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Original Research Article

The Effect of Administration of Sapodilla Leaf Extract Cream (*Manilkara Zapota (L.) P. Royen*) On the Expression of PDGF And Il-10

Sahara Maharani[1*], Titiek Sumarawati[1], Dedi Hermansyah[1]

[1]*Department of Postgraduate Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Indonesia*

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Abstract

Background: *Sunburn* is an acute inflammatory skin condition caused by exposure to UV rays. Excessive exposure increases the production of ROS which, if accumulated, can lyse *growth factors*, one of which is PDGF and also form IL-10 immune suppression. This condition can be influenced by providing antioxidants and anti-inflammatories such as those contained in sapodilla leaf extract (*Manilkara zapota (L.) P. Royen*) which has many benefits such as anti-inflammatory, anti-pyretic, anti-tumor, antioxidant, anti-microbial, anti- diabetic, anti-lipid and anti-aging. In previous research, the polyphenol content in Sapodilla leaves was a potential source of inhibiting ROS. However, until now the role of Sapodilla leaf extract on UVB burns has not been studied.

Objective: The aim of this research is to determine the effect of administering Sapodilla leaf extract cream on PDFG and IL-10 in Wistar rats that experienced burns due to exposure to UVB.

Method: Experimental research with a *posttest only control group design* approach. This research used 24 Wistar rats exposed to UVB rays which were divided into 4 groups (normal control, control with cream, 25% Sapodilla leaf extract cream, and 50% Sapodilla leaf extract cream). The ELISA (Enzyme-Linked Immunosorbent Assay) method was used to analyze PDGF and IL-10 levels in skin tissue.

Results: The highest ratio of PDGF levels was found in (K3) 2.915 ± 0.368. The results of the *one-way Anova* analysis had a *p value* of 0.024 ($p<0.05$) which stated that there were significant differences between treatment groups. In IL-10 levels there was an increase in K3 255.9 ± 35,563. In IL-10, the results of *one-way Anova* analysis had a *p value* of 0.240 ($p>0.05$), which stated that there were no significant differences between treatment groups.

Conclusion: Administration of sapodilla leaf extract cream at a dose of 25% had a significant effect on increasing PDGF levels and a slight increase in IL-10 in mice that experienced burns due to exposure to UVB.

Keywords: *Manilkara zapota (L.) P. Royen*, PDFG, IL-10, ELISA Method
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INTRODUCTION

Indonesia is a country with a tropical climate with quite a lot of exposure to sunlight. Ultraviolet light consists of 3 zones according to its wavelength, namely UVA (UVA 315-400 nm), UVB (280-315 nm), and UVC (100-280 nm).[1] Exposure to UV light can cause acute skin inflammation.[2] Excessive exposure will cause skin damage, including immunosuppression.[3] When cells and tissues are exposed to UVB rays, molecular dissociation (radiolysis) occurs and free radicals are produced in the form of *Reactive Oxygen Species* (ROS). The accumulation of ROS by UVB exposure can cause inflammation which can lyse several *growth factors* including *Platelet Derived Growth Factor* (PDGF) and also form interleukin-10 (IL-10) anti inflammation.

* Corresponding author:
E-mail: *drsaharamaharani@gmail.com*
(Sahara Maharani)

Indonesia is rich in natural resources, researchers have turned to using it as an alternative medicine, one of which is sapodilla leaf extract which has been proven to contain active polyphenol substances and has anti-inflammatory properties.[4] In previous research, ethanol sapodilla leaf extract reduced edema in mice given carrageenan, activity significantly anti-inflammatory.[5] However, research on sapodilla leaf extract which is linked to burns due to UVB exposure is still scarce, so further research is needed.

Based on previous research in the US in 2015, of 31,162 US citizens, 34% of respondents had experience of experiencing *sunburn*. The highest incidence in Fitzpatrick types I-III is at a young age of around 18-29 years.[6] Sunburn due to exposure to UV rays and DNA damage will increase the risk of melanoma and non-melanoma skin cancer.[7] Most of the Indonesian population does a lot of outside activities, around 57.3 % focuses on UV light exposure.[8]

An imbalance in ROS production initiates inflammation and activates proinflammatory cytokines from epidermal keratinocytes such as PDGF.[9] In injured areas PDGF is an important chemokine and mitogen for fibroblasts, keratinocytes and vascular endothelium, and also stimulates macrophages to produce and secrete growth factors such as TGF-β.[10] PDGF can influence the function of dendritic cells and induce regulatory T cells via C-type lectin like receptor member 2 (CLEC-2) expression. Furthermore, regulatory B cells interact with pro-inflammatory mediators, platelet-activation factor and produce Interleukin-10 as an immunosuppressive reaction to UVB exposure.[11] *Sunburn* conditions by UVB induced skin inflammation. Its potential to recover after eliminate the cause and get appropriate therapy. Polyphenols are found in many types of plants. Polyphenols are used as antioxidants, anti-inflammatory and anti-tumor. There is a lot of research on the benefits of polyphenols in reducing damage caused by UVB exposure.[12]

The *Manilkara zapota (L.) P. Royen* plant has many benefits such as anti-inflammatory, anti-pyretic, anti-tumor, antioxidant, anti-microbial, anti-diabetic, anti-lipid and anti-aging.[13] In previous research, the polyphenol content in *Manilkara zapota leaves (L.) P. Royen* is a potential source in inhibiting ROS.[4] However, until now the role of Sapodilla leaf extract in burns caused by UVB has not been studied, so this research was conducted to determine the effect of administering Sapodilla leaf extract cream at concentrations of 25% and 50% against PDGF and IL-10 in Wistar rats that experienced UVB burns.

MATERIALS AND METHODS

This research is an experimental study using *a post test only control group design* which was carried out at *the Integrated Biomedical Laboratory* IBL, Faculty of Medicine, Universitas Islam Sultan Agung from December to January 2024. Ethical clearence of research was given by the bioethics commission of medical/health research, Faculty of Medicine Sultan Agung Islamic University. The research subjects were male Wistar rats aged 2-3 months with body weight 190-210 grams which was declared healthy and suitable for use for research by veterinarians from the Animal House Integrated Biomedical Laboratory-IBL, Faculty of Medicine, Universitas Sultan Agung, Semarang. Wistar rats underwent adjustment for 7 days. Mice were placed in separate cages at a fixed temperature and given a normal diet and access to water.

Sampling uses *probability sampling* techniques, namely by taking samples from a population that has the same opportunity to be selected as a sample. The system used is very simple random sampling (*simple random sampling*). All 28 Wistar rats that met the criteria for the study were divided into 4 treatment groups randomly. There is one control group and another as a treatment group.

The sample used was 1 kg sapodilla leaf (*Manilkara zapota (L.) P. Royen*), taken from Tuban city. The samples were first cleaned of adhering dirt, then dried in an oven at 40oC. The results are checked for water content using a *moisture balance*. If the water content results are below 10% then the drying results are considered good. Simplicia is then dry sorted to remove any dirt remaining during the drying process, cut into small pieces and weighed. Then blend it into powder. Then sifted with a 20 mesh sieve. 450 grams of simplicia leaf powder was extracted using the maceration method with 1500 ml of 96% ethanol solvent. The simplicia leaf powder is put into a separate dark colored bottle. Then the simplicia is soaked using ethanol solvent for 3 days and occasionally stirred 3 times a day, after 3 days it is filtered and the dregs are macerated again for 2 days with 1500 ml of 96% ethanol. repetition is done twice. The collected filtrate is then thickened using a *rotary evaporator* at a temperature of 40ºC until a thick extract is obtained, 110 grams [14,15]

Making a 20-gram cream preparation is done by mixing 15 grams of cream base with 25% sapodilla leaf extract (5 grams) and 10 grams of cream base with 50% sapodilla leaf extract (10 grams). Stirring is done until homogeneous. Sapodilla leaf extract cream was used every day at 0.5 grams per rat, so the dose of sapodilla leaf extract used was 0.125 grams for a 25% dose and 0.25 grams for a 50% dose. Samples taken randomly came from 28 Wistar rats, which were divided into 4 groups. The mice were housed in 4 cages consisting of normal controls, controls with cream, 25% Sapodilla leaf extract, and 50% Sapodilla leaf extract. Each cage contains 7 mice.

The rats were then exposed to UVB 160 mj/cm2/day for 3 days and continued to apply sapodilla leaf extract cream. After treatment for 6 days, tissue was taken. Previously, all Wistar rats were euthanized using anesthesia. Make a tissue incision on the part of the skin exposed to UV B, using scissors and tweezers. Tissue samples were cut and weighed 1 gram, then the tissue was added with PBS (PH 7.4). Then sonicate the sample for 15 seconds or until the tissue melts. Take 1ml/1000uL and put it in a 1.5 ml tube to become supernatant. Next, a protein test is carried out by mixing 500 µL of the sample plus 500 µL of 10% NaOH and 500 µL of 0.1% $CuSO_4$ and then observing the color change to bluish purple if it is positive for protein. Next, the samples were frozen at -20ºC.[16]

Table 1. Results of measurements of sapodilla leaf extract flavonoids

ppm concentration	Absorbents	Initial total flavonoid content (mg/ml)	Average Total Flavanoid Content (mg/ml)
1000	0,894	55,9	
1000	0,950	59,4	56,8
1000	0,882	55,1	

Meanwhile, the average total phenol content in sapodilla leaf extract was 170.1 mg/ml ± 12.4.

Table 2. Results of Sapodilla Leaf Extract Phenol Measurements

ppm Concentration	Absorbents	Initial total flavonoid content (mg/ml)	Average Total Phenol Content (mg/ml)
500	0,989	184,4	
500	0,855	159,6	170,1
500	0,891	166,2	

The skin tissue samples that were obtained were then analyzed for PDGF and IL-10 levels using the ELISA method. PDGF and IL-10 ELISA analysis was carried out using a kit from BioEnzy . The working principle of the ELISA examination in this practicum is an antigen-antibody reaction using a quantitative technique based on the number of specific antigen-antibody bonds determined by the absorbance value from a spectrophotometer.

The collected data processed, edited and tabulated for descriptive tests, followed by data normality using the *Shapiro Wilk* test and data homogeneity testing using the *Levene* test. Data analysis was conducted using One Way Anova SPSS version 26 followed by the Post Hoc LSD and Duncan tests to determine the differences between each group.

RESULTS

This study used 24 samples, no one was excluded during the research. This study consisted of 4 groups consisting of normal control, positive control and 2 treatment groups. Normal control (K1) consists of 6 samples without intervention and treatment. The positive control (K2) consisted of 6 samples exposed to UVB light and applied with a cream base. The first treatment group (K3) consisted of 6 samples smeared with 25% sapodilla leaf extract cream and the second treatment group (K4) consisted of 6 samples smeared with 50% sapodilla leaf extract cream.

Assessment of Total Flavonoid and Phenol Content

Sapodilla leaf extract in this study was obtained by maceration using ethanol solvent and producing a sapodilla leaf extract bath. Results of the assessment of total flavonoids and phenol in sapodilla leaf extract using the spectrophotometric method. In 1 gr of sapodilla leaf extract there is an average total flavonoid content of 56.8 mg/ml ± 2.1.

Effect of UVB Illumination on the Microscopic Image of Sunburn Cells

The group of mice that were given UVB irradiation with an energy intensity of 160 mJ/cm2/day for 3 days and those without irradiation were then subjected to a *sunburn* cell histology validation test. The examination was carried out on day 4 with microscopic observation, the results obtained were as shown in Figure 1.

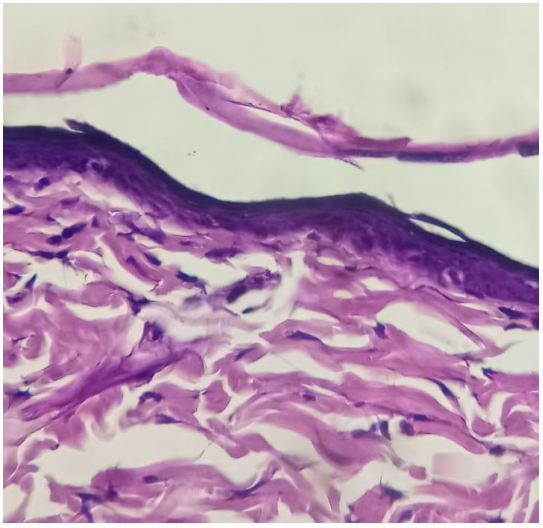

Figure 1.A Histology of healthy skin (Normal Control)

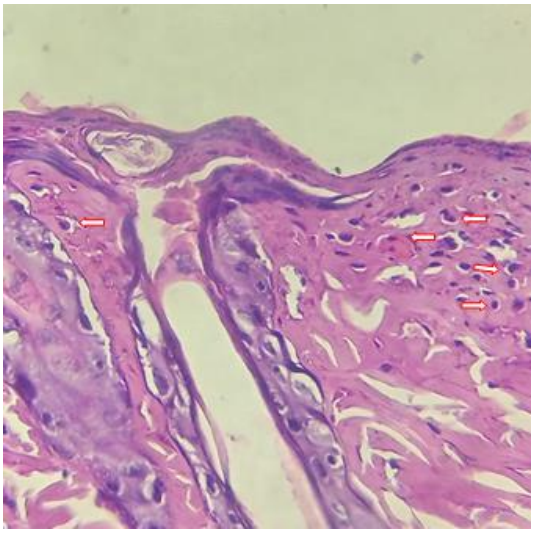

Figure 1.B Histology of exposed skin to UVB (Negative Control)

Table 3. Research Data the Effect of Giving Sapodilla Leaf Extract Cream on PDGF and IL-10 Levels

Variabel	Group				pvalue
	K1 n=6 Mean± SD	K2 n=6 Mean± SD	K3 n=6 Mean± SD	K4 n=6 Mean± SD	
Kadar PDGF	2.440±0.447	2.496±0.209	2.915±0.368	2.341±0.099	
Saphiro wilk	0.912	0.470	0.462	0.097	
Levene test					0.010
One way Anova					0.024
Kadar IL-10	214.142±38.707	220.713±31.147	255.9±35.563	217.615±47.027	
Saphiro wilk	0.817	0.947	0.819	0.936	
Levene test					0.659
One way Anova					0.240

Figure 1.A is a histological picture of healthy skin. The outermost layer is visible, namely the epidermis with a thin layer of keratin lying on top. The lower part of the epidermis is the dermis layer which consists of connective tissue and dense elastic tissue. Figure 1.B shows the histology of mouse skin exposed to UVB light. The main histological changes in the epidermis include *dyskeratotic* and *vacuolated* keratinocytes (*sunburn* cells) marked by red arrows. Microscopically, the two images show differences in the presence of *sunburn* cells.

In this study, the results of PDGF and IL-10 levels were obtained in a mouse model with *sunburn* burns due to UVB exposure treated with sapodilla leaf extract. In the normal control group (K1) the ratio of PDGF levels was 2,440 ± 0.447, in the positive control group (K2) 2,496 ± 0.209, in treatment group 1 (K3) 2,915 ± 0.368, in treatment group 2 (K4) 2,341 ± 0.099. Based on statistical tests, the level data for each group is normally distributed but not homogeneous with a significance value of the *Shapiro Wilk* test > 0.05 for each group and the *Levene test* of 0.010. The results of the *one way Anova* analysis had a *p value* of 0.024 (p<0.05) which stated that there were significant differences between treatment groups. Differences between groups were continued with the *Games-Howell post hoc* test.

In this study, IL-10 levels were also analyzed, in K1 it was 214,142 ± 38,707, for K2 it was 220,713 ± 31,147, there was an increase in K3 255.9 ± 35,563, in K4 it was 217,615 ± 47,027. Based on statistical tests, the IL-10 levels for each group were normally distributed and homogeneous with a significance value of the *Shapiro Wilk* test > 0.05 for each group and the *Levene test* of 0.659. The results of the *one way Anova* analysis had a p value of 0.240 (p>0.05) which stated that there were no significant differences between treatment groups.

Effect of Giving Sapodilla Leaf Extract Cream on PDGF Levels of Wistar Rats with UVB Burns

In the descriptive data, the PDGF levels of each group were tested for normality using Shapiro Wilk, obtaining a significance value for all groups of p>0.05. These results show that the data is normally distributed. In the *Levene test* assessment, a value of 0.010 (p<0.05) was obtained, which shows that the data is not homogeneous. Followed by the *one way Anova* test to assess significant differences between groups, a p value of 0.024 (p<0.05) was obtained, which stated that there were significant differences between groups. The results of further tests using *Games-Howell* are presented in graphical form in figure 2 and table 4. In this study, the results showed that the average PDGF levels between K3 (25% sapodilla leaf extract cream) and K4 (50% sapodilla leaf extract cream) had is significantly different because it has a significant value smaller than 0.05, namely 0.042. Based on the data above, it can be seen that administering a 25% dose of sapodilla leaf extract cream increases the expression of PDGF levels compared to a 50% dose of sapodilla leaf extract cream.

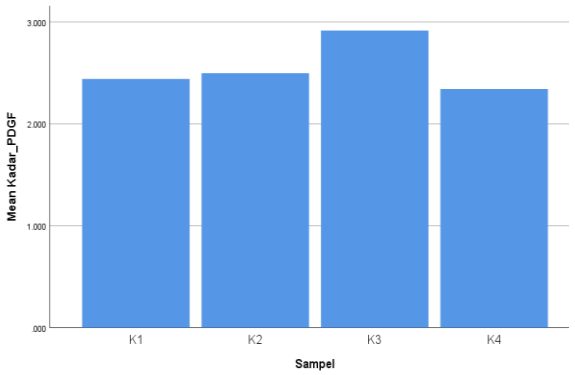

Figure 2. Graph The Effect of Giving Sapodilla Leaf Extract Cream on PDGF levels in all groups

Table 4. Games-Howell test for PDGF levels between research groups

Group	Comparison Group	Significance
K1	K2	0.992
	K3	0.250
	K4	0.950
K2	K1	0.992
	K3	0.152
	K4	0.417
K3	K1	0.250
	K2	0.152
	K4	0.042*
K4	K1	0.950
	K2	0.417
	K3	0.042*

*The mean difference is significant at the p < 0,05 level

Effect of Giving Sapodilla Leaf Extract Cream on IL-10 Levels of Wistar Rats with UVB Burns

In the descriptive data, the IL-10 levels of each group were tested for normality using Shapiro Wilk, obtaining a significance value for all groups of p>0.05. These results show that the data is normally distributed. In the *Levene test* assessment, a value of 0.659 (p>0.05) was obtained, which shows homogeneous data. Followed by *the one way Anova* test to assess significant differences between groups, a *p value* of 0.240 (p>0.05) was obtained, which stated that there were no significant differences between groups. In graph 3, there is a slight

increase in IL-10 levels in the K3 group who received 25% sapodilla leaf extract cream treatment. In the results of further tests using LSD in table 5, it was found that there was no real difference in IL-10 levels in the research samples, which was indicated by the larger sig value. of 0.05 across sample test comparisons.

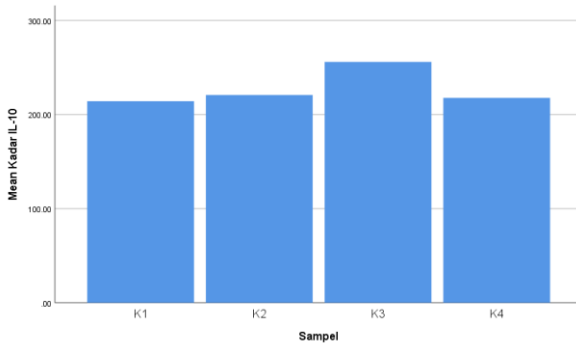

Figure 3. Graph The Effect of Giving Sapodilla Leaf Extract Cream on IL-10 levels in all groups

Table 5. Post Hoc LSD Test for IL-10 Levels Between Research Groups

Group	Comparison Group	Significance
K1	K2	0.771
	K3	0.075
	K4	0.878
K2	K1	0.771
	K3	0.130
	K4	0.891
K3	K1	0.075
	K2	0.130
	K4	0.101
K4	K1	0.878
	K2	0.891
	K3	0.101

*The mean difference is significant at the p <0,05 level

DISCUSSION

UVB exposure can cause *sunburn* which is characterized by redness, pain and inflammation of the skin. On the histological picture, *sunburn cells* were found. This is because UVB penetrates the outermost layer of the skin, the epidermis, there is an increase in *reactive oxygen species* (ROS) which activates the NF-kB pathway and leads to the release of inflammatory mediators and activation of immune cells.[17] The initial response to UVB exposure is the release of pro-cytokines inflammation, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which contribute to the redness, pain, and swelling associated with UVB exposure.[18] However 20, UVB exposure can also induce anti-inflammatory responses in the skin, such as the release of interleukin-10 (IL-10) and PDGF, which are anti-inflammatory and promote tissue repair.[19]

Flavonoid and phenolic compounds have benefits as antioxidants, they can induce the release of Lipooxygenase and cyclooxygenase enzymes which can suppress inflammatory reactions. The antioxidant content in the ability to prevent DNA damage and influence cellular signaling pathways is one of the properties of flavonoids. Other benefits include scavenging free radicals, as a UV absorber, and as a cytoprotective, anti-inflammatory, and anti-apoptotic factor. Previous research has shown that plants high in polyphenols are effective photoprotectants against UV carcinogenesis.[20] The results of this study found that the average total flavonoids per gram of extract was 56.8 mg/ml ± 2.1. Meanwhile, the average total phenol per gram of extract was 170.1 mg/ml ± 12.4. This is impressive as suppressing the NF-kB pathway is expected to reduce the inflammatory response.

This study determined the effect of sapodilla leaf extract cream on IL-10 and PDGF levels in Wistar rats that experienced *sunburn*. The sample of male Wistar rats was chosen as a model for analyzing the anti-inflammatory benefits of sapodilla leaf extract cream, because Wistar rats are mammals with a skin structure similar to humans.

This study analyzed the levels of IL-10 and PDGF, which are anti-inflammatory factors and *growth factors*. Molecular levels of PDGF and IL-10 in this study were checked using ELISA. The results showed that there was an increase in PDGF levels in the 25% sapodilla leaf extract treatment group compared to the control and 50% extract. The decrease in PDGF levels at a dose of 50% is thought to be because inflammation has been controlled. This is because PDGF is produced by macrophages, injured endothelial cells so that the inflammatory signal decreases, causing PDGF production to decrease. The structure of flavonoids also acts as a binder for hydroxyl free radicals, so that ROS produced due to UVB rays can be suppressed.[21] The PDGF receptor is activated by binding to the PDGF ligand, which is secreted by platelets, macrophages and other cells. PDGF signaling is also involved in physiological and pathological processes, including wound healing, tissue repair in *sunburn*.[22] In Konuku et al's research, high doses of sapodilla leaf ethyl acetate extract were more effective in suppressing inflammatory effects in a mouse model with leg swelling.[23] In Swarnakumari's research, The higher the dose of sapodilla leaf ethanol extract, the faster the repair of wounds on the cornea.[24]

In areas exposed to UV light, Langerhans cells will be lost because these cells migrate into the *draining lymph nodes* (DLN). These Langerhans cells lose their ability to present antigen because they cannot produce IL-12, but they can activate T-natural killer cells which trigger T-regulatory cells to produce IL-10 which is also an anti-inflammatory cytokine.[25] In this study, The results of the IL-10 study showed a slight increase in the 25% sapodilla leaf extract group compared to the control and 50% sapodilla leaf extract, but there was no significant difference between the groups. It is suspected that the inflammatory process has been controlled, the treated mice have entered the next phase, namely the proliferation and remodeling phase.

Overall, the results of this study show that sapodilla leaf extract cream has antioxidant activity and a dose of 25% induces growth factors and acts as an anti-inflammatory. This shows that sapodilla leaf extract has the potential to be developed as a *sunburn* therapy.

CONCLUSION

Based on the research results, it can be concluded that administering a 25% dose of sapodilla leaf extract cream had a significant effect on increasing PDGF levels in the skin of model mice that experienced burns due to exposure to UVB, while a 50% dose did not show a significant effect. The administration of 25% and 50% sapodilla leaf extract cream did not have a significant effect on increasing IL-10 levels in the skin of model mice that experienced burns due to exposure to UVB.

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