

Effect of Calendula officinalis L. extract emulgel on TNF- α and VEGF levels in UV-B-exposed male Wistar rats

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ABSTRACT

Background: Ultraviolet B (UV-B) exposure triggers photoaging through inflammatory mediators such as tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF). Calendula officinalis L. contains flavonoids, triterpenoids, and carotenoids with potential anti-inflammatory and angiogenic activity. This study evaluated the effect of Calendula officinalis L. extract emulgel on TNF- α and VEGF levels in UV-B-exposed Wistar rats.

Methods: An in vivo post-test-only control group experiment was conducted in June–July 2025 using 24 male Wistar rats (6 per group): healthy control (K1), UV-B with base emulgel (K2), UV-B with 10% calendula emulgel (K3), and UV-B with 20% calendula emulgel (K4). UV-B (302 nm, 400 mJ/cm²) was given daily for 14 days, and emulgel was applied once daily one hour after exposure. Blood was collected from the orbital sinus 24 hours after the last exposure, and TNF- α and VEGF were measured by enzyme-linked immunosorbent assay. Data were analyzed by one-way ANOVA and Tukey tests.

Results: TNF- α differed among groups ($p = .001$). It was higher in K2 than in K1 ($p = .001$), lower in K3 than in K2 (201.34 ± 11.79 vs. 236.97 ± 10.42 ng/mL; $p = .001$), and higher in K4 than in K2 (259.55 ng/mL; $p = .017$). VEGF did not differ among groups ($p = .258$), although K4 had the highest mean (1312.30 ± 220.13 ng/mL).

Conclusion: A 10% calendula emulgel lowered TNF- α in UV-B-exposed rats, whereas a 20% emulgel did not and raised it; no significant effect on VEGF was shown.

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INTRODUCTION

Excessive exposure to ultraviolet B (UV-B) radiation triggers inflammation and oxidative stress in the skin, which can damage tissue, thicken the epidermis, and accelerate skin aging (Michalak, 2023; Vitale et al., 2022). UV-B penetrates the epidermis, damages the extracellular matrix, disturbs normal skin structure and function, and impairs regeneration, a process known as photoaging (Tang et al., 2024). Indonesia is a tropical country in which photoaging due to UV-B exposure is common. Chemical treatments such as retinoids, alpha-hydroxy acids, glycolic acid, and hydroquinone are widely used but can cause irritation, redness, itching, peeling, and hypersensitivity with prolonged use, which motivates the search for safe and effective herbal topical agents (Campa & Baron, 2018). Photoaging is therefore not only a cosmetic concern but a process in which cumulative inflammatory and oxidative injury changes the structure of the dermis and epidermis over time. In tropical settings, where exposure is frequent and prolonged, the burden of this damage is considerable,

and the demand for topical products that prevent or reverse it is high. The question of how to limit this damage with accessible, well-tolerated topical agents therefore motivates the study of plant-derived preparations. Because the epidermis is the first tissue to absorb this radiation, the earliest signs of injury appear there and are followed by changes in deeper layers.

At the molecular level, UV-B exposure generates excess reactive oxygen species (ROS) that activate inflammatory pathways, including nuclear factor kappa B (NF- κ B), and raise the expression of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) (Ansary et al., 2021; Salminen et al., 2022). TNF- α further damages the extracellular matrix by increasing matrix metalloproteinase expression and degrading collagen and elastin (Hughes et al., 2021). ROS also cause local tissue hypoxia and activate hypoxia-inducible factor 1- α , which together with NF- κ B raises vascular endothelial growth factor (VEGF), an angiogenic mediator that responds to injury and environmental stress and whose excessive activity contributes to abnormal skin vascularization (Hartono et al., 2022). Raised TNF- α and VEGF together create an inflammatory, proangiogenic microenvironment that accelerates structural and functional skin aging. Because both mediators respond to the same upstream signals, measuring them together gives a more complete view of the inflammatory and angiogenic response to UV-B than either one would alone, and it allows an intervention to be judged on whether it dampens inflammation, modifies vascular signaling, or both. The relationship between the two is not simple, since TNF- α is mainly a marker of inflammatory activity while VEGF reflects vascular remodeling, and the two may change independently.

Calendula officinalis L. has long been used to treat wounds and skin inflammation, and its flavonoids, triterpenoids, and carotenoids have drawn scientific attention (Shahane et al., 2023). Calendula flower extract reduced inducible nitric oxide synthase and TNF- α expression and accelerated tissue recovery after UV-B exposure in macrophages and skin (Silva et al., 2021). A vesicular cream containing geranium and calendula essential oil improved biochemical parameters in rats exposed to UV-B (Lohani & Morganti, 2022), and a calendula cream improved functional skin parameters (Naveed et al., 2011). Calendula ethanol extract at 10% has also been used in a topical sunscreen formulation (Rohmani et al., 2024). Whether a topical calendula emulgel affects both TNF- α and VEGF in UV-B-induced photoaging, and at which concentration, has not been well described. This study therefore evaluated the effect of *Calendula officinalis* L. extract emulgel at 10% and 20% on TNF- α and VEGF levels in UV-B-exposed Wistar rats. Answering this question requires a controlled animal model in which UV-B exposure, treatment, and sampling are standardized, and in which a base emulgel group separates the effect of the extract from that of the vehicle. The present study addresses that gap by testing two concentrations of a calendula emulgel in an established rat model of UV-B photoaging, using blood levels of the two mediators as outcomes.

METHODS

Research Design and Animals

This was an in vivo experimental study with a post-test-only control group design. The animal facility work was done at the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, and the emulgel was formulated at PT Derma Elok Farma; biomarker analysis by ELISA was performed at the CITO Laboratory, Semarang, between June and July 2025. Ethical clearance was requested from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung. Male Wistar rats (*Rattus norvegicus*), 8–10 weeks old and weighing 150–200 g, healthy and active, were included; rats with wounds or skin damage before treatment were excluded, and rats that died during the treatment period were dropped out. Using the Federer formula, $(n - 1)(t - 1) \geq 15$ with four groups, at least six rats per group were required; with one reserve animal per group, 28 animals were prepared, and 24 rats (six per group) were analyzed. Rats were allocated by simple random sampling after a 7-day adaptation under a 12-hour light/dark cycle, 22–24 °C, and 40–60% humidity. The groups were: K1, healthy control (no UV-B, base emulgel); K2, negative control (UV-B, base emulgel); K3, UV-B with 10% *Calendula officinalis* extract emulgel; and K4, UV-B with 20% extract emulgel. Including a healthy group, a UV-B group treated with base only, and two extract concentrations allowed the effect of the extract and the effect of its concentration to be compared against clearly defined reference conditions.

Intervention Procedure

Calendula flower extract (70% ethanol extraction) was obtained commercially from PT INBI Nusantara Sejahtera with a certificate of analysis and safety data sheet. The extract was mixed into a cosmetic emulgel base (Carbopol 940, glycerin, and triethanolamine as pH balancer) at 10% and 20% (50 g and 100 g of extract per 500 g of formula) and stirred until homogeneous. The 10% concentration was chosen from earlier work on topical calendula against photoaging (Rohmani et al., 2024), and 20% was added to compare effectiveness. Photoaging was induced on the dorsal skin with a UV-B lamp (302 nm) at 400 mJ/cm² for 12 minutes per session, daily for 14 days, at a distance of 8 cm (Rosita & Prakoeswa, 2023). Emulgel was applied once daily, one hour after exposure, at 0.1 mL/cm² (about 1 g per rat) with a sterile spatula for 14 days (Naveed et al., 2011). All groups received the same base emulgel as vehicle, so that differences among the exposed groups could be attributed to the extract. Application one hour after exposure placed treatment in the period when the skin was responding to the radiation dose of the day, and the same daily schedule was kept for the full 14 days.

Measures and Data Analysis

On day 15, 24 hours after the last UV-B exposure, rats were euthanized with ketamine 60 mg/kg and xylazine 20 mg/kg intramuscularly, and 0.5 mL of blood was drawn from the orbital sinus (Teilmann et al., 2014). TNF- α and VEGF in blood supernatant were measured by sandwich ELISA. The skin was inspected macroscopically to validate the photoaging model. Normality was tested with Shapiro–Wilk and homogeneity of variance with Levene's test; when both were met, one-way ANOVA was used, followed by a post hoc test if significant (reported here as Tukey HSD). The significance level was 5%, and SPSS version 26.0 was used. Blood sampling rather than skin sampling was chosen because it is less invasive and gives a systemic reading, and the same collection procedure was used in every group. Results are reported as mean $\pm$ standard deviation, with p values for the omnibus test and for pairwise comparisons. Statistical decisions followed the assumptions checked beforehand, so that the parametric test was used only for data that met them. Standardizing the timing of sampling at 24 hours after the last exposure meant that all animals were compared at the same point in the response, and the use of a commercially available sandwich ELISA gave quantitative values in the same unit for every sample. The macroscopic condition of the dorsal skin was inspected to confirm that the model had been established before the biochemical results were interpreted.

RESULTS

Macroscopic Validation of the Model

Normal skin before treatment was smooth and pink, without lesions, erythema, or thickening. After 14 days of UV-B at 302 nm and 400 mJ/cm², exposed skin showed erythema, rough surface, thickening, brownish spots, and hyperpigmentation, consistent with photoaging. The healthy group (K1), which received base emulgel without UV-B, showed no such changes. These macroscopic observations were descriptive and were not scored or analyzed statistically, and no histological examination was performed, so they serve to confirm that the exposure protocol produced visible skin changes in the exposed groups rather than to quantify the degree of damage. The biomarker analysis described next therefore carries the quantitative comparison among groups, and it was carried out on blood collected on day 15, 24 hours after the last UV-B session, from all 24 animals with six rats per group. Taken together, these features were taken as evidence of photoaging in the exposed rats and allowed the biomarker results to be read in the context of a functioning model. Because the same protocol of wavelength, dose, distance, and duration was applied to every exposed animal, the model was applied consistently across groups K2, K3, and K4. A limitation of this validation is that the changes were recorded by observation only. Even so, the consistent appearance across exposed animals supports the use of the model.

TNF- α Levels

TNF- α was normally distributed in every group (Shapiro–Wilk $p = .388-.822$) with homogeneous variances (Levene $p = .145$), and one-way ANOVA showed a significant difference among groups ($p = .001$; Table 1). The highest mean was in K4 (259.55 ng/mL), followed by K2 (236.97 $\pm$ 10.42), K3 (201.34 $\pm$ 11.79), and K1 (193.62 $\pm$ 8.09). The ordering of the means indicates that UV-B raised TNF- α in the group treated with base alone relative to the healthy group, that 10% extract brought the level back close to the healthy value, and that 20% extract was associated with the highest level of all. The table gives the full set of means and standard

deviations for both biomarkers. The overall difference among groups was therefore statistically clear, and the pairwise tests reported next identify where it lies. The differences in mean among the groups are the basis for the pairwise findings described below, and they are expressed in the unit used in the results of the source study.

Table 1. TNF- α and VEGF levels by group (ng/mL)

Variable	K1 Healthy	K2 UV-B + base	K3 UV-B + 10%	K4 UV-B + 20%	p (ANOVA)
TNF- α	193.62 $\pm$ 8.09	236.97 $\pm$ 10.42	201.34 $\pm$ 11.79	259.55 $\pm$ 15.63	.001
VEGF	1148.47 $\pm$ 146.96	1149.34 $\pm$ 106.49	1197.89 $\pm$ 130.26	1312.30 $\pm$ 220.13	.258

Note. Values are mean $\pm$ SD (n = 6 per group). Levene test p = .145 for TNF- α and .324 for VEGF; Shapiro–Wilk p > .05 in all groups for both variables. Units are as reported in the results of the source study.

Tukey's test showed that K2 had higher TNF- α than K1 (p = .001), and K4 was also higher than K1 (p = .001). K3 did not differ from K1 (p = .675). K3 was significantly lower than K2 (p = .001) and K4 (p = .001), whereas K4 was higher than K2 (p = .017). In other words, the 10% emulgel was the only exposed group whose TNF- α was indistinguishable from healthy rats and significantly lower than that of the exposed group treated with base emulgel, while the 20% emulgel was significantly higher than both the base group and the 10% group. Every pairwise comparison except one, K1 versus K3, reached significance, which shows that the concentration of the extract mattered for the direction of the response. The pairwise pattern is also consistent with the descriptive means in the table, in which K3 and K1 are close and K2 and K4 are far from them in opposite directions relative to the base group. No adjustment beyond the Tukey procedure itself was applied, and all p values reported are those of that procedure. The p values therefore reflect the comparison of each pair of group means under the pooled variance estimate of the analysis.

VEGF Levels

VEGF was normally distributed (Shapiro–Wilk p = .073–.900) with homogeneous variances (Levene p = .324). The means were 1148.47 $\pm$ 146.96 (K1), 1149.34 $\pm$ 106.49 (K2), 1197.89 $\pm$ 130.26 (K3), and 1312.30 $\pm$ 220.13 ng/mL (K4), and one-way ANOVA showed no significant difference among groups (p = .258), so no post hoc test was done (Table 1; Figure 1). The means rose numerically from the healthy and base groups to the 10% and 20% groups, but the standard deviations were large, particularly in K4 ($\pm$ 220.13), so the data do not allow a conclusion that either concentration changed VEGF. Figure 1 shows both biomarkers by group. Because the overall test was not significant, no post hoc comparison was performed, and none is reported, which avoids drawing conclusions about individual pairs from an analysis that did not indicate any group difference. The result is nevertheless informative, since it shows that the strong effect on TNF- α was not accompanied by a corresponding effect on VEGF within the 14-day period studied. The healthy and base-emulgel groups had almost identical means (1148.47 and 1149.34 ng/mL), which indicates that UV-B exposure itself did not raise blood VEGF in this experiment by a detectable amount. This is also reflected in the table, where the overall p value is shown together with the distribution and variance tests.

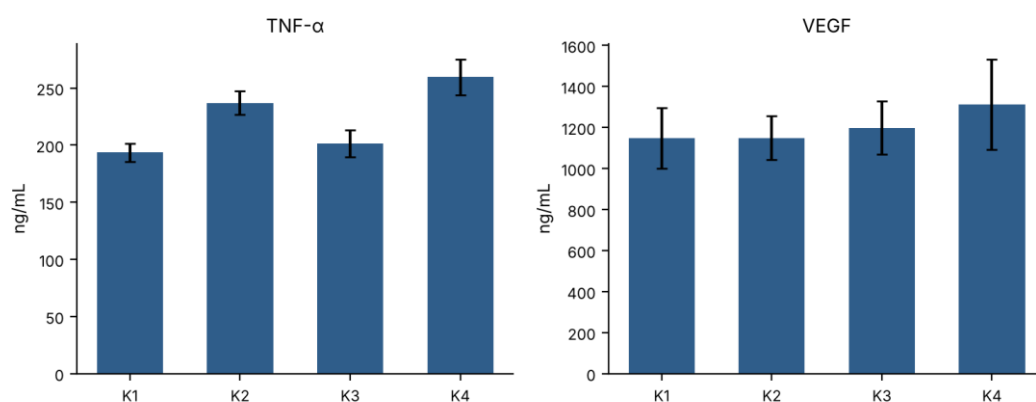


Figure 1. TNF- α and VEGF levels in blood by group (mean $\pm$ SD)

DISCUSSION

TNF- α

Blood was used to reflect the systemic state after treatment (Teilmann et al., 2014). UV-B exposure raised TNF- α in the untreated exposed group (K2) above the healthy group, in line with reports that UV-B induces oxidative stress that activates NF- κ B and AP-1 and increases proinflammatory cytokines, including TNF- α (Kang et al., 2019; Rosita & Prakoeswa, 2023). The 10% calendula emulgel lowered TNF- α significantly compared with the base emulgel, to a level not significantly different from healthy rats. Calendula flavonoids such as quercetin and rutin may suppress NF- κ B activation and thereby lower TNF- α (Fonseca et al., 2010; Guo et al., 2024). In contrast, the 20% emulgel did not lower TNF- α and was associated with a higher level than both the healthy and the base-emulgel groups. One explanation offered is a biphasic or pro-oxidant effect at higher concentrations, in which protective activity is lost and NF- κ B signaling is further activated (Fonseca et al., 2010). Calendula extract has been reported to be anti-inflammatory in a dose-dependent manner up to a limit, with toxicity or irritation at the highest concentrations, around 14.7% in one review (Shahane et al., 2023). Because irritation or toxicity at 20% was not examined here, this explanation remains a hypothesis; skin histology was not examined, and the systemic TNF- α measured in blood may not fully represent local skin inflammation.

VEGF

VEGF did not differ significantly among groups. The base-emulgel group had a mean similar to the healthy group, and the calendula groups had higher means, with the highest in K4, but these differences were within the variation of the data. Calendula triterpenoids and flavonoids have been linked to angiogenic activity through HIF-1 α and VEGF (Fonseca et al., 2010; Shahane et al., 2023), and a rise in VEGF after UV-B may represent an early compensatory response to tissue damage, which is not necessarily effective angiogenesis because vessel formation under excess oxidative stress can be disordered (Hartono et al., 2022). The present data cannot establish that calendula emulgel changes VEGF in photoaging. The wide variation within groups, especially in the 20% group, further reduces the chance of detecting a difference with six animals per group, and it is possible that a larger sample or a local measurement in skin tissue would show an effect that the blood measurement did not. Because VEGF was measured only once, on day 15, any earlier transient change would also have been missed. In summary, the VEGF data are best described as inconclusive rather than as evidence of no effect. This distinction matters because a non-significant result in a small sample cannot exclude a modest true effect.

CONCLUSION AND IMPLICATIONS

In UV-B-exposed Wistar rats, a 10% *Calendula officinalis* extract emulgel significantly lowered blood TNF- α compared with base emulgel, whereas a 20% emulgel did not lower TNF- α and was associated with a higher level. VEGF did not differ significantly among groups. A 10% concentration therefore appears more suitable than 20% as an anti-inflammatory topical agent in this model, while the effect on angiogenesis was not demonstrated. These conclusions answer the objective of the study directly: the extract emulgel influenced the inflammatory marker in a concentration-dependent way, whereas the angiogenic marker was not shown to change. For practice, the findings suggest that if a calendula emulgel is developed for photoaging, concentration should be chosen carefully and a lower concentration may be preferable, but this remains a preclinical result in rats and cannot be applied to people without further testing. For research, the results show that higher dose does not necessarily mean greater benefit for a topical botanical. They are also limited by the design, in which only two concentrations and one sampling time were used. Taken together, the data support a role for the 10% concentration as the more favorable option for dampening systemic inflammation after UV-B exposure in this model. Overall, the study met its objective of describing how the extract emulgel affects the two mediators.

Limitations and Future Research

Skin histopathology was not examined, which limits interpretation of tissue damage and angiogenesis, and the outcomes were biochemical and indirect. Treatment lasted 14 days, and only two concentrations were tested. Future studies should include skin histology, longer treatment, and more concentrations to find a dose that

lowers TNF- α without a pro-oxidant effect (Menzel et al., 2021). Blood levels may not reflect the local situation in the skin, only male rats of one strain were used, and the extract was applied for a single fixed period. Future studies should combine blood and skin measurements, include histological scoring of epidermal thickness and vessel density, test intermediate concentrations between 10% and 20%, and add irritation or toxicity assessment at the higher concentration so that the proposed pro-oxidant explanation can be tested directly rather than inferred from the literature. Reporting these limitations clearly helps readers judge how far the findings can be generalized beyond this model. The measurement of one inflammatory and one angiogenic marker also leaves other relevant mediators, such as other cytokines and matrix-degrading enzymes, unexamined. The findings should be confirmed in independent experiments before any recommendation is made. Such confirmation would also help to define the concentration range in which the extract is both effective and well tolerated. The study also did not measure the degree of oxidative stress, so the mechanisms discussed remain unverified by direct measurement.

DECLARATIONS

Ethics approval: Ethical clearance for the animal experiment was requested from the Health Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang.

Author contributions: Puty Annisa Prilina: conceptualization, methodology, investigation, data curation, formal analysis, writing – original draft. Joko Wahyu Wibowo: supervision, methodology, writing – review and editing. Prasetyowati Subchan: supervision, methodology, writing – review and editing. All authors have read and approved the final manuscript.

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