



Effect of *Calendula officinalis* L. extract emulgel on MMP-1 and MMP-9 levels in UV-B-exposed male Wistar rats

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ABSTRACT

Background: Ultraviolet B (UV-B) radiation increases matrix metalloproteinases (MMPs) such as MMP-1 and MMP-9, which degrade collagen and drive photoaging. *Calendula officinalis* L. has antioxidant and anti-inflammatory properties that may limit this damage. This study evaluated the effect of *Calendula officinalis* L. extract emulgel on blood MMP-1 and MMP-9 levels in UV-B-exposed Wistar rats.

Methods: An in vivo post-test-only control group experiment was conducted 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 after 24 hours, and MMP-1 and MMP-9 were measured by ELISA. Data were analyzed with one-way ANOVA and Tukey's post hoc test.

Results: MMP-1 differed among groups ($p = .004$). It was higher in K2 (4.787 ± 0.437 ng/mL), K3 (4.662 ± 0.205 ng/mL), and K4 (4.788 ± 0.360 ng/mL) than in K1 (3.935 ± 0.534 ng/mL), but K3 and K4 did not differ from K2 ($p = .949$ and 1.000). MMP-9 did not differ among groups ($p = .104$).

Conclusion: *Calendula* emulgel at 10% and 20% did not significantly change blood MMP-1 or MMP-9 levels compared with the UV-B group treated with base emulgel in this model.

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INTRODUCTION

Excessive exposure to ultraviolet B (UV-B) radiation is one of the main environmental causes of skin damage, because it has a significant impact on skin health through the destruction of the extracellular matrix (Kim et al., 2022). UV-B generates reactive oxygen species (ROS) that damage skin cells and trigger inflammation, and these events activate signaling pathways such as mitogen-activated protein kinase (MAPK), which in turn induce the production of matrix metalloproteinases (MMPs), especially MMP-1 and MMP-9 (Feng et al., 2024). MMP-1, known as collagenase, degrades type I collagen, whereas MMP-9 degrades type IV and V collagen, which are important for skin remodeling after injury (Feng et al., 2024). The increased activity of these enzymes is considered one of the main causes of skin aging and tissue damage after UV-B exposure (Hanai et al., 2025). Photoaging is therefore not only a cosmetic concern but a process in which cumulative oxidative and inflammatory injury changes the structure of the dermis over time, and the enzymes that remodel the matrix are central to this process. For this reason, MMP-1 and MMP-9 are widely used as biochemical

indicators of UV-induced matrix damage and of the response to treatment. Because the skin is the first organ to absorb this radiation, protecting the matrix from enzymatic breakdown is a central aim of photoprotective strategies.

Many studies have looked for natural solutions to reduce the impact of UV-B, and one of them uses *Calendula officinalis*, or marigold. The flower is widely known in traditional medicine for its anti-inflammatory and wound-healing properties, and its extract contains bioactive compounds such as flavonoids, triterpenoids, and phenolic acids that can counter oxidative damage and reduce inflammation in the skin (Shahane et al., 2023). Earlier studies reported that *Calendula* extract can lower the activity of MMPs, particularly MMP-2 and MMP-9, that is induced by oxidative stress after UV exposure (Fonseca et al., 2011). On this basis, *Calendula* has potential as a topical agent for protecting the skin against UV damage. A topical form is attractive because it can be applied directly to the exposed skin, and an emulgel combines the advantages of a gel and an emulsion, with a hydrophilic base suited to an ethanol extract. A plant extract of this kind can be formulated in a cosmetic base at defined concentrations, which allows the effect of the extract to be examined in a controlled way. Studies of this kind generally compare treated skin with untreated exposed skin and with healthy skin, which is the logic followed in the present experiment, and they allow the contribution of the extract to be separated from that of the vehicle and of the radiation itself. The availability of a certified commercial extract with a certificate of analysis also supports reproducibility.

The effect of a topical *Calendula* emulgel on MMP-1 and MMP-9 in UV-B-induced photoaging, and the concentration at which any effect appears, has not been well described. Blood measurement by enzyme-linked immunosorbent assay (ELISA) is a practical way to follow these enzymes, because blood is easier to collect and analyze than skin tissue, and biomarkers in blood may reflect biological processes in the skin such as inflammation and tissue damage (Matson, 2023). The present study therefore evaluated the effect of *Calendula officinalis* L. extract emulgel at 10% and 20% on MMP-1 and MMP-9 levels in the blood of UV-B-exposed male Wistar rats. The study used a healthy group, a UV-B group treated with the base emulgel, and two extract groups, so that the effect of UV-B, the effect of the vehicle, and the effect of the extract could each be identified. The answer to this question is relevant to the development of photoprotective topical products from a widely used medicinal plant. Such information would help to decide whether further development of this preparation is justified, and at which concentration. Because MMP-1 and MMP-9 represent two different parts of the matrix-degrading response, measuring both gives a broader view of the effect of the emulgel than either enzyme alone would provide, and it allows the study to describe whether the extract acts on one or both of them.

METHODS

Research Design and Animals

This was an in vivo experimental study with a post-test-only control group design in male Wistar rats exposed to UV-B. The rats were bred and kept at the Integrated Biomedical Laboratory (IBL), Faculty of Medicine, Universitas Islam Sultan Agung, Semarang. The extract was obtained commercially from PT INBI Nusantara Sejahtera, the emulgel was formulated at the laboratory of PT Derma Elok Farma, and blood samples were analyzed at the CITO Laboratory, Semarang, between July and August 2025. Ethical clearance was requested from the Health Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, as a requirement before the study. The population consisted of male Wistar rats (*Rattus norvegicus*) aged 8–12 weeks with a body weight of 150–200 g. Inclusion criteria were clinically healthy rats with intact skin and no previous exposure to UV light or other chemicals. Rats that became ill or stressed during the 7-day adaptation period, had skin damage before or during treatment, or showed an abnormal response such as allergy to the emulgel were excluded, and rats that did not survive until the end of treatment were dropped out. Sample size followed the Federer formula, which gave a minimum of 6 rats per group, so 24 rats were used. The rats were adapted for 7 days under controlled conditions (temperature 25°C, a 12-hour light–dark cycle, and humidity of about 50–60%) and then randomly assigned to four groups. 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. The independent variable was the *Calendula* emulgel, the dependent variables were MMP-1 and MMP-9 levels, and UV exposure duration, diet, environmental conditions, and sample size were controlled.

Intervention Procedure

Calendula flower extract was obtained from PT INBI Nusantara Sejahtera, prepared by industrial-standard extraction with 70% ethanol and supplied with a certificate of analysis (COA) and a material safety data sheet (MSDS) that document its safety, quality, and consistency. The emulgel base was obtained from PT Derma Elok Farma and contained Carbopol 940, glycerin, and triethanolamine (TEA) as pH stabilizer. The extract was weighed and added gradually to the base while being stirred slowly with a homogenizer mixer until evenly distributed, and viscosity was checked before the product was stored in a tightly closed sterile container away from direct sunlight. Each formula was made in 500 g, with 50 g of extract (10%) in the first formula and 100 g (20%) in the second, completed with the base to 450 g and 400 g respectively. The 10% concentration was chosen because earlier work showed a stable effect on photoaging symptoms, and 20% was added to compare the effectiveness of a higher concentration (Rohmani et al., 2024). Photoaging was induced on the shaved dorsal skin with a UV-B lamp at 302 nm and 400 mJ/cm² for 12 minutes per session, once 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) for 14 consecutive days (Naveed et al., 2011). K1 received base emulgel without UV-B, K2 received base emulgel after UV-B, and K3 and K4 received the 10% and 20% extract emulgels after UV-B. This schedule was kept identical across the exposed groups.

Measures and Data Analysis

After the last treatment, the rats were left for 24 hours and then euthanized with ketamine 60 mg/kg body weight and xylazine 20 mg/kg body weight intramuscularly (Krissanti et al., 2023). A 0.5 mL blood sample was taken from the orbital sinus, and MMP-1 and MMP-9 levels were measured with commercial ELISA kits according to the manufacturer's protocol and read with an ELISA plate reader. Skin condition was observed macroscopically from day 0 to day 14 to check that the UV-B exposure and the emulgel application were carried out consistently in all animals. Data were analyzed with descriptive statistics, the Shapiro–Wilk test for normality, and the Levene test for homogeneity of variance. Data that were normally distributed with homogeneous variance ($p > .05$) were analyzed by one-way ANOVA, followed by Tukey's post hoc test to identify which groups differed. Hypotheses were decided at the 5% significance level ($p < .05$), and all analyses were done with SPSS version 27. Results are reported as mean $\pm$ standard deviation in ng/mL, with p values for the omnibus test and for the pairwise comparisons. Blood was used rather than skin tissue because it is easier to obtain and allows a systemic reading of the response.

RESULTS

Macroscopic Findings

A total of 24 male Wistar rats aged 8–10 weeks with body weights of 150–200 g were used, with 6 rats in each of the four groups. Skin condition was observed from day 0 to day 14. The healthy group (K1) received neither UV-B nor extract, and its skin stayed normal and smooth with no signs of damage or inflammation, so it served as the normal reference. In the K2 group, which received UV-B and base emulgel, redness and irritation appeared on the first day, and skin damage became more evident on later days, with mild burn-like lesions and skin thickening, indicating that UV-B without treatment causes inflammation and damage to rat skin. In K3 (10% emulgel), the skin showed slight redness on the first day but then signs of healing, with less damage than in the base-emulgel group. In K4 (20% emulgel), the skin showed more damage than K3 early on, which was attributed to a stronger effect of 20% Calendula on the immune or inflammatory response, but over a longer period it showed healing and regeneration better than K2, although it still looked redder than K3. These observations were descriptive and were not scored statistically. Blood was then taken for the biomarker analysis.

MMP-1 Levels

MMP-1 was normally distributed in every group (Shapiro–Wilk $p = .949, .083, .462$, and $.683$ for K1 to K4) with homogeneous variances (Levene $p = .148$), and one-way ANOVA showed a significant difference among groups ($p = .004$; Table 1). The mean MMP-1 in the healthy group (K1) was 3.935 ± 0.534 ng/mL, and it rose to 4.787 ± 0.437 ng/mL in the negative control (K2), showing that UV-B exposure increased MMP-1 compared with unexposed skin. In the 10% (K3) and 20% (K4) emulgel groups, MMP-1 was 4.662 ± 0.205

and 4.788 ± 0.360 ng/mL, relatively close to the negative control. This indicates that the emulgel at these concentrations did not give a significant decrease in MMP-1 compared with the negative control. Table 1 gives the means, standard deviations, and test results for both biomarkers. The overall test was therefore statistically clear, and the pairwise comparisons that follow identify where the difference lies. Descriptively, the three exposed groups had means that were close to one another and all clearly above the healthy group, which already suggests that UV-B exposure was the dominant factor and that the emulgel did not move the mean far from the negative control. The standard deviations were similar in size across groups, in agreement with the homogeneity of variance reported by the Levene test, so the parametric analysis was appropriate for these data.

Table 1. MMP-1 and MMP-9 levels by group (ng/mL)

Variable	K1 Healthy	K2 UV-B + base	K3 UV-B + 10%	K4 UV-B + 20%	p (ANOVA)
MMP-1	3.935 ± 0.534	4.787 ± 0.437	4.662 ± 0.205	4.788 ± 0.360	.004
MMP-9	1.062 ± 0.089	1.173 ± 0.171	1.260 ± 0.114	1.228 ± 0.166	.104

Note. Values are mean $\pm$ SD ($n = 6$ per group). Shapiro–Wilk p for MMP-1: .949, .083, .462, .683; for MMP-9: .993, .340, .660, .372 (K1–K4). Levene test $p = .148$ for MMP-1 and .271 for MMP-9.

Tukey's test (Table 2) showed that the healthy group (K1) differed significantly from the negative control K2 ($p = .008$), from the 10% group K3 ($p = .025$), and from the 20% group K4 ($p = .008$). This means that UV-B exposure, with or without Calendula emulgel, raised MMP-1 significantly compared with normal skin. In contrast, there was no significant difference between K2 and K3 ($p = .949$) or between K2 and K4 ($p = 1.000$), and K3 did not differ from K4 ($p = .947$). Neither concentration of emulgel therefore lowered MMP-1 significantly compared with the negative control. The result suggests that the increase in MMP-1 in UV-B-exposed skin is driven mainly by the photodamage mechanism, and that any protective effect of Calendula at the tested concentrations would need a longer period of use, a higher dose, or combination with other photoprotective agents to become significant. The pairwise pattern agrees with the descriptive means in Table 1, in which K2, K3, and K4 are close to each other and K1 is lower. All p values given are those of the Tukey procedure, and the asterisk in Table 2 marks values at or below the .05 threshold. Only the three comparisons with the healthy group were significant, and none of the comparisons among the exposed groups was.

Table 2. Tukey HSD p values for MMP-1 between groups

Group	K1	K2	K3	K4
K1	-	.008*	.025*	.008*
K2		-	.949	1.000
K3			-	.947
K4				-

Note. Significant at $p^* \leq .05$.

MMP-9 Levels

MMP-9 was normally distributed in all groups (Shapiro–Wilk $p = .993$, .340, .660, and .372) with homogeneous variances (Levene $p = .271$). The mean MMP-9 was 1.062 ± 0.089 ng/mL in K1, with a narrow spread, and slightly higher at 1.173 ± 0.171 ng/mL in K2. The 10% and 20% emulgel groups had higher means than the other groups, at 1.260 ± 0.114 and 1.228 ± 0.166 ng/mL. One-way ANOVA gave $p = .104$, so there was no significant difference among the healthy, negative control, and extract groups, and no post hoc test was done. The emulgel at both concentrations therefore had no significant effect on blood MMP-9 in UV-B-exposed rats compared with the control. A box plot of the data showed the same picture: a lower and narrower distribution in healthy rats, a higher and wider range in the negative control, and extract groups within the range of the negative control. Figure 1 shows both biomarkers by group. The spread of the data was small in K1 and larger in the exposed groups, and the box plot presented in the source study shows the same difference in variation. The data therefore do not allow a conclusion that either concentration changed MMP-9, and the result is best read as a lack of detectable effect in this experiment, not as proof that no effect exists, because a small sample has limited power to detect modest differences. The authors noted that the higher means in the

extract groups might reflect stimulation of MMP-9 production as part of healing or adaptation to the changes caused by UV-B, but this was not supported statistically.

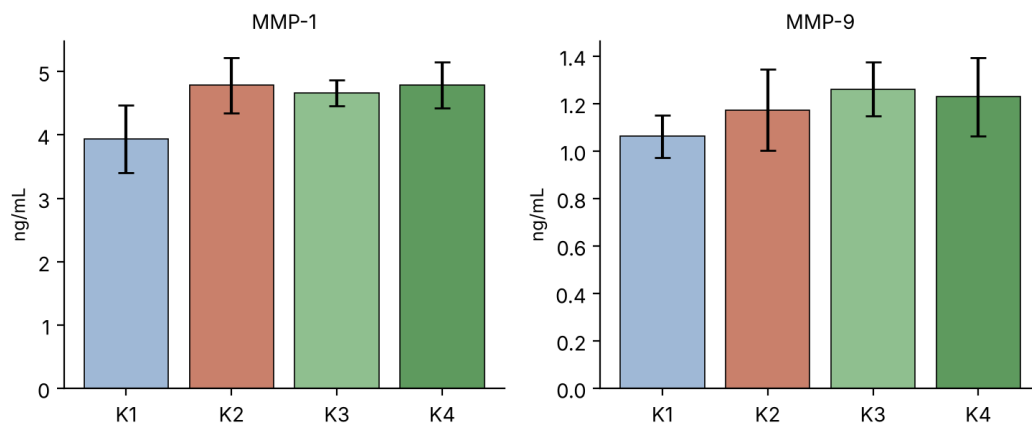


Figure 1. MMP-1 and MMP-9 levels in blood by group (mean $\pm$ SD)

DISCUSSION

MMP-1

UV-B exposure has a significant impact on skin health through damage to the extracellular matrix caused by increased MMP activity (Kim et al., 2022). UV-B induces ROS and inflammation, and activates MAPK signaling that induces MMP-1 and MMP-9 (Feng et al., 2024). In this study, MMP-1 in the UV-B group treated with base emulgel (K2) was higher than in healthy rats (K1), in line with the idea that UV-B raises MMP-1 as part of the response to damage (Lee et al., 2024). The 20% emulgel group (K4) had the highest MMP-1 mean of all groups, marginally above K2. The authors did not regard this increase as necessarily harmful: although *Calendula* is known for anti-inflammatory and wound-healing properties, at a higher dose it may stimulate MMP-1 production in connection with faster regeneration, which requires MMP-1 activity to repair a matrix damaged by UV-B, although further research is needed to confirm a positive effect on regeneration (Fonseca et al., 2010). The 10% group (K3) had lower MMP-1 than K4 but still higher than K1, so the lower dose appeared to give a more moderate effect. The statistical analysis, however, showed no significant difference between K2 and either extract group. Taken together, these observations indicate that UV-B raised MMP-1 in the exposed rats and that the emulgel did not reverse the increase within the period studied. Peripheral measurement by ELISA of blood is a practical way to follow the response, since biomarkers in blood may reflect processes in the skin such as inflammation and tissue damage (Matson, 2023), although it remains an indirect measure of what happens locally.

MMP-9

MMP-9 showed a pattern similar to MMP-1, with a higher level in the negative control than in healthy rats, which indicates a response to UV-induced tissue damage in which MMP-9, a gelatinase, degrades type IV collagen and other matrix components and contributes to tissue remodeling and inflammation (Feng et al., 2024). The 10% and 20% emulgels did not change MMP-9 significantly compared with the negative control. This is in line with the macroscopic observation that K3 showed slightly better skin repair than K4. The authors interpreted the lower dose as giving a better balance between repairing tissue damage and avoiding unwanted effects from excessive MMP-1 stimulation. The result does not mean that the outcome is poor: MMP-1 and MMP-9 act together to break down collagen into small fragments, which remains important for optimal tissue regeneration (Fonseca et al., 2010). The dose in this study was not effective enough to modulate MMP-9 significantly, since MMP-9 in K3 and K4 did not differ much from K2. Further research is needed to determine the optimal dose and duration that regulate MMP-1 and MMP-9 effectively without provoking excessive effects. In summary, the MMP-9 data show a response to UV-B in the direction expected from the literature, with no statistically detectable modification by the *Calendula* emulgel at either concentration, and the interpretation of the higher extract-group means as a repair-related response remains a hypothesis that needs direct testing in later studies with local tissue measurement.

CONCLUSION AND IMPLICATIONS

In UV-B-exposed male Wistar rats, *Calendula officinalis* L. extract emulgel at 10% and 20% did not significantly affect blood MMP-1 and MMP-9 levels compared with the negative control. The 10% and 20% emulgels were associated with significantly higher MMP-1 than in healthy rats, but did not differ from the negative control for either MMP-1 or MMP-9. The findings suggest that, at these concentrations and with 14 days of application, the emulgel did not reduce the blood levels of the two collagen-degrading enzymes after UV-B exposure. The authors recommend using more varied doses to find the dose that regulates MMP-1 and MMP-9 effectively, extending the observation period to assess long-term effects on skin regeneration, using other animal models, testing combinations with other active ingredients, and using larger samples to improve accuracy. For practice, the results do not support a claim of MMP-lowering photoprotection for these concentrations, and they remain preclinical results in rats that cannot be applied to people. These conclusions answer the objective of the study directly: UV-B raised MMP-1 relative to healthy skin, whereas the extract emulgel did not produce a statistically detectable change in either enzyme compared with the vehicle control. For research, the results show that a higher concentration does not necessarily mean a greater effect on matrix-degrading enzymes, and that outcomes should be interpreted together with measures of tissue repair.

Limitations and Future Research

The results may be influenced by individual variability, because physiological responses differ among animals even when procedures are uniform (Anagnostou et al., 2010). The doses used need further evaluation, since the 20% emulgel gave the highest MMP-1 level, which may indicate more intense tissue repair but also a risk of a stronger inflammatory effect. The observation duration needs to be reviewed to determine whether the period is adequate to assess longer-term effects of treatment, because skin repair takes longer (Raudenbush & Liu, 2001). Environmental conditions such as temperature, humidity, and diet could not be fully controlled and may influence biological responses (von Kortzfleisch et al., 2020), and a larger sample could reduce the possibility of statistical error and improve reliability (Netz et al., 2019). Future studies should use a wider range of doses and a longer observation period, and should be designed to test whether the higher MMP-1 level at the highest dose reflects tissue repair. Reporting these limitations clearly helps readers judge how far the findings can be generalized beyond this model. The study measured only two enzymes in blood and did not include histology of the skin, so tissue-level changes in collagen were not assessed directly, and the findings should be confirmed in independent experiments before any recommendation is made.

DECLARATIONS

Author contributions: Nadhilla Nurayu Lathifa: 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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