International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Administration of *Myrmecodia pendans* Ethanol Extract Cream Prevented Elevation of Keratinocyte Apoptosis and Number of Macrophages in Male Wistar Rats (*Rattus norvegicus*) Exposed to Ultraviolet B (UVB)

Diana Utami[1], Wimpie Pangkahila[2], I Gusti Made Aman[3]

[1, 2, 3]Master Program in Biomedical Science, Concentration in Anti - Aging Medicine, Medical Faculty Udayana University, Bali, Indonesia
Email: *dianautami.dr[at]gmail.com*

Abstract: ***Background: Aging is a natural process that is influenced by several factors, one of which is sunlight/ultraviolet (UV). Continuous exposure to UV radiation causes changes in the structure and function of the skin, ranging from sunburn, which is the apoptosis of keratinocyte cells; to increasing the formation of acute inflammatory cells such as macrophages. This study aimed to prove that administration of M. pendans 40% ethanol extract cream prevented elevation of keratinocyte apoptosis and number of macrophages in male Wistar rats (Rattus norvegicus) exposed to Ultraviolet B (UVB). Methods: This study was an animal experimental study using a randomized post - test only control group design. The subjects were rats (Rattus norvegicus), Wistar strain, male, 2 - 3 months old, 180 - 200 grams body weight, and healthy. The number of samples used was 36, divided into 2 groups (n = 18). The control group was exposed to UVB and treated with placebo cream, the treatment group was exposed to UVB and treated with M. pendans 40% ethanol extract cream. After 24 hours of exposure, the rats were anesthetized and a biopsy of the back skin tissue was performed to examine the apoptotic number of keratinocyte cells and the number of macrophage cells using histological methods. Results: The mean apoptotic number of keratinocyte cells in the control group was 25.1 ± 3.81 cells/field of view, while in the treatment group was 15.0 ± 3.10 cells/field of view (p <0.001). The mean number of macrophage cells in the control group was 3.73 ± 1.14 cells/field of view, while in the treatment group was 2.26 ± 1.09 cells/field of view (p <0.001). Conclusion: The study presented herein proved that administration of M. pendans 40% ethanol extract cream prevented elevation of keratinocyte apoptosis and number of macrophages in male Wistar rats (Rattus norvegicus) exposed to Ultraviolet B (UVB).***

Keywords: *M. pendans* 40% ethanol extract cream, apoptosis of keratinocyte, macrophages, Ultraviolet B (UVB)

1. Introduction

Aging is a natural process that occurs in all living things. Pangkahila (2017) summarizes the cause of the aging process and categorized it into external factors and internal factors. By manipulating these factors, it is believed that the aging phenotype will also be prevented.1 Based on the results of many laboratory and epidemiological studies, it is known that one of the causes of aging is free radicals. Free radicals are produced physiologically in the body as a result of the inflammatory process as well as the respiratory process in the mitochondria, and also arise from the environment such as due to ultraviolet light.2

The effects of skin aging caused by ultraviolet light include sunburn (skin inflammation), hyper pigmentation, and skin cancer.3, 4 Sunburn itself is the apoptosis of keratinocyte cells that occurs due to ultraviolet light, especially UVB.5 There has been a lot of research evidence on the role of UVB light exposure in causing apoptosis of keratinocyte cells.6 - 8 In addition to causing keratinocyte apoptosis, acute exposure to UVB radiation can also increase the homing of acute inflammatory cells such as macrophages and neutrophils, especially macrophage cells. Research has shown that in response to acute UVB radiation exposure, keratinocyte cells secrete various cytokines and chemokines that stimulate inflammatory cell infiltration, especially macrophages.9 - 12

Because chronic exposure to UVB light that causes apoptosis of keratinocytes and inflammation (increased number of macrophages) can cause photoaging skin, efforts to prevent keratinocyte apoptosis and an increase in the number of macrophages are included as steps in Anti - Aging Medicine (AAM), especially the skin aging.

Approaches that can be taken to prevent sunburn and increase the number of macrophages include protecting oneself from sun exposure and utilization of sunscreen. An ideal sunscreen should be safe, but some of the chemicals contained in sunscreens often cause side effects such as allergic reactions, irritation, and endocrine disorders.13 Currently, many photoprotective materials have been developed with formulations derived from natural ingredients. This is because natural compounds have relatively low toxicity with the same effectiveness as synthetic compounds.14

The *Myrmecodia pendens* plant or often referred to as a "tanaman sarang semut" by local Indonesians, is a plant that has been used traditionally in Indonesia, especially in Papua as a potential medicine and is considered relatively safe.15 The effect of this *M. pendens* plant for health has been scientifically proven through research results, namely to prevent inflammation - related diseases.16

The results showed that the *M. pendens* plant contains many bioactive compounds, such as total phenol of 658.08 mg/100g, flavonoids of 4718.05 mg/100g, and tannin of 43642.38 mg/100g. The total antioxidant capacity of the ethanol extract of the *M. pendens* plant is 2141.62 mg/L with an inhibitory concentration 50 (IC50) of 2008, 0139 ppm. With the content of various active compounds in the *M. pendens* plant to support its antioxidant content, it can be used as an antioxidant to overcome pathologies caused by UVB rays.1[7]

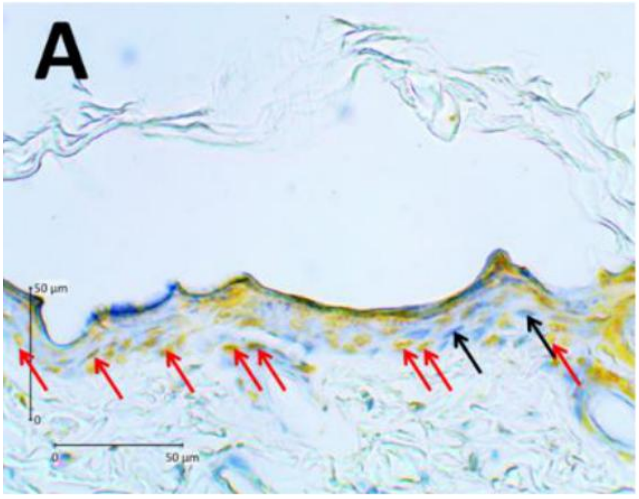
The aim of this study was to prove that administration of *M. pendans* 40% ethanol extract cream prevented elevation of keratinocyte apoptosis and number of macrophages in male Wistar rats (*Rattus norvegicus*) exposed to Ultraviolet B *(*UVB).

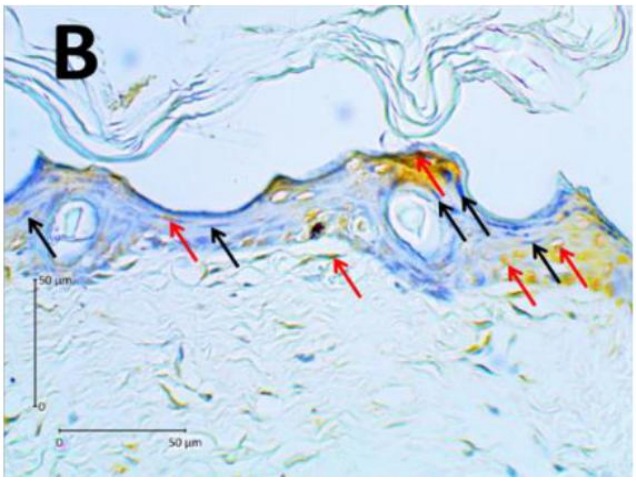
2. Methods

This study was an animal experimental study using a randomized post - test only control group design. The subjects were rats (*Rattus norvegicus*), Wistar strain, male, 2 - 3 months old, 180 - 200 grams body weight, and healthy. The number of samples used was 36, divided into 2 groups (n = 18). The control group was exposed to UVB and treated with placebo cream, the treatment group was exposed to UVB and treated with *M. pendans* 40% ethanol extract cream. After 24 hours of exposure, the rats were anesthetized and a biopsy of the back skin tissue was performed to examine the apoptotic number of keratinocyte cells and the number of macrophage cells using histological methods.

3. Results

The mean apoptotic number of keratinocyte cells in the control group was 25.1 ± 3.81 cells/field of view, while in the treatment group was 15.0 ± 3.10 cells/field of view (p <0.001). The mean number of macrophage cells in the control group was 3.73 ± 1.14 cells/field of view, while in the treatment group was 2.26 ± 1.09 cells/field of view (p <0.001).





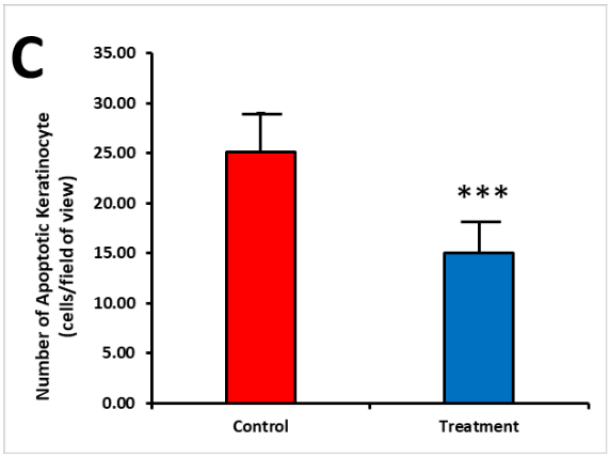
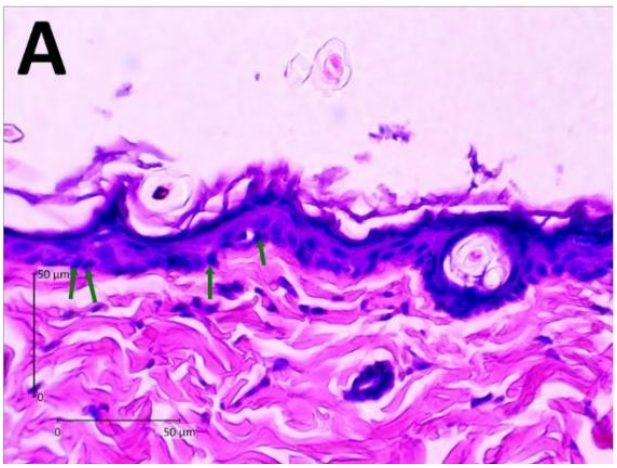


Figure 1: Histopathology and Comparison of Keratinocyte Apoptosis. (A) Control group, visible keratinocyte cells with flat - shaped nuclei with a yellow - brown color (red arrow) indicating apoptosis. (B) Treatment group, fewer apoptotic keratinocytes (red arrow) than the control group were seen. (C) Quantitative comparison of the number of apoptotic keratinocytes, ***p<0.001



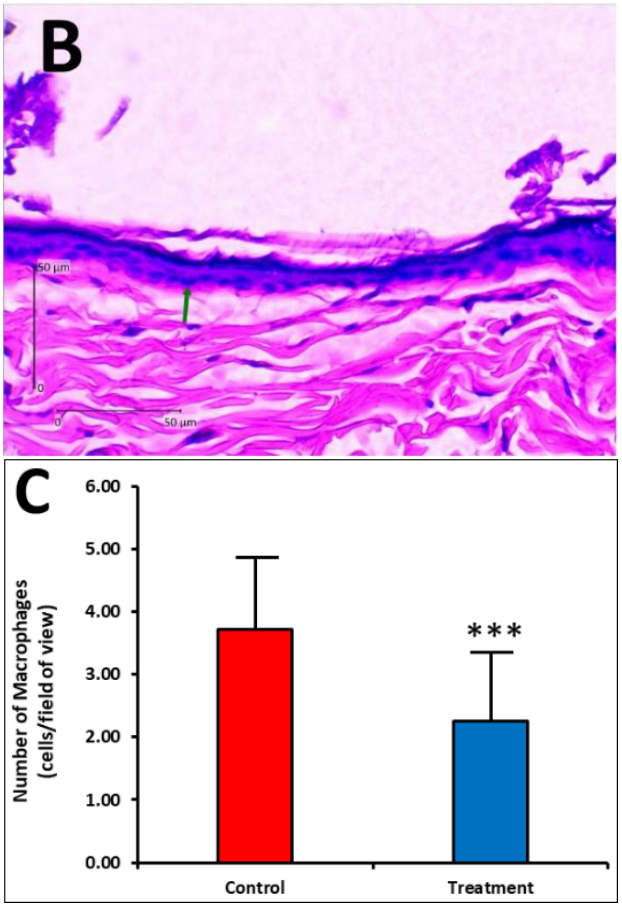


Figure 2: Histopathology and Comparison of the Macrophages. (A) Control group, macrophage cells with agranular cell cytoplasm (green arrows) indicate inflammation. (B) In the treatment group, fewer macrophages (green arrows) were seen than in the control group. (C) Quantitative comparison of the number of macrophages, ***p<0.001

4. Discussion

The results of this study showed that the average number of keratinocyte cell apoptosis in the control group was higher than in the treatment group. UVB - induced keratinocyte apoptosis involved the intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways.6 DNA is the most important intracellular chromophore for UVB absorption that can cause DNA damage (pyrimidine dimers). If UVB - induced DNA damage occurs in keratinocytes continuously without proper repair, it can lead to mutations. And mutations in this DNA will then cause cell death or apoptosis.1[8]

UVB light with a wavelength of 290 - 320 nm at a dose of 200 - 700 J/nm2 is capable of causing apoptosis within 24 - 48 hours, through intrinsic apoptotic pathways including mitochondrial leakage, the release of cytochrome C and activation of various caspases (capcase - 3, - 8, - 9). After acute UVB exposure, keratinocyte cells undergo morphological changes known as sunburn cells, including chromatin condensation and eosinophilic cytoplasm, which can be seen by staining with hematoxylin - eosin.1[9]

Research showed that the formation of ROS that occurs within 24 - 48 hours after UV exposure is the main mechanism that causes apoptosis of keratinocytes (sunburn cells).2[0] So, in this study, the administration of 40% ethanol extract cream of the *M. pendens* plant containing flavonoids and antioxidants could prevent the apoptosis of the keratinocytes. These results are supported by the previous finding, in which oral administration of *Polypodium leucotomos* which contains high phenolic compounds was found to be photoprotective and inhibited the formation of sunburn cells in male Wistar rats exposed to UVB rays.2[1] However, the above study only examined the content of Caffeic acid, ferulic acid, vanillic acid and p - coumaric, and also utilize oral treatment rather than topical so that it cannot be compared to this study which emphasizes the content of flavonoids, phenols and antioxidants and the treatment was carried out topically. In this study, the ethanol extract cream of the *M. pendens* plant acts as a sunscreen to protect the skin from the adverse effects of UV rays by acting as a chemical or physical barrier that absorbs or reflects UV rays and reduces the amount of UV reaching the skin so that it is designated as an anti - aging agent. Several studies on antioxidants showed a protective effect on the skin. Hence, it supports the results of this study that antioxidants derived from the ethanol extract of the *M. pendens* plant are protective against skin aging due to exposure to UVB rays.2[2]

The antioxidant content of the *M. pendens* plant can prevent damage to the skin caused by UVB by inhibiting the lipid peroxidation chain reaction. The content of flavonoids, phenols, tannins, and saponins can neutralize ROS including superoxide anions, hydroxyl radicals, singlet oxygen and H2O2 and also prevent NO synthesis by inhibiting iNOS expression.2[3] In addition, it was found that administration of antioxidants to UVB - exposed human skin fibroblasts resulted in faster DNA repair due to an increase in XPA and GADD45α transcripts in a p53 - dependent manner.2[4] Polyphenol antioxidants provide protection against cell death induced by UVB and ROS by modulating p53 and c - Jun N - terminal kinase (JNK).2[5] Polyphenols were found to inhibit tumor necrosis factor (TNF) - alpha - induced proliferation of matrix metalloproteinase (MMP) acquired via inhibition of nuclear factor - kappa B (NFkB) and activator protein - 1 (AP - 1) in smooth muscle cells.2[6]

In addition to preventing the formation of sunburn, the results of this study also clearly showed preventive effects of *M. pendens* on the acute inflammation caused by UVB which was characterized by a decrease in the number of macrophages in the treatment group compared to the control group. Within two hours after UVB exposure, skin cell damage can be seen, mast cells will release mediators such as histamine, serotonin and tumor necrosis factor (TNF) that cause the synthesis of prostaglandins and leukotrienes. The release of cytokines plays a role in the inflammatory reaction resulting in the infiltration of pro - inflammatory cells such as macrophages. Antioxidants contained in the ethanol extract cream of the *M. pendens* plant can modulate these cytokines, acting as an inhibitor of UV - induced oxidative damage depending on the regulation of JNK and p38 MAPK activities. Inhibition of JNK and p38 activity inhibits the secretion of IL - 6, TNF - a and COX - 2 cytokines so that the infiltration of inflammatory cells in skin exposed to UVB radiation can be reduced.2[7]

5. Conclusion

The study presented herein proved that administration of *M. pendans* 40% ethanol extract cream prevented elevation of keratinocyte apoptosis and number of macrophages in male Wistar rats (*Rattus norvegicus*) exposed to Ultraviolet B *(*UVB). Further research to examine the beneficial effects of the *M. pendans* 40% ethanol extract cream in addition to preventing the acute effects of UVB rays, such as chronic effects including melasma, decreased amount of collagen is necessary. Additionally, clinical trials on humans to determine the benefits and potentially toxic effects of *M. pendans* 40% ethanol extract cream after long - time exposure are warranted.

References

[1] Pangkahila, W.2017. Tetap Muda, Sehat dan Berkualitas: Konsep Anti - Aging Medicine.1st edition. Jakarta: Kompas.
[2] Kim, G. H., Kim, J. E., Rhie, S. J., and Yoon, S.2015. The Role of Oxidative Stress in Neurodegenerative Diseases. *Exp Neurobiol*.24 (4): 325 - 340.
[3] Bernerd, F., Marionnet, C., and Duval, C.2012. Solar Ultraviolet Radiation Induces Biological Alterations In Human Skin In Vitro: Relevance of A Well - Balanced UVA/UVB Protection. *Indian J Dermatol Venereol Leprol.7*8: 15 - 23.
[4] Pandel, R., Polsajak, B., Godic, A., and Dahmane, R.2013. Skin Photoaging and The Role of Antioxidants in Its Prevention. *International Scholarly Research Notices.* ISRN *Dermatology* Vol.2013 (2013), Article ID 930164.
[5] Thompson, A. E.2015. Suntan and Sunburn. *JAMA.*2015; 314 (6): 638.
[6] Lee, C. H., Wu, S. B., Hong, C. H., Yu, H. S. And Wei, Y. H.2013. Molecular Mechanism of UV - induced Apoptosis and Its Effects on Skin Residential cells: The Implication in UV - Based Phototherapy. *J Mol Sci*.2013: 14 (3): 6414 - 6435
[7] Park, Y., and Jang, B.2014. UVB - induced anti - survival and pro - apoptotic effects on HaCaT human keratinocytes via caspase - and PKC - dependent downregulation of PKB, HIAP - 1, Mcl - 1, XIAP and ER stress. *Int J Mol Med.*33 (3): 695 - 702.
[8] Siegenthaler, B., Defila, C., and Muzumdar, S.2018. Nrf3 promotes UV - induced keratinocyte apoptosis through suppression of cell adhesion. *Cell Death Differ* 25, 1749–1765.
[9] Moore, C., Cevikbas, F., Pasolli, H. A., Chen, Y., Kong, W., Kempkes, C., Parekh, P., Lee, S. H., Kontchou, N. A., Yeh, I., Jokerst, N. M., Fuchs, E., Steinhoff, M., dan Liedtke, W. B.2013. UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin - 1 signaling. *Proc Natl Acad Sci U S A*.110 (34): E3225 - 34.
[10] Banerjee, S., dan Leptin, M.2014. Systemic response to ultraviolet radiation involves induction of leukocytic IL - 1β and inflammation in zebrafish. *J Immunol*.193 (3): 1408 - 1415.
[11] Yoshihisa, Y., Norisugi, O., Matsunaga, K., Nishihira, J., dan Shimizu, T.2014. Involvement of MIF in basement membrane damage in chronically UVB - exposed skin in mice. *PLoS One*.2014 Feb 21; 9 (2): e89569.
[12] Pastore, S., Mascia, F., dan Girolomoni, G.2016. The contribution of keratinocytes to the pathogenesis of atopic dermatitis. *Eur. Dermatol*.16: 125–131.
[13] Lim, H., Arellano - Mendroza, M. I., and Stengel, F.2017. Current Challenges in Photoprotection. *J Am Acad Dermatol*.76 (3S1): S91 - S99.
[14] Valle, A. L. Current methodologies in assessing the toxicity of natural products. *International Journal of Phytocosmeticsand Natural Ingredients*.5 (3): 1 - 6.
[15] Dirgantara, S., Dewi, K., Raya, J. N., dan Simanjutak, T. L.2015. Studi Botani dan Fitokimia Tiga Spesies Tanaman Sarang Semut) asal Kabupaten Merauke, Papua. *Jurnal Farmasi Sains dan terapan.2* (2): 20 - 22.
[16] Dirgantara, S., Nawawi, A., dan Insanu, 2013. Uji Aktivitas Antioksidan Tiga Spesies Tanaman Sarang Semut (Famili: Ruiaceae) asal Kabupaten Merauke, Papua. *Jurnal Biologi Papua.5* (1): 10 - 14.
[17] Epsilawati, L., Satari, M., and Azhari, A.2019. Analysis of Myrmecodia Pendens in Bone Healing Process to Improve the Quality of Life: Literature Review. *IOP Conf. Series: Earth and Environmental Science*.248: 012052a.
[18] Bulat, V., Situm, M., Dediol, I., Ljubicić, I., and Bradić, L.2011. The mechanisms of action of phototherapy in the treatment of the most common dermatoses. *Coll Antropol*.35 Suppl 2: 147 - 51.
[19] Dixon, K. M., Tongkao - On, W., Sequeira, V. B., Carter, S. E., Song, E. J., Rybchyn, M. S., Gordon - Thomson, C., dan Mason, R. S.2013. Vitamin D and death by sunshine. *Int J Mol Sci*.2013; 14 (1): 1964 - 77.
[20] D'Orazio, J., Jarrett, S., Amaro - Ortiz, A., dan Scott, T.2013. UV radiation and the skin. *Int J Mol Sci*.14 (6): 12222 - 48.
[21] Ariana, P.2018. Pemberian Oral Bubuk *Polypodium leucotomos* Menghambat Pembentukkan Sel *Sunburn* pada Tikus Wistar Jantan (*Rattus norvegicus*) yang Dipapar UVB. *Tesis.* Program Magister Biomedik Universitas Udayana.
[22] Saric, S., dan Sivamani, R. K.2016. Polyphenols and Sunburn. *Int J Mol Sci*.2016; 17 (9): 1521.
[23] Gonzales, S., Yolanda, G. dan Philips, N.2010. Mechanistic Insight in the use of a Polypodium leucotomos Extract as an Oral and Topical Photoprotective Agent. *Photochem Photobiol Sci*.9 (4): 559 - 63.
[24] Guillermo - Lagae R., Deep G., Ting H., Agarwal C., and Agarwal R.2015. Silibinin enhances the repair of ultraviolet B - induced DNA damage by activating p53 - dependent nucleotide excision repair mechanism in human dermal fibroblasts. *Oncotarget* 6, 39594–39606.
[25] Sharma, P., Montes de Oca, M. K., Alkeswani, A. R.2018. Tea polyphenols for the prevention of UVB - induced skin cancer. *Photodermatol Photoimmunol Photomed*.34 (1): 50 - 59.
[26] Dunaway, S., Odin, R., Zhou, L., Ji, L., Zhang, Y., dan Kadekaro, A. L.2018. Natural Antioxidants: Multiple

Mechanisms to Protect Skin from Solar Radiation. *Front Pharmacol*.9: 392.

[27] Shin, S. W., Jung, E., Kim, S., Kim, J. H., Kim, E. G., Lee, J., and Park, D. 2013. Antagonizing effects and mechanisms of afzelin against UVB - induced cell damage. *PLoS One.8* (4): e61971.