International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 7, Issue 2, pp: (449-453), Month: October 2019 - March 2020, Available at: www.researchpublish.com

The Effect Of Duwet Extract *(Syzygium Cumini)* On Langerhans Cells As Result Of Ultraviolet B Exposure On The Dorsal Skin Of Male Wistar Rat

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Abstract: **One of the main contributing factor to skin aging is oxdative stress. Previous studies showed that antioxidants are necessary for inhibiting the process of skin aging due to oxidative stress through antioxidant properties and inflammation inhibition. Duwet *(Syzgium Cumini)* which is planted in Indonesia is know to have high concentration of antioxidant. The aim of this study to assess Duwet ethanol extract cream effects on number of Langerhans cell on the dorsal skin of male Wistar rats exposed to UV-B. This study is purely experimental research. A post-test only control group design research was conducted on 30 male rats. Group were divided into control (P1), placebo (P2), 5% (P3) , 10% (P4) and 20% (P5) Duwet ethanol extract concentrations. The control, placebo, 5%, 10% and 20% shows significant result to (p>0.05). The mean difference of P1, P2, P3, P4 and P5 was 26.00, 50.00, 48.0, 52.67 and 58.00. This study show that Duwet ethanol extract cream can preserve the number of Langerhans cell.**

Keywords: **Number of Langerhans Cell, Duwet, UV-B, Antioxidant.**

1. INTRODUCTION

Sun exposure can have both positive and negative effects, especially toward the skin which is an organ that directly contact with the sun. Various mechanism involved in photoaging process that cause by UV-B. One the mechanisms involved is a decrease in the Superoxide Dismutase (SOD) level. SOD is a compund that has an antioxidant properties on the skin. Antioxidant are molecules that has an antioxidant properties on the skin. Antioxidant are molecules that inhibit the oxidation of other molecule. Oxidation is a chemical reaction that can produce free free radical which can lead to cell damages[1]. Antioxidant can help preventing futher cell damage that cause by oxidantion. Human body can naturally produce antioxidant, but normally the amount of free radical is greater than the amount of antioxidant produced. To balance it, external supply of antioxidant is needed to get maximum antioxidant supply.[2]

Photaging is complex progessive process that cause functional and aesthetic changes of the skin. This process occur intrinsically and extrinsically. Photoaging can be caused by UV-B exposure with length of 300-400 nm. This cause a decrease in Langerhans cell number.[3] Langerhans cell is a member dendritic cell family that can be found in epidermis area. It specialize in antigen presentation and are important cells of the immune system which also involved in photoaging process.[4] When exposed to UV-B Langerhans cell will undergo an activation or maturation process that leads to their migration to the lymph nodes draining through the lymphatic vessels.[3]

Duwet or it scientific name *Syzygium Cumini* come form the guava tribe.[5] It is high with anthocynin such as cyanidin due to its purple skin.[6] Anthocynin has a high antioxidant role that prevent oxidation process. Duwet has antioxidant activity of 64.75% which almost the same as Buthloted hydroxitoluene activity. This shows the ability of duwet anthocyanin in

preventing photoaging.[7] Therefore in this study the researcher wants to examine the duwet potiential in preventing phtoaging.

2. METHODOLOGY

This study is purely experimental research. A post-test only control group design research was conducted on 30 male rats. Groups were divided into control (P1), placebo (P2), 5% ethanol extract of duwet (P3), 10% ethanol extract of duwet (P4) and 20% ethanol extract of duwet (P5). Duwet is collected from Desa Noelbaki, Kecamtan Kupang Tengah, Kabupaten Kupang, Nusa Tenggara Timur, Indonesia. It was sorted and processed to get 60g of extract. Duwet ethanol extract is processed in oil phase and water phase to form 5%, 10% and 20% of duwet ethanol extract at Pharmaceutical Labarotory Mahasaraswati University. Rats will be shaved of the dorsal skin with measurement of 4x4 cm. The shaved skin of the rats are exposed under UV-B one by one with the MED of 2000mJ/cm^2 for 400 seconds. Skins of rat in P1 are not applied with any substances before exposured to UV-B. skins of rat in P2 is applied with palcebo 1 hour before exposure to UV-B, skins of rat in P3 is applied with 5% duwet ethanol extract 1 hour before exposure to UV-B, skins of rat in P4 is applied with 10% duwet ethanol extract 1 hour before exposure to UV-B and skins of rat in P5 is applied with 20% duwet ethanol extract 1 hour before exposure to UV-B. After exposure, the rats are applied with their respective creams again by group and left. The skin biopsy is taken after 24 hours and stained by haemotoxylin eosin (HE) and observed under light of microscope with 400x magnification. The results is observed and recorded using optilab and image raster. The methods are conducted in Integrated Biomedical Labarotory, Faculty of Medicine Udayana University. Descriptive analysis is conducted to obtain a central tendency value and standard deviation (SD) of the dependent variable. Besides, statistical analysis is conducted to test the normality of the data using the Shapiro-Wilk test, homogeneity by Levene's test and proceed with test parametric statistics with one-way ANOVA. Lastly, proceed with Least Significance Difference (LSD) test.

3. RESULTS AND DISCUSSION

3.1 Descriptive Test

Table 1 shows descriptive test results of mean, standard deviation, lower and upper limits of 95% confidence interval and the maximum and minimum values of the number of Langerhans cell on the dorsal skin of male wistar rats. The highest average of Langerhans cell count found in group P5 with score 58.00 $\pm$ cell/mm^2 followed by P4 with score 52.66 $\pm$ 14.02 cell/mm^2 and P2 with score 50.00 $\pm$ 13.79 cell/mm^2.

TABLE 1: Descriptive test result of Number of Langerhans Cell on the Dorsal Skin of Wistar Rat

				95% Confidence Interval			
	N	Mean	Standard Deviation	Lower	Upper	Min	Max
P1	6	26.0000	8.29458	17.2954	34.7046	16.00	40.00
P2	6	50.0000	13.79855	35.5193	64.4807	32.00	68.00
P3	6	48.0000	15.59487	31.6342	64.3658	28.00	68.00
P4	6	52.6667	13.48579	38.5142	66.8191	40.00	76.00
P5	6	58.0000	14.02854	43.2779	72.7221	36.00	72.00
Total	30	46.9333	16.64007	40.7198	53.1468	16.00	76.00

3.2 Normality & Homogeneity Test

Normality and homogeneity test are useful to determine the type of mean comparative test that will be continued after. In this study, the normality test was performed using the Shapiro-Wilk test for studies with a small total sample. It was found that all treatment groups had mean value of normality distributed Langerhans cells with a p value greater than 0.05. While based on the Levene test it was found that the average data on the number of Langerhans cells in this study was homogeneuos ($p>0.05$). Table 2 shows normality and homogeneity results.

TABLE 2: Normality and Homogeneity test

Normality Test *Saphiro Wilk*		Homogeneity Test *Levene Test*		
Group	Significant		Statistic	Significant (p)
P1 (Control)	0.700	Based on Mean	0.671	0.619
P2 (Placebo)	0.644			
P3 (5%)	0.715			
P4 (10%)	0.286			
P5 (20%)	0.396			

3.3 Comparability Test

The mean number of Langerhans cells in the skin of rats in each treatment group was proven to be normally distributed being compared with One Way ANOVA test where significant results would be symbolized by p value < 0.05. In this study it was found that average number of Langerhans cells in each treatment group had significant difference (p = 0.04). Table 3 shows the complete data.

TABLE 3: Result of Number of Langerhans Cell on the Dorsal Skin of Wistar Rat Analysed by Using One-Way ANOVA Method

	Sum of Squares	Mean of Square	Sig (p).
Between Groups	3624.533	906.133	0.004*
Within Group	4405.333	176.213	
Total	8029.867		

3.4 Post-Hoc Test

Because the mean difference was found to be statically significant, the comparison of mean values between groups was continued with the Post-Hoc test using the Least Significant Difference (LSD). in this study it was found that there were statistically significant differences in the average number of Langerhans cell between P1 and other group P2, P3, P4, P5 ($p<0.05$). However there were no significant mean differences between groups P2, P3, P4, and P5. Table 4 shows the complete data.

TABLE 4 : Post-Hoc Result of Number of Sunburn Cell on the Dorsal Skin of Wistar Rat Analysed by Using Least Significant Difference (LSD) Test

	P1	P2	P3	P4	P5
P1		0.004*	0.008*	0.002*	0.000*
P2	0.004*		0.796	0.731	0.307
P3	0.008*	0.796		0.548	0.204
P4	0.002*	0.731	0.548		0.493
P5	0.000*	0.307	0.204	0.493	

3.5 Discussion

UV-B exposure contributes in the occurence of photoaging events. Various mechanisms are know to explain the pathogenesis of photoaging due to UV-B exposure. One mechanism that is often discussed is through the oxidative stress process. Previous research has shown that chronic UV-B exposure has been shown to reduce levels of Superoxide Dismutase (SOD), a compund that has antioxidant properties on the skin.[1] UV-B exposure is also know to reduce the expression of the enzyme Matrix Metalloproteiase (MMP) -1 in skin fibroblasts which in turn will increase oxidative stress in the skin through increased levels of Reactive Oxygen Species (ROS).[1] Histological photoaging process, is characterized by a decrease in the number of Langerhans cell in the skin. Langerhans cell were a members of the dendritic

cell family that plays a role in antigen presentation so that when exposed to UV-B these cell respond by emigrating to lymph node drainage channels.[2]

Duwet is a fruit that contain antioxidant, anti-inflammatory, anti-bacterial properties and can act as a scavenger of free radicals such as ROS. Duwet contain antioxidant in a form of Anthocyanin derived from the purple color of the duwet fruit and several other substances such as delphinidin, petunidin and mavidin-diglucosida which are anti-neoplastic agents.[8] Previous study shows that anthocyanin rich duwet extracts have an effectivenessof 90.6% in taking free radical where these results are evaluated with the auto-oxidation of β-carotene and linoleic acid assays. A study that tested the antioxidant activity of duwet and compared with Ardisia elliptica extract show that antioxidant in duwet had a higher free radical scavenger activity 1.9 times compared to Ardisia elliptica.[9]

Based on the descriptive test data in this study, it can be seen that the number of Langerhans cell in the group of the rats only get UV-B exposure at a dose of 2000mJ/Cm^2 (P1) has the lowest mean number of Langerhans cell compared to other groups. Whereas the highest number of Langerhans cell was found in the group of rats given duwet ethanol extracts with 20% concentration (P5), followed by group of rats give duwet ethanol extracts with 10% concentration (P4), and group of rats given palcebo (P2). Based on ANOVA analysis test it was found that the mean difference between group was stastically significant ($p=0.004$), but after multivariate analysis using Post-Hoc LSD is proceed it was found that only P1 had a mean difference between the other four group P2,P3,P4 and P5 ($p<0.005$). This shows that in this study, the duwet ethanol extract had a significant effect on increasing the number of the Langerhans cells in rats given exposure to UV-B light although the effectiveness between doses could not be determined.

In this study was found that the placebo group had a higher average number of Langerhans cell compared to group given duwet ethanol extract with 5% concentration. This study used a placebo in the form of combination cram consisting of 145 grams of stearic acid, 15 grams of triethanolamine, 30 grams of the adepsine, 250 grams of paraffin, 550 grams of aquadest and 0.18% of nipagine. The base ingredient for this placebo cream is lanolin which is a sheep's fat that is high in cholesterol in form of esters and alcohol that help increase in absorption of water which provide moisturizing effect on skin.[10] In sunscreen lanolin works by forming an oil film that lines the skin's stratum corneum so that the skin is better protected from sunlight. Lanolin that currently used as a base for sunscreen is lanolin that has been stabilized by using the antioxidant taponol has a lower UV absorption power.[11]

4. CONCLUSION

Based on the results of studies conduct, it can be concluded that the duwet ethanol extract can increase the number of Langerhans cell, but the effectiveness of duwet cannot be ascertained since there is no significant difference in the average number of Langerhans cell in giving duwet ethanol extracts in various concentration and placebo. Further studies with a larger sample size might be possible to show the different in effectiveness at each dose of duwet ethanol extract.

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