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Topical Ajwa date (Phoenix dactylifera L.) extract cream modulates fibroblast proliferation in UV-B-Exposed Wistar rats: Effects on TNF-α and SOD levels

Sri Mahoni [1], Sri Priyantini Mulyani [2*], Siti Thomas Zulaikhah [2]

[1] Program Study of Magister Biomedical Sciences, Post Graduate School, Universitas Islam Sultan Agung, Semarang, Indonesia
[2] Department of Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia

Abstract: Humans always want to look attractive and youthful, in order to increase self-confidence, so they make efforts to avoid the appearance of signs of aging by slowing down and preventing the aging process. One of the important factors that affects the skin aging process is ultraviolet (UV) rays. The effects of UV-B exposure on the skin include changes in its structure and function. The purpose of this study was to determine the effect of administering Ajwa date extract cream (Phoenix dactylifera L) on TNF-α and SOD levels in Wistar rats exposed to UV-B. This study was an in vivo experimental design using a post-test only control group design. The study used 30 Wistar rats, divided into five groups: the normal group (K1), negative control (K2), positive control (K+), treatment 1 (K1) with 4% Ajwa date extract cream, and treatment 2 (K2) with 8% Ajwa date extract cream. After UV-B exposure for seven days, TNF-α and SOD levels were measured using the ELISA method. Statistical analysis included the Shapiro-Wilk test for normality, Levene's test for homogeneity, and One-way ANOVA to assess differences between groups if needed. The results showed no significant difference in the average SOD levels between groups ($p = 0.262$), nor in TNF-α levels ($p = 0.106$). The highest average SOD level was observed in the group treated with 8% Ajwa date extract cream. Although the statistical analysis did not show significant differences between groups, a positive trend was observed in the treatment groups, including increased SOD levels, reduced TNF-α expression, and notably, a trend of increased fibroblast proliferation in the 8% treatment group. This study provides novel evidence regarding the topical application of Ajwa date extract cream in modulating oxidative stress, inflammation, and fibroblast activity in UV-B-exposed skin.

Keywords: Ajwa date extract cream; SOD levels; TNF-α levels; UV-B.

INTRODUCTION

Humans always want to look attractive and youthful in order to increase self-confidence, so they make efforts to avoid the appearance of signs of aging by slowing and preventing the aging process [1]. Aging is a natural process that occurs in the body; all organs, including the skin, will eventually age. One of the most important factors influencing skin aging is ultraviolet (UV) radiation. UV exposure causes changes in the structure and function of the skin [2,3], leading to oxidative stress and inflammation that accelerate premature aging [4]. The rapid accumulation of *reactive oxygen species* (ROS) in skin cells can lead to photoaging [5]. ROS produced by UV-B rays activates the MAPK pathway, resulting in increased inflammatory mediators, such as *tumor necrosis factor-α* (TNF-α) [6].

Topical antioxidants are commonly used in skincare products to combat UV-induced damage [7,8]. Natural ingredients are favored due to their minimal side

Corresponding author.
E-mail address: sripriyantini@unissula.ac.id (Sri Priyantini Mulyani)
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effects and better availability compared to synthetic compounds [6]. One such ingredient is Ajwa dates (*Phoenix dactylifera L*), known for their high antioxidant content[9]. However, research on their topical use for skin protection especially in vivo is still limited.

The skin is aesthetically important, making photoaging a major concern [4,6]. Continuous exposure to stressors such as oxidative stress, DNA damage, and telomere shortening impairs cellular replication and promotes aging [10]. Clinically, UV radiation causes wrinkles, thickening, dryness, and pigmentation [11]. Subcutaneous changes, such as nasolabial folds and sagging, affect perceived facial age [12].

Understanding wrinkle pathophysiology is essential for developing effective anti-aging treatments, which are in high demand within the cosmetic industry [13]. However, many topical agents still cause side effects, such as irritation or retinoid dermatitis [14 15]. Therefore, there is a growing need for alternative therapies that are both effective and safe [16].

Natural antioxidants like Ajwa dates offer potential alternatives. Technological advances have enabled the development of anti-aging creams using date extracts[9]. Dates have traditionally been used to treat various diseases and possess antifungal and antibacterial properties. They contain sugars, vitamins (A, C, B-complex), fiber, minerals, and phenolic compounds with strong antioxidant activity [17]. Ajwa dates also contain flavonoids with anti-cancer, antimicrobial, anti-inflammatory, and cardioprotective effects.

Several natural antioxidants such as vitamin E, green tea polyphenols (particularly EGCG), and resveratrol have been extensively evaluated for their skin-protective roles against UV-induced damage. Vitamin E, particularly α-tocopheryl acetate, is a fat soluble antioxidant that acts as a free radical scavenger, offering photoprotective and antitumorigenic benefits, though controlled clinical evidence is limited[18]. Green tea catechins, especially EGCG, have demonstrated inhibition of UV-induced MAPK and NF-κB signaling, reduction of leukocyte infiltration and inflammatory mediators, and protection against oxidative stress in skin models[19]. Resveratrol has been shown to activate Nrf2-mediated antioxidant defenses (e.g., HO-1, SOD1), suppress UVB-induced MMP expression and inflammation, and enhance dermal repair in vitro and in vivo[20,21]. Nevertheless, limitations such as formulation instability, low bioavailability, or skin irritation hinder their broader topical applications. In contrast, Ajwa date extract with its rich composition of polyphenols, flavonoids, vitamins, minerals, and natural sugars may offer a more stable and dermally compatible alternative, combining multiple bioactivities (antioxidant, anti-inflammatory, moisturizing, regenerative). This unique profile renders it a compelling candidate for multifunctional topical dermato-protection.

Studies have shown Ajwa dates possess strong antioxidant capacity, with an IC_{50} of 26.14 µg/mL and an LC_{50} of 126.610 µg/mL[19]. Other date varieties also show high phenolic and flavonoid content with potent antioxidant activity[20]. These findings highlight their medicinal and pharmacological potential. Dates have also demonstrated anti-wrinkle effects and are widely accepted in topical form due to their moisturizing properties [22].

Despite the known antioxidant and anti-inflammatory properties of Ajwa dates, evidence on their topical application against UVB induced skin damage particularly their effect on inflammatory markers like TNF-α and oxidative markers like SOD remains limited [23]. This study was conducted to address this gap by evaluating the topical effect of Ajwa date extract cream in UV-B-exposed Wistar rats.

MATERIAL AND METHOD

The materials used in making the extract require Ajwa dates, 70% ethanol. The 4% and 8% concentrations were selected based on stability and efficacy considerations. Green tea extract creams at 3–6% have shown significant antioxidant and skin-improving effects without irritation [24,25]. Likewise, a 4% Phoenix dactylifera (Ajwa date) cream significantly improved skin characteristics related to aging and health [26]. Thus, 4% was chosen as a safe minimal effective dose, while 8% was tested as a higher dose expected to enhance bioactivity while maintaining formulation quality.

Whie Materials during the treatment used Wistar rats, feed and drinking water for rats. Materials used in ELISA microplate ELISA examination, standard diluent, blocking buffer, diluent sample, biotinylated antibody, antibody capture, ABC, HRP,TMB, stop solution, wash solution.

Animals and Grouping

This research was classified as an in vivo experimental study using a post-test only control group design. The experimental subjects consisted of Wistar rats aged 2–3 months with body weights ranging from 190 to 250 grams. All rats underwent a 7-day acclimatization period under controlled environmental conditions with adequate ventilation and temperatures between 28–32°C. They were given pellet food and water ad libitum. All animal handling and experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia (Approval No. 56/II/2024/Komisi Bioetik).

After adaptation, a total of 30 rats were randomly divided into five groups, each containing six animals: the KN group (normal control), the K− group (UV-B exposure without treatment), the K+ group (UV-B exposure with base cream), the K1 group (UV-B exposure with 4% Ajwa date extract cream), and the K2 group (UV-B exposure with 8% Ajwa date extract cream). Randomization was performed using a simple random sampling method, and blinding was applied during data analysis to reduce bias.

Cream Formulation

The cream was formulated by preparing a vanishing cream base consisting of stearic acid, triethanolamine, glycerin, borax, and distilled water. Stearic acid (14.5 g) was melted in a porcelain cup over boiling water. Once liquefied, borax (125 mg) was added and stirred until homogeneous, followed by the addition of triethanolamine (1.5 mL), glycerin (10 mL), and distilled water (25 mL) while maintaining continuous stirring until a uniform cream base was formed. To produce the Ajwa date extract cream, 0.6 grams of ethanol extract was placed in a mortar, mixed with sufficient Tween until homogeneous, and then added to 20 grams of the prepared vanishing cream base. The cream was stirred until fully mixed and stored in a sealed container for application [27]. The resulting formulation was a water-in-oil (W/O) emulsion and was not subjected to any microbiological stability testing.

The Ajwa date extract used in the cream formulation was prepared from 2 kg of Ajwa dates. The seeds were removed, and the flesh was oven-dried at 50°C. Moisture content was checked using a moisture balance, and samples with moisture content below 10% were processed further. The dried flesh was cut, ground into powder, and sieved through a 20 mesh filter. A total of 500 grams of powder was extracted using 3,750 mL of 70% ethanol via the maceration method. The powder was soaked in ethanol for five days in a dark container and shaken three times daily. After initial filtration, the residue was re-macerated for two additional days with 1,250 mL of ethanol. This process was repeated three times. The collected filtrates were concentrated using a rotary evaporator at 50°C to obtain a thick extract.

UVB Exposure Protocol

Following the acclimatization period, rats in the K−, K+, K1, and K2 groups were exposed to broadband UV-B light (302 nm). The UV-B source used was a Philips TL 20W/12 RS UVB Broadband lamp. Prior to exposure, rats were anesthetized using a combination of ketamine (60 mg/kg body weight) and xylazine (20 mg/kg body weight). The dorsal fur of each rat was shaved to create a 4 × 4 cm treatment area. The UV-B radiation was administered at a minimum erythema dose of 160 mJ/cm²/day, with a distance of 12 cm between the light source and skin surface, for seven consecutive days. The lamp's irradiance was calibrated prior to use, and appropriate shielding was applied to protect surrounding body areas during exposure. One hour after each exposure session, topical treatments were applied: base cream for the K+ group, 4% Ajwa extract cream for the K1 group, and 8% Ajwa extract cream for the K2 group. All treatments were applied once daily for 7 days.

Histopathological Examination

Tissue samples were fixed in 10% neutral buffered formalin at a ratio of 1:10 for 24 hours. The fixed tissues were dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin blocks. Thin sections of 5 µm thickness were obtained using a microtome, then mounted on glass slides and dried. The sections were stained with hematoxylin and eosin (H&E) and observed under a light microscope at 400× magnification. Fibroblast density was counted in five randomly selected visual fields per sample. Histological evaluation was performed by a trained pathologist blinded to group assignment.

Biomarker Analysis (ELISA)

On the eighth day, the rats were euthanized under anesthesia, and skin tissue samples were collected from the previously irradiated dorsal area. The tissue was excised using sterile instruments, weighed, and placed in phosphate-buffered saline (PBS, pH 7.4). Samples were homogenized under cold conditions (4°C) and centrifuged at 2,000–3,000 rpm for 20 minutes. The resulting supernatant was collected and used for biochemical analysis or stored at –20°C until use.

The levels of tumor necrosis factor-α (TNF-α) and superoxide dismutase (SOD) were analyzed using the enzyme-linked immunosorbent assay (ELISA) method. TNF-α was measured using a 96-well Bioenzyme ELISA kit (product code: BZ-08184670-EB), and SOD was measured using a 48-well ELISA kit (catalog code: E0168Ra-). All procedures were performed according to the manufacturers' instructions. Absorbance readings were measured using a microplate reader at a wavelength of 450 nm.

RESULTS AND DISCUSSION

The results of the test and analysis of the average SOD level in each group of research subjects are shown in table 1.

Table 1. Analysis of Superoxide Dismutase (SOD) Expression Levels Across Treatment Groups Using One-Way ANOVA

Group	KN Healthy Mice	K- No Intervension	K+ Cream Base	K1 Dosage 4%	K2 Dosage 8%	*P value*
SOD Expression (ng/mL)						
Mean±SD	4.38±0.565	4.90±0.603	4.68±1.037	4.48±0.986	5.43±0.705	
Shapiro-Wilk	0.812*	0.250*	0.320*	0.446*	0.699*	
Levene Test						0.541*
One way Anova						0.262

Description: **Shapiro-Wilk* = Normal (p>0.05)
* *Levene Test* = Homogen (p>0.05)

* *One way Anova* = Significance (p<0,05)

The highest mean SOD level was observed in the K2 group (8% Ajwa extract) at 5,436 ± 0,705 ng/mL, followed by K- (UVB only) at 4,90±0,603, K+ (base cream) at 4,68±1,037, and K1 (4% Ajwa cream) at 4,48±0,986, while the lowest can be found on KN group (healthy control) at 4,338 ± 0,565 ng/mL. The difference in SOD levels across the five groups was analyzed using a one-way ANOVA test after confirming normality (Shapiro–Wilk test, $p > 0.05$ for all groups) and homogeneity of variance (Levene test, $p = 0.541$)..

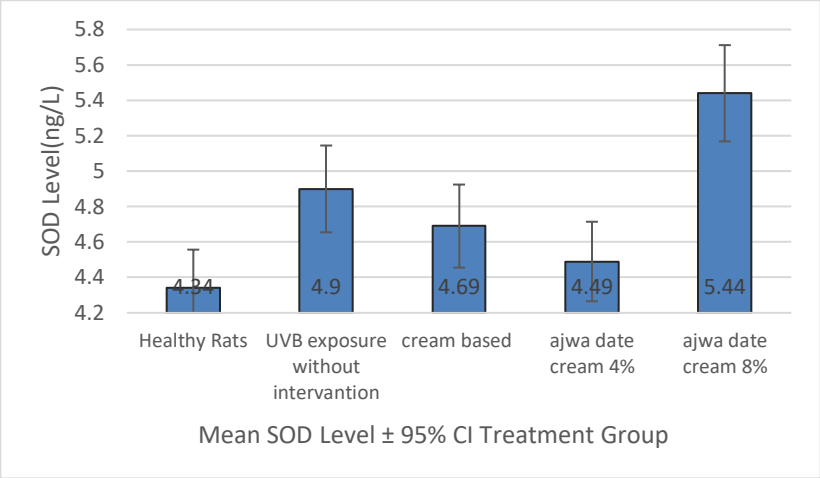


Figure 1. Effect of Ajwa Date Cream on Superoxide Dismutase (SOD) Levels in UVB-Exposed Mice

Analysis of SOD expression across the five experimental groups revealed observable differences, although the overall variation was not statistically significant. The group receiving 8% Ajwa date extract cream (K2) exhibited the highest mean SOD level (5.436 ± 0.705 ng/mL), while the lowest was observed in the healthy control group (KN) at 4.338 ± 0.565 ng/mL. One-way ANOVA indicated no statistically significant difference among the groups ($F(4,20) = 1.425$; $p = 0.262$). However, the observed partial eta squared ($\eta^2p = 0.222$) suggested a large effect size, with 22.2% of the variance in SOD levels attributable to treatment conditions.

Table 2. LSD Post Hoc Comparisons of Superoxide Dismutase (SOD) Levels Between Groups

Group	**Comparison Group**	***p-value***	**95% Confidence Interval**	
SOD expression (ng/mL)			Lower Bound	Upper Bound
Healthy mice (KN)	No intervention (K-)	0.278	-1.6257	.4937
	Cream base(K+)	0.499	-1.4097	.7097
	Ajwa date cream 4% (K1)	0.774	-1.2077	.9117
	Ajwa date cream 8% (K2)	0.043	-2.1577	-.0383
No intervention (K-)	Cream base (K+)	0.675	-.8437	1.2757
	Ajwa date cream 4% (K1)	0.420	-.6417	1.4777
	Ajwa date cream 8% (K2)	0.308	-1.5917	.5277
Cream base (+)	Ajwa date cream 4% (K1)	0.695	-.8577	1.2617
	Ajwa date cream 8% (K2)	0.156	-1.8077	.3117
Ajwa date cream 4% (K1)	Ajwa date cream 8% (K2)	0.076	-2.0097	.1097

Further evaluation using LSD post hoc analysis revealed a statistically significant difference between the KN and K2 groups (mean difference = −1.098 ng/mL; p = 0.043), with a 95% confidence interval ranging from −2.1577 to −0.0383 ng/mL. This CI does not include zero, indicating a true effect with increased precision. The difference between K1 and K2 (Ajwa 4% vs. 8%) approached significance (mean difference = −0.950 ng/mL; p = 0.076; 95% CI: −2.0097 to 0.1097).

The results of the test and analysis of the average TNF- α level in each group of research subjects are shown in table 1 as follow

Table 3. Analysis of Tumor Necrosis Factor-Alpha (TNF-α) Expression Levels Across Treatment Groups Using One-Way ANOVA

Group	KN Healthy Mice	K- No Intervension	K+ Cream Base	K1 Dosage 4%	K2 Dosage 8%	*P-value*
TNF-α Expression (ng/mL)						
Mean±SD	339.51± 34.51	410.93± 83.79	332.28± 32.51	343.24± 36.86	390.01± 57.74	
Shapiro-Wilk	0.611*	0.644*	0.755*	0.123*	0.839*	
Levene Test						0.150*
One way Anova						0.106

Description: **Shapiro-Wilk* = Normal (p>0.05)
* *Levene Test* = Homogen (p>0.05)
* *One way Anova* = Significance (p<0.05)

The UVB-only group (K−) exhibited the highest TNF-α level (410.93 ± 83.80 ng/mL), while the lowest was observed in the base cream group (K+) at 332.28 ± 32.52 ng/mL. The healthy control group (KN) had a mean TNF-α level of 339.51 ± 34.52 ng/mL. Groups treated with Ajwa extract cream at 4% and 8% (K1 and K2) showed intermediate means of 343.24 ± 36.86 and 390.01 ± 57.74 ng/mL, respectively.). The results of the data were normally distributed and homogeneous, eligible for a parametric test, to compare the average TNF-α levels between groups was carried out a one-way anova test.

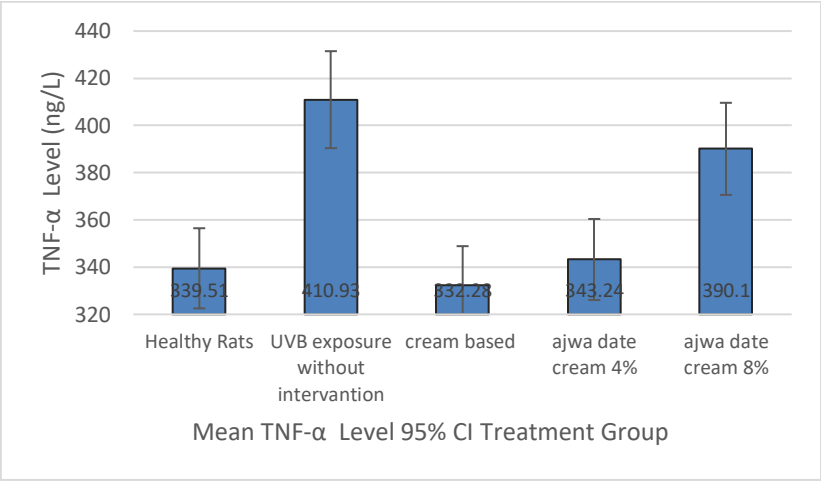


Figure 2. Effect of Ajwa Date Cream on TNF-α Levels in UVB-Exposed Mice

A one-way ANOVA test was performed to assess group differences. The resulting p-value was 0.106, indicating no statistically significant difference in TNF-α levels among the five groups. However, there was a trend toward reduced TNF-α expression in the K1 (343.24 ± 16.48 ng/mL) and K2 (390.10 ± 25.82 ng/mL) treatment groups compared to K−. The largest absolute difference was between

K− and K+ (−78.65 ng/mL.) with the partial eta squared (η^2p = 0.305) indicated a large effect size, with 30.5% of the variance in TNF-α levels attributed to treatment supporting underlying biological variation.

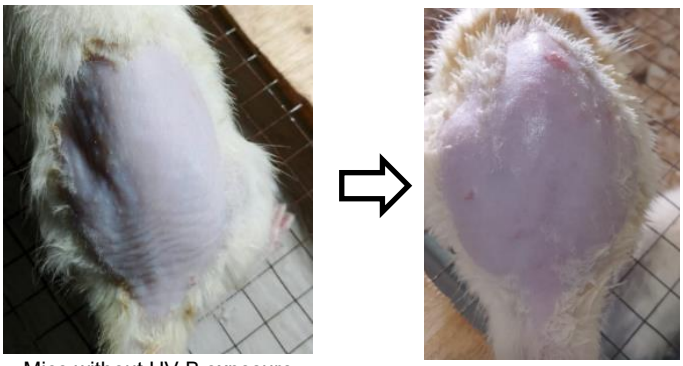
Table 4. LSD Post Hoc Comparisons of Tumor Necrosis Factor-Alpha (TNF-α) Levels Between Groups

Group	Comparison Group	*p-value*	95% Confidence Interval	
TNF-α expression (ng/mL)			Lower Bound	Upper Bound
Healthy mice (KN)	No intervention (K-)	0.045	-141.1309	-1.6971
	Cream base(K+)	0.831	-62.4829	76.9509
	Ajwa date cream 4% (K1)	0.912	-73.4429	65.9909
	Ajwa date cream 8% (K2)	0.146	-120.2109	19.2229
No intervention (K-)	Cream base (K+)	0.029	8.9311	148.3649
	Ajwa date cream 4% (K1)	0.056	-2.0289	137.4049
	Ajwa date cream 8% (K2)	0.538	-48.7969	90.6369
Cream base (K+)	Ajwa date cream 4% (K1)	0.746	-80.6769	58.7569
	Ajwa date cream 8% (K2)	0.177	-127.4449	11.9889
Ajwa date cream 4% (K1)	Ajwa date cream 8% (K2)	0.177	-116.4849	22.9489

LSD post hoc analysis showed that TNF-α levels in the KN group were significantly lower than in the K− group (mean difference = 71.41 ng/mL; p = 0.045; 95% CI: 141.13 to 1.70), consistent with UVB-induced inflammation. Likewise, TNF-α levels in the base cream group (K+) were significantly lower than those in the K− group (mean difference = 78.65 ng/mL; p = 0.029; 95% CI: 148.36 to 8.93).

Although not statistically significant, Cohen's calculation between K− and K1 give result approximately 1.05, reflecting a large effect size. the comparison between KN and K2 showed a moderate increase in TNF-α levels (mean difference = −50.49 ng/mL; p = 0.146; 95% CI: −120.21 to 19.22) while The comparison between K− and K1 (Ajwa 4%) shown the value between group approacing significance (mean difference = 67.69 ng/mL; p = 0.056; 95% CI: 137.40 to -2.03), suggesting a trend toward TNF-α reduction at moderate concentrations.

Macroscopic observations of the subject describe a mice model that was exposed to UV-B light with an emission of 302 nm and an erythema dose of 160 mJ/cm². On the first day, the macroscopic appearance of the skin in mice exposed to UV-B was observed, as shown in Figure 3 below.



Mice without UV-B exposure. Mice after UV-B exposure on day 1.

Figure 3. Comparison of mice skin without exposure to UV-B exposure on day 1.

On the first day of macroscopic observation, no significant inflammatory reaction was observed. Observations on the third day showed inflammation and the appearance of erythema, and on the seventh day of exposure, widespread erythema was observed on the UV-B exposed rat skin. Images of rat skin exposed to UV-B rays showed signs of skin burning, irritation, erythema/redness, and excessive pigmentation.

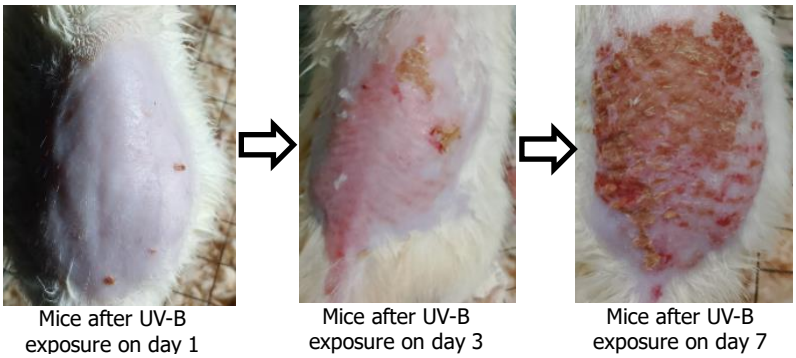


Figure 4. Macroscopic photo of mice skin exposed to UV-B light

The increase in erythema by the seventh day of treatment resulted in vasodilation of blood vessels, which triggered a mast cell reaction to ROS and the release of histamine, causing skin redness. UV-B rays have the most damaging effects on skin, triggering immune responses that destroy damaged cells naturally. This process also leads to redness and skin peeling. [28]

The study subjects were conditioned with UV light exposure treatment (broadband with peak emission of 302 nm) at a minimum dose of erythema of 160 mJ/cm2/day for 15 minutes daily, followed one hour later by topical treatment of ajwa date extract cream for 7 days. On the 8th day, skin tissue samples were collected to prepare for HE staining anatomical histopathology, the results of observations were carried out by anatomical pathology experts.

The results of HE staining analysis by calculating the number of fibroblast cells as a marker of inflammation from UVB exposure, are shown in figure 5 and table 3. The average number of fibroblast cells was obtained by calculating 5 fields of view in each HE staining preparation, thehealthy group (KN) obtained the highest total number of fibroblasts which was 60.5 per field of view, the group without intervention (K-) with the least total number of fibroblasts which was 12 per field of view, the cream-based group with the number of fibroblasts 23.5 per field of view, the 4% dose group (K1) with the number of fibroblasts 19.5 per field of view and the 8% dose group (K2) with the number of fibroblasts 37.5 per field of view.

The results of the validation of the mean number of fibroblasts showed a difference in the healthy group (KN) compared to the group without intervention (K-) with a significant decrease in the number of fibroblasts due to UVB exposure, indicating the validation of UVB exposure conditioning in confirmed mice subjects.

Table 5. Mean Number of Fibroblasts in Hematoxylin–Eosin (H&E) Staining Across Treatment Groups

Group	KN Healthy Mice	K- No Intervention	K+ Cream Base	K1 Dosage 4%	K2 Dosage 8%
Number of fibroblast					
Average	60.5	12	23.5	19.5	37.5

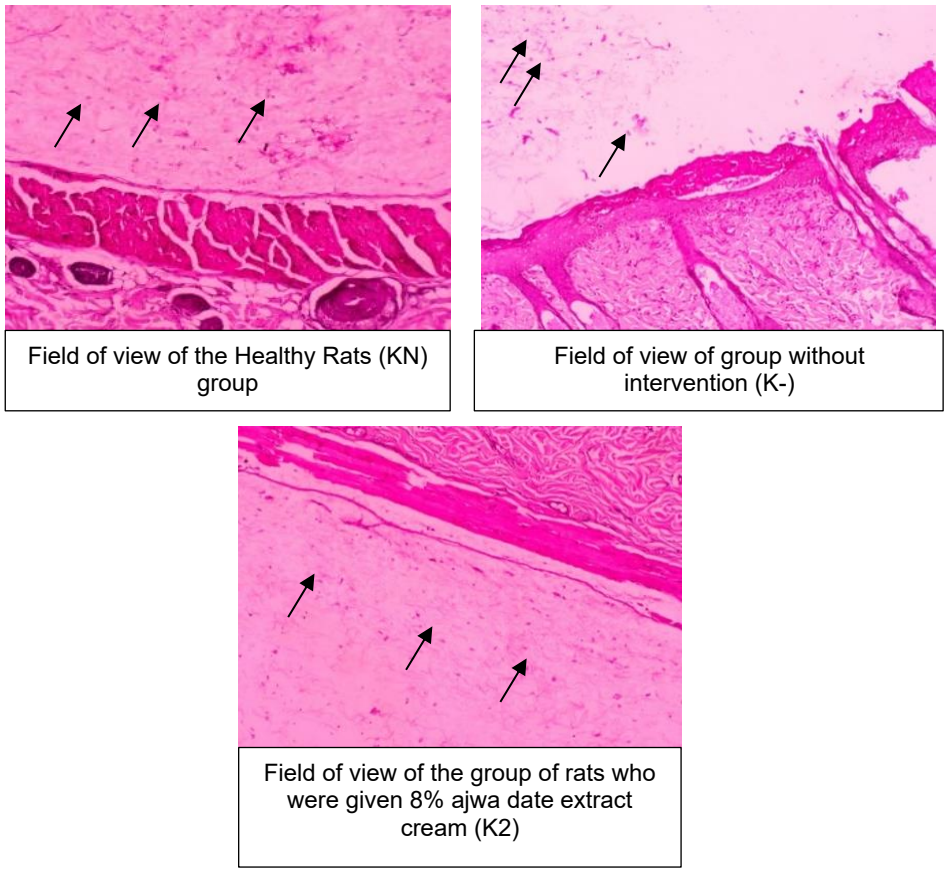


Figure 5. Distribution of fibroblast cells in the field of view of HE staining

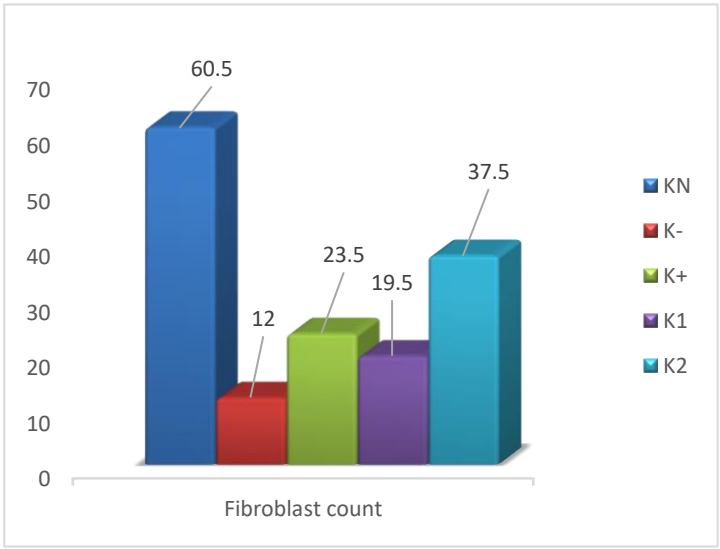


Figure 6. Mean Number of Fibroblasts Across Treatment Groups

The difference in the number of fibroblasts in the healthy mice group (KN) when compared to the K+, K1, and K2 treatment groups also decreased. There was a difference in the increase in the number of fibroblasts in the 8% (K2) date cream dose group when compared to the group without intervention (K-). The analysis of HE stains showed significant differences in results between the control group and the treatment group.

Table 6. Analysis of Mean Body Weight Before and After Treatment in Mice

Group	KN Healthy Mice	K- No Intervention	K+ Cream Base	K1 Dosage 4%	K2 Dosage 8%
Average Weight Before Treatment	222	204	222	212	212
Average Weight After Treatment	219	201	211	204	189
Paired T test	0.573	0.07	0.468	0.097	*0.016

Description: * T-test = Significance (p<0.05)

A comparative test of the rat group before and after the treatment showed the results of the t test in the KN group of healthy rats 0.573, the K-rat group with UV-B light exposure without treatment obtained a value of 0.07, the K+ group of rats with UV-B light exposure and the administration of base cream with a value of 0.468, The K1 group of rats with exposure to UV-B rays and the administration of base cream with a value of 0.097 which means that there was no significant difference between 4 groups of rats (KN, K-, K+, and K1) with a pre- and post-treatment value (p<0.05) and the K2 group of rats with exposure to UV-B light and 8% ajwa extract date cream treatment with a value of 0.016 (p<0.05) showed a significant difference between the K2 group before and after the treatment.

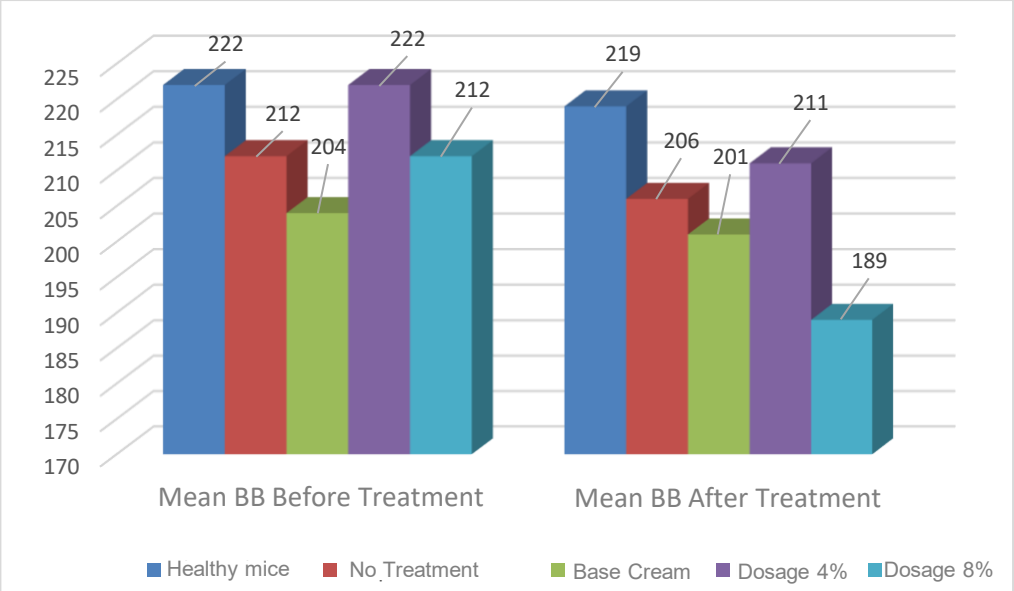


Figure 7. Mean Body Weight of Mice Before and After Treatment

This study investigated the topical effects of Ajwa date (*Phoenix dactylifera L.*) extract cream on skin damage caused by UV-B radiation. Although the observed changes in SOD and TNF-α levels were not statistically significant, the results demonstrated positive directional trends, particularly in the treatment groups, suggesting potential therapeutic activity of the extract. It shown Notably, effect size analysis and confidence intervals provided important insights beyond p-values. Several comparisons, while not reaching statistical significance, demonstrated large effect sizes and confidence intervals that suggested biologically meaningful differences. This underscores the potential relevance of the observed trends, especially in small sample studies.

The highest average SOD level was observed in the 8% Ajwa extract group (K2), while the lowest was in the healthy control group (KN). Interestingly, the untreated UV-B group (K−) also showed an increase in SOD levels, which may reflect an endogenous antioxidant response to UV-induced oxidative stress. [29] However, these changes did not reach statistical significance, possibly due to short treatment duration, inter-individual biological variation, or insufficient dosage to yield a more consistent response. The expression of antioxidant enzymes like SOD is known to be cell-type specific and may vary between keratinocytes and fibroblasts [30]. Additionally, under conditions of mild oxidative stress, cells may upregulate antioxidant defenses independently of external intervention [31], as likely occurred in the K− group. Nevertheless, the comparison between K2 and KN showed a statistically significant post hoc difference with a 95% confidence interval of −2.1577 to −0.0383 ng/mL. Furthermore, Cohen's d for this comparison was 1.71, indicating a very large effect size and reinforcing the extract's potential antioxidant activity despite the lack of overall significance in ANOVA.

Similarly, the changes in TNF-α levels across treatment groups showed a non-significant but biologically meaningful trend. Both the base cream (K+) and 4% extract cream (K1) groups had lower TNF-α levels than the untreated UV-B group, suggesting some anti-inflammatory effect of the formulation. However, TNF-α expression was elevated in the 8% extract group (K2). This may reflect the dual role of TNF-α, which, beyond its function as a pro-inflammatory cytokine, also participates in tissue remodeling and cell repair by activating transcription factors such as NF-κB and regulating matrix metalloproteinases (MMPs) [31, 32]. For instance, TNF-α stimulates the release of MMP-2 and MMP-9 in dermal fibroblasts, enabling extracellular matrix turnover and tissue regeneration [32]. Furthermore, TNF-α activation has been linked to pathways involving hypoxia-inducible factor-1α (HIF-1α), Nrf2, and AP-1, all of which mediate oxidative stress and inflammatory gene expression [33]. TNF-α also signals through specific receptors (TNFR1, TNFR2), recruiting intracellular mediators like TRADD and TRAF2, which influence downstream gene regulation and apoptosis. [34 , 35] If antioxidant levels remain insufficient relative to ROS accumulation, this repair mechanism may become dysregulated, resulting in sustained or elevated TNF-α levels [36]. Although not statistically significant, the comparison between K− and K1 revealed a 95% confidence interval of −137.40 to 2.03 ng/mL and a Cohen's d of 1.05, again indicating a large effect size. The molecular mechanisms underlying the observed effects can be partly explained by the modulation of signaling pathways involved in photoaging. UVB radiation activates MAPK pathways (ERK, JNK, and p38), leading to increased AP-1 activity and the subsequent induction of MMPs, which accelerate collagen degradation. It also stimulates NF-κB translocation, upregulating pro-inflammatory cytokines such as TNF-α and IL-6, thereby amplifying oxidative and inflammatory stress [37]. Antioxidant-rich extracts, including polyphenols from Ajwa dates, may inhibit NF-κB activation and attenuate MAPK signaling, reducing cytokine release and MMP expression. Furthermore, enhanced fibroblast proliferation in the 8% extract group suggests a possible involvement of the TGF-β/Smad pathway, which is known to stimulate collagen synthesis and tissue remodeling [38]. Taken together, these findings indicate that Ajwa date extract may exert dermato-protective effects not only through direct antioxidant activity but also via regulation of NF-κB, MAPK, and TGF-β pathways. This supports the potential of moderate-dose Ajwa extract to suppress TNF-α levels and modulate inflammatory pathways, warranting further investigation.

The increase in SOD activity and modulation of TNF-α observed in our study aligns with known effects of classical antioxidants such as Vitamin E who has been shown to scavenge free radicals and provide photoprotection, although high-quality clinical data remain sparse [39] Some study shown EGCG from green tea exhibits significant anti-inflammatory and antioxidant effects, blocking UVB-induced MAPK and NF-κB activation, reducing leukocyte infiltration, prostaglandin production, and preserving enzymatic antioxidant levels like glutathione peroxidase and catalase [19]. Resveratrol induces Nrf2-dependent antioxidant pathways (e.g., HO-1, SOD1), inhibits

UVB-induced MMPs and inflammatory mediators, and facilitates skin renewal in vivo [20,21]. Shown Ajwa date extract may offer advantages over these agents by integrating a diverse spectrum of bioactive compounds capable of simultaneously targeting oxidative stress, inflammation, and dermal regeneration.

Beside that The observed protective effects of Ajwa date extract cream are consistent with findings from other polyphenol-based topical agents. For instance, Resveratrol-containing formulations demonstrated photoprotective effects by activating Nrf2-mediated antioxidant pathways and inhibiting NF-κB signaling, thereby suppressing inflammation and enhancing collagen synthesis [40].While grape seed, another polyphenol, have been shown to inhibit UVB-induced oxidative stress and suppress MAPK and NF-κB activation in human epidermal keratinocytes.[41] This multifaceted mechanism might underpin the favorable trend toward fibroblast recovery seen in this study, pointing to Ajwa's potential superiority as a dermato-protective topical agent.

Although no significant changes were observed in TNF-α and SOD levels, histological analysis showed enhanced fibroblast activity, which may indicate Ajwa extract promotes dermal remodeling independent of direct cytokine modulation. The increase in fibroblast numbers observed in the histological analysis adds further insight into the extract's activity. Despite the absence of statistically significant biochemical changes, the 8% extract group (K2) showed a marked increase in fibroblast density compared to the untreated group, supporting its role in dermal regeneration.The increase in fibroblast density may be partially explained by indirect modulation of the TGF-β/Smad pathway or inhibition of MMP-mediated ECM degradation, although further investigation is warranted, Fibroblasts itself are essential for tissue repair, collagen production, and wound healing, and their proliferation following Ajwa extract application suggests an underlying stimulatory effect on skin remodeling, even in the presence of ongoing inflammation. Previous studies have reported polyphenol-rich plant extracts to stimulate fibroblast proliferation and modulate oxidative signaling in UV-damaged skin [42].

In line with previous research, macroscopic observation showed erythema and skin irritation in UV-B-exposed rats, consistent with the induction of sunburn, inflammation, and pigmentation [28, 43]. While these observations were not quantified, the visual improvement in treated animals indicates potential protective or reparative effects of the extract. UV-B radiation is known to induce DNA damage via the formation of cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts, leading to apoptosis and photoaging [43]. It also triggers ROS generation through pathways like MAPK, leading to lipid peroxidation and membrane disruption [28].

Regarding systemic effects, the significant weight loss in the K2 group may reflect a compensatory immune or stress response, although environmental factors, reduced intake, or inflammatory-mediated anorexia cannot be ruled out. Elevated TNF-α has been associated with cachexia and metabolic imbalance, which may contribute to this observation.

Several limitations should be acknowledged. The short duration of treatment (7 days) may have been insufficient to observe more pronounced effects. Moreover, the absence of pre-treatment data limited the ability to analyze within-group changes over time. Only two biomarkers (SOD and TNF-α) were analyzed, and additional markers like MMPs, IL-1β, or collagen levels may have provided a more comprehensive understanding of skin healing. Lastly, the study lacked dose-ranging analysis, which would be necessary to determine optimal therapeutic concentrations of Ajwa extract. In addition, the cream formulation was not subjected to any microbiological stability testing, which limits the assessment of long-term product safety and integrity.

In conclusion, Ajwa date extract cream demonstrated potential dermatoprotective properties, particularly in enhancing fibroblast proliferation and modulating oxidative and inflammatory pathways. While TNF-α and SOD levels did not differ significantly, observed trends and histological improvements suggest a need for

further investigation using longer treatment durations, expanded biomarker panels, and prepost intervention designs.

CONCLUSION

This study provides early evidence that Ajwa date (*Phoenix dactylifera L.*) extract cream may offer dermato-protective effects against UVB induced skin damage through its potential to support antioxidant mechanism, inflammatory modulation, and fibroblast regeneration. Although changes in TNF-α and SOD levels did not reach statistical significance, the observed positive biological trends, along with histological improvements, suggest that the extract may enhance tissue repair and skin resilience. These findings underscore the need for future studies employing longer treatment durations, larger cohorts, and comprehensive molecular profiling to fully elucidate the mechanisms involved. With further validation, Ajwa extract cream holds potential as an affordable and natural adjunct in preventive or therapeutic strategies against UV-induced skin damage.

AUTHORS CONTRIBUTIONS

Sri Mahoni: Investigation, Data curation, Writing-Original draf, Conceptualization. Sri Priyantini Mulyani: Reviewing, Validation, Supervision Siti Thomas Z: Supervision, Validation.

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DATA AVAILABILITY STATEMENT

The data supporting findings of this study are available from the corresponding author.

DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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