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The Effect of Sapodilla Leaf Extract on IL-6 and TGF- β Levels of Skin Tissue (Experimental Study on Male White Rats of Wistar Strain Exposed to UV Light)

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ABSTRACT: Excessive exposure to ultraviolet light affects cell and tissue homeostasis which has a damaging effect on DNA characterized by the formation of reactive oxygen species (ROS). Inflammation due to UV rays is the main cause of oxidative stress in the skin. Healing of wounds caused by sunburn through a complex process consisting of inflammatory cell infiltration, cell proliferation, and tissue remodeling phases to restore normal skin integrity and function, rapid healing and epithelialization to prevent infection and minimize functional and aesthetic complications. The researchers aimed to see the effect of administering sapodilla leaf extract cream (*Manilkara zapota*) on IL-6 and TGF-β levels in Wistar rats with sunburn. Experimental study with a post test only control group design using a total of 30 mice as experimental animals, divided randomly into 4 treatment groups consisting of a control group, a placebo group, a sapodilla leaf cream group with a dose of 25% and a 50% cream group. Mouse skin tissue exposed to UV was analyzed using the ELISA method to determine the levels of IL-6 and TGF-β. There was no significant difference in the average IL-6 levels between treatment groups with the one way anova difference test, a p value of 0.25 ($p<0.05$), there was a significant difference in the average TGF-β levels for each group with a p value of 0.005 ($P<0.05$), *Manilkara zapota* leaf extract cream had a significant effect on TGF-β levels at a dose of 50%. Administration of *Manilkara zapota* leaf extract cream has a dose-dependent effect on IL-6 and TGF-β levels in skin tissue of mice exposed to UV.

KEYWORDS: *Manilkara zapota* extract cream, IL-6, TGF-β, UV

I. INTRODUCTION

Excessive exposure to ultraviolet light in humans can cause health problems. UV light affects the homeostasis of cells and tissues providing a DNA-damaging effect characterized by the formation of reactive oxygen species (ROS). ROS increases the peroxidation of lipid components of cell membranes, alters the structure and increases oxidation resulting in oxidative stress. Inflammation due to UV rays is a major cause of oxidative stress on the skin. [1] Acute inflammation associated with *helper T* (Th)1 cells, *activates trasforming growth factor beta* (TGF-β) signaling and contributes to collagen degradation, causing gene mutations and genome instability.[2] The ability to fight oxidative stress decreases, resulting in an increase *in Interleukin* 6 (IL-6), acting as a pro-inflammatory mediator released in times of inflammation. IL-6 signaling blockade has been shown to be effective in treating conditions characterized by chronic inflammation.[3] Sapodilla leaf extract contains flavonoids that are very significant known to have anti-inflammatory effects.[4]

Indonesia is a country located on the equator, with tropical climatic conditions as well as scorching sunlight and a fairly high level of humidity. *Sunburn* is caused by excessive exposure to the sun's UV rays.[5] Excessive sun exposure presents risks and side effects to human health. According to *the World Health Organization* (WHO), it is estimated that around 11 million people each year experience sunburn.[6] Solar radiation is becoming the most important environmental risk factor for skin cancer, a major cause of *photoaging*, and also an exacerbation factor for some dermatoses.[7] Clinical and epidemiological data link cumulative sun exposure intensity and number of sunburns to skin damage.[8] The increase in the number of sunburns experienced is directly correlated with an increased risk of skin cancer.[5] The incidence of cancer in Indonesia (136.2/100,000 population) ranks 8th in Southeast Asia,

while in Asia ranks 23rd. The development of traditional medicine today is greatly increased so that the use of traditional medicine is preferred and the price is more affordable, as well as the side effects caused are not dangerous to life.

Wound healing caused by *sunburn* goes through a complex process consisting of inflammatory cell infiltration, cell proliferation, and tissue *remodeling* phases to normalize skin integrity and function. Sapodilla leaf extract can result in a decrease in IL-6 and an increase in TGF-β production. [9] Sapodilla has recently been shown to trigger apoptosis in various cell lines by activating mitochondrial pathways. Manila leaves contain phytochemical compounds of alkaloids, flavonoids, saponins, tannins, terpenoids and glycosides. Lavonoids can inhibit the formation of arachidonate acid and the secretion of lysosomal and endothelial enzymes so that proliferation and exudation from the inflammatory process are inhibited. [4]

The ultimate goal of sunburn management and treatment is rapid healing and epithelialization to prevent infection and minimize functional and aesthetic complications.[10] Proper wound care results in therapeutic success.[6] Natural compounds have been used for thousands of years to treat wounds. Natural compounds found in many plants and animals are widely available sources for wound care including the treatment of burns due to sun exposure.[11] *Manilkara zapota* (L.) is a plant from the *Sapotaceae* family widely cultivated throughout the tropical world. Extract from *Manilkara zapota* (L.) is known to have a high antioxidant content that has the potential to be used as a natural therapy in wound healing.[12] Therefore, researchers intend to examine the effect of sapodilla leaf extract on IL-6 and TGF-β levels in UV-induced wistar rats.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

The study is an experimental study *Post Test Only Control Group Design*. The study subjects used 30 male rats of *wistar* strain aged 10-12 weeks, with a body weight of 190-210 grams that met the inclusion and inclusion criteria, adapted for 7 days. The study subjects were randomly divided into 4 groups, namely the group of rats without treatment (K1), the group of rats exposed to UV light for 3 days and given placebo cream (base cream) for 3 days (K2), the group of rats exposed to UV light for 3 days and given 25% sapodilla leaf extract cream for 3 days (K3), and the group of rats exposed to UV light for 3 days with 50% sapodilla leaf extract cream for 3 days (K4). The examination was carried out on day 7 using skin tissue with the ELISA method to measure IL-6 and TGF-β levels.

Research Materials

Research materials include *Manilkara zapota* sapodilla leaf extract, aquabides, ketamine, ingredients for preparation preparation: 70% alcohol, 80%, paraffin, Fine test ELISA kit Rat IL-6, Fine test ELISA kit Rat TGF-β.

Research Equipment

This study used several equipment, namely a cage measuring 55 x 22 x 22 cm equipped with a feed and drinking bin, UV-B lamp, digital scale, PVC pipe, scalpel knife, oven, maceration bottle, rotary evaporator, porcelain dish, water heater, mortar, specimen tube, and name tag.

How to Make *Manilkara zapota* Leaf Ethanol Extract Works

(1) 1 kg of fresh *Manilkara zapota* leaves, washed thoroughly using running water. **(2)** *Manilkara zapota leaves* are then dried in an oven at 40°C. **(3)** Simplisia is checked for moisture content with moisture *balance* (Ministry of Health RI, 1985). The drying result of simplisia is considered good if the moisture content is below 10%. **(4)** Simplisia is made powder by blending, then sifted with a 20 mesh sieve. **(5)** The preparation of *Manilkara zapota* leaf extract is carried out by maceration method. **(6)** A total of 450 grams of *Manilkara zapota* leaf simplisia powder was weighed, put into a jar, then added 96% ethanol filter liquid as much as 1,500 mL. **(7)** The mixture is stirred until all powder is wetted, covered, and left for 3 days protected from light. **(8)** The next stage is routine stirring 3 times a day. **(9)** After 3 days the maceration results are filtered, the pulp is remacerated for 2 days with a volume of 96% ethanol as much as 1,500 mL. **(10)** Maserat is thickened using a rotary evaporator at 40°C until a thick extract is obtained. **(11)** The thick extract of *Manilkara zapota* leaves obtained is calculated in yield, then made in the form of 25% and 50% cream.[13]

Treatment of Experimental Animals

(1) Rats that have been adapted for 7 days are anesthetized with a mixture of ketamine (60 mg / kgbb) and xylazine (20mg / bb), then the hair on the dorsal part of the mouse is shaved clean with a size of 5x5 cm. **(2)** The rat's back was exposed to UV light (broadband with peak emission 302 nm) with a minimum dose of erythema 160 mJ/cm2/day for 3 days. **(3)** The rats were then given treatment according to their group. **(4)** Topical treatment is given once a day for 3 days after UV B irradiation.

III. RESULT

Results of analysis of flavonoid levels and total phenol of sapodilla leaf extract

Test results of flavonoid content of sapodilla leaf extract (*Manilkara zapota)* by testing flavonoid levels and total phenols. The average flavonoid content of sapodilla leaf extract *(Manilkara zapota)* was 56.8 mg / ml, while the average phenol content of *Manilkara zapota* leaf extract preparation was 170.1 mg / ml. The phytochemical screening results of Sapodilla leaf extract are positive for alkaloids, flavonoids, terpenoids, glycosides, tannins and saponins. [14]

Validation results of Hematoxilin eosin (HE) staining of rat skin tissue that experienced *sunburn*

Results of observations on samples with mouse models given exposure to UVB light, UV light exposure (broadband with peak emission 302 nm) with erythema doses of 160 mJ / cm2 / day for 3 days. The next day (day 7) skin tissue samples were taken to make H&E staining. Analysis results in the treatment group there were sunburn cells on the yellow arrow while in the control group no sunburn cells were found, as in figure 1 below:

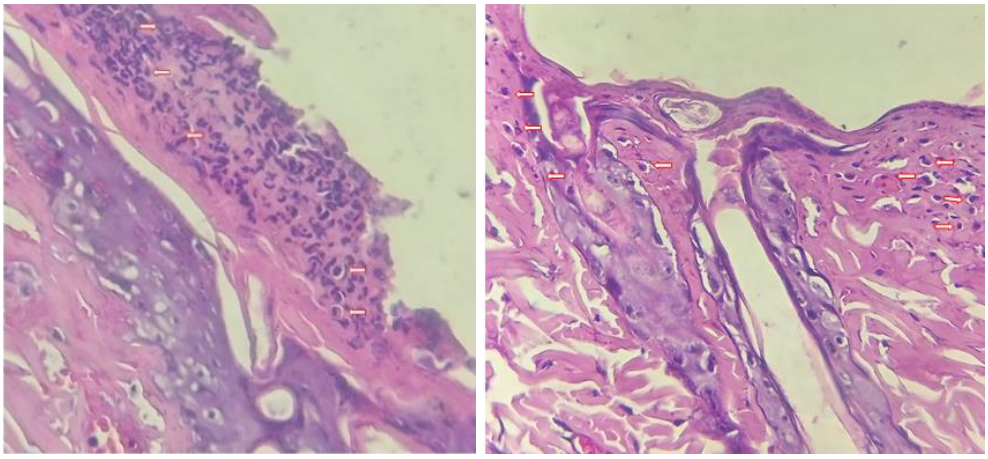

Figure 1. H&E staining results of treatment group with 40x magnification

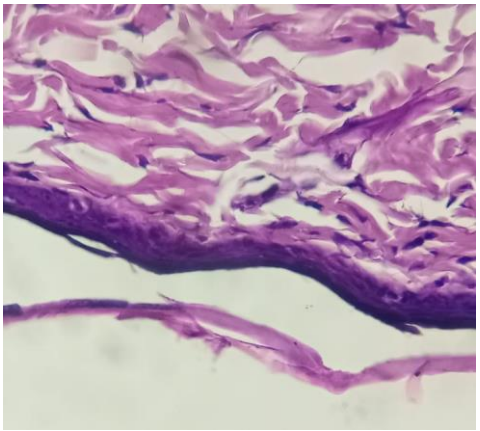

Figure 2. Control group H&E staining results with 40x magnification

Results of IL-6 Rate examination in UVB-shown mice

The results of the examination of average IL-6 levels, after treatment with the administration of *Manilkara zapota* leaf extract *cream* for 3 (day 4 to day 6), day 7 Wistar rat skin tissue was taken to be analyzed IL-6 levels by the ELISA method. The average test results of IL-6 levels in each group are shown in table 1 below:

Table 1. Descriptive test results and *One way Anova* IL-6 levels

Group	K1 Without Treatment	K2 *Base cream*	K3 *Cream* Dosage 25%	K4 *Cream* Dosage 50%	*P value*
Kadar IL-6 (ng/L)					
Wistar 1	9.59	10.23	12.45	8.61	

Wistar 2	10.76	10.34	9.83	8.94	
Wistar 3	11.16	8.37	8.79	8.37	
Wistar 4	9.07	10.05	10.89	10.39	
Wistar 5	11.9	11.48	9.75	9.71	
Wistar 6	9.91	10.16	9.25	9.26	
Mean	10,39	10,10	10,16	9,21	
SD	±1.06	±0.99	±1.32	±0.74	
Shapiro-Wilk	*0,89	*0.25	*0.42	*0.83	
Leuvene Test					*0.54
One way Anova					0,25

Description: * *Shapiro-Wilk* = Normal (p>0.05)

* *Leuvene Test* = Homogen (p>0,05)

* *One way Anova* = Signifikan (p<0,05)

The average IL-6 levels with the ELISA method, the highest levels were found in the K1 group and the lowest levels in the K4 group. There is a downward trend in IL-6 levels with the administration of 50% dose of *Manilkara zapota* leaf extract cream which still has to be proven by statistical tests to see the difference significantly or not.

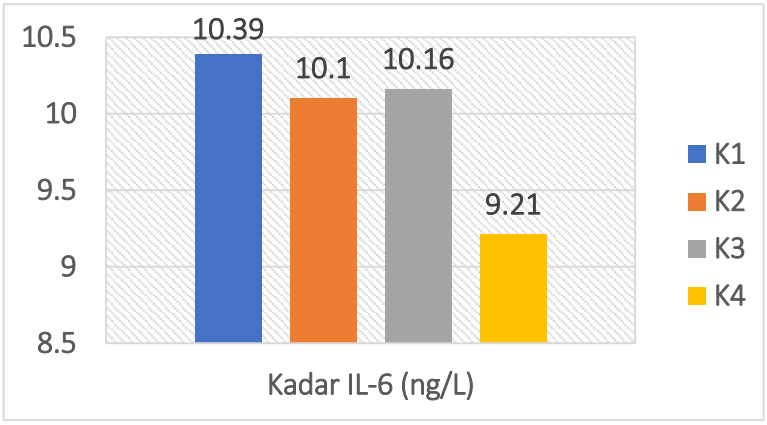

Figure 3. Graph of average IL-6 levels between groups

Descriptive analysis to determine the normality of data with the Shapiro-Wilk test known to all normally distributed groups (p>0.05), and determine the homogeneity of data with the Leuvene Test obtained results of 0.54 (p>0.05) which means that the distribution of IL-6 level data between groups has homogeneous data variance. The average normal and homogeneous IL-6 levels carried out by One way *Anova* difference test obtained results of 0.25 (P<0.05). It can be concluded that there is no significant difference in average IL-6 levels between treatment groups

Results of TGF-β rate examination in UVB-shown mice

The results of the average examination of TGF-β levels in each group using the ELISA method using rat skin tissue samples, shown in Table 2 below:

Table 1. Descriptive test results and *One way Anova* TGF-β levels

Group	**K1 Without Treatment**	**K2 *Base cream***	**K3 *cream* Dosage 25%**	**K4 *cream* Dosage 50%**	***P value***
Up to TGF-β (ng/L)					
Rat 1	506.39	460.07	436.22	387.13	
Rat 2	567.10	428.01	440.02	490.39	
Rat 3	500.76	485.14	518.29	459.91	

Mouse 4	527.34	509.21	430.69	360.16	
Rat 5	511.37	477.15	424.87	463.05	
Rat 6	518.71	499.36	469.76	471.05	
Mean	521,94	476,49	453,30	438,61	
SD	24,01	29,29	35,44	52,13	
Shapiro-Wilk	*0,13	*0.77	*0.07	*0.16	
Leuvene Test					*0.11
One way Anova					0,005

Description: * *Shapiro-Wilk* = Normal (p>0.05)
* *Leuven Test* = Homogen (p>0,05)
* *One way Anova* = Signifikan (p<0,05)

The average TGF-β levels obtained the highest levels in the K1 group and the lowest in the K4 group. There was a decrease in TGF-β levels by giving *Manilkara zapota* leaf extract cream at a dose of 50.

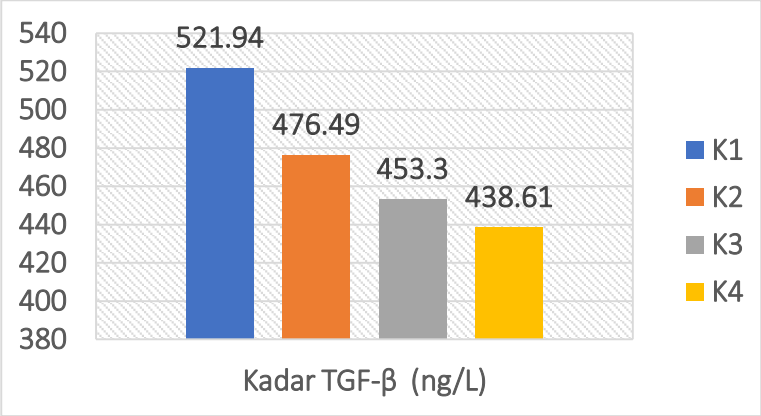

Figure 4. Graph of average TGF-β levels between treatment groups

The descriptive test of determining the normality of the average TGF-β levels with the *Shapiro-Wilk test showed the results of each normally distributed group (p > 0.05), and the determination of data homogeneity with the* Leuvene Test *obtained results of 0.106 (p > 0.05*) the results showed that the data on TGF-β levels between groups had a homogeneous distribution of data. Average normally distributed and homogeneous TGF-β levels are eligible for *the One-way Anova test.* One way anova *test results* show a significant value of 0.005 (P<0.05). So it was concluded that there was a significant difference in the average TGF-β levels between groups.

The results of *the One way anova* test are meaningfully carried out *Post Hoc Tests* to see the differences between the two groups and get the most influential dose between the treatment group and the control group. Comparison of each group with *Post Hoc Tests* as follows:

Table 2. Hasil uji *Post Hoc Tests* at TGF-β

Group	K1	K2	K3	K4
K1		*0,045	*0,004	*0,001
K2			0,228	0,090
K3				0,497

Description: * Means p<0.05

The results of the *Post Hoc LSD* test showed that the difference in the average TGF-β levels between groups in graph 5.3, obtained the results of giving *Manilkara zapota* leaf extract *cream* dose of 50% had the most significant influence on TGF-β levels with a value of 0.001 (p<0.05). It can be concluded that *Manilkara zapota* leaf extract *cream* has an effect on TGF-β levels between

control groups compared to the treatment group at a dose of 50% which has the most significant effect.

IV. DISCUSSION

Analysis conducted to observe the effect of *sapodilla leaf extract* cream (*Manilkara zapota*) in improving the condition of skin that experiences *sunburn* due to UV exposure. *Sunburn cells* (SCs) are radiation-induced burns to the skin caused by too much UV exposure, The biggest risk factor for sunburn is the amount of time the skin is exposed to UV rays, plus its intensity. Skin keratinocytes in SCs undergoing apoptosis show morphological features such as picnotic nuclei with eosinophilic cytoplasm, mitochondrial swelling and rupture.[15]

UV-B exposure increases ROS which plays an important role in activating the MAPK pathway, leading to increased infiltration of inflammatory mediators and epidermal hyperplasia. Inflammatory mediators are released from keratinocytes, fibroblasts, tumor cells, leukocytes and the endothelial lining of blood vessels.[16,12] Excess UV radiation secretes pro-inflammatory cytokines (IL-1, IL-6 and TNF-α), and IL-6 levels in serum peak about 12 hours after UVB irradiation. The role of IL-6 in inflammation caused by UV-B light, is involved in the production of various types of matrix metalloproteinases (MMPs) by stimulating fibroblasts. Increased production of IL-6 after UV-B irradiation leads to increased expression of MMP1 and MMP9, thereby contributing to the degradation of the extracellular matrix.[15]

The results showed that IL-6 levels did not experience significant differences between treatment groups, giving sapodilla leaf extract cream (*Manilkara zapota*) did not affect IL-6 levels after UVB exposure for three days followed by cream for three days. The use of local skin tissue samples also did not express IL-6 significantly, based on the length of UVB exposure, skin tissue collection was carried out on the seventh day which showed improvement was not in the inflammatory stage but had moved to the proliferation stage so that it became the cause of insignificant IL-6 levels.[17,18] The antioxidant content of the sapodilla leaf extract cream also intervened for a decrease in IL-6 levels.

In contrast to TGF-β levels, the results showed a decrease in TGF-β levels in the *cream* dose group of 25% and 50%. Synergistic modulation by increasing antioxidant capacity, modulating MAPK/AP-1/MMP-1 and TGF-β/SMAD signaling pathways, stimulating type I procollagen synthesis, and restoring damaged architectural structures.[15] UV-B radiation decreases the extracellular matrix content of the skin either due to changes in collagen synthesis or collagen degradation.[19,20] Activation of the TGF- β gene is responsible for dermal collagen synthesis.[15] The mechanism behind dermal collagen synthesis involves the interaction of TGF-β with the TGF-β cell surface receptor complex ((TβR I-III). The interaction of TGF-β with TβR II results in activation of TβRI serine/threonine kinase activity, resulting in phosphorylation and activation of Smad2 and Smad3 (transcription factors). Smad4, another transcription factor, combined with phosphorylated Smad2 and Smad3, forms complexes that translocate to the nucleus. It activates the TGF-β gene by binding to its promoter region and inducing collagen synthesis. UV-B radiation results in the activation of another transcription factor, SMAD 7, which synergizes the action of TGF-β, thereby inhibiting the TGF-β/SMAD 2-3 signaling pathway and blocking collagen synthesis, thereby increasing the skin collagen produced and loss of skin integrity.[15,19]

This study showed that the administration of sapodilla leaf extract cream had an effect on TGF-β levels in the skin tissue of male Wistar rats exposed to UV-B light and described a decrease in IL-6 levels but not significant. Further research can be carried out simultaneously between UVB exposure and the administration of sapodilla leaf extract cream, so that it can compare TGF-β and IL-6 levels in *sunburn* intervened by sapodilla leaf extract cream with a shorter treatment time. Design treatment with pre and posh tests needs to be done to ensure differences between groups before and after in order to unravel the causes of other influencing factors such as genetic factors of mouse subjects and their environment.

V. CONCLUSION

1. Giving sapodilla leaf extract cream (*Manilkara zapota*) had no effect on IL-6 levels in UVB-induced wistar rats.
2. Giving sapodilla leaf extract cream (*Manilkara zapota*) affects TGF-β levels in UVB-induced wistar rats.

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