MEDISAINS
Jurnal Ilmiah Ilmu - Ilmu Kesehatan

Original Article

Kelakai serum reduces UVB-induced vascular endothelial growth factor and interleukin-6: in vivo study

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ARTICLE INFORMATION

Received: February 25, 2025
Revised: September 11, 2025
Accepted: September 11, 2025

KEYWORDS

Anti-inflammatory agents; Interleukin-6; Plant extracts; Reactive oxygen species; VEGF

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A B S T R A C T

Background: UVB exposure increases reactive oxygen species leading to higher expression of vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), which accelerate photoaging. Kelakai leaf (Stenochlaena palustris) contains flavonoids and phenolic compounds with antioxidant and anti-inflammatory potential, yet its efficacy in serum formulations has not been evaluated.

Purpose: To determine the effect of kelakai leaf extract serum on VEGF and IL-6 expression in BALB/c mice exposed to subchronic UVB radiation.

Method: This experimental post-test only control group study used 30 BALB/c mice assigned to five groups: healthy control (K1), UVB-exposed negative control (K2), UVB + 0.025% retinoid serum (K3), UVB + 2% kelakai serum (K4), and UVB + 4% kelakai serum (K5). UVB exposure (160 mJ/cm²) was given for 14 days followed by topical treatment. Skin tissues were examined using immunohistochemistry to assess VEGF and IL-6 expression. Data were analyzed using the Kruskal–Wallis test followed by Mann–Whitney.

Results: VEGF and IL-6 expression were highest in K2 and lowest in K1. Both retinoid and kelakai serum significantly reduced expression levels ($p<0.05$). The 4% kelakai serum (K5) produced greater reductions than the 2% dose, approaching the effect of retinoid serum.

Conclusion: Kelakai leaf extract serum at 4% effectively decreases VEGF and IL-6 expression in UVB-exposed mice, supporting its potential as a natural anti-photoaging agent.

INTRODUCTION

UVB radiation is a major extrinsic factor contributing to photoaging through increased production of reactive oxygen species (ROS) and activation of inflammatory pathways in the skin. Excessive UVB exposure disrupts collagen structure, damages elastin fibers, and alters dermal architecture, leading to wrinkles and reduced skin elasticity.[1-4] Oxidative stress induced by UVB stimulates the release of pro-inflammatory cytokines such as interleukin-6 (IL-6), enhances matrix metalloproteinase (MMP) activity, and upregulates vascular endothelial growth factor (VEGF) as a compensatory response to tissue injury.[5-7]

Natural antioxidant sources are increasingly explored as topical agents to mitigate UV-induced skin damage by neutralizing free radicals and modulating inflammatory cascades.[6,7] One promising botanical candidate is kelakai (*Stenochlaena palustris*), a traditional medicinal fern from Kalimantan known to contain phenolic compounds, flavonoids, and other bioactive constituents with strong antioxidant and anti-inflammatory properties.[8] Previous studies have reported that kelakai leaf extract possesses antioxidant activity comparable to ascorbic acid and can protect skin tissue from UV-induced oxidative injury.[9]

Despite these findings, scientific evidence regarding the effectiveness of kelakai extract in topical formulations particularly in serum form and its impact on key photoaging biomarkers such as VEGF and IL-6 remains limited. To date, no in vivo study has evaluated the biological effects of kelakai serum on molecular markers associated with UVB-induced skin damage.[10-14] This study aims to investigate the effects of kelakai leaf extract serum on VEGF and IL-6 expression in BALB/c mice exposed to subchronic UVB radiation, thereby providing a scientific basis for the development of natural, plant-based anti-photoaging skincare formulations.

https://doi.org/10.30595/medisains.v23i3.25906
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METHOD

Study Design

This study was an experimental investigation using a randomized post-test only controlled group design.[15]

Study Site

The research was conducted from November 2024 to January 2025. Male BALB/c mice were obtained from Kemuning, Solo City, a certified provider of laboratory animals. All laboratory procedures were carried out at the Integrated Biomedical Laboratory (IBL), Faculty of Medicine, Sultan Agung Islamic University, Semarang.

Materials

Materials used in this study included fresh kelakai leaves, serum base components (propylene glycol, triethanolamine, and sodium benzoate), 70% ethanol, dry gauze, cotton swabs, standard BALB/c mouse feed, and distilled water. Additional chemicals included buffer reagents, formalin solution, xylazine, and ketamine.

Kelekai leaf Extraction Process

A total of 500 g of kelakai leaf powder was extracted using the maceration method with 3,750 mL of 70% ethanol. The simplicia powder was placed into a dark glass container and soaked for 5 days with occasional shaking three times daily. After 3 days, the mixture was filtered, and the residue was re-macerated for 2 days using 1,250 mL of 70% ethanol. This process was repeated three times. All filtrates were combined and concentrated using a rotary evaporator at 50°C until a thick extract was obtained.

Kelakai Leaf Extract Serum Preparation

Serum preparation began by dispersing xanthan gum in distilled water and heating the mixture until a mucilaginous mass formed. After cooling, propylene glycol, kelakai leaf extract, and triethanolamine were added and stirred until homogeneous. The mixture was then incorporated into the xanthan gum base and stirred again until uniformly blended. Distilled water was added to adjust the final serum volume to 20 mL.

In Vivo Procedure

Animal Preparation

Twenty-five male BALB/c mice (3 months old, 25–30 g) were acclimatized for seven days before random assignment into five groups. The healthy control group (K1) received no UVB exposure. The negative control group (K2) was exposed to UVB and treated with serum base, while the positive control group (K3) received UVB followed by 0.025% retinoid serum. Treatment groups K4 and K5 were exposed to UVB and given 2% and 4% kelakai leaf extract serum, respectively, with all treatments administered once daily for 14 days.

Dosage Determination

The extract used in the serum was produced using the same maceration procedure described earlier: 500 g of kelakai leaf simplicia soaked in 3,750 mL of 70% ethanol, shaken periodically, filtered, and re-macerated using 1,250 mL ethanol for 2 days. The process was repeated three times, and the filtrate was concentrated using a rotary evaporator at 50°C.

Experimental Procedure

After a 7-day acclimatization period, the dorsal fur of each mouse was shaved to create a 3 × 4 cm area. UVB irradiation was administered from a distance of 20 cm at a minimal erythema dose (MED) of 160 mJ/cm² for approximately 15 minutes per day over 14 days. The development of erythema on the dorsal skin served as confirmation of cumulative UVB absorption. Following UVB exposure, kelakai leaf extract serum (2% or 4%) or retinoid serum was applied topically once daily for 14 consecutive days, depending on the group allocation. At the end of the treatment period, skin tissue samples were collected. All mice were euthanized under anesthesia. Incisions were made on the UVB-exposed dorsal skin to obtain tissue samples, which were weighed and homogenized in phosphate-buffered saline (PBS, pH 7.4) under cold conditions (4°C). Homogenates were centrifuged at 2,000–3,000 rpm for 20 minutes, and the resulting supernatant was used for analysis.

VEGF and IL-6 Expression Analysis Using the Immunohistochemistry (IHC) Method

Paraffin-embedded skin sections (5 µm thick) were heated at 60°C and deparaffinized in three changes of xylene (30 minutes each). Sections were rehydrated through graded alcohols (100%, 95%, 70%, 50%, 30%) followed by two rinses in deionized water (20 minutes each). Tissue sections were digested with hyaluronidase (1 mg/mL in 0.1 M sodium acetate and 150 mM sodium chloride, pH 5.5) at 37°C for 30 minutes. After washing in PBS (three times, 10 minutes each), sections were incubated with 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity.

Following PBS washes, sections were incubated with primary polyclonal antibody (1:30 dilution; 10 µg/mL) for 4 hours at room temperature. Slides were then rinsed and incubated with biotinylated secondary antibody (2 µg/mL; Dako, Carpinteria, CA) for 30 minutes, followed by streptavidin–peroxidase conjugate (2 µg/mL; Dako) for another 30 minutes. After three additional PBS washes, antigen–antibody complexes were visualized using 3-amino-9-ethylcarbazole (AEC) substrate with 3% hydrogen peroxide for 15 minutes. Counterstaining was performed using Smith hematoxylin for 1 minute. All samples were coded and evaluated semi-quantitatively by a trained anatomical pathologist.

Statistical Analysis

VEGF and IL-6 expression data were tested for normality using the Shapiro–Wilk test and for homogeneity using Levene's test. Since the data were not normally distributed ($p < 0.05$), the Kruskal–Wallis test was used to determine differences among groups, followed by the Mann–Whitney

post-hoc test for pairwise comparisons ($p < 0.05$). Statistical analyses were conducted using SPSS for Windows with a significance level of $\alpha = 5\%$.

Ethical Consideration

This study received ethical approval from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang (No. 318/VIII/2024/ Komisi Bioetik).

RESULTS

VEGF expression based on IHC staining is shown in Figure 1, with arrows indicating VEGF-positive brown-stained cytoplasm. UVB exposure substantially increased VEGF levels in the Negative Control group (K2), while the Healthy Control group (K1) showed only minimal expression. Retinoid treatment (K3) reduced VEGF expression compared with K2. Kelakai serum also lowered VEGF levels, with the 4% dose (K5) producing a greater reduction than the 2% dose (K4) and approaching the effect observed in the retinoid group. The IHC staining results for IL-6 expression are shown in Figure 2. UVB exposure markedly increased IL-6 expression in K2, consistent with the expected inflammatory response. Retinoid serum (K3) and kelakai leaf extract serum (K4 and K5) both reduced IL-6 expression, with the most notable effect observed at the 4% concentration in K5.

The quantitative analysis of VEGF expression is presented in Figure 3. The highest mean VEGF expression occurred in K2 (18.88 ± 0.27%). Expression levels were lower in K4 (5.12 ± 0.23%) and further decreased in K5 (4.40 ± 0.24%). Retinoid serum in K3 produced an even greater reduction (2.68 ± 0.11%), while K1 showed the lowest expression (0.08 ± 0.11%). The quantitative IL-6 data are summarized in Figure 4. The highest IL-6 expression was recorded in K2 (17.92 ± 0.18%). Expression decreased to 4.92 ± 0.23% in K4 and to 4.44 ± 0.26% in K5. The retinoid group (K3) again showed lower expression (2.68 ± 0.11%), while K1 demonstrated the lowest IL-6 value (0.08 ± 0.11%).

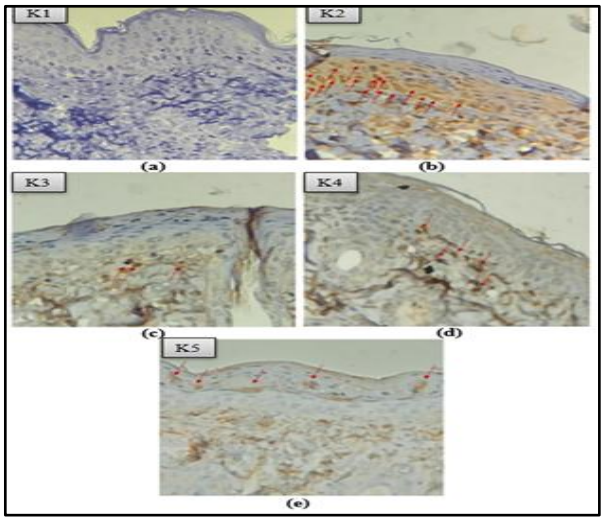

Figure 1. comparasion VEGF expression in various treatment groups: (a) K1, (b) K2, (c) K3, (d) K4, (e) K5.

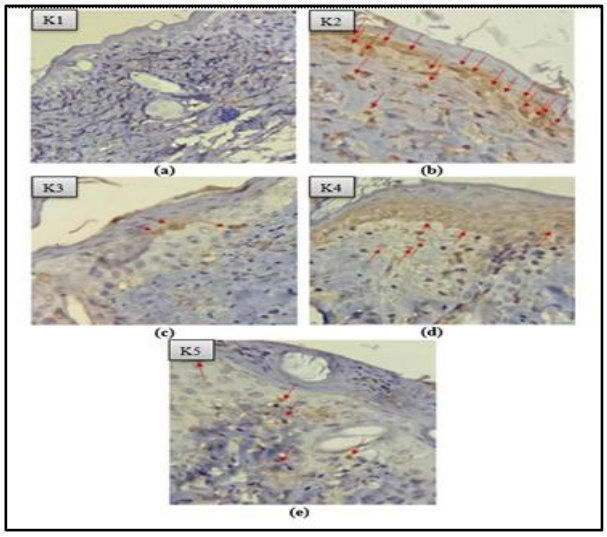

Figure 2. IL-6 expression in various treatment groups: (a) K1, (b) K2, (c) K3, (d) K4, (e) K5. Arrows indicate darkly stained cytoplasm indicating IL-6 expression.

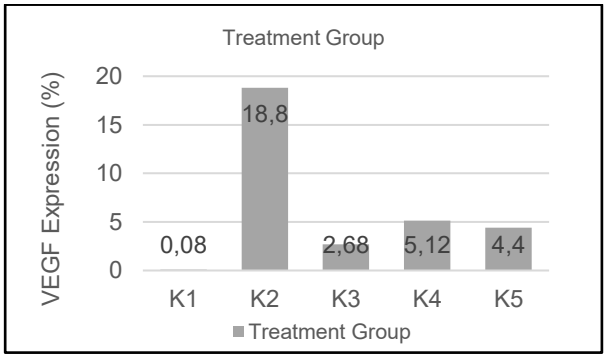

Figure 3. Comparation VEGF expression in each treatment group

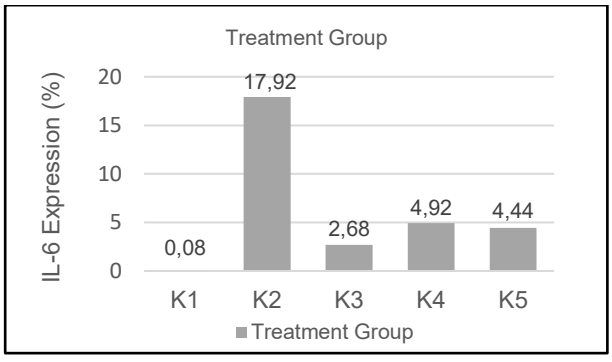

Figure 4. IL-6 expression in each treatment group

DISCUSSION

This study demonstrated that topical administration of kelakai leaf extract serum effectively modulated VEGF and IL-6 expression in BALB/c mice exposed to subchronic UVB radiation. The results showed that the 2% kelakai serum decreased VEGF expression, while the 4% serum produced a more substantial reduction in IL-6 compared with the negative control group. These findings indicate that kelakai extract exerts dose-dependent antioxidant and anti-inflammatory effects against UVB-induced skin damage.

The healthy control group exhibited the lowest VEGF expression, consistent with the absence of UVB exposure and the resulting lack of ROS accumulation.[16] UVB irradiation is known to induce oxidative stress, which activates intracellular pathways that increase VEGF expression as part of tissue repair and angiogenic responses. This mechanism explains the pronounced VEGF elevation observed in the negative control group. Antioxidant and anti-inflammatory agents have been shown to inhibit VEGF upregulation under oxidative conditions, supporting the ability of kelakai extract and retinoids to reduce VEGF expression in this study.[17,18] Retinoid serum produced the strongest suppression of VEGF, likely due to its established antiproliferative and anti-angiogenic effects. In contrast, kelakai extract exerted a more moderate yet beneficial response by lowering VEGF without excessively inhibiting angiogenesis, which is essential for tissue regeneration.[19,20]

A similar pattern was observed for IL-6. Retinoid serum (0.025%) was more effective in reducing IL-6 expression than kelakai extract, reflecting its potent anti-inflammatory mechanism. Nonetheless, the 4% kelakai serum significantly reduced IL-6 levels compared to both K2 and the 2% dose, consistent with the higher availability of flavonoids capable of suppressing NF-κB activation.[21] This reduction supports the role of kelakai extract in controlling inflammation while preserving the tissue repair process. Unlike retinoids, which may cause irritation or slow healing when overused, kelakai extract provides a gentler, plant-based alternative.[22,23]

The biological effects observed in this study align with the known phytochemical properties of kelakai leaves. Flavonoids act as strong antioxidants and reduce IL-6 production, while saponins and phenolic compounds support wound healing by promoting fibroblast proliferation and regulating inflammation.[24] These combined actions help accelerate tissue recovery while maintaining inflammation at a controlled, beneficial level.[25] Previous research has also highlighted the high antioxidant and flavonoid content of kelakai extract, reinforcing its potential as a natural anti-photoaging agent.[26]

Taken together, the findings of this study show that kelakai leaf extract serum can reduce key molecular markers of photoaging (VEGF and IL-6) and may serve as a promising botanical ingredient for topical skin protection.[27,28] Although retinoids remain more potent, kelakai extract offers a safer alternative for individuals with sensitive skin or those who experience irritation from conventional retinoid therapy. This study has several limitations that should be considered when interpreting the findings. First, the experiment was conducted using a relatively small sample size, which may limit the generalizability of the results. Second, only two concentrations of kelakai serum (2% and 4%) were tested, leaving the optimal therapeutic dose undetermined. Third, the study focused solely on VEGF and IL-6 as biomarkers, without evaluating other important markers of photoaging such as MMPs, collagen degradation, or oxidative stress parameters.

CONCLUSIONS AND RECOMMENDATION

This study demonstrated that kelakai leaf extract serum effectively modulated VEGF and IL-6 expression in BALB/c mice exposed to subchronic UVB radiation. The 2% kelakai serum reduced VEGF expression compared with the UVB-exposed control group, while the 4% formulation produced a significant decrease in IL-6 expression, indicating a dose-dependent anti-inflammatory and antioxidant effect.

Further research is needed to determine the optimal therapeutic dose of kelakai extract. Future studies should include dose-response testing with concentrations above 4% and evaluate whether higher doses can enhance the suppression of IL-6 and VEGF without causing irritation. Investigating combination formulations using low-dose retinoids with kelakai extract serum may also provide synergistic benefits for improving skin regeneration and reducing UVB-induced damage.

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