ANTI-PIGMENTATION EFFECTS OF SALACCA ZALACCA'S SKIN ON WISTAR RATS UNDER UV-LIGHT EXPOSURE

Ulfah Octavia Saragih[1], Novita Yuliana[1], Ermi Girsang[1], Maya Sari Mutia[1]

Article History: **Received:** 12.04.2023 **Revised:** 23.05.2023 **Accepted:** 17.06.2023

Abstract

This research aims to determine the effect of salak skin against UV exposure due to oxidative stress and hyperpigmentation. Salak skin contains antioxidants that can neutralize free radicals, prevent inflammation, and have anti-tyrosinase effects so that it can brighten the skin. This study used a post-test-only control group design using 24 white rats divided into four groups of treatments. There was positive control, and others were treated by salak skin extract in various concentration as 210 mg/kg BW, 420 mg/kg BW, and 840 mg/kg BW respectively. Histopathological staining was using Masson-Fontana. Melanin was added based on five fields of view per-sample. The results of the study were discussed altogether with descriptive analytics, normality, homogeneity, and ANOVA tests. We obtained that the data were normally distributed. The average amount of melanin was highest in K1 and the lowest was in K3 ($p < 0.05$). This research suggested that salak skin extract is effective in reducing pigmentation in animals model.

Keywords*: Salak skin, Antioxidants, UV exposure, Melanin, Hyperpigmentation*

[1]Department of Biomedical Sciences, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Corresponding Email: ermigirsang@unprimdn.ac.id

DOI:10.48047/ecb/2023.12.9.254

1. Introduction

Excessive exposure to daily sunlight might harm the health of human skin. Sunlight contains ultraviolet; UV-A, UV-B, and UV-C. The impact of exposure such as DNA damage, and redness of the skin, even in the long term results in excessive free radicals and increased activity of tyrosinase enzymes that play a role in melanogenesis, the premature aging process, and skin cancer (Brenner & Hearing, 2008).

Salacca zalacca (namely as salak) skin is empirically used by the community as a traditional treatment, namely for beauty, antimicrobial, and antidiabetic for centuries (Kanon et al., 2012). Salak skin contains antioxidants that can be used as lightening and anti-aging agents. Salak peel contains flavonoids, saponins, tannins, and alkaloids (Adjeng et al., 2019). Antioxidant levels in salak skin are active (Fitrianingsih et al., 2014). Flavonoids have an effect as antioxidants to fight free radicals that cause inflammation and hyperpigmentation by donating 1 hydrogen electron to eliminate Radical Oxygen Species (ROS) such as O2-, H2O2, HO, and ONOO-. Because of Salacca zalacca skin has rich of the bioactive compounds, it is important to explore its potential effect on protecting skin under UV-irradiation regarding the hyperpigmentation process (Hernández et al., 2006).

This research was conducted by making ethanol extract from salak skin. Experimental animals were divided into four groups, each consisting of six white adults rats. Experimental animals were exposed to UV light and then the treatment group was smeared with salak skin extract, this procedure was carried out for 7 days. After the experimental animals were terminated, a histopathological preparation of skin tissue with Masson-Fontana staining was made. The results of histopathology readings were described and analyzed using the ANOVA statistical test.

The tools used are rat cages and equipment, oral sondes, analytical scales, extract-making equipment, masks (Onemed), handscoen (Onemed), glass objects, and electron microscopes (Olympus). The ingredients used are white rat wistar strains, standard rat feed, water, salak skin extract, and UV light. Salak skin extract was obtained by maceration method with alcohol. This study used a post-test-only control group design using 24 adult white rats (Rattus novergicus) Wistar strains. The white rats were divided into four groups, consisting of 6 experimental animals. Group 1 (K1) was a UV-irradiated control and fed only ad libitum. Group 2 (K2), group 3 (K3), and Group 4 (K4) were treatment groups that were exposed to UV light and then smeared with salak skin extract at levels of 210 mg/kg BW, 420 mg/kg BW, and 840 mg/kg BW respectively. After 7 days the rats were terminated. Histopathological examination using Masson-Fontana staining. Calculate the amount of melanin in each sample by calculating melanin in five fields of view. The data obtained were tested for normality and homogeneity, then tested for differences with ANOVA.

2. Methods

The tools used are rat cages and equipment, oral sondes, analytical scales, extract-making equipment, masks (Onemed), handscoen (Onemed), glass objects, and electron microscopes (Olympus). The ingredients used are white rat wistar strains, standard rat feed, water, salak skin extract, and UV light. Salak skin extract was obtained by maceration method with alcohol. This study used a post-test-only control group design using 24 adult white rats (Rattus novergicus) Wistar strains. The white rats were divided into four groups, consisting of 6 experimental animals. Group 1 (K1) was a UV-irradiated control and fed only ad libitum. Group 2 (K2), group 3 (K3), and Group 4 (K4) were treatment groups that were exposed to UV light and then smeared with salak skin extract at levels of 210 mg/kg BW, 420 mg/kg BW, and 840 mg/kg BW respectively. After 7 days the rats were terminated. Histopathological examination using Masson-Fontana staining. Calculate the amount of melanin in each sample by calculating melanin in five fields of view. The data obtained were tested for normality and homogeneity, then tested for differences with ANOVA.

3. Results and Discussion

Melanin Contents

Exposure to UV light and administration of salak skin extract has affected the amount of melanin in experimental animals. The highest amount of melanin was found in K1 while the lowest was in K3. as shown in Figure 1:

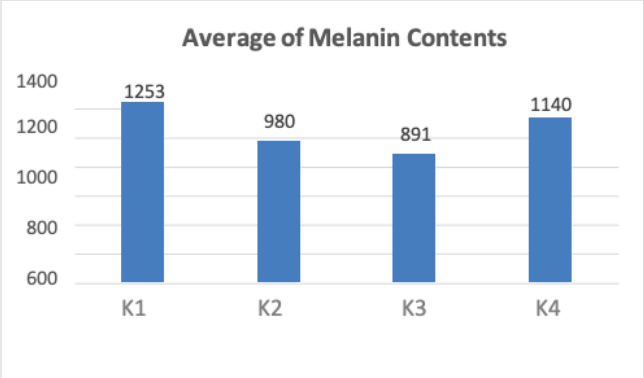

Figure 1. Melanin counted in the five fields of view.

These results showed a decreased amount of melanin between the control group and the treatment group K2 and K3, but a slight decreased was found in group K4 compared to control. In average, the Mean ± SD of K1, K2, K3 and K4 were 210.5±15.2, 163.3±12.4, 147.1±15.1, and 191.6±15.3, respectively. The data obtained in the normality and homogeneity test and obtained the results of normally distributed data (p >0.05). Then tested the average difference in melanin between groups with ANOVA and obtained p <0.05. This showed that salak skin extract significantly decreased pigmentation in experimental animals.

Table 1. Shapiro-Wilk, Levene Test, and ANOVA

Group	Shapiro-Wilk	Levene test	ANOVA
K1	0.631	0,398	< 0.001
K2	0.994		
K3	0.517		
K4	0.432		

The ANOVA test then continued with a posthoc test to see the differences between groups. Based on the table above, it showed that all doses significantly reduced the amount of melanin in experimental animals exposed to UV light (p <0.05). However, the most effective dose of salak peel extract is the medium dose (K3) 420 mg/kg BW because it can reduce melanin.

Table 2. Post-hoc test between groups

Group	K1	K2	K3	K4
K1				
K2	<0.001			
K3	<0.001	0.070		
K4	0.037	0.003	<0.001	

The ANOVA test was followed by a post hoc test to examine the differences between groups. Based on the table above, it appears that all doses significantly reduced the amount of melanin in experimental animals exposed to UV light (p <0.05). However, the most effective dose of salak peel extract is the medium dose in K3, which is 420 mg/kg BW because with that dose it can reduce more melanin.

In this study, the highest average amount of melanin was obtained in the UV light control group. The highest amount of melanin in this group is associated with UV exposure which triggers melanocytes to form more melanin to

reduce the effect of free radicals on tissues. UV exposure causes oxidative stress with an increase in free radicals that disrupt the physiological and biochemical processes of the body. Oxidative stress also triggers inflammation and causes damage to skin tissue (Nobile et al., 2021).

Elevated inflammatory mediators such as nitric oxide, prostaglandins, leukotrienes, and cytokines trigger melanin synthesis because keratinocytes and melanocytes have receptors to respond to those mediators. Inflammatory mediators such as IL-18, IL-33 increase tyrosinase activity and trigger TYrP-1 and TYrP-2 expression (Zhou et al., 2013). The inflammatory mediator GM-cSF triggers melanocyte proliferation and melanin synthesis. Keratinocytes respond to PGe2 and PGF2α by stimulating the formation of dendrite melanocytes via a caMP-dependent pathway. So, melanin production increases as an inflammatory response (Videira et al., 2013). Inflammatory processes indicate an imbalance of antioxidants in the body, such as when sunburn, atopic dermatitis, as well as in scarring processes, where increased oxidative stress can inhibit skin repair, especially in times of acute inflammation (Addor, 2017).

The treatment groups of K2, K3, and K4 that were exposed to UV light and smeared with salak peel extract stratified doses ranging from 210 mg/kg BW, 420 mg/kg BW, and 840 mg/kg BW respectively showed an antioxidant effect on salak skin extract that reduced the amount of melanin in experimental animal skin. Among the three groups, the lowest amount of melanin was shown in group 3 given a salak skin extract dose of 420 mg/kg BW. Previous research mentions the benefits of antioxidant supplementation such as reducing the depth of wrinkles, increasing elasticity and firmness, increasing skin moisture, and significantly reducing dark melanin (Nobile et al., 2021).

The difference in the amount of melanin between treatment groups was related to the dose of salak peel extract given to the body's cell response. The administration of salak skin extract dose of 840 mg/kg BW was the highest dose of extract in the treatment group, but the decrease in melanin was smaller than the other 2 treatment groups. This is related to the physiological process of the body producing endogenous antioxidants naturally (Bánhegyi et al., 1997). When given high doses of exogenous antioxidants above the need, it causes inhibition of endogenous antioxidant synthesis. Giving high doses of antioxidants causes cell damage it has a bad effect on the body (Bjelakovic et al., 2014).

Histopathology Analysis

Histology data showed that K1 (UV light control) has the highest amount of melanin, melanin accumulates in the basal stratum, and the stratum corneum thickens. The K2 (Salak skin extract, 210 mg/kg BW) exhibited melanin accumulates and the stratum corneum thickens. The K3 (Salak skin extract, 420 mg/kg BW) indicated the lowest melanin distribution. The K4 (Salak skin extract, 840 mg/kg BW) denoted that stratum corneum thickened and melanin accumulates second-highest

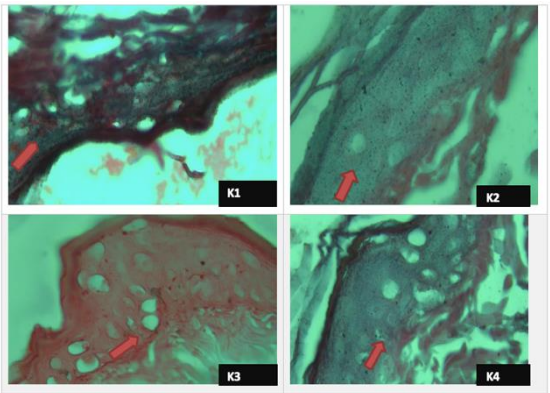

Figure 2. Histology observation of white rat skin (Masson Fontana, 1000x).

Experimental animals exposed to UV light showed a thickening of the epidermis and an increase in the amount of dark melanin in the basal stratum. Group K3 which was treated with salak skin extract at a dose of 420 mg/kg BW appeared to less thickening of the epidermis and the amount of melanin. These changes are in line with studies in mice exposed

to UV light of epidermal hyperplasia and changes in keratinocytes, melanocytes, and fibroblasts.

Thickening of the epidermis (hyperplasia) occurs due to growth factor activity in the skin, so the proliferation of the epidermis layer increases to inhibit damage to the function of the layer inside (Saxena et al., 2014). According to histological analysis, a previous study on olive leaf extract concluded that the preventative effects of olive leaf extract and oleuropein on chronic UVB-induced skin damage, carcinogenesis, and tumor growth may be due to inhibition of the expression of VEGF, MMP-2, MMP-9, and MMP-13 via a decrease in COX-2 levels (Kimura & Sumiyoshi, 2009).

Conclusion

Salak skin extract has an anti-hyperpigmentation effect with a decrease in the amount of melanin. The most effective dose of salak skin extract is the medium dose of 420 mg /kg bb because it mostly reduces the amount of melanin. We also found that UV exposure caused hyperplasia of the epidermis, and changes in keratinocytes, melanocytes, and fibroblasts. This result has suggested that salak skin extract could be useful to protect skin from the negative effect of UV exposure.

References

Addor, F. A. S. A. (2017). Antioxidants in dermatology. *Anais brasileiros de dermatologia*, *92*, 356-362.

Adjeng, A., Hairah, S., Herman, S., (2019). Phytochemical Screening and Evaluation of Liquid Soap Preparation of 96% Ethanol Extract of Salak Pondoh Fruit Skin as an Antioxidant. *Pharmauho. 5*(2).

Bánhegyi, G., Braun, L., Csala, M., Puskás, F., & Mandl, J. (1997). Ascorbate metabolism and its regulation in animals. *Free Radical Biology and Medicine*, *23*(5), 793-803.

Bjelakovic, G., Nikolova, D., & Gluud, C. (2014). Antioxidant supplements and mortality. *Current Opinion in Clinical Nutrition & Metabolic Care*, *17*(1), 40-44.

Brenner, M., & Hearing, V., (2008). The Protective Role of Melanin Against UV Damage in Human Skin. *NIH Public Access. 84*(3). 539-49.

Fitrianingsih, S. P., Lestari, F., & Aminah, S. (2014). Uji Efek Antioksidan Ekstrak Etanol Kulit Buah Salak [Salacca Zalacca (Gaertner) Voss] Dengan Metode Peredaman DPPH. *Prosiding SNaPP: Sains, Teknologi*, *4*(1), 49-54.

Hernández, Y., Lobo, M. G., & González, M. (2006). Determination of vitamin C in tropical fruits: A comparative evaluation of methods. *Food chemistry*, *96*(4), 654-664.

Kanon, M. Q., Fatimawali, & Bodhi, W. (2012). Test of the Effectiveness of Salak Fruit Skin Extract (Salacca thorny palm (gaertn.) voss) on Sucrose-Induced Blood Sugar Reduction of Male White Rats Wistar Strains (Rattus norvegicus l.). *Pharmacon, 1*(2), 52–58.

Kimura, Y., & Sumiyoshi, M. (2009). Olive leaf extract and its main component oleuropein prevent chronic ultraviolet B radiation-induced skin damage and carcinogenesis in hairless mice. *The journal of nutrition*, *139*(11), 2079-2086.

Nobile, V., Schiano, I., Peral, A., Giardina, S., Spartà, E., & Caturla, N. (2021). Antioxidant and reduced skin-ageing effects of a polyphenol-enriched dietary supplement in response to air pollution: a randomized, double-blind, placebo-controlled study. *Food & nutrition research*, *65*.

Saxena, P. N., Khanam, N. S., & Saxena, N. (2014). Anatomical changes in the skin of Rattus norvegicus after artificial UV exposure. *Journal of Advanced Laboratory Research in Biology*, *3*, 71-78.

Videira, I. F. D. S., Moura, D. F. L., & Magina, S. (2013). Mechanisms regulating melanogenesis. *Anais brasileiros de dermatologia*, *88*, 76-83.

Zhou, J., Shang, J., Song, J., & Ping, F. (2013). Interleukin-18 augments growth ability of primary human melanocytes by PTEN inactivation through the AKT/NF-κB pathway. *The international journal of biochemistry & cell biology*, *45*(2), 308-316.