Medical Laboratory Technology Journal
12(1), 2026, 36-49
Received 2025-18-11; Revised 2026-26-01; Accepted 2026-15-04
Available online at : http://ejurnal-analiskesehatan.web.id

The Effect of Periwinkle Flower Extract Gel (*Catharanthus roseus)* on MITF and GPx in C57BL/6 Mice Exposed to Sub-chronic UVB

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Abstract: Ultraviolet B (UVB) exposure induces oxidative stress, which decreases antioxidant enzyme activity such as Glutathione Peroxidase (GPx) and activates the transcription factor Microphthalmia-associated Transcription Factor (MITF), a key regulator of melanocyte survival and melanogenic adaptation. Natural antioxidants such as *Catharanthus roseus* (Madagascar periwinkle) extract possess the potential to enhance endogenous antioxidant defences and modulate UVB-induced melanocyte responses. This study tries to determine the effect of *Catharanthus roseus flower* extract gel on MITF expression and GPx levels in the skin tissue and GPx levels in the serum of C57BL/6 mice exposed to subchronic UVB radiation. This experimental study employed a post-test only control group design with five groups: K1 (normal), K2 (negative control, gel base), K3 (positive control, vitamin E), K4 (Catharanthus roseus Extract gel 15%), and K5 (Catharanthus roseus Extract gel 30%). The mean GPx levels differed significantly (p=0.001): K1=10.66±0.85 ng/mL, K2=2.60±1.22 ng/mL, K3=3.70±1.00 ng/mL, K4=8.29±1.95 ng/mL, and K5=9.15±0.91 ng/mL. MITF expression also showed significant differences (p=0.003): K1=27.47±3.62%, K2=31.82±8.11%, K3=51.06±8.16%, K4=34.49±7.91%, and K5=40.49±3.74%. Based on these observations, it can be inferred that the administration of *Catharanthus roseus* flower extract gel significantly increased GPx levels and modulated MITF expression in UVB-exposed mouse skin. The 30% concentration demonstrated the most optimal antioxidant and protective effects, approaching physiological antioxidant levels, while maintaining controlled MITF activation, highlighting its potential as a natural photoprotective and antioxidant agent.
Keywords: *Catharanthus roseus*; glutathione peroxidas; hyperpigmentation; ultraviolet B.

INTRODUCTION

Ultraviolet B (UVB) exposure triggers the formation of free radicals that induce oxidative stress in the skin. This condition activates the melanogenesis pathway through the upregulation of *Microphthalmia-associated Transcription Factor* (MITF), a key regulator of melanocyte survival, activity, and adaptive pigmentation responses to UV radiation. (Nishio et al., 2016) Excessive melanin accumulation leads to hyperpigmentation, characterised by dark spots, uneven skin tone, and a dull appearance. (Fabian et al., 2023) Transient MITF activation represents an important early protective mechanism by enhancing melanin production to absorb UV radiation and limit oxidative damage (J. J. Liu & Fisher, 2010). Hyperpigmentation itself has become a major concern in the cosmetic field due to its direct relationship with aesthetics and self-confidence. (Moolla & Miller-Monthrope, 2022; Nautiyal & Wairkar, 2021) Growing public awareness of the adverse effects of synthetic chemicals has

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driven a shift toward natural or herbal cosmetic ingredients, which are considered safer, more skin-friendly, and less toxic. (Tanveer et al., 2023) One promising natural ingredient for development as a skin-lightening agent is the extract of *Catharanthus roseus* (Madagascar periwinkle). (Najukha et al., 2025; Pham et al., 2020) This extract exhibits antioxidant activity that may help reduce excessive melanin production and prevent skin hyperpigmentation. (Goboza et al., 2020)

MITF is the principal transcription factor regulating genes involved in melanin synthesis (Bastos & Chool Boo, 2022), and its expression is essential for maintaining melanocyte function and skin photoprotection. (Nguyen & Fisher, 2018) Therefore, regulation rather than complete inhibition of MITF expression is critical in maintaining physiological pigmentation balance and preventing UVB-induced skin damage. (Y. Zhang et al., 2024) On the other hand , Glutathione Peroxidase (GPx) can inhibit the activity of tyrosinase, the key enzyme in melanin production, thus helping prevent hyperpigmentation. (Nazhan Mahmood, 2022) By shifting melanin synthesis from dark eumelanin to lighter pheomelanin, GPx contributes to protecting the skin from UVB-induced damage. (Etnawati et al., 2019; Petruk et al., 2018; Tanveer et al., 2023) GPx also assists in regenerating other antioxidants, such as vitamins C and E, back to their active forms, enhancing the skin's overall antioxidant defence. Antioxidant use is therefore considered an effective approach for preventing or addressing pigmentary disorders. (Weschawalit et al., 2017)

Catharanthus roseus is known for its diverse health benefits due to its antioxidant, anti-inflammatory, and anticancer properties. Alkaloids such as vincristine and vinblastine are known to inhibit melanocyte proliferation, and the resulting reduction in melanocyte number may decrease melanin production, making this plant potentially useful for hyperpigmentation therapy.(Bunyanis & L.Ode, 2023; Leny et al., 2023) Extracts of *Catharanthus roseus* (ECR) also enhance the expression of antioxidant enzymes such as glutathione reductase and glutathione synthetase, which play key roles in glutathione (GSH) regeneration through indirect mechanisms involving activation of endogenous antioxidant gene expression. (Q. Zhang et al., 2017) These mechanisms help maintain cellular antioxidant balance, reduce oxidative stress, and prevent glutathione depletion. (Pham et al., 2020) The protective effects of *s* occur through enhancement of the cellular antioxidant defence system, neutralising UVB-induced ROS, supporting skin regeneration, inhibiting hyperpigmentation, and protecting skin tissue from damage. (Saeedi et al., 2021; Scaria et al., 2020)

The plant contains bioactive compounds such as alkaloids, flavonoids, and phenolics, which exhibit various biological activities, including potential roles in managing hyperpigmentation. Flavonoids from *Catharanthus roseus* may suppress MITF expression by modulating MAPK signalling, particularly through inhibition of p38 MAPK or ERK phosphorylation, which directly affects MITF stability and activity. Additionally, the extract may modulate the cAMP–PKA pathway, influencing MITF regulation through alterations in phosphorylation levels. ECR have been shown to reduce skin pigmentation by inhibiting melanogenesis signalling pathways and downregulating MITF expression. (Prabha Lahare et al., 2021)

Several preliminary studies indicate the potential of *Catharanthus roseus* as a skin-lightening agent through mechanisms involving tyrosinase inhibition and regulation of transcription factors associated with melanogenesis. (Pham et al., 2020) The selection of C. roseus flower extract as a cosmetic alternative for hyperpigmentation is supported by its bioactive content capable of reducing MITF expression while combating oxidative stress and inflammation. With further research

and development, *Catharanthus roseus* may serve as a safe, effective, and natural primary ingredient in skin-lightening formulations. (Leny et al., 2023)

Catharanthus roseus also contains strong antioxidants (Bunyanis & L.Ode, 2023; Leny et al., 2023), and antioxidant activity is known to support GPx synthesis, maintain redox balance, and reduce oxidative stress. By neutralising UVB-induced ROS, supporting skin regeneration, preventing hyperpigmentation, and protecting the skin from damage (J. K. Liu, 2022; Zhao et al., 2025), the extract demonstrates potential as a multifaceted skin protective agent. However, to date, the studies have specifically evaluated the effects of ECR on MITF expression and glutathione-related pathways. Evidence regarding its specific effects on microphthalmia-associated transcription factor (MITF) expression and glutathione-related antioxidant pathways in UVB-exposed skin remains limited, indicating a need for further investigation.

Therefore, this study aimed to evaluate the effects of topical ECR gel on MITF expression in skin tissue and serum glutathione peroxidase (GPx) levels in C57BL/6 mice exposed to sub-chronic UVB radiation.

MATERIALS AND METHODS

Research Design

This study employed an experimental laboratory design using a post-test only control group approach to assess the effect of ECR gel on MITF expression and GPx levels in UVB-exposed C57BL/6 mice. Male mice aged 6–8 weeks with body weights of 20–25 g were selected through simple random sampling. All mice were declared healthy during the initial examination and underwent a seven-day acclimatisation period in the Integrated Biomedical Laboratory (IBL) of Universitas Islam Sultan Agung Semarang. A total of 30 mice were randomly allocated into five groups, consisting of a normal control group without UVB exposure, a negative control group with UVB exposure and gel base application, a positive control group with UVB exposure and vitamin E application, and two treatment groups receiving UVB exposure along with either 15% or 30% ECR.

Throughout the research, plant materials included C. roseus flowers, while chemicals consisted of ethanol 70% and 96%, propylene glycol, HPMC (Hydroxypropyl Methylcellulose), PVA (Polyvinyl Alcohol), preservatives, and PBS (Phosphate-Buffered Saline). The equipment used in this study included a rotary evaporator (Rotary Evaporator RV 10 Digital, IKA, Germany) for concentrating plant extracts. Drying of plant materials was carried out using a laboratory drying oven (25 L drying oven, BIOBASE, China). Histological tissue preparation was performed using a rotary microtome (RM2235, Leica Biosystems, Germany). Absorbance measurements for ELISA analysis were conducted using a microplate reader (Multiskan™ FC Microplate Photometer, Thermo Fisher Scientific, USA).

Quantification of glutathione peroxidase (GPx) levels was performed using a Rat Glutathione Peroxidase (GSH-Px) ELISA Kit (BT LAB, China). Additional equipment included a narrowband UVB irradiation lamp with a peak wavelength of 311 nm (Philips TL/01 UVB Narrowband, Philips, Netherlands) and adjustable micropipettes (Research Plus, Eppendorf AG, Germany). Extraction of C. roseus was performed at STIFAR Semarang Laboratory, and all animal treatment, UVB exposure, and laboratory analyses were conducted in the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, between August and November 2025. Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung Semarang, No.366/VII/2025/komisi bioetik.

Preparation of Extract and Gel Formulation of Catharanthus roseus

Fresh flowers of *Catharanthus roseus* were sorted, cleaned, and dried in an oven at 50°C until the moisture content was below 10%. The dried materials were then ground and sieved to obtain a uniform powder. The powder was extracted through maceration using 70% ethanol over several days with intermittent shaking. All filtrates were collected and concentrated under reduced pressure using a rotary evaporator to produce a thick extract.

The gel formulation was prepared by dissolving PVA in a water bath until fully melted, hydrating HPMC in cold distilled water, and dissolving methyl and propyl paraben in propylene glycol. These components were gradually combined with continuous mixing until a uniform gel base was obtained. The *Catharanthus roseus* extract, previously dissolved in distilled water, was incorporated into the base to produce gels with concentrations of 15% and 30%. The mixture was stirred thoroughly to ensure homogeneity before use.

Treatment Procedure

All mice were housed in ventilated cages maintained at 28–32°C, with free access to pellet feed and drinking water throughout the study. After acclimatisation, the dorsal fur of each mouse was shaved in a circular area of approximately 3 cm to prepare for UVB exposure. UVB irradiation was performed once daily for fourteen consecutive days using a broadband UVB lamp with a peak emission at 302 nm, positioned 20 cm above the dorsal skin. Each irradiation session lasted ten minutes and delivered a minimal erythema dose of 0.5 J/cm². One hour after irradiation, each treatment group received its designated topical application, consisting of gel base, vitamin E, 15% extract gel, or 30% extract gel. The normal control group received neither UVB exposure nor topical treatment.

Sample collection was performed on day fifteen following euthanasia under anaesthesia. Skin tissue samples from the dorsal region were excised, rinsed with PBS, homogenised on ice, and centrifuged to obtain a supernatant for analysis, while additional samples were stored at −20°C. Blood samples for GPx measurement were collected from the orbital sinus and centrifuged to separate serum, reflecting systemic antioxidant status associated with UVB-induced oxidative stress.

Measurement and Analysis Procedures

MITF expression in skin tissue was assessed using the immunohistochemistry method. Tissue sections were deparaffinized, rehydrated through graded alcohols, and treated with hyaluronidase for antigen retrieval. Endogenous peroxidase activity was blocked before incubation with primary antibodies. Afterwards, slides were treated with biotinylated secondary antibodies and streptavidin-HRP, followed by colour development using AEC substrate, and finally counterstained with hematoxylin. Evaluation was performed semi-quantitatively by a pathology expert based on staining intensity and the percentage of positively stained cells.

Serum GPx levels were quantified using a commercial ELISA kit. Serum samples and standards were added to antibody-coated wells and processed according to the manufacturer's instructions. HRP-conjugated detection antibodies and TMB substrate were applied sequentially, and absorbance was measured at 450 nm using a microplate reader. GPx concentrations were determined from the standard curve generated in the assay.

Data analysis was conducted using SPSS. The Shapiro–Wilk test was applied to evaluate data normality, followed by Levene's test for homogeneity of variance. GPx data with normal and homogeneous distribution were analysed using one-way ANOVA with LSD post hoc testing, whereas MITF expression data, which were not normally

distributed, were examined using the Kruskal–Wallis test followed by Mann–Whitney pairwise comparisons. A p-value of less than 0.05 was considered statistically significant for all analyses.

RESULTS AND DISCUSSION

Microscopic observation showed clear differences in the intensity of MITF staining across the treatment groups. Brown staining (indicated by black arrows) marked the location and level of MITF protein expression within the basal layer of the epidermis, the area where melanocytes reside. Variations in staining intensity reflected differences in melanocyte activity following UVB exposure and subsequent treatments with vitamin E and Catharanthus roseus extract gel at varying concentrations (Figure 1).

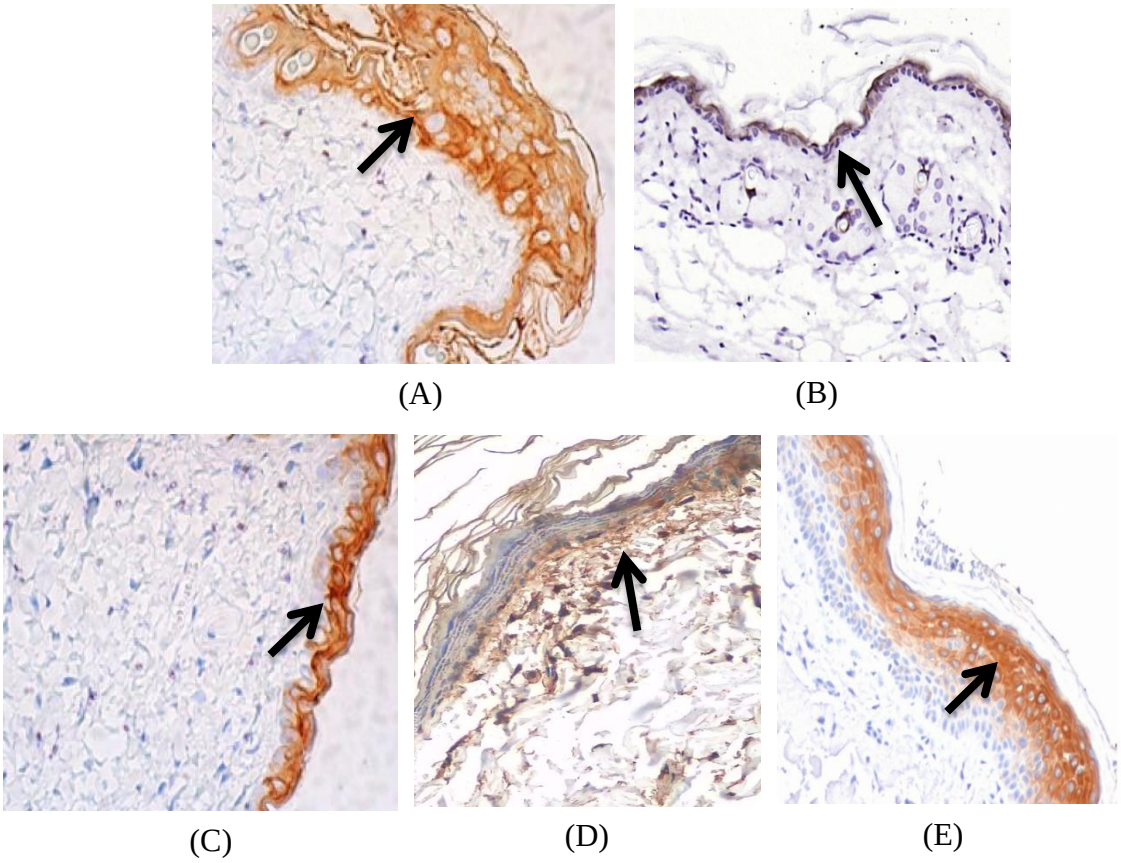

Figure 1. Microscopic expression of MITF in each treatment group (normal control group (A), negative control group (B), positive control group (C), treatment group 1 extract gel Catharanthus roseus 15% (D) dose, and treatment group 2 extract gels Catharanthus roseus Dosage 30% (E).

The expression of MITF in mouse skin tissue demonstrated distinct patterns between groups, as shown in Figure 1. In the normal control group (A), an even brown colouration was visible along the basal epidermal layer, indicating normal MITF expression without UVB exposure. Melanocytes appeared well arranged with clear nuclear structures, suggesting optimal cell function. In contrast, the negative control group (B), which received UVB exposure and only a gel base, showed a marked reduction in MITF expression, with very weak brown staining. This suggested that UVB

exposure reduced melanocyte activity and caused cellular structural damage due to oxidative stress and degradation of MITF protein.

In the positive control group (C), which received UVB exposure and vitamin E, staining intensity increased compared with K2. The clearer brown colouration of the basal layer indicated that vitamin E provided a protective effect on melanocytes and helped maintain MITF expression through antioxidant activity that reduced free radical damage. In treatment group K4 (D), which received 15% *C. roseus* extract gel (ECR), moderate brown staining was observed. MITF expression increased compared with the negative control, indicating a protective effect at 15%, although not as strong as vitamin E.

The K5 treatment group (E), which received 30% ECR gel, showed the strongest and most uniform brown staining in the basal layer, nearly resembling the normal group. This suggested that the 30% concentration provided the most optimal protective effect against UVB-induced damage and significantly enhanced MITF expression.

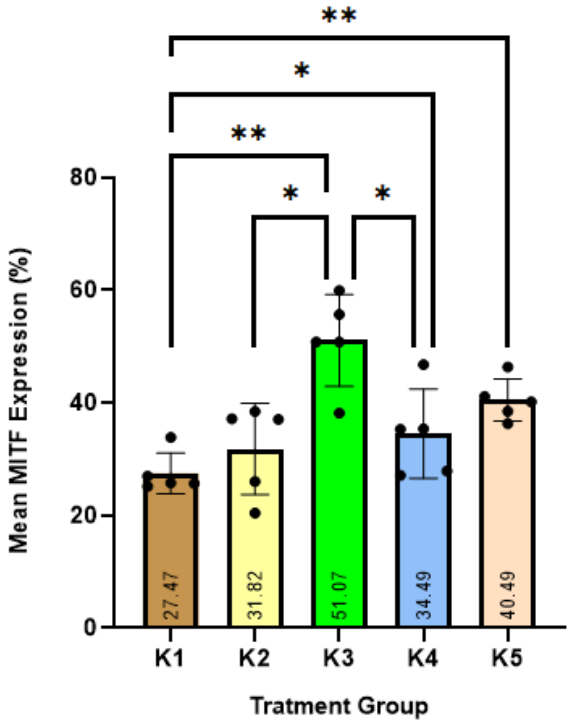

Figure 2. Mean MITF Expression for each group

This pattern aligns with the quantitative data shown in Table 1 and Figure 2. The highest MITF expression was observed in the K3 group (51.07%), followed by K5 (40.49%), K4 (34.49%), K2 (31.82%), and K1 (27.47%). These results indicate that both vitamin E and *C. roseus* extract increased MITF expression compared with the UVB-only group, with the 30% concentration providing an effect closest to the positive control.

Based on Table 1 and Figure 1, the normal group (K1) showed the lowest MITF expression of 27.47 ± 3.62%, representing baseline skin conditions without UVB exposure. The negative control group (K2) showed an increase to 31.82 ± 8.11% due to UVB-induced activation of melanogenesis in the absence of protective treatment. The positive control group (K3) demonstrated the highest expression (51.06 ± 8.16%), indicating heightened melanocyte activity supported by vitamin E. Treatment with 15%

ECR gel (K4) resulted in an average MITF expression of 34.49 ± 7.91%, whereas the 30% ECR gel group (K5) showed a higher value of 40.49 ± 3.74%, indicating a dose-dependent protective effect against UVB-induced oxidative damage.

Table 1**.** Mean MITF Expression for Each Group

Group	**Average±SD (%)**
K1(Normal Group)	27.47±3.62
K2(Negative Control)	31.82±8.11
K3(Positive Control)	51.06±8.16
K4(15% ECR gel)	34.49±7.91
K5(Gel ECR 30%)	40.49±3.74

SD = standard deviation

The analysis continued with data normality and homogeneity tests, summarised in Table 2. Most groups showed normally distributed MITF expression data except K1, which had a p-value of 0.011. The Levene homogeneity test yielded a p-value of 0.294, indicating homogeneous variance across groups. Due to one non-normal data group, the Kruskal–Wallis test was used, revealing a significant difference in MITF expression among groups (p = 0.003).

Table 2. Result of Normality Test, Homogeneity, and Kruskal–Wallis Test

Treatment Groups	MITF Expressions		
	Normality (Saphiro Wilk)	Homogeneity (Levene'sTest)	Kruskal Wallis
K1(Normal Group)	0,011	0.294*	0.003*
K2 (Negative Control)	0.110*		
K3(Positive Control)	0.527*		
K4(15% ECR gel)	0.331*		
K5(Gel ECR 30%)	0.728*		

**Saphiro Wilk test* (*p* > 0.05 = normal)
**Levene's Test* (*p* > 0.05 = homogeneous)
**Kruskal Wallis* (*p* <0.05 = significant)

Table 3. Mann–Whitney Inter-Group MITF Expression Test Results

Group	K2 (Negative Control)	K3(Positive Control)	K4(15% ECR gel)	K5(Gel ECR 30%)
K1(Normal Group)	0,310	0.008**	0.032*	0.008**
K2 (Negative Control)	-	0.016*	1,000	0,056
K3(Positive Control)	-	-	0.016*	0,095
K4(15% ECR gel)	-	-	-	0,151

*p < 0.05; **p < 0.01; ***p < 0.001 (significant based on Mann–Whitney U test).

The results in Table 3 further detail the Mann–Whitney post-hoc comparisons. Significant differences were found between several paired groups, including between K3 and K1 (p = 0.008), K4 and K1 (p = 0.032), and K5 and K1 (p = 0.008). These findings confirm that both vitamin E and Catharanthus roseus extract gel significantly increased MITF expression compared with the normal baseline and UVB-only exposure.

The next analysis focused on the concentration of Glutathione Peroxidase (GPx) in mouse serum following subchronic UVB exposure. The average GPx levels, measured using ELISA, showed substantial differences across groups.

Table 2. Mean GPx Level on Intergroup Mouse Serum

Treatment Groups	GPx level (ng/mL)	Value *p*		
		Saphiro Wilk	Levene's Test	Oneway Anova
K1 (Normal Group)	10.66 ± 0.85	0,442		
K2 (Negative Control)	2.60 ± 1.22	0,121		
K3(Positive Control)	3.70 ± 1.00	0,230	0,329	0,001
K4 (15% ECR gel)	8.29 ± 1.95	0,892		
K5 (30% ECR gel)	9.15 ± 0.91	0,715		

**Saphiro Wilk test* (*p* > 0.05 = normal)
**Levene's Test* (*p* > 0.05 = homogeneous)
**Oneway Anova* (*p* <0.05 = significant)

The normal group (K1) presented the highest GPx level (10.66 ± 0.85 ng/mL), whereas the negative control (K2) had the lowest (2.60 ± 1.22 ng/mL). GPx levels increased in mice treated with vitamin E (K3: 3.70 ± 1.00 ng/mL), as well as in the 15% (K4: 8.29 ± 1.95 ng/mL) and 30% (K5: 9.15 ± 0.91 ng/mL) extract gel groups.

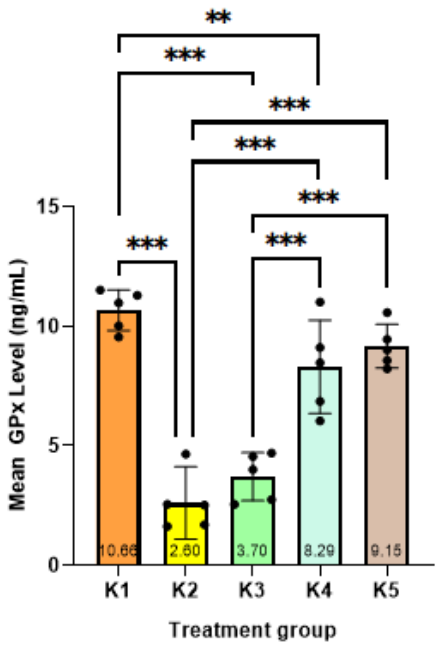

Figure 3. Average GPx Levels in Mouse Blood Serum in Each Treatment Group

Normality testing using Shapiro–Wilk indicated that all GPx data were normally distributed (p > 0.05), while the Levene test confirmed homogeneous variance (p = 0.329). One-way ANOVA revealed significant differences between groups (p = 0.001), indicating that Catharanthus roseus extract gel improved GPx levels in UVB-exposed mice, particularly at the 30% concentration, which approached values seen in the normal group.

Post-hoc LSD analysis results are summarised in Table 5.5. Significant differences were observed between the normal group (K1) and all UVB-exposed groups. GPx levels differed significantly between K2 and both extract groups (K4 and K5), indicating that the extract effectively increased GPx activity. No significant difference was observed between K2 and the vitamin E group (K3), nor between K4 and K5, suggesting that the 30% extract concentration produced an antioxidant effect comparable to vitamin E.

Table 3. Test Posh Hoc LSD GPx Level in Each Group

Group		Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound
K1 (Normal Group)	K2 (Neg Control)	0.000	6,407	9,718
	K3 (Pos Control)	0.000	5,304	8,616
	K4 (15% ECR gel)	0.007	0,719	4,031
	K5 (30% ECR gel)	0,072	-0,147	3,164
K2 (Neg Control)	K3 (Pos Control)	0,180	-2.758	0,553
	K4 (15% ECR gel)	0.000	-7.343	-4,032
	K5 (30% ECR gel)	0.000	-8.210	-4,898
K3 (Pos Control)	K4 (15% ECR gel)	0.000	-6.241	-2,929
	K5 (30% ECR gel)	0.000	-7.107	-3,796
K4 (15% ECR gel)	K5 (30% ECR gel)	0,288	-2.522	0,789

Overall, the findings of this study showed that Administration of *Catharanthus roseus* extract gel significantly increased GPx levels in the K4 and K5 treatment groups compared with the negative control. This increase indicates that the bioactive compounds in the extract, particularly flavonoids, phenolics, and alkaloids, have a strong capability to stimulate antioxidant enzyme activity and reduce oxidative damage caused by UVB exposure. (Zahra et al., 2024) The protective effect generated by the 30% *C. roseus* extract gel produced the most optimal result, approaching the physiological level of the normal group. This finding suggests that increasing the concentration of the extract enhanced its effectiveness in counteracting oxidative stress. These findings suggest that increasing the concentration of the extract enhanced its effectiveness in counteracting oxidative stress. The observed antioxidant effects may be associated with known redox-regulatory and anti-inflammatory pathways reported for Catharanthus roseus in previous studies (Interdonato et al., 2023), although the specific involvement of these molecular mechanisms was not directly examined in this study.

These results demonstrate that *C. roseus* extract gel was more effective than vitamin E in increasing GPx levels and protecting skin against oxidative stress induced by UVB exposure. This superiority is likely attributed to its diverse phytochemical profile, including flavonoids, phenolics, and alkaloids such as vinblastine and vincristine, which work synergistically to neutralise free radicals and activate the Nrf2–ARE transcription pathway. Activation of this pathway upregulates endogenous antioxidant enzymes, including GPx, thereby strengthening the overall redox defence system. (Hira et al., 2024) In contrast, vitamin E mainly functions as a lipophilic antioxidant that interrupts lipid peroxidation chains in cell membranes but does not stimulate the expression of antioxidant enzymes. (Chen et al., 2025) Meanwhile, *C. roseus* extract exerts a multi-target protective effect by increasing antioxidant activity, reducing oxidative stress, and suppressing inflammatory processes. This combination

of mechanisms makes the extract more effective than vitamin E in providing comprehensive protection against UVB-induced skin damage. (Hashim et al., 2024; Hira et al., 2024)

Meanwhile, the increase in MITF expression following UVB exposure represents an early adaptive response of the skin. The increase in MITF expression observed after UVB exposure represents a normal and beneficial early adaptive response of the skin. MITF regulates several essential genes in melanogenesis, such as *TYR*, *TYRP1*, and *DCT*, which contribute to melanin production. Activation of MITF enhances melanin synthesis as a protective mechanism because melanin absorbs UV radiation and neutralises free radicals produced during UVB exposure. (Gelmi et al., 2022; Nguyen & Fisher, 2018) In this context, the initial rise in MITF is part of the skin's natural defence system designed to limit DNA damage and oxidative stress.

The difference between the 15% extract group (K4) and the vitamin E group (K3) indicates variation in the strength of this adaptive response. Higher MITF expression in the vitamin E group reflects a stronger early-phase protective melanogenic response. Although vitamin E is widely known as an antioxidant, under certain oxidative conditions, a portion of vitamin E may be converted into tocopheroxyl radicals, which modestly enhance ROS signalling and activate the MAPK–p38/CREB pathway. This activation stimulates MITF more strongly, signalling that cells perceive the oxidative environment as requiring increased melanin-based protection. (Kaye et al., 2025) This response remains physiological and beneficial in the short term because it supports UV defence.

Similarly, the MITF expression in the 30% extract group, which showed no significant difference from the vitamin E group, suggests that melanocytes reached a balanced or plateau state in their redox response. At this stage, cells may have achieved an optimal level of melanogenesis-related signalling to counteract UVB-induced oxidative stress. (Kaye et al., 2025) The comparable expression between K5 and K3 reflects effective photoprotective adaptation rather than excessive activation.
It is important to note that MITF activation is advantageous during acute or short-term UV exposure. It triggers protective pigmentation and enhances cellular resilience. The potential for adverse effects, such as prolonged melanocyte activation or hyperpigmentation, applies primarily to *chronic* or *excessive* MITF signalling. (Yajima et al., 2011) In the context of this study's sub-chronic 14-day UVB exposure, MITF expression remained within a physiological range and did not indicate pathological activation.

The increased MITF in the vitamin E group can also be explained by the dual behaviour of vitamin E under oxidative conditions. While vitamin E functions primarily as an antioxidant, part of the molecule may undergo oxidation at higher stress levels and form tocopheroxyl radicals. These radicals participate in lipid peroxidation chains and activate MAPK and CREB signalling, thereby sustaining MITF expression. Activation of endothelin-B receptors due to UVB exposure also contributes to p38/CREB pathway stimulation. (Shetty et al., 2014) Uneven absorption of vitamin E or redox imbalance may further enhance these adaptive signals, contributing to the MITF increase observed in group K3. This mechanism does not imply damage but rather reflects a robust melanogenic defence response. (Ngo & Duennwald, 2022)

The lack of a significant difference in MITF expression between the normal and negative control groups suggests that the 14-day UVB exposure was not long enough to induce full melanogenic activation. The skin's endogenous antioxidant systems—including SOD, catalase, and epidermal GPx were still capable of managing the ROS load generated during this sub-chronic exposure period. As a result, MAPK–AP1 and

CREB signalling did not reach the threshold required for strong upregulation of MITF. Therefore, the MITF responses observed in this study are consistent with early-phase physiological adaptation rather than long-term melanogenic remodelling.

All these findings demonstrate that Catharanthus roseus extract gel enhanced both MITF expression and GPx levels in mice subjected to subchronic UVB radiation. The 30% extract concentration consistently showed the most optimal protective effect, closely resembling the physiological conditions of the normal control group.

Regardless, several limitations should be considered when interpreting the findings. The relatively short, sub-chronic UVB exposure of 14 days may not fully represent the long-term oxidative and melanogenic responses that occur under chronic photodamage. The sample size, although statistically acceptable, may not capture broader biological variation among individuals. Only two concentrations of *Catharanthus roseus* extract gel were evaluated, leaving the full dose–response relationship unexamined. The analysis focused solely on MITF and GPx, without including additional oxidative or melanogenic markers that could offer a more complete mechanistic picture. Finally, the use of a mouse model limits direct translation to human skin physiology and potential clinical applications.

CONCLUSION

The findings of this study demonstrate that topical administration of Catharanthus roseus extract gel exerts significant protective effects on mouse skin exposed to sub-chronic UVB radiation. The extract enhanced both Microphthalmia-associated Transcription Factor (MITF) expression and Glutathione Peroxidase (GPx) levels, indicating improvements in melanogenic adaptation and antioxidant defence. The increase in MITF reflects an effective early protective melanocyte response, while the elevation of GPx signifies strengthened endogenous redox regulation against UVB-induced oxidative stress. Together, these results show that Catharanthus roseus extract gel supports skin resilience by modulating molecular pathways involved in pigmentation and oxidative balance, highlighting its potential as a natural photoprotective and antioxidant agent.

ACKNOWLEDGEMENT

The author extends sincere appreciation to Universitas Islam Sultan Agung Semarang and the Faculty of Medicine for providing facilities and institutional support throughout the research process. Gratitude is also expressed to the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, for technical assistance, laboratory access, and support in the provision and handling of experimental animals. Appreciation is further conveyed to STIFAR for their assistance in supplying research materials. The author also thanks all laboratory staff, analysts, and individuals who contributed their time, expertise, and support to ensure the smooth completion of this study.

FUNDİNG

This research did not receive any specific grant or external financial support. All research expenses were funded independently.

CONFLICT OF INTEREST

The author declares no conflict of interest related to the conduct of this research

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