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Effect of Serum Breadfruit Leaf Extract *(Artocarpus Altilis*) on TNF-A and Sod Levels (Experimental Study on Guinea Pigs Exposed to UVB Light)

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ABSTRACT: Continuous exposure to ultraviolet B (UVB) light can increase the amount of melanin, causing hyperpigmentation on the skin. This process involves the important role of *Tumor Necrosis Factor-α* (TNF-α) and *Superoxide Dismutase* (SOD). *Artocarpus Altilis* contain flavonoids that have anti-inflammatory potential, can reduce TNF-α levels and increase SOD which affects the melanogenesis process. To determine the effect of administering Artocarpus Altilis extract serum on TNF-α and SOD levels in guinea pigs exposed to UVB. This research is an *in vivo* experimental study with *Post Test Only Group design*, which was conducted at the IBL FK Laboratory (*Integrated Biomedical Laboratory* Faculty of Medicine) Unissula. A total of 30 male guinea pigs were divided into 5 groups, namely: Group 1 sham, group 2 negative control, group 3 treated with 2% Artocarpus Altilis extract serum, group 4 treated with 4% serum and group 5 treated with 6% serum. Then analyzed using the One-way ANOVA test to determine differences between groups. The lowest mean TNF-α levels were found in group P2 (4.958), other values were P1 (5.820), P3 (5.964), KS (6.069) and KN (5.811). Meanwhile, the average SOD levels in all groups were not much different, P2 (5.802), P1 (5.975), P3 (5.906), KS (6.054) and KN (6.000). In the One-way ANOVA test on TNF-α and SOD there was no significant difference, p>0.05. Administration *Artocarpus Altilis* extract serum, especially 4%, caused a decrease in TNF-α levels but was not significant, and there was no increase in SOD levels, in guinea pigs exposed to UVB light.

KEYWORDS: *Artocarpus Altilis* extract, TNF-α, SOD

I. INTRODUCTION

Environmental factors that are very influential in the aging process of the skin are ultraviolet light radiation. Continuous exposure to ultraviolet B (UVB) light can cause skin changes, such as hyperpigmentation.[1] Hyperpigmentation is one of the signs of aging of the skin, which occurs due to an increase in the amount of melanin.[2–4] Melanogenesis that triggers pigmentation cannot be separated from the role of *Necrosis Tumor Factor-α* (TNF-α) which is formed due to inflammation in the aging process due to sun exposure. Proinflammatory cytokines such as TNF-α play an important role in inflammation-aging caused by chronic inflammation. The interrelationship between proinflammatory cytokines and cellular aging exacerbates inflammatory aging.[5]

Hyperpigmentation is also influenced by an increase in *Reactive Oxygen Species* (ROS) due to UVB radiation, DNA damage, activating p53, and triggering melanogenesis.[6] Superoxide Dismutase (SOD) is one of the enzymatic antioxidants, which works to capture free radicals ROS.[7] Research conducted by Kim HY, *et al*, 2018, revealed that SOD suppresses UVB-induced ROS production, and inhibits melanin synthesis in melanocyte cells.[8]

Currently, skin lightening ingredients that are widely used to treat hyperpigmentation problems work as *tyrosinase inhibitors*,[9] among them are ascorbic acid, arbutin, kojik acid, and hydroquinone. 4% hydroquinone cream has been the gold standard for the treatment of hyperpigmentation for more than 50 years,[10] however, hydroquinone has side effects such as allergic or irritant dermatitis and ochronosis.[11–13] Therefore, the use of hydroquinone has now begun to be severely restricted.[6] Based on this, it is necessary to look for other skin lightening ingredients that are natural with minimal side effects.[10]

One of the natural skin lightening ingredients that can affect skin pigmentation is breadfruit plants, because these plants produce antioxidant compounds.[14] Breadfruit plants are one of the plants that are easily obtained and empirically have been used in certain

communities in Indonesia as traditional medicine.[10] Breadfruit leaves (*Artocarpus altilis*) contain flavonoid compounds,[15] which acts as an antioxidant inhibitor of ROS, anti-inflammatory and inhibits the activity of tyrosinase enzyme which is efficacious as a skin lightener.[16] Flavonoids are natural phenolic compounds from secondary metabolites found in many other green plants.[17]
Several studies show the potential of flavonoid compounds with their ability as anti-inflammatory to reduce TNF-α levels through its mechanism as an anti-oxidant. Based on research conducted by Yang CY *et al.*, 2022 : *Artocarpus Altilis woody bark extract* contains flavonoid compounds, can reduce intracellular ROS overproduction, reduce the expression of inflammatory proteins, including TNF-α and TNF-1 receptors (TNFR1).[18]
Experimental studies in *vivo and in* vitro *conducted by Tiraravesit* et al., *2015, reported:* Artocarpus altilis wood stem extract *contains flavonoid compounds, topically can suppress structural changes in skin damaged by UVB radiation, decreased production of MMP-1 in fibroblasts, and decreased production of TNF-α and IL-6 in keratinocytes* .[19]
Research by Yang *et al.*, 2022, reports : topical *Artocarpus Altilis* extract has the potential to be developed as an anti-oxidant active ingredient to prevent skin oxidation, inflammation and aging, and restore the skin's protective function.[18]
The use of serum preparations as cosmetic products to lighten the skin is widely found, because serum has the advantages that the active substances contained in it are more, high absorption, and official spreadability. Serum has small particles, comfortable, and easy to apply, so serum is faster and more effective in overcoming skin problems.[20]
So in this study will be examined on the influence of sukun leaf extract (*Artocarpus Altilis*) on the rate of TNF-α and SOD on UVB ray-shown marmots, which are given in the form of topical preparations in the form of serum.

II. MATERIAL AND METHOD

Study Design and Experimental Animals

This research is an *in vivo* experimental study with *Post Test Only Group design*, which was conducted at the IBL FK Laboratory (*Integrated Biomedical Laboratory* Faculty of Medicine) Unissula. Study subjects used guinea pigs (*Cavia porcellus*) males, aged 2-3 months with a body weight of 300-350 grams that fit the criteria of inclusion and inclusion, adapted for 7 days. This study used 5 groups, namely: Sham control group 1, *healthy* guinea pigs that were not exposed to UVB and were not treated (KS); group 2 negative control, guinea pigs applied serum base 20 minutes before exposure to UVB light, dose 65 mJ/cm^2 for 130 seconds, after 4 hours of exposure reapplied serum base, UVB exposure was carried out 3x a week for a period of 28 days, so that the total dose is 780 mJ/cm^2; group 3 P1 treatment, such as the process on KN, which uses 2% breadfruit leaf extract serum; group 4 P2 treatment, using 4% breadfruit leaf extract serum; and group 5 P3 treatment, using 6% breadfruit leaf extract serum. On the 29th day, a temination was carried out to take samples of skin tissue.

Research Materials

The research material used parafilm, 3% H2O2 solution, ethanol concentration 70%, 80%, 90%, 100% I, 100% II, xylene, PBS, citrate buffer, ELISA Kit SOD, ELISA Kit TNF-α, guinea pig skin tissue samples, and aquades.

Research Equipment

This research uses equipment consisting of *microplate reader*, 37°C incubator, micropipette, tip, beaker glass, 1.5 ml microtube, tissue, *marker ink*, slides rack, dip rack, *humidity chamber,* microwave*, deck glass, entellan, tissue, and timer*.

Screening and Flavonoid Test

Flavonoid screening and testing are carried out by: **(1)** Flavonoid analysis is carried out using quercetin standard solution reagents Concentrations of 2ppm, 4ppm, 6ppm, 8ppm, and 10 ppm. **(2)** Absorbance measurement using UV-Vis spectrophotometer with wavelength 415 nm. **(3)** Absorbance carried out for flavonoid testing was carried out with variations in ethanol concentrations, namely 60%, 70%, 80%, 90% and 99.8% and variations in massification time of 24 hours, 30 hours, 36 hours, 42 hours and 48 hours. **(4)** The result of the absorbance value is entered as the value of x in the standard curve regression equation. **(5)** Obtained flavonoid levels from breadfruit leaves.

How to Make Breadfruit Leaf Extract

Making breadfruit leaf extract is massed with 70% ethanol. The procedure is as follows: **(1)** Selection of breadfruit leaves to be extracted, drying with a 35^{o}C temperature oven. **(2)** Making masseration by grinding breadfruit leaves with a blender up to 40 messes. **(3)** After obtaining breadfruit leaf powder, extraction is carried out using 70% ethanol. **(4)** Filtering process with filter paper until filtration is obtained. **(5)** Evaporation of ethanol with *a rotary vacuum evaporator* to separate flavonoids from ethanol. **(6)** Obtained flavonoid results from breadfruit leaves.

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How to Make Breadfruit Leaf Extract Serum,[21]

Procedures : (1) Carbomer (base) is weighed according to the amount and then developed with hot water temperature 80 ° C for 5 hours. (2) The fluffy carbomer is stirred and added TEA until thickened. (3) Sodium benzoate and disodium EDTA are dissolved into glycerin and then mixed into the base. (4) Breadfruit leaf extract (made *base serum and* preparation of 3 serum formulas of breadfruit leaf extract with concentrations of 2%, 4% and 6%), dissolved with 5 mL of 70% ethanol then added to the base mixture and stirred until homogeneous. (5) The serum formed is carried out various tests, namely pH, viscosity, dispersion and adhesion

Dosage Assignment

The dose of topical breadfruit leaf serum extract was determined before the study was conducted based on literature studies. Previous research stated: giving breadfruit leaf extract cream (*Artocarpus altilis)* 3% can prevent an increase in skin melanin in guinea pigs (*Cavia procellus*) exposed to UVB light. The application of breadfruit leaf extract cream (*Artocarpus altilis) 3% is as* effective as the 4% hydroquinone cream in preventing an increase in the amount of melanin in guinea pig skin.[10] In another experimental in *vitro* study conducted by Sholikha M, et al., confirmed that: breadfruit extract gel levels of 2%, 4%, and 6% inhibited tyrosinase with L-Dopa substrate, with formaula respectively 40.35%, 39.11% and 37.21%.[16] In this study using doses of 2%, 4% and 6% breadfruit leaf extract serum applied topically on guinea pig skin exposed to UVB light.

Network Sampling for ELISA Examination

Guinea pigs on the 29th day are terminated using chloroform liquid that has been moistened on a cotton swab, then put in a closed container. Furthermore, tissue samples were taken by biopsy on the back with a size of 1x1 cm to subcutaneous, thickness of approximately 2 mm.[2,22] The tissue sample is put into a tube and soaked with PBS liquid, then stored in a freezer at -80^{o} so that the ELISA analysis process can be carried out.

ELISA TNF-α and SOD Inspection Procedures

Procedures : (1) Prepare all reagents, standard solutions and samples according to instructions. Bring all reagents to room temperature before use. Tests are carried out at room temperature. (2) Determine the number of strips required for testing. Put the remaining strips in a zipped aluminum for storage. (3) Unused strips should be stored at 2-8°C. (4) Add standard 50µl to standard wells. Note: Do not add antibodies to the standard because standard solutions contain biotin labeled antibodies. (5) Add 40µl of sample that has been sonicated, and lysed with PBS to the sample well and then add 10µl of anti-TNF-α or SOD antibodies to the sample well, then add 50µl of streptavidin-HRP to the sample well and standard well (Not a blank well). Mix well. Cover the plate with a sealer. Incubation 60 minutes at 37°C. (6) Remove sealer and wash well 5 times with wash buffer of at least 0.3 ml for 30 seconds to 1 minute for each wash. (7) Add 50µl of substrate A solution to each well and then add 50µl of substrate B solution to each sump. (8) Incubation plate covered with new sealer for 10 minutes at 37°C in a dark place. (9) Add 50µl Stop Solution to each sump, the blue color will instantly change to yellow. (10) Determine the OD value of each well using a microplate reader set at 450 nm within 10 minutes after adding the stop solution.

III. RESULT

Determination and Extraction of Breadfruit Leaf Serum

The breadfruit leaves (*Artocarpus Altilis*) used in this study were obtained from local plantations in the city of Semarang. Material determination was carried out at the Biology Laboratory of Semarang State University.

Breadfruit leaves are dried and macerated with 70% ethanol for 3 days, then filtered using filter paper, the residue obtained is remacerated with the remaining ethanol solvent for 2 days to extract the active substance in breadfruit leaves. The filtrate obtained is evaporated using a *rotary evaporator* and *waterbath* so that a thick extract of breadfruit leaves is obtained. At the end of the formulation the oil phase chosen is *carbomer*, because it has good base-forming properties in the process of making serum. The preparation is made into 1 *serum base and* 3 other doses, namely 2%, 4% and 6%. Phytopharmaceutical tests have been carried out, the results show: breadfruit leaf extract serum is positive for alkaloids, flavonoids, saponins and tannins.

The effect of giving breadfruit leaf extract serum (Artocarpus Altilis) on TNF-α levels in guinea pig skin exposed to UVB light

After 28 days of UVB exposure and treatment, after another 24 hours on the 29th day, guinea pigs were terminated and skin tissue samples were taken and analyzed using the ELISA examination method to measure TNF-α and SOD levels in each group.

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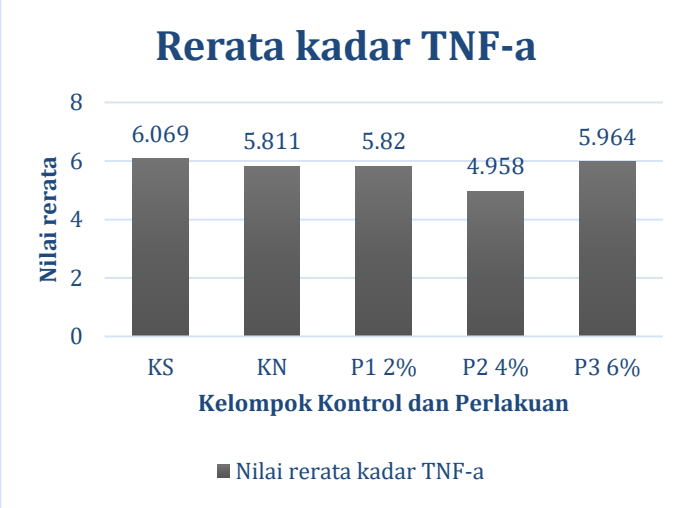
Table 1. Test of normality and homogeneity of TNF-α levels between research groups after treatment

Group	Average	SD	*Shaphiro Wilk*	*Levene test*	Oneway ANOVA
KS	6,069	1,40	0,200	0,725	0,508
KN	5,811	1,04	0,200		
P1	5,820	0,88	0,200		
P2	4,958	1,04	0,200		
P3	5,964	1,42	0,096		

Mark * = Significant meaning (p<0.05)



Graph 1. Mean value of TNF-α levels between study groups after treatment

Based on table 1. and Graph 1. above, the lowest average value of TNF-α levels in the P2 treatment group (serum breadfruit leaf extract 4%) was 4.958 ± SD 1.04; while the average TNF-α levels in the *Sham* Group (KS) were 6.069 ± SD 1.40; and Negative control group (KN) 5.811 ± SD 1.04. Normal distributed data in all study groups with a value of p>0.05, homogeneous data variant with a *Levene test value of 0.725* (p>0.05). In the One-way ANOVA test, a value of 0.508 (p>0.05) was obtained, meaning that although TNF-α levels in the P2 treatment group (4% breadfruit leaf extract serum) were the lowest, there was no significant difference in TNF-α levels between research groups.

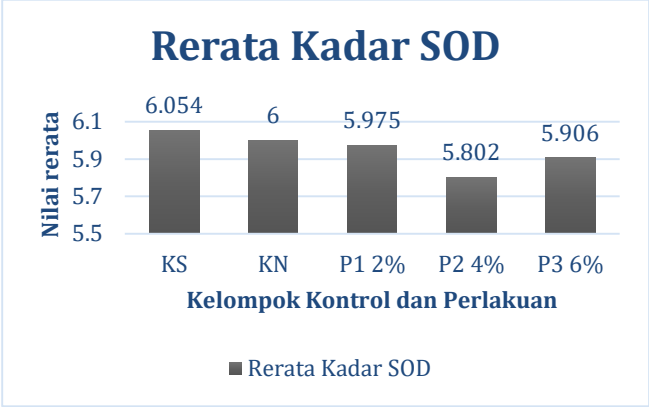
The effect of serum giving of sukun leaf extract (Artocarpus Altilis) on SOD levels on the skin of marmots exposed to UVB rays

Results of ELISA test analysis of SOD levels between study groups after treatment as seen in the table below.

Table 2. Test of normality and homogeneity of SOD levels between study groups after treatment

Group	**Average**	**SD**	***Shaphiro Wilk***	***Levene test***	**One-way ANOVA**
KS	6,054	0,84	0,895	0,709	0,979
KN	6,000	0,79	0,967		
P1	5,975	0,45	0,901		
P2	5,802	0,81	0,980		
P3	5,906	0,68	0,898		

Mark * = Significant meaning (p<0.05)



Graph 2. Average values of SOD levels between study groups after treatment

Based on table 2. and Graph 2. above, the average value of SOD levels was not much different in all groups, both in the control and treatment groups. The average SOD levels in the P2 group (4% breadfruit leaf extract serum) were 5.802 ± SD 0.81; while the average SOD level in the *Sham* Group (KS): 6,054 ± SD 0.84; and the KN Group: 6,000 ± SD 0.79. Normal distributed data in all groups with p>0.05 value, homogeneous data variant with *Levene test value* 0.709 (p>0.05). In the One-way ANOVA test, a value of 0.979 (p>0.05) was obtained, meaning that there was no significant difference in SOD levels between research groups.

IV. DISCUSSION

Continuous exposure to UVB rays can cause skin changes, namely epidermal thickness, dermal elastase, decreased amount of ECM protein, increased MMP activity and collagen fragmentation, increased inflammatory infiltrates and *telangiectasia*. In addition, UVB can trigger keratinocyte DNA damage that initiates the release of inflammatory mediators, such as cytokines IL-1α, IL-6, and TNF-α. UVB also directly induces p53 which will affect the amount of skin melanin.[1]

UVB radiation also triggers increased ROS causing an increase in inflammatory cytokines, *Growht Factor* and reseptop activator (increase in NF-kB and AP1, decrease in TGF-β), resulting in decreased collagen production, increased collagen breakdown, increased elastin accumulation characterized as photoaging, solar elastosis, wrinkel, telangiectasis and pigmentation.[23]

UVB radiation induces melanogenesis via p53 triggering increased proopiomelanocortin (POMC) expression to secrete αMSH which regulates MITF expression, further triggering the enzymes tyrosinase, Tyrp1 and Tyrp2. In addition, UVB radiation increases the production of reactive oxygen species (ROS) in keratin and melanocyte cells, and at high concentrations ROS causes DNA damage, activates p53 further, and thus triggers melanogenesis.[6]

An important thing to pay attention to, in the process of increasing skin melanin pigment (melanogenesis) as revealed in the article above is the presence of UVB radiation will trigger an increase in ROS and inflammatory mediators such as TNF-α. Therefore, this study specifically examined two relevant parameters, namely SOD as an inhibitor of ROS, and inflammatory mediators in the form of TNF-α, both of which are directly involved in the process of melanogenesis. The active ingredient object that we chose is breadfruit leaf extract serum because, which is believed to have a natural effect on inhibiting the process of melanogenesis.

Breadfruit leaves (*Artocarpus altilis*) contain flavonoid compounds that can act as antioxidants inhibitors of ROS, anti-inflammatory and inhibit the activity of tyrosinase enzymes which are efficacious as skin lighteners. Breadfruit leaf extract contains saponins, flavonoids, triterpenoids and steroid compounds. The presence of phenolic compounds and flavonoids that have been reported such as flavonols, stilbens, phenolic acids, and quercetin contribute to inhibiting tyrosinase activity so as to prevent skin hyperpigmentation.[16]

This study uses preparations in the form of serum, because one of the advantages is that the active substances contained in serum can be more than other cosmetic preparations so that serum is faster and more effective in overcoming skin problems.[24]

UVB exposure causes increased secretion of IL-1 and TNF-α, stimulating tyrosinase through a specific intracellular signaling cascade, which is activated after binding of endothelin-1 (EDN1*) or stem* cell factor (SCF) to either endothelin B receptor (EDNRB) or stem *cell growth factor* receptor, known as *proto-oncogen c-KIT* (c-KIT), then melanocyte activation occurs resulting in stimulation of epidermal pigmentation.[25]

The results of this study showed that the lowest average value of TNF-α levels in the P2 treatment group (serum breadfruit leaf extract 4%) was 4.958 ± SD 1.04; however, it was not significantly different when compared to the control group of both KS and KN. While the average value of TNF-α levels in the P1 and P3 treatment groups was almost the same as the control group (KS and KN), and did not differ significantly. All treatment groups both P1, P2, and P3 when compared with each other were also not

significantly different.

The decrease in TNF-α levels due to the influence of breadfruit leaf extract serum in this study is in accordance with the theory expressed by Imokawa G, regarding the mechanism of inhibition of intracellular signaling pathways causing depigmentation.[25] The results of this study are in accordance with in *vivo and in* vitro *experimental studies conducted by Tiraravesit* et al*., in 2015, on the use of* Artocarpus altilis wood stem extract *topically can reduce TNF-α and IL-6 in keratinocyte tissue* .[19] In another study by Yang *et al*., 2022, also reported that *Artocarpus Altilis extract* has anti-aging and anti-inflammatory effects by regulating phosphorylation of MAPK and NF-kB signaling proteins.[18]

This study also examined tissue SOD levels, The result was that the average value of SOD levels in all groups both control and treatment was almost the same, and there was no significant difference. The average value of SOD levels in the P2 group (serum breadfruit leaf extract 4%) was 5.802 ± SD 0.81; not significantly different when compared to the control group both KS and KN. All treatment groups both P1, P2, and P3 when compared with each other were also not significantly different.

The decrease in TNF-α levels in this study occurred in the P2 treatment group, while in the other treatment groups (P1 and P3) there was no decrease. While SOD did not change in all treatment groups (P1, P2, and P3). This is a limitation in research, insignificant changes in TNF-α and SOD may be caused by: the small number of research samples, or the sensitivity of measuring instruments using ELISA examination skin tissue is less sensitive in detecting changes, especially SOD levels.

V. CONCLUSION

1. Serum administration of breadfruit leaf extract (*Artocarpus Altilis*) can reduce TNF-α levels in guinea pig skin exposed to UVB rays, but not significantly.
2. Serum administration of breadfruit leaf extract (*Artocarpus Altilis*) did not have a significant effect on increasing SOD levels in guinea pig skin exposed to UVB rays.
3. Giving serum breadfruit leaf extract (*Artocarpus Altilis*) 4% can reduce TNF-α levels but not significantly, but there is no increase in SOD levels in guinea pig skin exposed to UVB light.

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