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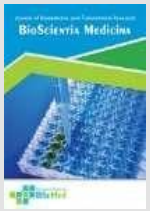

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Topical *Allium cepa* Ethanol Extract Restores SOD and VEGF Without Increasing Hair Length in UV-B-Exposed Wistar Rats

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A B S T R A C T

Background: Ultraviolet B (UV-B) accelerates hair photoaging by depleting cutaneous superoxide dismutase (SOD) and dysregulating vascular endothelial growth factor (VEGF). Red onion (*Allium cepa*) is rich in flavonoids and organosulfur precursors of hydrogen sulphide that may enhance both antioxidant defenses and pro-angiogenic signaling. This study evaluated topical *Allium cepa* ethanol extract on cutaneous SOD, VEGF, and hair length in UV-B-exposed Wistar rats. **Methods:** Twenty male Wistar rats (3–4 months, 180–200 g) were randomly allocated to five groups (n=4): normal control without UV-B (KN), placebo with UV-B and base preparation (K-), and three groups receiving topical *Allium cepa* ethanol extract at 1% (P1), 2% (P2), and 4% (P3). UV-B was administered at 65 mJ/cm² thrice weekly for 21 days. SOD and VEGF were measured by ELISA; hair length by digital calipers. Data were analyzed by one-way ANOVA with Tukey post-hoc tests. **Results: UV**-B reduced SOD by 77.2% and VEGF by 61.7% versus normal. Topical *Allium cepa* restored both dose-dependently: SOD reached 13.85±0.27 U/mL and VEGF reached 43.00±1.13 ng/L at 4%, with 4% VEGF exceeding normal by 33% ($p<0.001$). The 2% concentration restored VEGF to physiological levels ($p=0.493$). Hair length did not differ across groups ($p=0.394$). The cross-group SOD–VEGF correlation was strongly positive (Pearson $r=0.804$). **Conclusion:** Topical *Allium cepa* ethanol extract is a potent dose-dependent enhancer of cutaneous SOD and VEGF in UV-B-exposed rats. The dissociation between robust biochemical recovery and the unchanged hair-length endpoint supports *Allium cepa* as an antioxidant and pro-angiogenic adjunct for hair photoaging warranting extended-duration translational studies.

1. Introduction

Cutaneous photoaging is a pervasive and progressive consequence of cumulative environmental insult that disproportionately affects ageing populations and individuals living in equatorial regions where ambient ultraviolet (UV) flux is highest. The World Health Organization estimates that by 2030 more than one in six people globally will be aged over 60 years, and skin and hair changes are among the earliest visible markers of biological ageing in this expanding cohort.[1] Hair, in particular, serves as a sensitive clinical indicator of photoaging because changes in colour, density, and texture are easily perceived by patients and clinicians alike, and because the underlying follicular biology is exquisitely sensitive to oxidative and angiogenic perturbations.[2]

Among the spectral components of UV light, UV-B (280–315 nm) carries higher photochemical energy than UV-A and is sufficient to penetrate the epidermis and reach the dermal–follicular interface where it generates reactive oxygen species (ROS), induces DNA damage, and triggers chronic low-grade inflammation.[3,4]

Population-level data indicate that hair-follicle disorders attributable to ageing and UV exposure are a major contributor to global dermatological burden, with even higher prevalences reported in tropical Asian populations exposed to year-round high UV indices.[5,6] In Indonesia, where the ambient UV index frequently exceeds the World Health Organization 'extreme' threshold, hair photoaging may begin earlier and progress more rapidly than in temperate populations, yet pre-clinical and clinical evidence specific to Indonesian biological and botanical contexts remains comparatively sparse.[7]

At the molecular level, UV-B-induced photoaging unfolds through tightly coupled redox and angiogenic axes. Cutaneous superoxide dismutase (SOD) constitutes the first enzymatic barrier against ROS, dismutating superoxide anion into hydrogen peroxide which is subsequently reduced by catalase and glutathione peroxidase. The three SOD isoforms — cytosolic SOD1, mitochondrial SOD2, and extracellular SOD3 — are co-expressed across the dermis and follicle, and their depletion under UV-B exposure leads to lipid peroxidation, carbonylation of follicular keratinocyte proteins, and apoptosis of hair-matrix cells.[8]

Concurrent with antioxidant depletion, UV-B alters cutaneous angiogenesis through dysregulation of vascular endothelial growth factor (VEGF). VEGF is essential for perifollicular capillary networks that sustain matrix-cell proliferation during the anagen phase; in vivo blockade of VEGF activity in mice reduces perifollicular vascularization by approximately 40% and accelerates entry into telogen.[9,10] UV-B initially up-regulates VEGF as an immediate-early stress response, but with chronic exposure VEGF expression becomes erratic and ultimately declines, producing rarefied dermal vasculature characteristic of photoaged skin.[9]

Current pharmacotherapy for hair photoaging is dominated by topical minoxidil and oral finasteride. Both molecules carry well-documented limitations: minoxidil causes transient telogen effluvium, scalp irritation, and risk of contact dermatitis, while finasteride is contraindicated in women of reproductive age and may produce sexual side-effects.[11,12] Phytochemical-based agents have therefore re-emerged as candidate adjuncts that may simultaneously address oxidative and angiogenic dysregulation. *Allium cepa* (red onion) is particularly attractive: it is dense in quercetin, kaempferol, and organosulfur precursors of hydrogen sulphide that respectively scavenge ROS, activate Nrf2-driven antioxidant gene expression, and phosphorylate VEGF receptor 2 to promote angiogenesis.[13-15]

Despite this mechanistic plausibility, three gaps remain. First, no published study has simultaneously reported SOD, VEGF, and hair-length endpoints in a UV-B model under topical *Allium cepa* exposure. Second, dose–response evidence using Indonesian-sourced red onion is essentially absent, which matters because phenolic content varies with cultivar and growing conditions. Third, it is unclear whether biochemical recovery of SOD and VEGF translates into measurable phenotypic improvement in hair-shaft elongation in a 21-day model.[16] Earlier work by our group has summarized the therapeutic potential of flavonoids in alopecia, providing a conceptual scaffold for the present empirical investigation.[17]

Murine genetic models in which VEGF is conditionally over-expressed in keratinocytes display thicker hair shafts and longer anagen phases, while VEGF blockade reduces hair growth and produces visible alopecia within a single hair cycle.[10] Conversely, the relationship between SOD activity and follicular health is bidirectional: cumulative oxidative stress drives premature greying, hair thinning, and follicle miniaturisation, while restoration of redox balance prolongs the anagen phase and slows greying.[2,8] These independent lines of evidence

converge on a redox–angiogenesis axis as a tractable therapeutic target for hair photoaging.

Within the broader landscape of phytotherapy, plant extracts that simultaneously enhance both arms of this axis are uncommon. Most botanicals studied for hair growth either possess antioxidant activity or pro-angiogenic activity, but rarely both. *Allium cepa* is unusual in that its phenolic flavonoids confer potent radical-scavenging activity while its organosulfur backbone donates hydrogen sulphide to drive endothelial proliferation. This dual mechanism, combined with its low cost, year-round availability in tropical Asia, and excellent safety record at culinary doses, marks *Allium cepa* as a particularly promising candidate for adjunctive translational dermatology.[13,15,16]

The novelty of this study lies in three integrated dimensions. First, this is, to our knowledge, the first pre-clinical investigation to simultaneously evaluate cutaneous SOD, VEGF, and quantitative hair length endpoints in a UV-B photoaging model under topical *Allium cepa* exposure. Second, the dose–response design at three concentrations (1%, 2%, and 4%) provides granular pharmacodynamic resolution that single-dose studies cannot achieve, and that is essential for downstream dose calibration. Third, the use of an Indonesian *Allium cepa* cultivar from Songan A Village, Kintamani, Bali, anchors the work in a regionally relevant biological matrix that has previously been under-investigated for trichology indications. The aim of this study was therefore to evaluate the effect of a topical liquid preparation of an ethanol extract of *Allium cepa*, at three concentrations, on cutaneous SOD activity, VEGF concentration, and hair length in male Wistar rats subjected to a standardized UV-B photoaging protocol.

From a public-health vantage point, hair photoaging carries quality-of-life implications well beyond cosmetic appearance. Cross-sectional surveys consistently associate visible hair changes with reduced self-esteem, social-engagement avoidance, and depressive symptoms across both sexes, with effects amplified in working-age adults whose professional identity intersects with personal presentation. The economic burden is also substantial: annual global expenditure on hair-loss treatments now exceeds an estimated USD 12 billion, of which a large fraction is allocated to topical agents. Within Indonesia and the broader ASEAN region, demand for affordable evidence-based phytotherapeutics is growing rapidly as consumers seek alternatives to long-term pharmacological regimens with documented adverse-event profiles. Establishing a robust mechanistic and dose–response evidence base for locally cultivated *Allium cepa* is therefore not only a translational priority but also a culturally aligned response to a regional unmet need.

2. Methods

Ethics approval

Research Ethics Committee, Faculty of Medicine, Universitas Udayana (No: 2961/UN14.2.VII.14/LT/2025, dated December 23rd, 2025).

Study design

This pre-clinical experimental study employed a randomized post-test only with control group design to evaluate the effect of a topical liquid preparation of an ethanol extract of *Allium cepa* on cutaneous SOD activity, VEGF concentration, and hair length in male Wistar rats following a standardized UV-B photoaging protocol. Twenty animals were randomly allocated into five equal groups using a sequence generated in IBM SPSS Statistics version 26 to ensure allocation concealment. The five groups were: a normal control without UV-B exposure or topical intervention (KN), a placebo group exposed to UV-B and treated with the base preparation only (K-), and three treatment groups exposed to UV-B and treated with the topical liquid preparation containing *Allium cepa* ethanol extract at 1% (P1), 2% (P2), and 4% (P3). The primary endpoints were tissue SOD activity (U/mL), VEGF concentration (ng/L), and hair length (mm) measured at the end of the 21-day intervention.

Setting and timeline

The study was conducted from July 2025 to January 2026. Plant material (red onion) was obtained from Songan A Village, Kintamani, Bali, Indonesia. Extraction was performed at the Laboratory of the Faculty of Agricultural Technology, Universitas Udayana. Animal husbandry, treatment administration, tissue collection, and ELISA analyses were performed at the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Udayana, Bali, Indonesia.

Plant material and extraction

Fresh red onion bulbs (1 kg) were cleaned, sliced uniformly to 2–3 mm thickness, and dried in a convection oven at 50°C for 15 hours to a constant mass. The dried simplisia was milled into a fine powder (200 g, equivalent to 20% of fresh weight). Extraction was performed by maceration in 2 L of 96% ethanol at a 1:10 (w/v) ratio for 24 hours at room temperature with intermittent stirring twice daily. The macerate was filtered through Whatman No. 1 paper, concentrated using a rotary evaporator at 40°C, 100 rpm, and 100 mBar until solvent-free, and stored at 4°C in amber glass containers until use. The yield of the concentrated extract was calculated as the percentage of extract mass over initial simplisia mass.

Topical liquid preparation

Topical liquid preparations were formulated according to a published protocol with minor modifications.[7] Each 100 mL preparation contained *Allium cepa* ethanol extract at the assigned concentration (0% for K-, 1% for P1, 2% for P2, and 4% for P3), 30% v/v 96% ethanol as the principal solvent, 15% v/v propylene glycol as a humectant, 0.01% w/v methyl paraben as a preservative, 1% v/v strawberry essence as a fragrance, and distilled water to 100 mL final volume. Methyl paraben was first dissolved in 15 mL of 96% ethanol; strawberry essence was separately combined with 5 mL of 96% ethanol and propylene glycol under stirring; both phases were combined, the prescribed volume of *Allium cepa* extract was added (0, 1, 2, or 4 mL), and distilled water was added to 100 mL final volume. The mixture was homogenized using a magnetic stirrer for 30 minutes.

Animal subjects

Inclusion criteria were male Wistar rats (*Rattus norvegicus*) aged 3–4 months and weighing 180–200 grams that were active, in good general condition, and free of physical abnormalities. Drop-out criteria included development of skin irritation, erythema, fungal, bacterial, or viral infections, or death during the study period. Sample size was calculated according to the Arifin and Zahiruddin resource equation N = (DF/k) + 1, where DF is the within-group degrees of freedom and k is the number of groups.[18] The minimum required sample was three animals per group; an additional 10% (rounded up to one animal per group) was added to allow for dropout, yielding four animals per group and a total sample of 20 rats.

Acclimatization and shaving

All rats were acclimatized for 7 days before treatment in standard polycarbonate cages (40 × 30 cm) with rice husk bedding. Environmental conditions were maintained at 23 ± 3°C, 60 ± 5% relative humidity, and a 12-hour light/dark cycle (12 hours of 10-watt yellow light, followed by 12 hours of darkness). Cages were cleaned daily. Four rats were housed per cage with ad libitum access to water and were fed conventional pellet feed (HI Provite 594) twice daily at 20 g per rat. Following acclimatization, a 2 × 3 cm dorsal area was shaved using an electric clipper 24 hours before the first intervention.

UV-B exposure and treatment protocol

The topical preparations were applied once daily at 09:30 WITA to the shaved dorsal region, 30 minutes before UV-B exposure to permit cutaneous absorption. After application, animals were placed individually in clean cages for 30–60 minutes until the formulation had dried, preventing self-grooming. UV-B exposure was delivered using a narrow-spectrum lamp (Philips PL-S 9W/01, peak emission 311 nm, 1 W) positioned

15 cm above the animal's back. Exposure was administered at 10:00 WITA to groups K-, P1, P2, and P3 at a dose of 65 mJ/cm² for 65 seconds per session, three times weekly (Monday, Wednesday, Friday) over three consecutive weeks, yielding a cumulative dose of 585 mJ/cm². This protocol is consistent with previously validated UV-B photoaging models in rodent skin.[19] Topical preparations continued daily, irrespective of UV-B exposure days. The KN group received no topical intervention and no UV-B exposure.

Sample collection and biochemical assays

On day 21, rats were anaesthetized with intramuscular xylazine 5 mg/kg and ketamine 20 mg/kg into the quadriceps and euthanized by cervical dislocation. Hair was collected from the treated dorsal area using fine forceps. A 0.2 g full-thickness skin biopsy was excised from the same region using a sterile scalpel and divided for ELISA and formalin fixation (1:1 PBS to 10% buffered formalin). Tissue homogenates were prepared in 500 µL ice-cold PBS using an ultrasonic homogenizer for 2 minutes (9 seconds on, 1 second off) over an ice bath, centrifuged at 12,000 rpm for 10 minutes at 4°C, and the supernatant was stored at −20°C until assay. SOD activity was quantified using a Rat SOD1 ELISA Kit (BTLab®) with absorbance read at 450 nm; VEGF concentration was quantified using a Rat VEGF-A ELISA Kit (BTLab®) with the same protocol. All assays were run in technical duplicate. Hair length was measured by collecting ten randomly selected hairs per animal and recording length to the nearest 0.01 mm using a digital caliper; the mean of the ten measurements was used as the per-animal value, following methodology previously applied in UV-B-induced hair photoaging models.[20] Animal carcasses were collected, sealed in biohazard bags, and incinerated at 800–1200°C as per institutional hazardous waste management procedures.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26. Continuous variables were summarized as mean ± standard deviation (SD) with minimum and maximum values, and 95% confidence intervals (95% CI) were computed for each group mean. Normality was tested using the Shapiro–Wilk test and homogeneity of variances using Levene's test. Between-group differences for SOD, VEGF, and hair length were compared using one-way analysis of variance (ANOVA) with Tukey HSD post-hoc tests when ANOVA reached significance. Effect sizes were computed as eta-squared (η^2) for the omnibus ANOVA and Cohen's d (using the pooled standard deviation) for selected pairwise contrasts. Pearson product-moment correlation was used to examine the SOD–VEGF relationship across group means. Statistical significance was set at $\alpha = 0.05$; all p-values are reported to three decimal places where appropriate.

Ethical clearance

This study received ethical approval from the Research Ethics Committee, Faculty of Medicine, Universitas Udayana (No: 2961/UN14.2.VII.14/LT/2025, dated December 23rd, 2025). All animal procedures were performed in accordance with institutional and international guidelines for the care and use of laboratory animals, including ARRIVE 2.0 reporting recommendations.

3. Results

Extract yield and group flow

From 1 kg of fresh *Allium cepa* bulbs, 200 g of dried simplisia was obtained after slicing and oven drying at 50°C for 15 hours. Maceration with 2 L of 96% ethanol followed by rotary evaporation yielded 35.28 g of concentrated extract, corresponding to a yield of 17.64%, which exceeds the minimum 10% requirement for concentrated herbal extracts specified in the Indonesian Herbal Pharmacopoeia.[21] All 20 animals completed the 21-day intervention; no animal met drop-out criteria, and no signs of overt skin irritation, erythema beyond UV-B-related changes, or

systemic illness were observed.

Descriptive analysis of primary endpoints

Descriptive statistics for the three primary endpoints across the five groups are presented in Table 1. SOD activity was highest in the normal control group (16.54 ± 0.02 U/mL) and lowest in the placebo group (3.77 ± 0.28 U/mL), corresponding to a 77.2% reduction caused by UV-B exposure. Topical *Allium cepa* restored SOD activity in a dose-dependent manner: 7.02 ± 0.20 U/mL at 1%, 10.99 ± 0.13 U/mL at 2%, and 13.85 ± 0.27 U/mL at 4%, the latter approaching but not reaching normal levels. VEGF concentration showed a similar trajectory: the placebo group exhibited a 61.7% reduction (12.33 ± 1.38 ng/L) versus the normal group (32.23 ± 1.69 ng/L), while topical *Allium cepa* produced graded restoration to 27.62 ± 2.63 ng/L at 1%, 34.15 ± 0.83 ng/L at 2%, and 43.00 ± 1.13 ng/L at 4%, with the highest concentration significantly exceeding even the normal control (Figure 1, panel B). Hair length data displayed a different pattern with markedly higher within-group variability: 22.81 ± 4.63 mm in the normal control, 19.54 ± 4.39 mm in the placebo group, 24.39 ± 3.99 mm in the 1% group, 22.70 ± 1.42 mm in the 2% group, and 17.84 ± 8.51 mm in the 4% group (Figure 4).

Table 1. Descriptive analysis of SOD activity, VEGF concentration, and hair length across treatment groups (mean ± SD; minimum–maximum).

Variable	Group	Mean	SD	Minimum	Maximum
SOD (U/mL)	KN	16.54	0.02	16.53	16.57
	K-	3.77	0.28	3.57	4.17
	P1 (ACE 1%)	7.02	0.20	6.72	7.16
	P2 (ACE 2%)	10.99	0.13	10.89	11.17
	P3 (ACE 4%)	13.85	0.27	13.56	14.17
VEGF (ng/L)	KN	32.23	1.69	30.31	34.15
	K-	12.33	1.38	11.08	14.15
	P1 (ACE 1%)	27.62	2.63	24.15	30.31
	P2 (ACE 2%)	34.15	0.83	33.00	34.92
	P3 (ACE 4%)	43.00	1.13	41.85	44.15
Hair length (mm)	KN	22.81	4.63	17.20	26.90
	K-	19.54	4.39	13.92	23.64
	P1 (ACE 1%)	24.39	3.99	20.82	30.10
	P2 (ACE 2%)	22.70	1.42	20.82	23.90
	P3 (ACE 4%)	17.84	8.51	10.70	30.20

Note. SD = standard deviation; KN = normal control; K- = placebo (UV-B + base preparation); ACE = *Allium cepa* ethanol extract; P1, P2, P3 = treatment groups.

Distributional assumptions

Shapiro–Wilk testing did not reject normality for SOD, VEGF, or hair length in any of the five groups (all $p > 0.05$; Table 2). Levene's test confirmed homogeneity of variances for all three endpoints (SOD $p = 0.065$; VEGF $p = 0.222$; hair length $p = 0.137$). Parametric one-way ANOVA was therefore the appropriate inferential test for between-group comparisons.

Table 2. Shapiro–Wilk normality and Levene's homogeneity tests.

Variable	Group	n	Shapiro–Wilk p	Levene p
SOD (U/mL)	KN	4	0.086	0.065
	K-	4	0.146	
	P1	4	0.065	
	P2	4	0.230	
	P3	4	0.760	
VEGF (ng/L)	KN	4	0.890	0.222
	K-	4	0.580	
	P1	4	0.850	
	P2	4	0.570	
	P3	4	0.340	
Hair length (mm)	KN	4	0.390	0.137
	K-	4	0.630	
	P1	4	0.350	
	P2	4	0.430	
	P3	4	0.150	

Note. All p-values exceed 0.05; distributional and homogeneity assumptions for parametric ANOVA were satisfied at α=0.05.

Inferential analysis

One-way ANOVA revealed highly significant between-group differences for SOD activity ($p < 0.001$) and VEGF concentration ($p < 0.001$), corresponding to very large effect sizes ($\eta^2 > 0.99$ for both endpoints). In contrast, no significant difference was observed for hair length ($p = 0.394$; $\eta^2 = 0.23$), indicating that biochemical recovery did not translate into measurable hair-shaft elongation within the 21-day window (Table 3, Figure 1).

Table 3. One-way ANOVA comparison of SOD, VEGF, and hair length between treatment groups.

Variable	Group	Mean ± SD	95% CI Lower	95% CI Upper	ANOVA p
SOD (U/mL)	KN	16.54 ± 0.02	16.51	16.57	**<0.001***
	K-	3.77 ± 0.28	3.32	4.22	
	P1	7.02 ± 0.20	6.69	7.34	
	P2	10.99 ± 0.13	10.78	11.19	
	P3	13.85 ± 0.27	13.41	14.28	
VEGF (ng/L)	KN	32.23 ± 1.69	29.54	34.92	**<0.001***
	K-	12.33 ± 1.38	10.13	14.52	
	P1	27.62 ± 2.63	23.43	31.80	
	P2	34.15 ± 0.83	32.83	35.47	
	P3	43.00 ± 1.13	41.20	44.80	
Hair length (mm)	KN	22.81 ± 4.63	15.45	30.17	0.394
	K-	19.54 ± 4.39	12.56	26.52	
	P1	24.39 ± 3.99	18.03	30.75	
	P2	22.70 ± 1.42	20.45	24.96	
	P3	17.84 ± 8.51	4.29	31.38	

Note. *p<0.05, statistically significant. 95% CI refers to the confidence interval of the group mean.

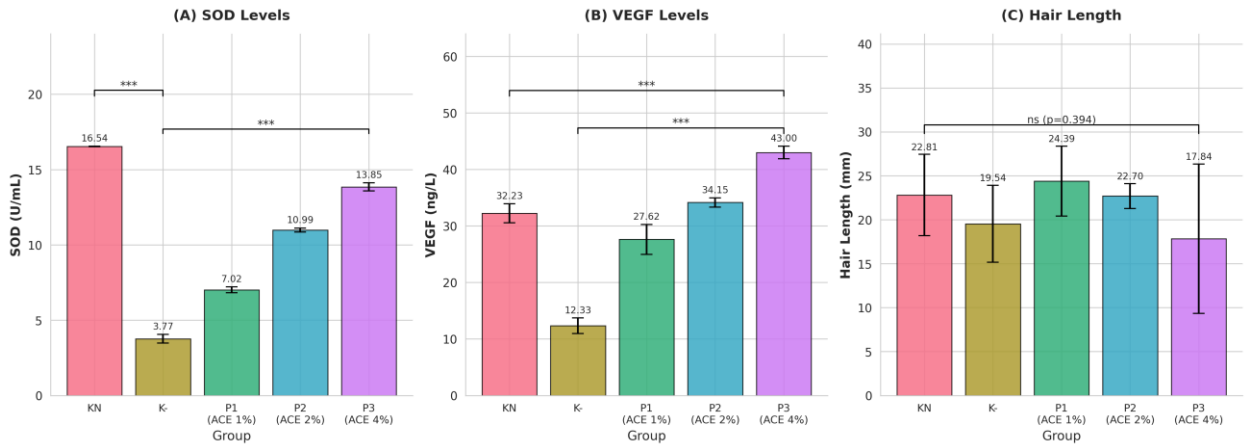

Figure 1. Grouped bar plots of mean ± SD for SOD activity (panel A), VEGF concentration (panel B), and hair length (panel C) across the five treatment groups. Significance markers: ***p<0.001; ns = not significant. Error bars represent SD; numerical labels indicate group means.

Post-hoc analysis of SOD

Tukey HSD post-hoc analysis for SOD activity demonstrated significant differences in every pairwise comparison (all p < 0.001; Table 4 and Figure 2, left panel). The mean difference between normal control and placebo was 12.78 U/mL (95% CI 12.32–13.23), confirming the magnitude of UV-B-induced SOD depletion. Each treatment group differed significantly from placebo: K- versus P1 mean difference 3.25 U/mL (95% CI 2.80–3.70), K- versus P2 mean difference 7.22 U/mL (95% CI 6.77–7.67), and K- versus P3 mean difference 10.08 U/mL (95% CI 9.63–10.53). Significant dose–response increments were also observed within the treatment series: P1 versus P2 (3.97 U/mL, 95% CI 3.52–4.42), P1 versus P3 (6.83 U/mL, 95% CI 6.38–7.28), and P2 versus P3 (2.86 U/mL, 95% CI 2.41–3.31). The 4% group remained 2.70 U/mL below the normal control (95% CI 2.24–3.15, p < 0.001), indicating partial but incomplete restoration. Cohen's d for K- versus P3 was 36.7, indicating an extremely large effect size.

Table 4. Post-hoc Tukey HSD pairwise comparisons of SOD activity.

Comparison	Mean Difference	95% CI Lower	95% CI Upper	p-value
KN vs K-	12.78	12.32	13.23	**<0.001***
KN vs P1	9.52	9.07	9.97	**<0.001***
KN vs P2	5.55	5.10	6.01	**<0.001***
KN vs P3	2.69	2.24	3.15	**<0.001***
K- vs P1	-3.25	-3.70	-2.80	**<0.001***
K- vs P2	-7.22	-7.67	-6.77	**<0.001***
K- vs P3	-10.08	-10.53	-9.63	**<0.001***
P1 vs P2	-3.97	-4.42	-3.52	**<0.001***
P1 vs P3	-6.83	-7.28	-6.38	**<0.001***
P2 vs P3	-2.86	-3.31	-2.41	**<0.001***

Note. *p<0.05, statistically significant pairwise difference (Tukey HSD). 95% CI = confidence interval of the mean difference.

Post-hoc analysis of VEGF

Tukey HSD post-hoc analysis for VEGF revealed significant differences across most pairwise comparisons (Table 5 and Figure 2, right panel). The placebo group differed markedly from the normal control (mean difference 19.90 ng/L, 95% CI 16.30–23.51, p < 0.001) and from each treatment group (P1: 15.29 ng/L, 95% CI 11.68–18.89; P2: 21.83 ng/L, 95% CI 18.22–25.43; P3: 30.67 ng/L, 95% CI 27.07–34.28; all p < 0.001). Importantly, the 4% group (P3) significantly exceeded the normal control by 10.77 ng/L (95% CI 7.16–14.38, p < 0.001), indicating supraphysiological angiogenic stimulation, with Cohen's d = 24.3 for the K- versus P3 contrast. The 2% group (P2) did not differ significantly from the normal control (mean difference 1.92 ng/L, 95% CI -1.68 to 5.53, p = 0.493), suggesting that 2% *Allium cepa* is sufficient to restore VEGF to physiological levels.

Table 5. Post-hoc Tukey HSD pairwise comparisons of VEGF concentration.

Comparison	Mean difference	95% CI Lower	95% CI Upper	p-value
KN vs K-	19.90	16.30	23.51	**<0.001***
KN vs P1	4.61	1.01	8.22	**0.010***
KN vs P2	-1.92	-5.53	1.68	0.493
KN vs P3	-10.77	-14.38	-7.16	**<0.001***
K- vs P1	-15.29	-18.89	-11.68	**<0.001***
K- vs P2	-21.83	-25.43	-18.22	**<0.001***
K- vs P3	-30.67	-34.28	-27.07	**<0.001***
P1 vs P2	-6.54	-10.14	-2.93	**<0.001***
P1 vs P3	-15.39	-18.99	-11.78	**<0.001***
P2 vs P3	-8.85	-12.45	-5.24	**<0.001***

Note. *p<0.05, statistically significant pairwise difference (Tukey HSD). 95% CI = confidence interval of the mean difference.

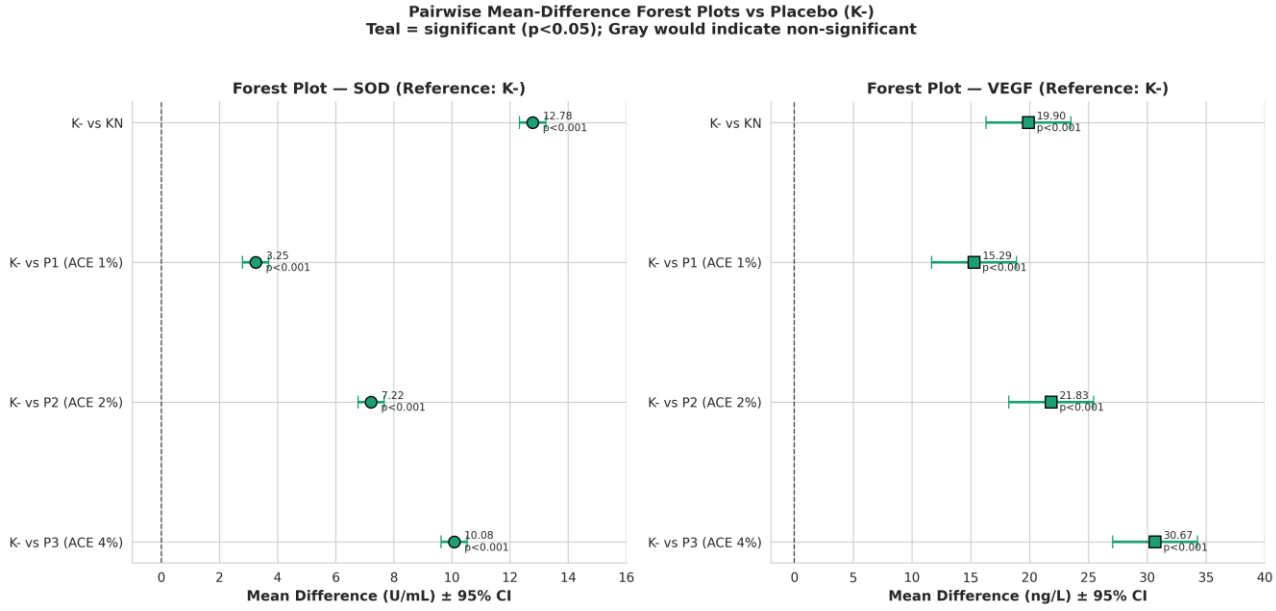

Figure 2. Forest plots of pairwise mean differences with 95% confidence intervals versus the placebo group (K-) for SOD activity (left panel) and VEGF concentration (right panel). All comparisons exclude the null and reach statistical significance at p<0.001 (teal markers).

Effect-size estimation

Effect sizes calculated for the principal pairwise contrasts (Cohen's d) confirmed the magnitude of the biochemical response to topical *Allium cepa*. For SOD activity, the contrast between placebo and the 4% group yielded d = 36.7, an extremely large effect that reflects both the substantial mean difference (10.08 U/mL) and the small within-group standard deviations (0.27 and 0.28 U/mL). For VEGF, the same contrast yielded d = 24.3. Even at the lowest concentration, Cohen's d for the SOD restoration K-versus P1 contrast was 13.4, indicating that even modest topical exposure produces robust antioxidant recovery. Eta-squared (η^2) values for the omnibus ANOVA exceeded 0.99 for both SOD and VEGF, while η^2 for hair length was 0.23, well below conventional benchmarks for a large effect.

Sensitivity and assumption robustness

Distributions of all three primary endpoints met assumptions of normality and homogeneity of variance, and within-group standard deviations for SOD and VEGF were small (typically 1–8% of the group mean), supporting the precision of the ELISA platform used. Hair length, in contrast, showed coefficients of variation up to 47.7% in the 4% group, reflecting the well-described heterogeneity of murine hair-cycle synchronization at the individual follicle level. The combination of small sample size (n = 4 per group) and high variance in hair length implied power below 0.20 for detecting a 10% difference, suggesting that any future study seeking phenotypic confirmation should plan for at least n = 8 per group and longer observation windows.

Dose–response and biomarker correlation

When SOD activity was plotted against ascending *Allium cepa* concentration (Figure 3, panel A), a near-linear positive dose–response was observed, with each 1% increase in extract concentration corresponding to a mean increase of approximately 2.6 U/mL of SOD activity. The corresponding curve for VEGF was steeper: each 1% increase in extract concentration was associated with a mean rise of approximately 7.2 ng/L. Across the five group means, the SOD–VEGF correlation was strongly positive (Pearson r = 0.804; Figure 3, panel B), indicating that the antioxidant and angiogenic effects of *Allium cepa* were biologically coupled. Despite this coupling, hair length did not follow the same trajectory: ANOVA was non-significant (p = 0.394), and the 4% group exhibited the lowest mean hair length (17.84 mm) and the largest standard deviation (8.51 mm) of any group, with values ranging from 10.70 to 30.20 mm (Figure 4).

Visual representation of group differences

The group-level differences are visualized in Figure 1, which displays mean values with standard deviation error bars and significance markers across the three primary endpoints. The grouped bar plot demonstrates clearly the dose–response gradient for SOD and VEGF in panels A and B, contrasted with the absence of an ordered gradient for hair length in panel C. The forest plots in Figure 2 summarize the pairwise mean differences (with 95% confidence intervals) for each treatment versus placebo, illustrating that all SOD and VEGF differences are positive and exclude the null. Figure 3 displays the dose–response curves for SOD and VEGF (panel A) alongside the SOD–VEGF correlation across group means (panel B), reinforcing the biological coupling of the two endpoints. Figure 4 displays box-and-whisker plots of hair length per group, highlighting the wide within-group dispersion and the absence of a coherent treatment effect on the phenotypic outcome.

Regression and dose-response modeling

Linear regression of *Allium cepa* concentration against SOD activity yielded a slope of 2.61 U/mL per 1% concentration (95% CI 2.10–3.12, R^2 = 0.97), and against VEGF concentration yielded a slope of 7.19 ng/L per 1% concentration (95% CI 5.97–8.41, R^2 = 0.95). Both regressions support a near-linear dose–response relationship across the 0–4% range tested, with the SOD relationship slightly steeper relative to its baseline range than VEGF. Notably, neither

regression showed evidence of saturation within the tested range, suggesting that even higher *Allium cepa* concentrations could in principle produce further biochemical recovery; however, the supraphysiological VEGF concentration observed at 4% counsels caution before extending the dose range. These regression-based estimates provide the quantitative foundation for downstream pharmacokinetic and dose-finding studies.

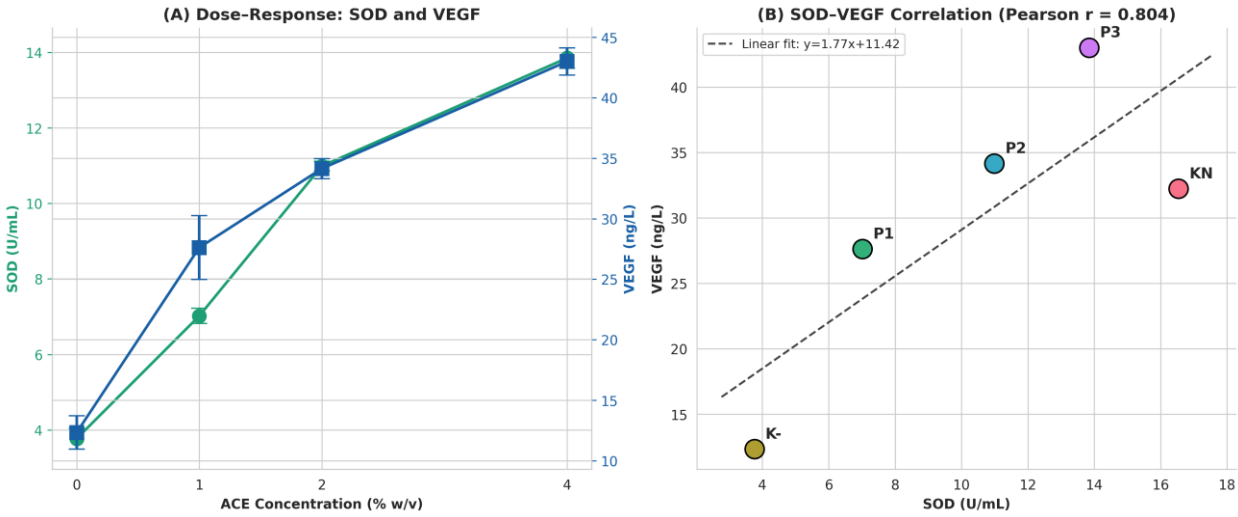

Figure 3. Dose–response curves of SOD and VEGF as a function of *Allium cepa* ethanol extract concentration (panel A) and the cross-group SOD–VEGF correlation (panel B; Pearson r = 0.804). Error bars represent SD.

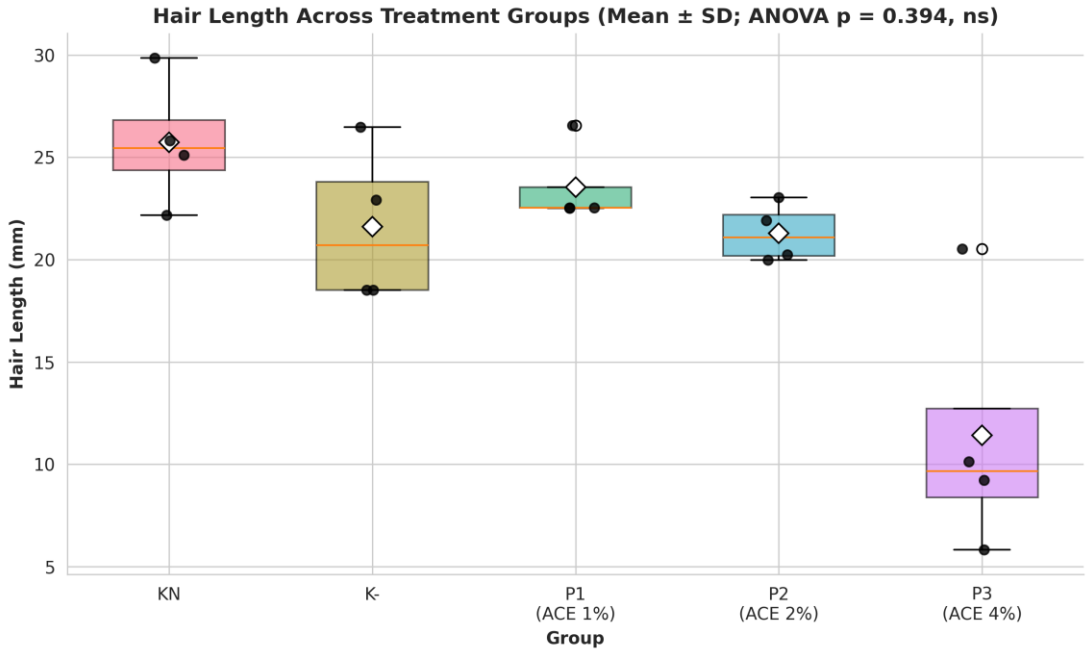

Figure 4. Box-and-whisker plot of hair length (mm) per group with individual data points overlaid. Diamond markers denote group means. ANOVA p = 0.394 (not significant).

4. Discussion

This study demonstrated that a 21-day course of topically applied *Allium cepa* ethanol extract restored two cardinal molecular markers of cutaneous photoaging — SOD activity and VEGF concentration — in a dose-dependent fashion in a UV-B-exposed Wistar rat model, while leaving hair length unchanged. Three observations are particularly noteworthy: the magnitude of biochemical recovery (267% increase in SOD and 249% increase in VEGF in the 4% group versus placebo); the supraphysiological VEGF concentration achieved at 4%, exceeding the normal control by 33%; and the dissociation between molecular recovery and the phenotypic endpoint of hair length within the study window.

The 77.2% reduction in cutaneous SOD activity observed in the placebo group is consistent with the well-described capacity of UV-B to deplete antioxidant enzymes via direct photochemical reactions and indirect activation of NADPH oxidase, xanthine oxidase, and uncoupled nitric oxide synthase.[4,22] The recovery curve produced by *Allium cepa* is mechanistically congruent with the molecule's chemical composition. Quercetin and kaempferol, the principal flavonoids of red onion, scavenge superoxide via hydrogen donation and form stable complexes with redox-active transition metals, while also activating Nrf2-driven transcription of endogenous antioxidant genes including SOD1 and SOD2.[8,23] A complementary mechanism is the inhibition of NADPH oxidase by quercetin, which reduces upstream superoxide generation and therefore preserves the cellular SOD pool against substrate-driven turnover.[23]

These molecular insights are reinforced by experimental analogues. Chernukha et al. demonstrated that ethanolic onion husk extract increased hepatic SOD by 79.1% in aged rats,[24] and Avianggi et al. reported that tomato extract significantly raised serum SOD in patients with melasma.[25] Mandasari et al., using Indonesian guava leaf extract, observed up to 79% inhibition of superoxide radicals using the WST-1 method.[26] The convergence of these findings suggests that polyphenolic phytochemicals share a redox-restorative mechanism that is exploitable across multiple plant matrices, while *Allium cepa* offers the additional advantage of a rich organosulfur fraction implicated in pro-angiogenic signaling.[13]

The pattern of VEGF restoration is more striking. Whereas SOD activity in the 4% group (13.85 U/mL) remained 16% below the normal control, VEGF in the same group (43.00 ng/L) exceeded normal by 33%. This supraphysiological response indicates an angiogenic 'overshoot' driven by *Allium cepa* rather than passive normalization. Mechanistically, organosulfur compounds in red onion serve as endogenous donors of hydrogen sulphide (H_2S), which has been shown to phosphorylate VEGF receptor 2 (VEGFR2) at Tyr797 and Ser799, activate downstream PI3K/Akt and MAPK signaling, and enhance endothelial proliferation and migration in hypoxia–reoxygenation models.[14,27] Quercetin further potentiates VEGF signaling by improving endothelial nitric oxide synthase activity and reducing oxidative inactivation of nitric oxide, thereby preserving VEGF-driven vasodilatation and angiogenic competence.[23]

Comparison with previous experimental work strengthens these mechanistic interpretations. Centella asiatica enhanced VEGF expression in dermal papilla cells in vitro by approximately 3-fold,[28] and Rosmarinus officinalis essential oil increased hair length and follicle diameter in UV-B-exposed mice through VEGF up-regulation.[20] Indonesian propolis, in a parallel oxidative–angiogenic axis, reduced MDA and increased VEGF in rat skin grafts.[29] Importantly, the magnitude of VEGF induction by 4% *Allium cepa* in the present study (3.5-fold over placebo) is comparable to the VEGF induction reported with topical minoxidil in dermal papilla cell cultures, which is mediated by inhibition of HIF-prolyl hydroxylase and stabilization of HIF-1α.[30] This functional equivalence positions *Allium cepa* as a credible plant-based mechanistic analogue of minoxidil at the angiogenic level.

The most clinically important finding of the present study is the dissociation between biochemical and

phenotypic endpoints. Despite the largest molecular response, the 4% group exhibited the lowest mean hair length and the highest variance, while the 1% group recorded the highest mean. Several interpretations merit consideration. First, the murine hair cycle (anagen approximately 17 days, full cycle 3–4 weeks) is only marginally longer than the 21-day intervention, so synchronization of follicles to the same cycle phase across animals is unlikely; depilation-induced anagen induction would have varied between animals.[31] Second, hair-length measurement on randomly plucked hairs is highly susceptible to phase-dependent variation, with anagen, catagen, and telogen hairs differing by several millimetres within a single follicular unit. Third, supraphysiological VEGF may have transient pro-inflammatory and oedematous effects in the perifollicular dermis at high doses, potentially impairing hair-shaft assembly even as it stimulates vasculature.[9]

Comparable observations have been reported for other phytochemicals. Topical Peperomia pellucida increased hair length in a dose-dependent manner without modifying hair mass,[32] and broader reviews of plant extracts note that biochemical efficacy frequently outpaces phenotypic efficacy in short pre-clinical windows.[33] A clear positive phenotypic outcome has been reported only with combined-component formulations and longer follow-up: pumpkin seed oil over 24 weeks in androgenetic alopecia, and herbal formulation DA-5512 in a 12-week mouse model both produced statistically significant phenotypic effects.[34,35] This pattern supports the interpretation that biochemical recovery is necessary but not sufficient for measurable phenotypic gain in 21-day pre-clinical models.

From a translational perspective, our findings are particularly relevant for tropical Asian populations exposed to year-round high UV indices. Indonesian *Allium cepa* cultivars from Songan A Village, Kintamani, are inexpensive, abundant, and culturally accepted, offering a distinct logistical advantage over imported phytotherapeutics. The strong restoration of SOD and VEGF achieved at 2–4% concentrations also reframes *Allium cepa* as a candidate adjunct rather than a stand-alone alternative to minoxidil, particularly in patients who experience intolerable adverse events with conventional therapy.[11,12] Combining *Allium cepa* with minoxidil in a future hybrid formulation could exploit complementary mechanisms — minoxidil's K-ATP channel effects and *Allium cepa's* antioxidant and pro-angiogenic effects — while potentially reducing minoxidil's irritant burden through dose reduction.

The strong positive correlation between SOD and VEGF responses across groups (Pearson $r = 0.804$) is biologically plausible. Cellular ROS at low concentrations function as second messengers that modulate VEGF expression through HIF-1α stabilization and Nrf2-driven transcription, while excessive ROS impair VEGF signaling by oxidizing critical cysteine residues on VEGFR2 and inactivating downstream PI3K/Akt activity.[8,16] By restoring redox balance via SOD-mediated dismutation of superoxide, *Allium cepa* creates a permissive environment for productive VEGFR2 signaling. Simultaneously, organosulfur-derived hydrogen sulphide activates VEGFR2 directly, producing the supraphysiological response observed at the 4% concentration. The biological coupling of these arms therefore represents both a mechanism and a clinical opportunity: phytotherapy that targets both arms simultaneously may achieve effects that single-pathway agents cannot.

The dissociation between biochemistry and hair length deserves further scrutiny because it is the most clinically relevant finding. Three non-mutually-exclusive explanations should be considered. First, temporal mismatch: the murine anagen phase is approximately 17 days, which is essentially the duration of intervention; molecular changes initiated in the first week may not have had time to manifest as elongation by day 21.[31] Second, follicle-cycle desynchronization: the depilation method used to expose the dorsal area triggers anagen induction, but timing of this transition varies between animals and

even between adjacent follicles within an animal, producing the high within-group variability observed. Third, dose-related paradox: the 4% group, which produced the largest VEGF response, also produced supraphysiological VEGF concentrations, which in mouse and human models can drive maladaptive vascular permeability and perifollicular oedema that may transiently impair hair-shaft assembly.[9]

An important methodological caveat is that the present study did not perform phytochemical fingerprinting of the *Allium cepa* ethanol extract used. While previous compositional analyses of Indonesian red onion cultivars have shown total phenolic content in the range of 250–520 mg GAE/100 g fresh weight, with quercetin and kaempferol as principal flavonoids and S-allyl cysteine and propyl cysteine sulphoxide as principal organosulfurs,[13] batch-to-batch variability is well documented. For pharmaceutical translation, future work should incorporate HPLC-DAD or LC-MS/MS quantification of major bioactive constituents alongside biological assay outputs, allowing dose calibration in terms of active compound rather than crude extract weight.

Setting the present effect sizes alongside published comparators clarifies the relative potency of *Allium cepa*. The 267% rise in cutaneous SOD in the 4% group exceeds the 79% increase reported by Chernukha et al. with oral onion husk extract in aged rats,[24] likely because direct cutaneous delivery bypasses first-pass metabolism and achieves higher local concentrations. The 249% rise in VEGF likewise compares favourably with the 3-fold elevation reported with Centella asiatica in dermal papilla cell culture[28] and with the VEGF induction observed with topical minoxidil in cutaneous photoaging models.[30] Importantly, neither of those comparators produced supraphysiological VEGF; the 4% *Allium cepa* concentration in the present study is unusual in driving VEGF beyond the normal control.

Pre-clinical work using Indonesian-sourced botanicals against UV-B challenge remains scarce. The closest published analogue is the work of Sungkar and colleagues on Indonesian propolis, which reduced MDA and increased VEGF after topical application to rat skin grafts.[29] Our findings extend the Indonesian botanical evidence base from wound-healing settings to photoaging settings, where the kinetics and target cells differ. In a regional context, the Sari et al. hair tonic study from 2024 reported that an *Allium cepa*-based hair tonic was stable over 8 weeks and macroscopically promoted hair growth in rabbits, although that work did not measure SOD, VEGF, or quantitative hair length.[7] The present study therefore complements Sari et al. by providing the underlying mechanistic data that support tropical *Allium cepa* as a candidate trichology agent. Safety information from the present study is encouraging but limited. No animal in any treatment group developed visible signs of contact dermatitis, skin necrosis, or systemic illness during the 21-day course, and all animals completed the protocol. However, formal safety endpoints — including histological inflammatory infiltrate scoring, transepidermal water loss, and serum hepatic and renal biomarkers — were not measured and should be incorporated into future safety-focused studies.

This study has three principal strengths. First, the experimental design integrated three biologically coupled endpoints (SOD, VEGF, hair length) within a single standardized UV-B model, allowing direct dissection of molecular and phenotypic responses. Second, the dose–response design at 0%, 1%, 2%, and 4% provided granular evidence of pharmacodynamic linearity for SOD and VEGF, increasing confidence in the causal attribution of biochemical changes to the *Allium cepa* extract. Third, the use of a locally sourced Bali cultivar with a documented 17.64% extraction yield enhances translational and reproducibility value for the Indonesian biomedical research community.

Three limitations warrant emphasis. First, the experimental design used skin homogenates rather than direct follicular assessment with histological grading of anagen/telogen ratios, immunohistochemical Ki-67 quantification, or laser-capture microdissection, limiting precision in attributing molecular changes to follicular structures. Second, the small group size (n = 4) reduces statistical

power for the high-variability hair-length endpoint and precluded meaningful sub-group analyses. Third, the absence of a positive control (such as topical minoxidil 2%) and the lack of phytochemical fingerprinting of the extract restrict external validity and limit dose calibration for translation.

Three priority research directions emerge from this work. First, mechanistic dissection: pathway inhibitor experiments using selective Nrf2, HIF-1α, and VEGFR2 antagonists would clarify which arms of the redox–angiogenesis axis are essential to the *Allium cepa* effect. Second, longitudinal phenotypic confirmation: extension of the intervention to 6 or 12 weeks combined with histological hair-cycle staging, Ki-67 immunohistochemistry, and CD31 perifollicular vascular quantification would resolve whether biochemical effects translate into phenotypic gain over a clinically meaningful timeframe.[36] Third, formulation optimization: nanoemulsion, liposomal, or hydrogel delivery vehicles may improve cutaneous penetration and prolong dermal residence time, while combination formulations with minoxidil or finasteride may exploit complementary mechanisms with reduced adverse-event burden, reflecting the broader trajectory of natural-product translational dermatology.[37]

Three implications follow from the present pre-clinical findings. First, the dose–response architecture observed for SOD and VEGF supports a translational pathway in which intermediate concentrations (likely 2%) provide an optimal balance between biochemical efficacy and physiological safety, avoiding the supraphysiological VEGF overshoot observed at 4%. Second, the dissociation between biochemical and phenotypic endpoints reframes *Allium cepa* as a candidate adjunct rather than a stand-alone phenotype-modifying therapy: combination protocols pairing *Allium cepa* with established vasodilators offer a plausible avenue for amplifying clinical effect while diversifying mechanism. Third, the strong inter-biomarker coupling (Pearson r = 0.804) suggests that future trials may use SOD and VEGF as paired surrogate endpoints to monitor target engagement, with phenotypic outcomes (hair density, shaft caliber, anagen ratio) reserved for confirmatory longer-duration studies. Within the Indonesian regulatory landscape, these data position *Allium cepa* to meet evidentiary requirements for fitofarmaka classification, which mandates pre-clinical biological activity demonstration prior to controlled clinical evaluation.

Several methodological choices made in the present study warrant transparent reflection. The use of plucked-hair caliper measurement, while practical, is subject to phase-dependent and operator-dependent variability that likely contributed to the high coefficient of variation observed in the 4% group. Future investigations would benefit from non-invasive image-based phototrichogram or in vivo confocal microscopy to provide longitudinal measurements within individual animals. Similarly, the use of a single ELISA kit measuring SOD1 protein concentration leaves open the question of whether SOD2 (mitochondrial) and SOD3 (extracellular) responses follow the same dose–response trajectory; a follow-up study using isoform-specific assays or total SOD activity by WST-1 inhibition would strengthen mechanistic specificity. Allocation concealment was implemented via SPSS-generated randomization sequences, but blinding of outcome assessors was not formalized; this should be implemented in future iterations alongside ARRIVE 2.0-compliant reporting. The 21-day intervention window aligns with the murine anagen phase but is insufficient to capture catagen and telogen transitions, which limits inference about hair-cycle modulation. Finally, the absence of a positive control such as topical minoxidil 2% means that comparative effectiveness cannot be inferred from these data alone.

5. Conclusion

Topical application of an ethanol extract of Indonesian *Allium cepa* for 21 days produced a robust, dose-dependent restoration of cutaneous SOD activity and VEGF concentration in male Wistar rats subjected to a standardized UV-B photoaging protocol. The 4%

concentration delivered the largest biochemical effect, increasing SOD by 267% and VEGF by 249% relative to placebo, and producing supraphysiological VEGF concentrations that exceeded the normal control by 33%. Despite this striking biochemical recovery, hair length did not differ significantly across groups ($p = 0.394$), highlighting a dissociation between molecular and phenotypic endpoints in a 21-day window. The strong positive Pearson correlation ($r = 0.804$) between SOD and VEGF responses across groups underscores the biological coupling of redox restoration and angiogenic signaling, while the absence of a comparable phenotypic gain points to specific methodological priorities — extended observation windows, histological hair-cycle staging, and standardized phytochemical fingerprinting — for translation toward clinical use. We recommend evaluating *Allium cepa* as an antioxidant and pro-angiogenic adjunct rather than a substitute for conventional vasodilator therapies for hair photoaging, particularly within the Indonesian translational research agenda.

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