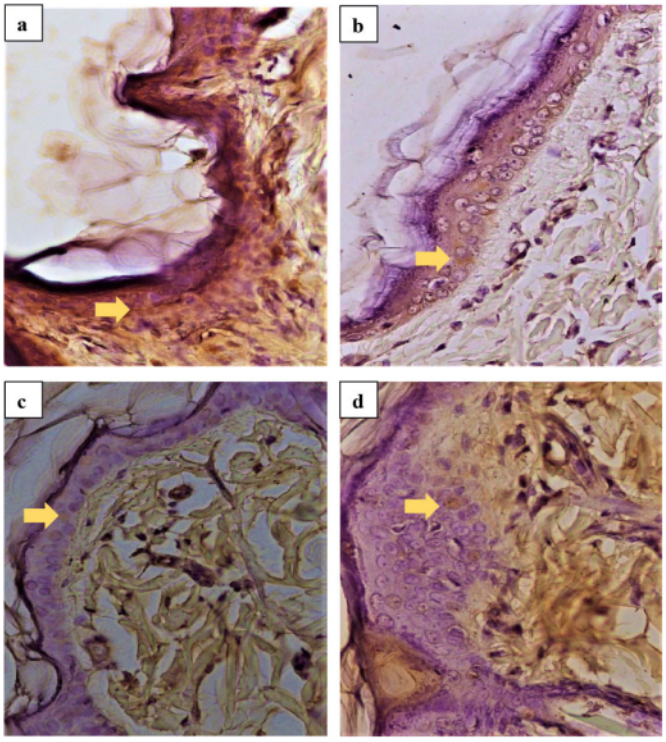

Figure 4. Immunohistochemistry result of TIMP-1 expression in (a) Group P1; (b) Group P2, (c) Group P3; (d) Group P0. (400x magnification). *p<0.05.

(ECM) including collagen. Secretome of ASC produced in a hypoxia condition had great benefit for skin especially in dermal regeneration.[17–19] This study also found that secretome could increase human keratinocyte proliferation and also affect expression of 290 regulated genes which contributed to cell proliferation and cell cycle. Our study showed that secretome of ASC could significantly improve epidermal and dermal thickness in UV irradiated group ($p<0.05$). Similar with our result, Amirthalingam and colleagues (2019) reported reduced value of epidermal thickness after using secretome of bone marrow stem cell. Secretome of ASC increases regenerative capacity of epidermal and dermal cells by increasing keratinocytes and fibroblasts proliferation.[16]

The UV signal acts directly on the fibroblasts and signals from the damaged keratinocytes, causing the release of MMP-1, which degrades collagen.[20] Other MMPs that are induced and activated rapidly by UV radiation, namely MMP-3, and MMP-9, are strongly regulated by the AP-1 transcription factor. The combined activation of these three MMPs can degrade most of the dermal ECM proteins.[3,8,21] An increase in MMP levels is not accompanied by an increase in TIMP. This imbalance causes progressive collagen fragmentation in the dermis, thereby accelerating skin aging.[11,22] An increase in expression of TIMP-1 will prevent collagen and ECM degradation by suppress the production of tumour necrosis factor (TNF)-α. Yokose and colleagues found that TIMP-1 expression decreased in both intrinsic and extrinsic aging.[23] Our study showed that topical secretome of ASC increased MMP-1 expression, but it was not significant difference ($p>0.05$) and increased TIMP-1 expression ($p<0.05$) in UV irradiated group with topical secretome of ASC (Group P1). Ultraviolet irradiation will induce ROS synthesis which will activate MMP transcription and synthesis. From those events, it will increase the collagen degradation. Secretome of ASC will balance this condition with increasing of TIMP-1 which also can reduce the collagen degradation.

The balance of MMP-1 and TIMP-1 will reduce degradation of ECM include

collagen so that the dermal collagen density will increase. Our study showed that dermal collagen density in UV irradiated with topical secretome of ASC group (Group P1) was significantly higher than other groups ($p<0.05$). Dermal collagen density of UV irradiated group with topical secretome of ASC group (Group P1) was significantly higher than topical vehicle alone (Group P2) and non-UV irradiated with topical secretome of ASC (Group P3) ($p<0.05$). Similar with our study, Pricillia and colleagues reported that collagen density in secretome of ASC group was significantly increased and denser than control group after injection of secretome of ASC ($p<0.05$) in aging skin. Similar with ours, their secretome was made in hypoxia condition.[24]

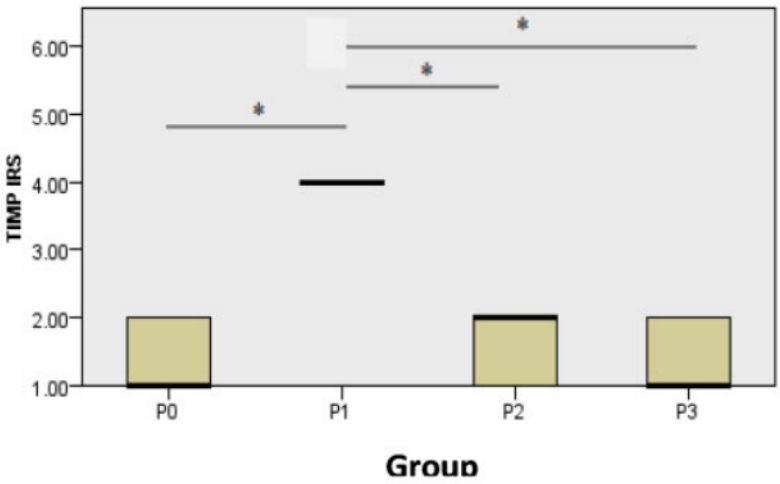

Figure 5. Effect of topical secretome of ASC on MMP-1 and TIMP-1 expression in UVB irradiated rats.

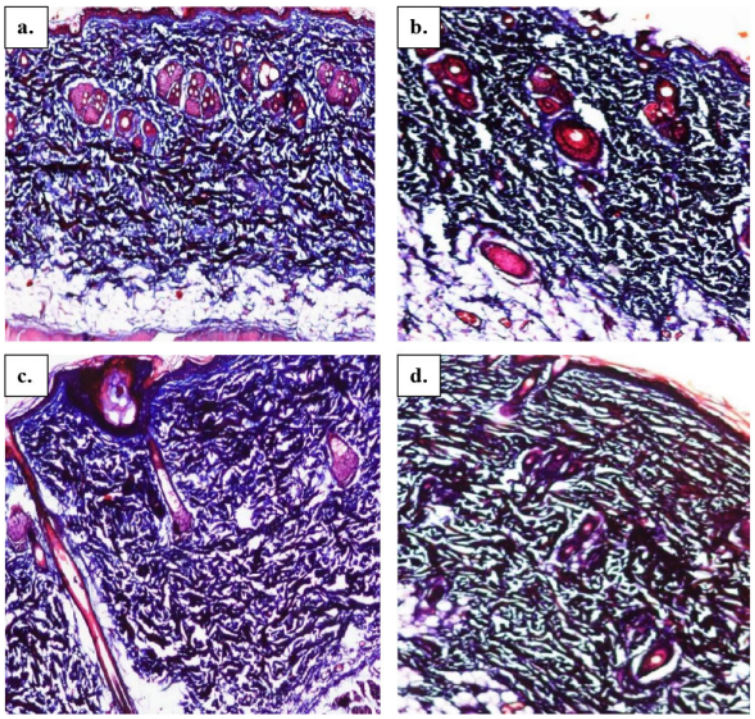

Figure 6. Dermal collagen density in (a) Group P1; (b) Group P2, (c) Group P3; (d) Group P0. (400x magnification).

CONCLUSION

We showed that four weeks of treatment of the secretome of ASC improved photoaged skin via a paracrine mechanism by increasing epidermal and dermal layer thickness, MMP-1 and TIMP-1 expression, as well as dermal collagen density. This secretome has potential effect to improve photoaged skin. Therefore, further research is needed with different study designs and larger samples to determine other factors that influence the effect of secretome of adipose stem cells (ASC) in photoaging skin.

AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

FUNDING

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CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

ETHICAL CONSIDERATION

This study has been declared ethical by the Ethical Commission for Health Research of Universitas Airlangga.

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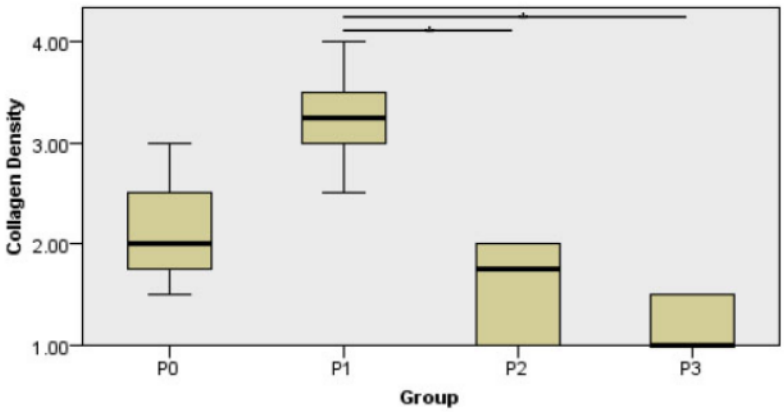

Figure 7. Effect of topical secretome of ASC on dermal collagen density in UVB irradiated rats.

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