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The Effect of Dayak Onion (*Eleutherine Palmifolia*) Extract Cream Application on Serum Interleukin-6 Levels: An Experimental Study in UVB-induced Male Wistar Rats

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ABSTRACT

Background: Indonesia receives intense ultraviolet B (UVB) exposure. Prolonged exposure to (UVB) radiation is a major environmental factor contributing to oxidative stress and skin inflammation through the overproduction of reactive oxygen species (ROS) and subsequent upregulation of pro-inflammatory cytokines, including interleukin-6 (IL-6). *Eleutherine palmifolia*, traditionally used in Indonesian herbal medicine, contains abundant flavonoids and phenolic compounds with documented antioxidant and photoprotective properties. Its ability to absorb UV rays and neutralizing ROS suggests potential efficacy as a topical agent for mitigating UVB-induced inflammatory responses.
Objective: This study aimed to evaluated the effect of *Eleutherine palmifolia* extract cream at various concentrations (10%, 15%, 20%) on serum IL-6 levels in UVB-induced male Wistar rats.
Methods: : A true experimental design with post-test only control group was conducted using 36 male Wistar rats randomly assigned to four groups: control (placebo), P1 (10%), P2 (15%), and P3 (20%). The cream was applied 20 minutes before UVB exposure and again 4 hours after irridation, three times per week for 30 days. Serum IL-6 levels were measured using ELISA. Statistical analysis was performed using Shapiro–Wilk, Levene's test, one-way ANOVA, and LSD post-hoc test.
Results: Mean IL-6 levels (pg/mL): control 1.63; P1 1.59; P2 1.65; P3 1.57. ANOVA indicated a significant differences among groups ($p = 0.047$). Post-hoc analysis showing a significant difference only between P2 and P3 groups ($p = 0.012$).
Conclusion: The 20% *Eleutherine palmifolia* cream showed the strongest anti-inflammatory effect, significantly lowering IL-6 compared with 15%, while other group differences were not significant.

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1. Introduction

Indonesia has a high level of sunlight exposure, and most work is performed outdoors. Consequently individuals are often exposed to high levels of sun. Sunshine exposure offers important health bonuses to the human body, especially in promoting the production of vitamin D, which has a part in bone turnover and immune function.[1] Conversely, excessive exposure to sunlight is a prominent exogenous skin damage mediator.[2]

Ultraviolet (UV) radiation is a component of the electromagnetic spectrum present in sunlight. UV radiation is classified according to its wavelength into UVA (320–400 nm), UVB (290–320 nm), and UVC (<290 nm). Excessive exposure to UV radiation on the skin increases the risk of skin damage.[3] Several conditions associated with such damage include sunburn, tanning, edema, erythema, photoaging, skin cancer, and hyperplasia.[4–6] Other consequences of UVB exposure include redness, wrinkling, dryness, burning, and irritation of the skin.[7] UVB penetration into the epidermis can also reduce collagen levels and generate reactive oxygen species (ROS), leading to DNA damage and the release of interleukin-6 (IL-6). Excessive ROS production causes redox imbalance, resulting in elevated expression of pro-inflammatory mediators such as IL-2, IL-6, and tumor necrosis factor-α (TNF-α). The normal serum concentration of IL-6 ranges from 1–5 pg/mL.[8] Values above this threshold indicate an inflammatory process. To mitigate the harmful effects of UV radiation, one of the preventive measures is the use of sunscreen cream. Sunscreens function by absorbing, scattering, and reflecting UV radiation to protect the skin from damage.[9]

Flavonoids are compounds capable of protecting the skin from UV exposure by absorbing UV radiation and scavenging ROS. Their aromatic ring structure enables absorption of both UVA and UVB, thereby functioning as an optical shield.[10] One plant with a high flavonoid content is the Dayak onion (*Eleutherine palmifolia*), which

originates from Kalimantan and has long been used as traditional medicine by the Dayak ethnic community.[11–13] *Eleutherine palmifolia* contains various secondary metabolites, including phenols, tannins, flavonoids, steroids, alkaloids, proteins, reducing sugars, terpenoids, and exhibits antioxidant activity with an IC_{50} value of 45.33 ppm.[11,12] Its flavonoid and phenolic contents have been reported to reduce IL-6 levels through anti-inflammatory, antioxidant, and immunomodulatory mechanisms.[14]

This study aims to evaluate the effect of topical application of *Eleutherine palmifolia* extract cream on serum interleukin-6 levels in male Wistar rats induced by UVB radiation.

2. Methods

This study was conducted at the Cendekia Nanotech Hutama Laboratory, the Biology Laboratory of Universitas Negeri Semarang, and the CITO Clinical Laboratory between May and June 2025. The research employed an experimental design using a Post-Test Only Control Group Design. A total of 36 male Wistar rats were obtained from the Biology Laboratory of Universitas Negeri Semarang as research subjects.

The inclusion criteria were male Wistar rats aged 8–12 weeks, weighing 200–250 grams, and in healthy and active condition. The exclusion criteria included Wistar rats with skin or anatomical abnormalities, wounds, signs of inflammation, or illness. The independent variable in this study was the topical application of *Eleutherine palmifolia* (Dayak onion) extract cream at concentrations of 10%, 15%, and 20%. The dependent variable was the serum IL-6 levels.

The 36 rats were randomly allocated into four groups, each consisting of nine rats that met the inclusion and exclusion criteria. Prior to treatment, the animals underwent a seven-day acclimatization period. The control group (K) received placebo cream (base cream without *Eleutherine palmifolia* extract), while the treatment groups (P) received extract creams according to their assigned concentrations. UVB irradiation was administered for 60 minutes using a narrowband UVB lamp with an irradiance of 3 mW/cm², corresponding to a total dose of 180 mJ/cm², and irradiance calibration was performed prior to the experiment to ensure consistent exposure across all subjects. The extract cream was applied 20 minutes before UVB exposure and again 4 hours after irradiation, with each treatment session conducted once every two days (three times per week) for a total duration of 30 days, providing a consistent and reproducible treatment schedule throughout the study. From each group, six rats were randomly selected by lottery for blood sampling. This sample size was determined based on WHO guidelines, which recommend a minimum of 5–6 animals per group to ensure statistical validity.

The primary data collected were the serum IL-6 levels, measured from blood samples. Statistical analysis began with a normality test using the Shapiro–Wilk test. Once normal distribution was confirmed, homogeneity of variance was assessed with Levene's test. If variances were homogeneous, parametric analysis was performed using one-way ANOVA to compare mean values across groups, followed by a Post Hoc test to determine significant differences between groups. One of the Post Hoc methods applied was the Least Significant Difference (LSD) test.

3. Results

During the treatment period, two rats from the control group (K), one rat from group P2, and one rat from group P3 dropped out due to death. Serum IL-6 levels were measured using the ELISA method.

Table 1. Comparative Analysis of Serum Interleukin-6 Levels

Treatment Group(s)	Mean ± *Standard Deviation*	Median (min-max)	*P Value*
K	1.63± 0.02	1.62 (1.57-1.69)	0.93*
P1	1.59 ± 0.02	1.58 (1.50-1.65)	0.89*
P2	1.65 ± 0.03	1.63 (1.58-1.76)	0.61*
P3	1.57 ± 0.02	1.57 (1.50-1.64)	0.88*

Note: Normal distribution, $p > 0.05$

Based on the Shapiro-Wilk test, all groups (K, P1, P2, and P3) showed significance values of $p > 0.05$. This indicates that the data were normally distributed within each group and therefore met the assumption of normality, allowing further analysis using parametric tests.

Table 2. Levene's Test Results on Serum Interleukin-6 Levels

	Levene Statistic	P Value
Based on Mean	0.22	0,88*

Note: Homogeneous if $p > 0.05$

After confirming that the data were normally distributed, homogeneity of variance was then tested using Levene's test. As shown in Table 6, Levene's test yielded a significance value of 0.884, well above the threshold of 0.05, indicating that the variances among groups were homogeneous. Hence, the data fulfilled the assumptions required for ANOVA.

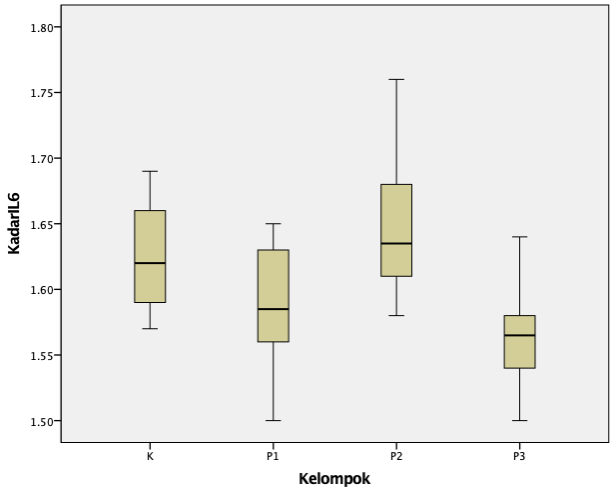

Figure 1. Boxplot of Differences in Serum Interleukin-6 Levels

Since both assumptions for parametric testing were met, comparative analysis was performed. This study employed univariate comparative analysis, as only one dependent variable (serum IL-6 levels) was assessed across four groups. The analysis aimed to determine whether there were significant differences in mean IL-6 levels among groups. Therefore, a one-way ANOVA was applied.

Table 3. Results of ANOVA on Serum Interleukin-6 Levels

	Sum of Squares	df	Mean Square	F	P Value
Between Groups	0.03	3	0.009	3.15	0.047
Within Groups	0.06	20	0.003		
Total	0.08	23			

Note: Significant if $p < 0.05$

The ANOVA test showed a significance value of 0.047 ($p < 0.05$), indicating a statistically significant difference in mean serum IL-6 levels among groups. Post hoc analysis using the Least Significant Difference (LSD) method was then conducted.

Table 4. Results of Least Significant Difference (LSD) Test on Serum Interleukin-6 Levels

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K	P1	.04000	.03056	.205	-.0238	.1038
	P2	-.02500	.03056	.423	-.0888	.0388
	P3	.06000	.03056	.064	-.0038	.1238
P1	K	-.04000	.03056	.205	-.1038	.0238
	P2	-.06500*	.03056	.046	-.1288	-.0012
	P3	.02000	.03056	.520	-.0438	.0838
P2	K	.02500	.03056	.423	-.0388	.0888
	P1	.06500*	.03056	.046	.0012	.1288
	P3	.08500*	.03056	.012	.0212	.1488
P3	K	-.06000	.03056	.064	-.1238	.0038
	P1	-.02000	.03056	.520	-.0838	.0438
	P2	-.08500*	.03056	.012	-.1488	-.0212

Note: Significant if $p < 0.05$

The LSD test revealed that only the comparison between groups P2 and P3 showed a significant difference ($p = 0.01 < 0.05$). This indicates that rats treated with *Eleutherine palmifolia* extract cream at 20% concentration (P3) had significantly lower serum IL-6 levels compared to those treated with 15% concentration (P2). Meanwhile, all other pairwise comparisons, including P1 (10%) and P2 (15%) versus the control group, did not demonstrate significant differences ($p > 0.05$).

4. Discussion

This study evaluated the effect of Dayak onion (*Eleutherine palmifolia*) extract cream on serum IL-6 levels in male Wistar rats following 30 days of UVB exposure. During the experiment, a small proportion of rats dropped out due to mortality, which may reflect individual variations in sensitivity.[15] Dropouts may also have resulted from UVB-induced suppression of cutaneous and systemic immunity, leading to a decline in immune status that varies between individuals.[16]

The results demonstrated variation in mean IL-6 levels among the groups, although not all differences were statistically significant. Group P3 (20% extract) showed the lowest IL-6 levels (1.57 pg/mL) and was the only group with a statistically significant reduction compared to P2 (15%) ($p = 0.012$). In contrast, differences involving the control, P1, and P2 groups were not significant ($p > 0.05$), despite consistent descriptive reductions suggesting a dose-response pattern.

The dose-dependent effect observed aligns with prior research demonstrating that plant-based topical extracts tend to reduce inflammatory cytokines even when significance is not consistently achieved.[17,18] Thallib et al. (2023) reported IL-6 reductions with 7.5% red dragon fruit extract cream in acute wounds, although intergroup differences were not statistically significant.[19] Similarly, Ekasari et al. (2023) examined 10% *Physalis angulata* leaf extract cream in BALB/c mice with atopic dermatitis and found reductions in IL-6 and IgE levels in the treatment group compared with controls, although these were not statistically significant.[20] They attributed this to the anti-inflammatory properties of flavonoids, while acknowledging that biological variability and immune status may influence IL-6 responses, leading to heterogeneous results. These findings are comparable to the non-significant but directionally consistent reductions in P1 and P2 of this study. Conversely, Hendrayanta et al. (2024) demonstrated that topical application of *Annona squamosa* L. leaf extract cream in UVB-exposed Wistar rats for four weeks significantly reduced TNF-α levels and increased TIMP-1, with the 20% concentration being most effective.[21] These effects were attributed to high flavonoid and phenolic content, which suppress pro-inflammatory pathways, support tissue regeneration, and provide strong antioxidant activity.[22]

Collectively, previous literature indicates that plant-derived topical formulations generally lower inflammatory mediators, though statistical outcomes

depend on concentration, timing, and inflammatory phase. The present findings reinforce that the 20% *Eleutherine palmifolia* extract concentration yields the most robust anti-inflammatory effect, supporting its potential as a topical photoprotective agent. This supports the hypothesis that Dayak onion extract, rich in naphthoquinones and flavonoids, possesses strong topical anti-inflammatory potential, particularly when applied at adequate doses and for sufficient duration to suppress peak inflammatory responses.

This study specifically focused on the effects of *Eleutherine palmifolia* extract cream on serum IL-6 in UVB-induced sunburn in male Wistar rats. UVB radiation is known to induces the overproduction of reactive oxygen species (ROS), which activate MAPK pathways and stimulate IL-6 secretion. Elevated IL-6 promotes fibroblast activity and MMP production, contributing to extracellular matrix degradation.[23]

The extract cream contains flavonoids and naphthoquinones with well-established antioxidant and anti-inflammatory activity.[24] Flavonoids, with their aromatic ring structure, are capable of absorbing UVA and UVB, thereby functioning as natural photoprotectants while suppressing ROS formation and subsequent inflammation.[10,25] This mechanism underlies the potential of Eleutherine palmifolia extract in preventing UVB-induced IL-6 elevation.

The lack of statistical significance in groups P1 and P2 may be partly explained by the timing of sample collection in the late afternoon, when IL-6 levels are physiologically lower due to circadian variation, thereby reducing statistical sensitivity.[26–28] Moreover, at this time point, the inflammatory response may have already shifted into the proliferative phase, during which IL-6 naturally declines. This is consistent with previous reports showing that UVB-induced inflammation is transient, typically resolving into tissue repair between days 4 and 7 after irradiation.[29,30] Biological variability among rats, including immune status, individual metabolism, and differential sensitivity to UVB, may also account for heterogeneity in IL-6 expression and the absence of statistical significance in some comparisons.

The Synchro®-based cream contains multiple bioactive compounds, including calendula oil, beeswax, sweet almond oil, B-complex vitamins, vitamins A, C, E, and H, *Hypericum* extract, fatty acid esters, biological extracts, amino acids (glutamine, arginine, lysine), magnesium, and potassium, which collectively exert antioxidant and anti-inflammatory effects[31]. Vitamins A, C, and E, together with essential minerals such as zinc, are crucial for collagen synthesis and tissue repair, with vitamin C in particular demonstrating strong antioxidant capacity in modulating immune responses and neutralizing free radicals. *Hypericum* extract has been reported to suppress pro-inflammatory cytokines such as IL-6 and TNF-α during wound healing, and recent in vitro studies confirmed that Calendula officinalis possesses significant anti-inflammatory and antioxidant activity.[32,33] Consequently, Synchro® cream should not be regarded as a passive vehicle, since its intrinsic antioxidant components may modulate therapeutic outcomes and act synergistically with *Eleutherine palmifolia* extract in suppressing IL-6 expression.[31,32] Conversely, repeated shaving of the dorsal skin may induce mild irritation and contribute to subtle increases in cytokines such as IL-6.[34,35]

Despite these limitations, the overall downward trend in IL-6 across treatment groups supports the anti-inflammatory potential of Eleutherine palmifolia extract cream, particularly at the 20% concentration. This study provides preliminary evidence for its use as a topical agent to mitigate UVB-induced inflammatory responses.

5. Conclusion

The application of *Eleutherine palmifolia* extract cream influenced serum IL-6 levels in UVB-induced male Wistar rats, although most intergroup differences were not statistically significant. While the 10% and 15% extract concentrations showed only descriptive changes compared with the control group, the 20% concentration produced the lowest IL-6 level and demonstrated a significant difference when compared with the 15% group, indicating a dose-dependent trend. Overall, the findings suggest that higher concentrations of *Eleutherine palmifolia* extract, particularly the 20% formulation, have greater potential in reducing UVB-induced inflammatory responses.

Ethical Approval

This study received ethical clearance from the Health Research Ethics Commission (KEPK), Faculty of Medicine UNDIP, under approval No. 044/EC/KEPK/FK-UNDIP/III/2025.

Conflicts of Interest

The authors declare no conflicts of interest related to this study.

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Author Contributions

Conceptualization, AFC, LA, GSD; methodology, AFC, LA, GSD, W; validation, LA, GSD; data analysis, AFC; investigation, AFC, LA, W; resources, AFC; data curation, AFC; original draft preparation, AFC; review and editing, AFC, LA, GSD, W; supervision, LA, GSD, W; funding acquisition, AFC.

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