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Original Article

Topical *Catharanthus roseus* gel modulates TNF-α expression and SOD activity in UVB-induced skin: an in vivo experimental study

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A B S T R A C T

Background: Subchronic UVB exposure induces oxidative stress and skin inflammation, characterized by increased TNF-α expression and decreased antioxidant activity, including superoxide dismutase (SOD). *Catharanthus roseus* contains bioactive compounds with potential anti-inflammatory and antioxidant properties; however, evidence regarding its topical effects on UVB-induced skin damage remains limited.

Objective: To evaluate the effects of *Catharanthus roseus* extract gel on TNF-α expression and SOD activity in UVB-induced skin and to compare the effectiveness of 15% and 30% concentrations.

Methods: A post-test-only controlled experimental study was conducted using 30 mice divided into five groups: normal control, negative control, positive control (vitamin E), 15% extract gel, and 30% extract gel. TNF-α expression and SOD activity were measured using ELISA and analyzed with one-way ANOVA.

Results: UVB exposure significantly increased TNF-α expression and decreased SOD activity in the negative control group compared with the normal group. Treatment with Catharanthus roseus extract gel improved both parameters. No significant differences were observed between the 15% and 30% concentrations for TNF-α ($p = 0.376$) or SOD activity ($p = 0.237$), indicating comparable effects.

Conclusions: Topical *Catharanthus roseus* extract gel modulates TNF-α expression and SOD activity in UVB-induced skin. Comparable effects between 15% and 30% concentrations suggest that the lower concentration may be sufficient to achieve the desired biological response.

INTRODUCTION

Chronic ultraviolet exposure is a major contributor to photoaging, characterized by reduced collagen density, decreased skin elasticity, and the formation of wrinkles.[1] These structural changes are primarily driven by oxidative stress and chronic inflammation, in which excessive reactive oxygen species (ROS) stimulate pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) while suppressing endogenous antioxidant enzymes, including superoxide dismutase (SOD).[2,3] This imbalance accelerates dermal matrix degradation and impairs skin regeneration. Therefore, regulating inflammatory mediators and restoring antioxidant defenses are key strategies in preventing UV-induced skin damage.

The burden of photoaging is particularly significant in tropical regions with year-round high ultraviolet exposure. Populations with Fitzpatrick skin type IV, which are common in these regions, are susceptible to early manifestations of photoaging.[4] Epidemiological data from Jakarta indicate that nearly half of adolescents aged 18–21 years exhibit signs of premature skin aging, underscoring the need for safe, effective preventive interventions suitable for long-term use.

Conventional topical agents such as hydroquinone are effective in managing UV-related skin disorders but are limited by adverse effects, including irritation and potential genotoxicity.[5,6] This has led to increasing interest in plant-based therapies with antioxidant and anti-inflammatory properties. One promising candidate is *Catharanthus roseus* (periwinkle), which contains bioactive alkaloids and flavonoids, including vincristine, vinblastine, catharanthine, and vindoline.[7,8] These compounds have been reported to modulate inflammatory pathways and enhance tissue repair.[9,10]

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Previous studies have shown that C. roseus extract can reduce TNF-α expression and enhance collagen formation in wound-healing models.[11,12] However, most studies have focused on wound healing or systemic administration rather than UV-induced skin inflammation. Evidence regarding the topical application of C. roseus in gel formulations and its specific effects on TNF-α and SOD under subchronic UVB exposure remains limited.

Gel-based formulations offer advantages such as good skin adherence, non-greasy texture, and improved patient acceptability, making them suitable for the topical delivery of antioxidant compounds.[13] However, comparative data on different concentrations of C. roseus extract gel and their biological efficacy in UVB-irradiated skin are still lacking. Therefore, this study aims to evaluate the effects of C. roseus extract gel on TNF-α expression and SOD activity in UVB-induced skin and to compare the effectiveness of 15% and 30% concentrations.

METHOD

Study Design

This study was a true experimental investigation using a post-test-only control group design.[14]

Study Site

The study was conducted at the Department of Physiology, Faculty of Medicine, Universitas Brawijaya, Malang, from August to November 2025. Preparation of C. roseus extract was performed at STIFAR Semarang prior to the intervention phase.

Preparation of Plant Extract and Gel Formulation

C. roseus flowers were dried at 50°C, ground into powder, and sieved. Approximately 500 g of powder was macerated in 70% ethanol for five days with periodic agitation. The filtrate was collected, re-macerated twice, and concentrated using a rotary evaporator to obtain a viscous extract. Gel formulations were prepared using polyvinyl alcohol and HPMC as base materials. The extract was incorporated to obtain 15% and 30% gel formulations.

In Vivo Experimental Procedure

Thirty male C57BL/6 mice (6–8 weeks; 25–30 g) were acclimatized for seven days under controlled conditions with free access to food and water. Mice were randomly assigned into five groups: normal control, negative control (UVB + base gel), positive control (UVB + vitamin E), and treatment groups receiving 15% and 30% C. roseus extract gel.

Dorsal hair was shaved prior to UVB exposure. Subchronic UVB irradiation was administered once daily for 14 days using a broadband UVB lamp (302 nm). Mice were anesthetized, and UVB was delivered at 0.5 J/cm² for 10 minutes. Topical treatments were applied once daily, one hour after irradiation.

Sample Collection

Twenty-four hours after the final treatment, mice were anesthetized, and blood samples were collected via cardiac puncture. Serum was obtained by centrifugation and stored at −80°C until analysis.

Histological and Biochemical Analyses

Collagen structure was assessed using Masson's Trichrome staining on paraffin-embedded skin sections. Collagen fibers were visualized as blue staining and evaluated under a light microscope at 400× magnification. Serum TNF-α and SOD levels were measured using ELISA kits according to the manufacturer's instructions. Absorbance was read at 450 nm, and concentrations were calculated from standard curves.

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test and homogeneity using Levene's test. Parametric data were analyzed using one-way ANOVA followed by LSD post hoc tests. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

This study was approved by the Health/Medical Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia (No. 335/VI/2025/Komisi Bioetik).

RESULTS

Histological Findings

Masson's Trichrome staining demonstrated structural differences in dermal collagen between groups (Figure 1). The normal group (K1) showed dense, well-organized collagen fibers with intact dermal architecture. In contrast, the UVB-exposed group (K2) exhibited fragmented, disorganized collagen fibers, signs of matrix degradation, and dermal thickening.

Serum TNF-α and SOD levels

Serum TNF-α and SOD levels differed significantly among groups ($p < 0.05$) (Tables 1 and 2; Figures 2 and 3). TNF-α levels were highest in the negative control group (K2: 66.14 ± 10.81 ng/L) and lowest in the normal group (K1: 20.87 ± 9.64 ng/L). Treatment groups, including vitamin E and C. roseus extract gels, showed reduced TNF-α levels compared with the negative control.

SOD levels showed the opposite trend, with the lowest values observed in K2 (12.15 ± 2.24 ng/mL) and the highest in K1 (56.15 ± 4.37 ng/mL). Both concentrations of C. roseus extract gel significantly increased SOD levels compared with the negative control, indicating improved antioxidant capacity.

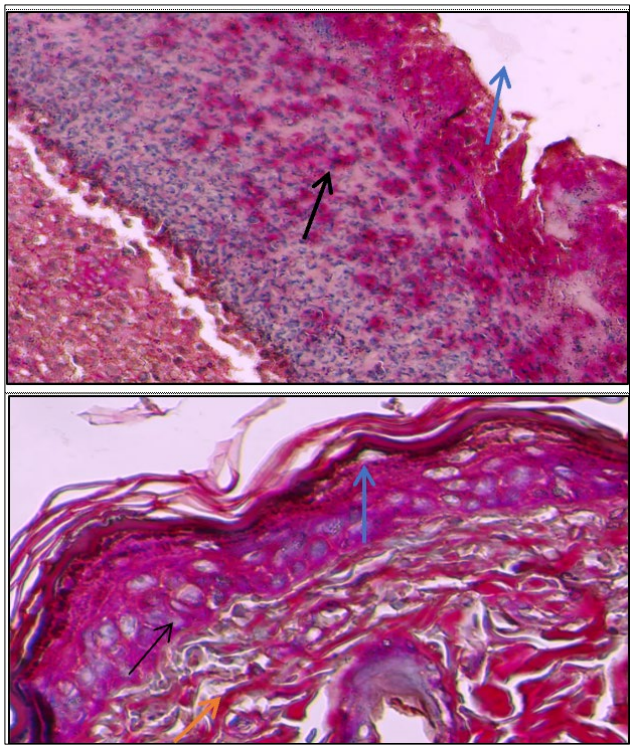

Figure 1. Masson's trichrome staining showing collagen density in the normal group (top) and the UVB-exposed negative control group treated with base gel (bottom).

Table 1. TNF-α Levels in Each Treatment Group

Group	Treatment Description	TNF-α (Mean ± SD)
K1	Normal	20.87 ± 9.64
K2	Negative Control	66.14 ± 10.81
K3	Positive Control	31.94 ± 7.54
K4	ETD Gel 15%	30.88 ± 13.49
K5	ETD Gel 30%	25.26 ± 5.77

Note: *Shapiro–Wilk* (p>0.05), *Levene's test* (p=0.277*), One-Way Anova* (p=0.000)

Table 2. SOD Levels in Each Treatment Group

Group	Treatment Description	SOD (Mean ± SD)
K1	Normal	56.15 ± 4.37
K2	Negative Control	12.15 ± 2.24
K3	Positive Control	18.68 ± 4.45
K4	ETD Gel 15%	43.97 ± 10.01
K5	ETD Gel 30%	48.41 ± 4.69

Note: *Shapiro–Wilk* (p>0.05), *Levene's test* (p=0,054*), One-Way Anova* (p=0.000)

Pairwise Comparisons

Post hoc analysis (Tables 3 and 4) confirmed that TNF-α levels in the negative control group were significantly higher than in all other groups ($p < 0.05$). No significant differences were observed between the 15% and 30% extract gel groups. Similarly, SOD levels in the negative control group were significantly lower than in all treatment groups ($p < 0.05$). Both extract concentrations produced comparable increases in SOD levels, with no significant difference between them.

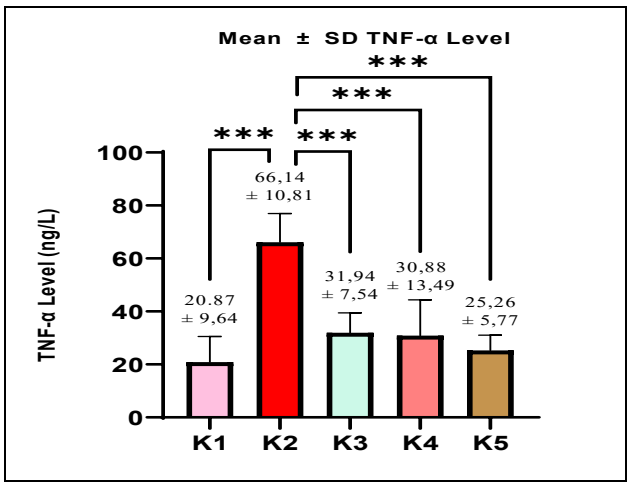

Figure 2. Mean ± SD of TNF-α levels in C57BL/6 mice across treatment groups (K1–K5). TNF-α levels were significantly higher in the negative control group compared with other groups ($p < 0.001$).

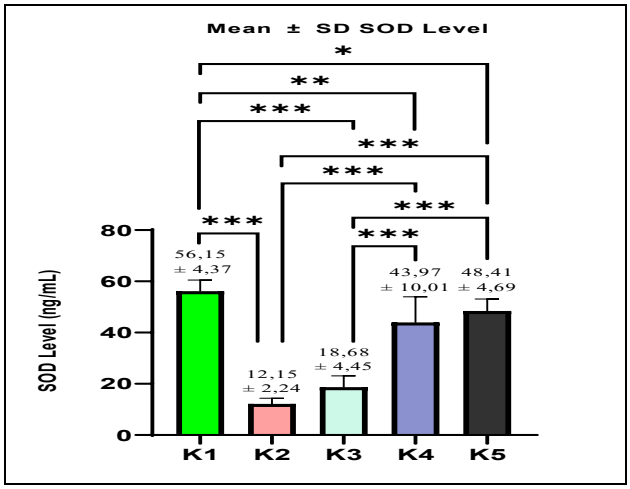

Figure 3. Mean ± SD of SOD levels in C57BL/6 mice across treatment groups (K1–K5). SOD levels were significantly lower in the negative control group compared with the treatment groups ($p < 0.001$).

Table 3. Post Hoc LSD Test for TNF-α Levels

Group	TNF-α				
	K1	K2	K3	K4	K5
K1		<0.001	0.090	0.123	0,488
K2	<0.001		<0.001	<0.001	<0.001
K3	0.090	<0.001		0.866	0.295
K4	0.123	<0.001	0.866		0.376
K5	0.488	<0.001	0.295	0.376	

Table 4. Post Hoc LSD Test for SOD Levels

Group	SOD				
	K1	K2	K3	K4	K5
K1		<0.001	<0.001	0.003	0.046
K2	<0.001		0.089	<0.001	<0.001

K3	<0.001	0.089		<0.001	<0.001
K4	0.003	<0.001	*0.000		0.237
K5	0.046	<0.001	*0.000	0.237	

DISCUSSION

This study demonstrates that repeated UVB exposure induces a strong inflammatory response in mouse skin, as reflected by the marked elevation of TNF-α levels in the negative control group. This finding is consistent with established mechanisms in which UVB radiation generates ROS and activates inflammatory signaling pathways, particularly NF-κB, leading to increased production of pro-inflammatory cytokines by keratinocytes and immune cells.[15] Such responses indicate an acute inflammatory state when endogenous cutaneous defenses are overwhelmed.

Histological findings further supported these results, showing marked disruption of dermal collagen fibers in the UVB-exposed group compared with the normal control. This structural damage reflects oxidative stress–induced degradation of the extracellular matrix, a hallmark of photoaging. ROS generated by UVB exposure activate matrix-degrading enzymes, resulting in collagen fragmentation and disorganization.[10] These findings are consistent with the observed increase in TNF-α and reduction in SOD levels, suggesting a link between inflammation, oxidative stress, and structural tissue damage

The positive control group treated with vitamin E showed reduced TNF-α levels compared with the negative control, although levels were not fully restored to normal. Vitamin E is known to suppress inflammation by inhibiting cyclooxygenase and lipoxygenase pathways, reducing prostaglandin and leukotriene synthesis, and acting as a photoprotective antioxidant that attenuates ROS production.[16-18] However, its partial effect in this study suggests limited efficacy under sustained UVB exposure.

Application of *C. roseus* extract gel at both 15% and 30% concentrations significantly reduced TNF-α levels. The flavonoids and alkaloids present in C. roseus are known to inhibit NF-κB activation, reduce ROS production, and promote tissue repair.[19] The absence of significant differences between the two concentrations suggests that the anti-inflammatory effect may reach a plateau at lower doses, possibly due to limitations in skin absorption or to a ceiling in biological activity. [20]

UVB exposure also impaired antioxidant defense, as reflected by the reduced SOD levels in the negative control group. This finding supports the concept that excessive ROS production overwhelms endogenous antioxidant systems.[21] Although vitamin E produced modest improvement, SOD levels remained below normal, indicating incomplete restoration of antioxidant capacity. [22]

Both concentrations of C. roseus extract gel significantly increased SOD levels, indicating enhanced antioxidant activity. This effect is likely mediated by flavonoids and terpenoids, which act as free radical scavengers and stimulate endogenous antioxidant enzymes such as SOD, catalase, and glutathione peroxidase.[10,23] These findings are consistent with previous studies demonstrating improved antioxidant status following C. roseus administration in oxidative stress models. [24] Similar to TNF-α, no significant difference was observed between concentrations, suggesting an optimal therapeutic threshold. [25]

Taken together, C. roseus extract gel effectively attenuated UVB-induced inflammation and oxidative stress, as evidenced by reduced TNF-α levels and increased SOD activity. Compared with vitamin E, C. roseus showed a stronger anti-inflammatory response, possibly due to more pronounced inhibition of NF-κB signaling. These findings extend previous research by demonstrating the effectiveness of topical C. roseus formulations in modulating both inflammatory and antioxidant pathways under subchronic UVB exposure.

This study has several limitations. The extract was not analyzed for specific bioactive compounds, limiting identification of active constituents. The absence of a UVB-only group without topical treatment restricts baseline comparison. Additionally, the relatively short exposure duration and reliance on serum biomarkers without quantitative histological analysis may limit interpretation of long-term protective effects. Future studies should include phytochemical characterization, molecular pathway analysis, and quantitative histological assessment.

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that topical application of *C. roseus* extract gel effectively attenuates UVB-induced skin inflammation and oxidative stress. Both 15% and 30% formulations significantly reduced TNF-α levels and increased SOD activity, indicating improved regulation of inflammation and enhanced antioxidant defense. No significant difference was observed between the two concentrations, suggesting that the lower concentration may be sufficient to achieve the desired biological effect.

Future studies should include quantitative phytochemical analysis to identify active compounds and their mechanisms of action. Incorporating a UVB-only control group and quantitative histological assessment would further strengthen the evaluation of therapeutic efficacy and provide a more comprehensive understanding of the protective effects of *C. roseus* extract gel.

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