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Research Article

Effect of Apple (*Malus domestica*) Stem Cells on UVB-Induced Damage Skin with Anti-Inflammatory Properties: An *In Vivo* Study

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Objective. Apple (*Malus domesticus*) stem cells have beneficial effects in preventing photodamages caused by UVB responsible for inflammation in different stages of various skin disorders. This study aimed to investigate Apple stem cells' anti-inflammatory and repairing effects on UVB-induced rat dorsal skin damage via microscopic and macroscopic analyses. *Materials and Methods*. Therapeutic effects of the apple stem cells (ASCs) extract were evaluated after UVB irradiation with macroscopic and microscopic studies, including pathological analysis, inflammatory cytokines measuring, and biometric studies containing investigation of thickness, density, erythema, melanin, sebum, and moisture content of epidermis and dermis layers in rat models. After biometric studies, skin samples were taken for histopathologic and biochemical analyses. *Results*. ASC extract could attenuate infiltration of inflammatory cells caused by UVB and ameliorate collagen regulation of the photodamaged skin. In addition, improved skin biometrics was considerable, such as reducing thickened epidermal and dermal layers compared to other rat groups. Furthermore, moisture content enhancement of the skin showed clinical advantages in treating damages and inflammation. Furthermore, TNF-α expression was downregulated after ASC application. ASC extract could treat UVB damages and indicate anti-inflammatory effects in animal models. *Conclusion*. The ASCs can be an appropriate candidate for treating inflammation and damages induced by UVB in clinical studies.

1. Introduction

About twenty-five percent of people suffer from different kinds of skin diseases. Inflammatory skin diseases are common forms of skin diseases, for example, atopic dermatitis, psoriasis, and allergic contact dermatitis [1]. One of the adaptive reactions is inflammation, which involves soluble mediators. Multiple defense cell types respond to harmful stimuli by removing infectious agents and regulating tissue homeostasis. These active defense cells release and produce inflammatory mediators, including interleukin IL-1β and TNF-α [2]. Also, one of the contributing factors to photodamage and skin carcinogenesis is exposure to ultraviolet (UV) radiation. Dysregulated inflammation has a vital role as a crucial mechanism in the destructive effects of UV irradiation [3]. These damages are known as photodamage and photoaging related to solar irradiation. Some damages characterize brown spots, irregular pigmentation, dryness, elasticity reduction, wrinkling, leathery look, and the development of coarse solar scars [4, 5]. The UV radiation, particularly UVB, has increased skin damage with long-term consequences such as skin inflammation,

photodamage, and photoaging, which are severe concerns for the health of humans due to the depletion of stratospheric ozone, which is the critical reason for incidents on the earth [6]. Acute inflammatory skin problems such as cellular apoptosis and erythema are also caused by UVB. This radiation can also cause the promotion of UVB-induced proinflammatory enzymes, and following activation of a related signaling pathway (COX-2), it produces specific inflammatory mediators containing various cytokines and prostaglandins (PGs). Cascades of COX-2 are reasons for pain, growth of cells, and progression of tumors.

Similarly, COX-2 cascades mediate the inflammatory process. It has elucidated the importance of inflammation in the pathogenesis of skin disorders caused by UVB exposure [7]. Today, the used drugs to treat skin inflammation are topical and systemic nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Despite this, the long-term duration of inflammatory disease treated with these agents leads to adverse reactions, including maceration, folliculitis, pruritus, irritations, dryness, military, hypertrichosis, acneiform eruptions, and allergic reactions contact dermatitis, stretch marks, and hypopigmentation. The side effects of current drugs cause to make an appropriate opportunity due to more minor toxic effects for numerous medicinal plants, which have an abundant biological function. The knowledge of using plants and plant extracts in disease prevention and treatment has been developed through continuous contact of humans with their natural environment since ancient times [2, 8]. Plants are cultivated on a large scale which is a systematic method for producing plant-derived compounds. However, high-priced harvesting and extraction methods make the procedure often economically unsustainable. In spite of this, most plants with valuable compounds have not been domesticated. Also, the wild population of these effective plants is limited. The low levels of target molecules and plant sluggish growth decline production potentiality [9].

Apple (*Malus* sp., Rosaceae) stem cells consist of many polyphenols, quercetin glycosides (flavonols), hydroxycinnamic acids, and oligomeric procyanidins, dihydrochalcones, and catechins, also anthocyanins in red apples and triterpenoids in peels of apples [10–12]. Several results proposed that apples have a broad spectrum of activities related to biological features. Production of apple compounds could be effective in respiratory problems (i.e., asthma), cancer prevention, diabetes mellitus, and cardiovascular disease. The compounds mentioned above contain antioxidants activity, anti-inflammatory mechanisms, carcinogenesis, antimutagenicity, antiproliferative pathways, changes of signal transduction activity, apoptosis-inducing, and new mechanisms on innate immunity [13]. The obtained active ingredients from apple (*Malus domesticus*) cells suspension cultures were included in the anti-aging ingredients. These plant stem cells regenerate the skin, are resistant to aging, and affect the viability and apoptosis of human stem cells, but the mechanism is unknown [9, 14]. In this study, the promising therapeutic effects of topical application of *Malus domesticus* were investigated on biophysical and biometrics skin analyses and histopathological studies in the dorsal skin of animal models exposed to acute UVB irradiation, which has been explored for the first time to evaluate the effect of apple stem cells extract on UVB-induced injury.

2. Materials and Methods

2.1. Materials. The current study purchased extracts of apple (*Malus domestica*) stem cells from Mibelle Biochemistry. Xylazine (Alfasan, Holland, 2%), ketamine (Bremer Pharma GmbH, Germany, 10%), and the rat tumor necrosis factor-α ELISA kit were purchased from ZellBio GmbH (Germany), and betamethasone topical cream 0.1% was purchased from Darou Pakhsh (Iran). Tewameter (TM300, Courage, and Khazaka, Germany), corneometer (CM825, Courage, and Khazaka, Köln, Germany), mexameter (MX18, Courage, and Khazaka, Köln, Germany), sebumeter (SM815, Courage and Khazaka, Köln, Germany), skin ultrasound imaging system (Skinscanner-DUB, taberna pro medium GmbH, Germany), and cutometer (MPA 580, Courage & Khazaka, Germany) are used for skin parameters analysis.

2.2. Animals. Twenty-four adult male rats (*Wistar*, weighing 275 ± 25 g) were obtained from Tehran University of Medical Sciences Laboratory Animal Center and were kept at the Animal Center. Animals were accustomed for two weeks and kept in polypropylene boxes with wood-lined bedding, which was changed daily, with 12 hours' light/dark cycle, and controlled temperature (22–24°C). Food and water were given *ad libitum* in experiment duration. In vivo studies were performed in compliance with National Institutes of Health guidelines for the welfare of experimental animals. The Ethics Committee approved the protocol of the Faculty of Pharmaceutical Science of Tehran University of Medical Science (IR.TUMS.VCR.REC.1399.455). The hair on the dorsal areas of the rats ($4 \times 4\,\text{cm}^2$) was removed by using a depilatory cream then. Irradiations started after 3 days. Finally, the animals were euthanized, and dorsal skin samples were taken for histopathological and biomarkers studies.

2.3. Experimental Design. The rats were randomly assigned into four groups (Sham, Control (-/UVB), betamethasone (betamethasone/UVB), and ASCs (ASCs/UVB)) ($N = 6$). The sham group animals were not exposed to UVB without any topical treatment. A pilot study was conducted to distinguish possible allergic reactions using two non-irradiated animals treated with ASCs extract. Sham groups are analyzed histopathologically and macroscopically, and TNF-α and TGF-β1 are assessed. The animals of other groups were exposed to UVB irradiation ($180\,\text{mJ/cm}^2$) applied once daily for one hour in 7 consecutive days. After UVB-induced damage, rats were treated for their group one daily for 7 days. As mentioned above, the control group was treated without any formulation. The betamethasone group was treated with Betamethasone 0.1% cream, and rats in the ASCs group were treated with the extract of apple stem cells. Biophysical and biometrics skin values were analyzed on days 0 (exactly before any topical treatments), 1, and 7. Moreover,

immunohistological investigation and histopathological studies were evaluated on days 1 and 7 in control, betamethasone, and ASC groups.

2.4. Irradiation. UVB radiation was conducted as mentioned above. The source of UVB irradiation (Philips TL20W/01 RS lamp, Holland) was placed 20 cm above rats, which emitted the peak wavelengths of 313 nm narrowband UVB [15, 16]. 80% production of total UV radiation was measured through the radiometer sensor (UV: SED005, and UVB: SED240). The irradiation rate of UVB was 0.18 mW/cm^2. Also, the dose of UVB utilized in the experiment was 0.3 J/cm^2. Before being exposed to UVB irradiation, rats were anesthetized with an intraperitoneal injection (Ketamine/Xylazine).

2.5. Administration. The ASC extract (20 mg with a concentration of 20 mg/ml) was topically used on the skin of rats once daily for 7 days in the ASC group. Moreover, betamethasone cream (500 μg with a concentration of 1 mg/g) was administered on the dorsal areas of rats in the betamethasone group once daily for 7 days. Pilot studies of our research group and previous studies showed that the amount of topical administration was following dose-response [17, 18]. All of the treatments were started after 7 days of UVB irradiation.

2.6. Histologic Analysis. The skin samples were fixed in buffered formaldehyde solution (10%), dehydrated in ethanol solutions, diaphenized in xylene, embedded in paraffin, and then cut into 5 μm sections stained with hematoxylin & eosin for a histological evaluation. The histopathological changes such as damages of different layers of skin also epidermis thickness were assessed randomly selected histological fields (optical microscopy, Olympus BX51, Tokyo, Japan) [19].

2.7. Tumor Necrosis Factor-α in the Cutaneous Tissue. Before the dorsal skin was surgically collected, animals were euthanized. Also, samples homogenated in 500 μL of phosphate-buffered saline (1.5 mM KH_2, 3 mM KCl, 10 mM Na_2HPO_4, and 137 mM NaCl, pH 7.4). Then, skin specimens were centrifuged (10.000 g, 4°C, 10 min). The produced supernatants were utilized for quantification of TNF-α with the ELISA Kit (ZellBio, GmbH, Germany).

2.8. Real-Time PCR Analysis. Total RNA Kit (RNA simple Total RNA Kit, CHN) divides total skin RNA, quantified by measuring the absorbance at 260 nm. Then, the cDNA Synthesis Kit (VILO™ cDNA Synthesis Kit, Thermo, USA) was used to reverse transcription RNA. Primers of TGF-β1 (forward primer (5′ to 3′) CGCCTGCAGAGATTCAAGTC and reverse primer (5′ to 3′) GCCCTGTATTCCGTCT CCTT) were designed using the sequence detection system. Despite accomplishing the quantitative expression of mRNA, real-time PCR elaboration was carried out on a sequence detection system (ABI Prism 7500, USA) with a Fast Start Universal SYBR Green Master (Rox, Roche, Switzerland) for amplification reaction assays at the optimal concentration (40 times cycles, 15 s at 95°C, 60 s at 60°C, and 2 min at 72°C). Data were analyzed and standardized by thermal cycler software, and each sample was tested three times.

2.9. Biometric Analysis of the Skin. Rats were anesthetized with an intraperitoneal injection (ketamine/xylazine) on days 0, 1, and 7 after induced damages by UVB irradiation. Afterward, the skin parameters of rats containing transepidermal water loss (TEWL), hydration of stratum corneum, melanin, erythema, sebum, thickness, density, and elasticity were assessed through particular apparatus such as the tewameter, corneometer, mexameter, sebumeter, and skin ultrasound system. Investigations are analyzed after collecting the results.

2.10. Statistical Analysis. Data were analyzed using analysis of variance, followed by Duncan's multiple range test for comparison between mean values at a significance level of $p < 0.05$ (SPSS Statistics 20 software, SPSS Inc., USA).

3. Results

3.1. Macroscopic Evaluation. Macroscopic evaluations of the UVB effect on rat dorsal skin are shown in Figure 1. The dorsal skin appeared healthy in the sham group without abnormality or erythema. The dorsal skin was irradiated for 7 days with UVB irradiation in other groups. The induced photodamage features were sunburns, deep-wrinkled, dark-brown color, and leathery skin (with flesh-colored lesions). The appearance of the skin was relatively improved after the first day of treatment with ASCs and betamethasone, but the signs of UVB remained.

Additionally, the undesirable skin appearance of UVB-induced was significantly improved in treated groups with apple stem cells and betamethasone. The skin was significantly softer in the ASCs group, and no signs of sunburns or erythematous changes were detected. The observation in the ASCs group was nearly close to the sham group on day 7. Moreover, in the betamethasone group, the improved dorsal skin was similar to treatment with ASCs (Figure 1).

3.2. Effect of Apple Stem Cells in Comparison with Betamethasone Cream on the UVB-Induced Skin Damage

3.2.1. Measurement of Transepidermal Water Loss (TEWL) on the UVB-Induced Skin Damage. TEWL was evaluated (Tewameter®, TM300, Courage, and Khazaka, Germany) to detect the baseline TEWL value (about 9 ± 2 g/h/m^2) and the skin barrier function [20]. TEWL was determined about $500 \pm 50\%$ after 7 days of the UVB irradiation. The extent of TEWL was gradually decreased in each group. On the other hand, TEWL levels had no significant difference in the ASCs group compared to the betamethasone and control groups on day 7. However, the TEWL level in the ASC group was decreased more than in other groups ($^* p \leq 0.05$, $^{**} p \leq 0.01$) (Figure 2(a)).

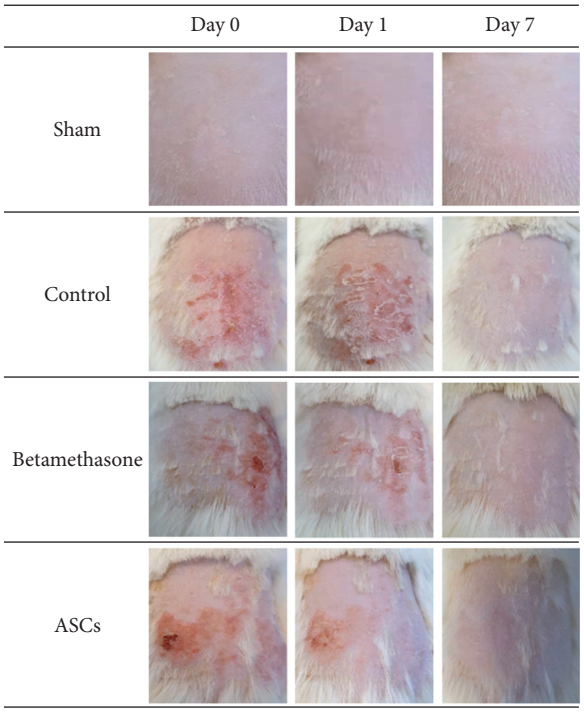

Figure 1: Macroscopic changes of rat skin in sham, control, betamethasone, and ASC groups on days 0, 1, and 7.

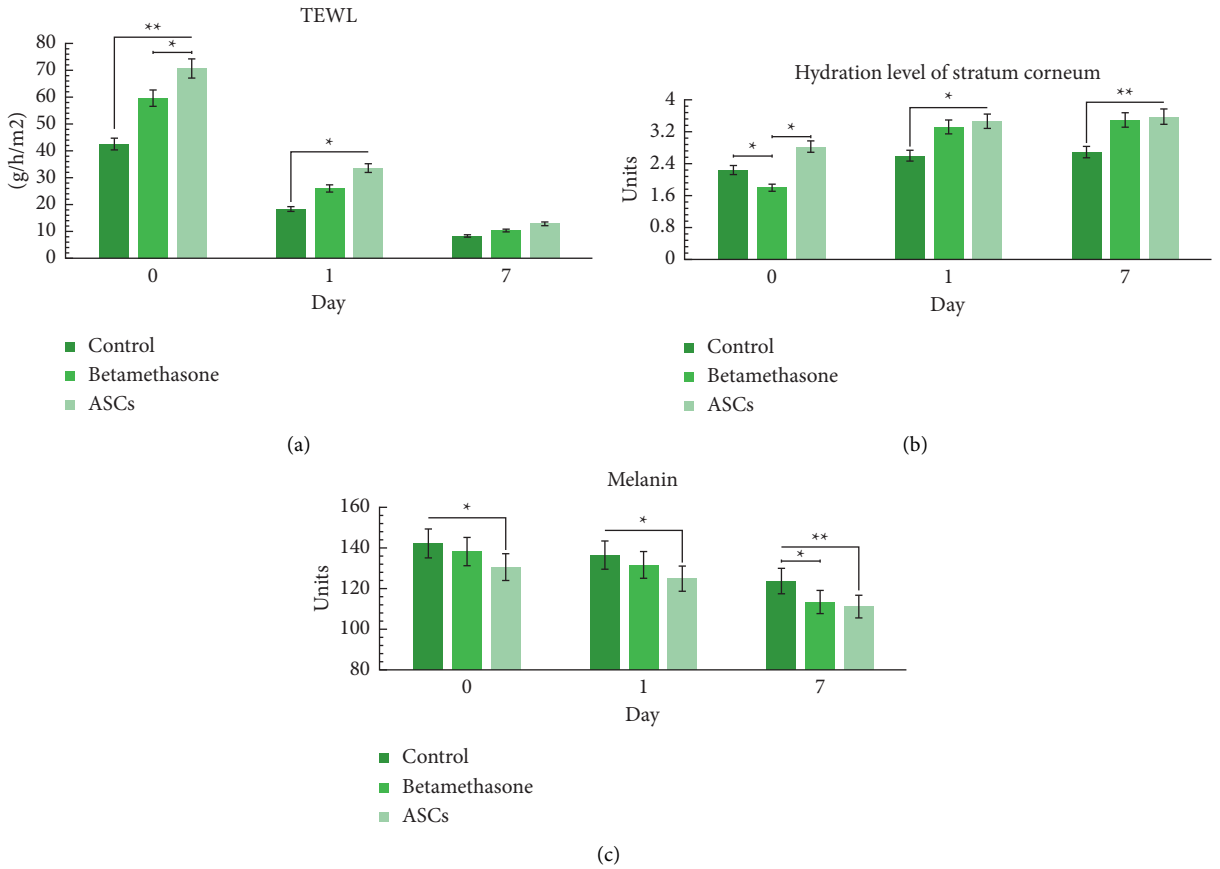

Figure 2: (a) TEWL level as an indicator of water loss, (b) hydration level in stratum corneum, (c) melanin content of UVB-irradiated skin in treated (betamethasone and ASC groups) and control groups on day 0, 1, and 7. Error bar: mean ± SD ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

3.2.2. Measurement of Skin Moisture Content on the UVB-Induced Skin Damage. Evaluation of moisture contents (Corneometer®, CM825, Courage, and Khazaka, Köln, Germany): in rats irradiated with UVB for 7 days (an hour per day), the moisture content of rat skin was steadily reduced in the betamethasone and control groups from day 0 to 7. Also, the topical application of ASCs extract slightly ameliorated the moisture loss of photodamaged skin, although TEWL was decreased in all treated groups ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$) (Figure 2(b)).

3.2.3. Measurement of Melanin on the UVB-Induced Skin Damage. Melanin values were measured (Mexameter®, MX18, Courage, and Khazaka, Köln, Germany) from days 0 to 7 in the treatment duration of treated groups. In 7 days duration of the experiment, melanin was decreased value in ASCs, betamethasone, and control groups.

The values attributed to melanin amount were declined in betamethasone and ASCs groups compared to the control group. The amount of the melanin was 113.2 ± 0.1 in the case of the betamethasone group, which was close to the ASC group (111.1 ± 0.1), but both values were lower than the control group with 124 ± 0.2 ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$) (Figure 2(c)).

3.2.4. Measurement of Erythema on the UVB-Induced Skin Damage. The measurement confirmed the observation of changes in the degree of erythema. Skin erythema was increased initially due to the UVB exposure in 7 days and had gradually reduced to a normal condition. Although the difference was not significant between groups in erythema with entire treatment duration in the control and ASCs groups, the values of the arbitrary units indicated the severity of erythema were lower in control and ASC groups than the betamethasone group ($^{*}p \leq 0.05$) (Figure 3(a)).

3.2.5. Measurement of Sebum Contents on the UVB-Induced Skin Damage. Skin sebum content was measured by a sebumeter® (SM815, Courage and Khazaka, Köln, Germany), which was substantially decreased after UVB exposure in 7 days. The betamethasone treatments slightly increased sebum content by about 30% without any significance. The sebum level dramatically increased after 7 days in the ASCs group, and also, 150% rise-up in sebum content was observed from a value of 3.8 to 9.7 $\mu g/cm^2$. A 130% increase of sebum could be detected in the control group in 7 days. It is noticeable that sebum amelioration was found in all treated groups but, the most significant induction showed in the ASC group ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$) (Figure 3(b)).

3.2.6. Measurement of Elasticity on the UVB-Induced Skin Damage. The standard deviations and means of the *R* parameters were obtained in three groups. Statistically, the elasticity differences were found between the control, Betamethasone, ASCs groups for every *R* parameter (R7: ratio of elastic recovery to the all deformation, R5: net elasticity, R2: overall elasticity of the skin). In particular, the R7 parameter was the most critical parameter of elasticity for measuring (Cutometer, MPA 580, Courage & Khazaka, Germany) skin aging. In the control and betamethasone groups, differences between changes of *R* parameters were not significant from day 0 to 7. On the other hand, the number of *R* parameters, particularly R7, was consistently increased in the ASC group compared to other groups ($p \leq 0.05$) (Table 1).

3.2.7. ASC Effect on the Decrement of TNF-α Activity in UVB-Induced damage on the Skin. UVB-irradiation significantly elevated the concentrations of cytokines such as TNF-α in the skin. After UVB-irradiation, ASC extract treatment indicated anti-inflammatory effects in topical administration such as inhibition of TNF-α expression. Also, the ASC group showed an anti-inflammatory effect on day 1, and the concentration of TNF-α was similar to day 7. However, it had a meaningful difference with the sham group. The TNF-α concentration in the skin was upregulated moderately in the control group. On the contrary, the expression of TNF-α in the betamethasone group was steadily decreased, but TNF-α expression was decreased significantly in the ASCs group in 1 day ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$) (Figure 4(a)).

3.2.8. Measurement of Skin Thickness and Density on the UVB-Induced Skin Damage. The skin thickness and density were measured by a skin ultrasound imaging system (Skinscanner-DUB, taberna pro medium GmbH, Germany). Total thickness and density of dorsal skin of rats were increased in all groups after 7 days of UVB exposure. The thickness and density of epidermis and dermis layers were measured on days 0, 1, and 7. The thickness and density were gradually decreased in all treated groups. Nevertheless, the thickness of the epidermal and dermal layer in the ASC group was thinner than the dorsal skin layers in the control and betamethasone groups. Also, the density of epidermis and dermis of the control and betamethasone groups were relatively declined, which was lower than the density of dorsal skin in the ASC group. To summarize, the results demonstrated that the density and thickness were decreased in all treated groups, but the reduction in the ASC group was more significant (Figure 4(b)). Thickness and density changes were evaluated in the treated group (Tables 2 and 3).

3.3. Pathological Analysis. Pathological analysis showed that the skin layers, without any UVB exposure, were observed in the dorsal area of the sham group as normal and healthy skin without any apparent abnormal structures. Moreover, no changes were detected in the epidermis, dermis, and hypodermis layers. Similarly, the thickness of the epidermis layer was standard. Moreover, the number of inflammatory cells and polymorphonuclear leukocytes were standard in the epidermal layer. Collagen fibers were normal in skin layers without any degradation. Histopathological results demonstrated that the thickness of the dermis and epidermis

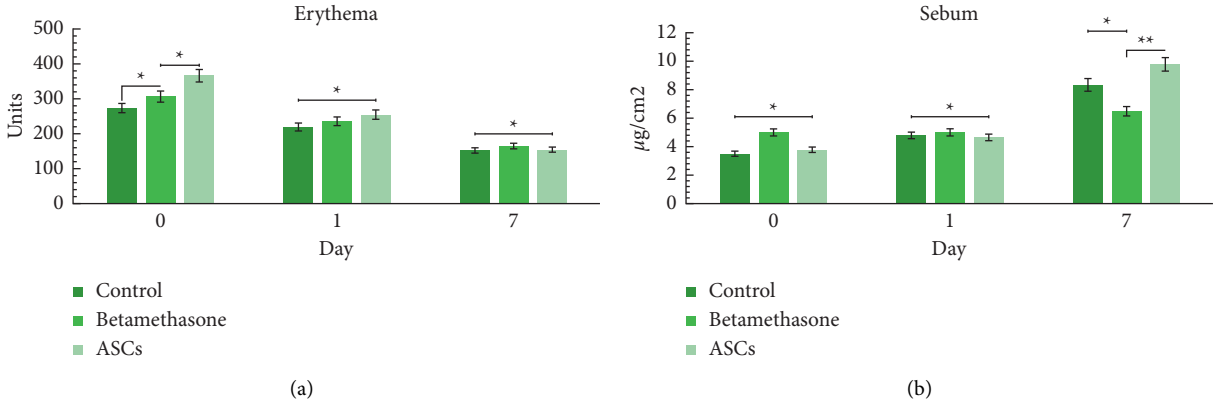

Figure 3: (a) Erythema formation and (b) sebum content of UVB-irradiated skin in treated (betamethasone and ASCs groups) and control groups on days 0, 1, and 7. Error bar: mean ± SD ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$).

Table 1: Percentage of overall elasticity (R2), net elasticity (R5), and the ratio of elastic recovery (R7) between three groups on days 0, 1, and 7 ($p \leq 0.05$).

Group	Elasticity (%)								
	Overall elasticity (R2)			Net elasticity (R5)			Ratio of elastic recovery (R7)		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
Control	0.514	0.571	0.576	0.4	0.402	0.381	0.239	0.209	0.203
Betamethasone	0.51	0.546	0.595	0.358	0.346	0.425	0.193	0.193	0.233
ASCs	0.508	0.572	0.75	0.356	0.386	0.575	0.203	0.216	0.327

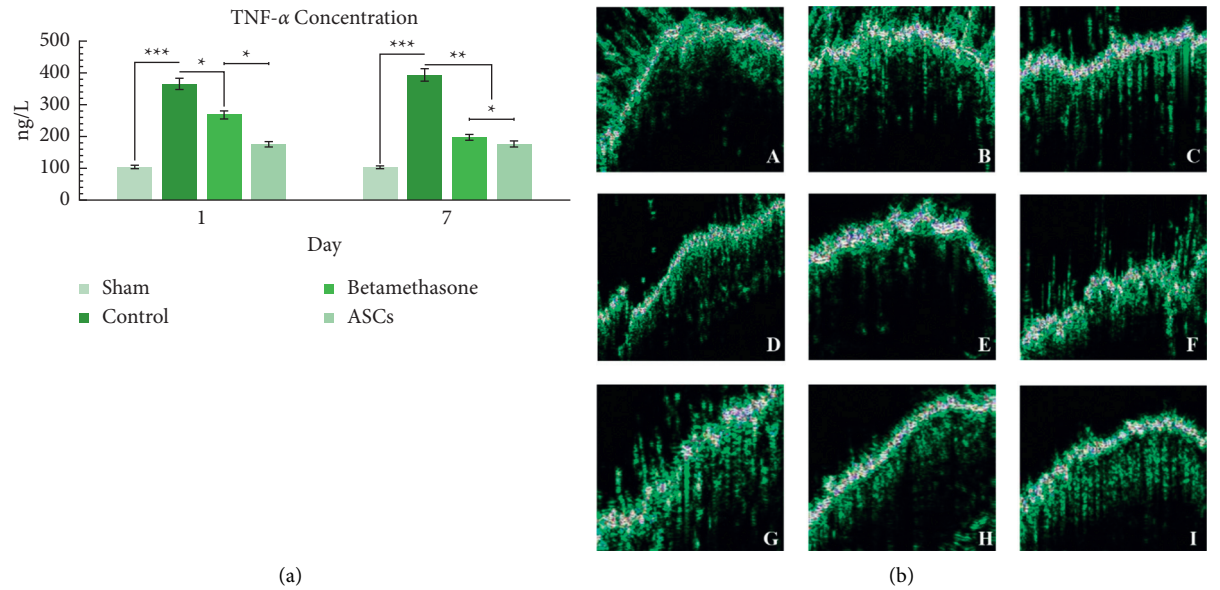

Figure 4: (a) Samples of sham, control, betamethasone, and ASC groups were collected and analyzed by TNF-α on days 1 and 7. Error bar: mean ± SD ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$). (b) Thickness and density were evaluated with ultrasonography images in treated (betamethasone and ASC groups) and control groups on days 0, 1, and 7. Ultrasonography image (A; 0, B; 1, and C; day 7) in control, (D; 0, E; 1, F; day 7) in betamethasone, and (G; 0, H; 1, I; day 7) in ASCs groups.

of the dorsal skin was significantly increased on day 1 after starting the treatment period in the control group. At the same time, the number of inflammatory cells and polymorphonuclear leukocytes were elevated substantially. Also, collagen fibers were irregularly arranged in the rat's dorsal skin in the control group.

In the control group, the thickness of the epidermis on day 7 was significantly enhanced more than the control group on day 1. The thickened epidermis on day 7 is approximately two times more than the control group on day 1. Moreover, the degraded collagen by UVB irradiation was substantially detected more than on day 1. Also,

Table 2: Thickness of epidermis, dermis, and complete skin in control, betamethasone, and treatment with ASC extract on days 0, 1, and 7 ($p \leq 0.05$).

Group	Density (%)								
	Epidermal density			Dermal density			Complete density		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
Control	41.2	40.2	40.5	7.61	6.98	5.15	13.74	12.94	10.67
Betamethasone	43.52	38.52	38.96	5.83	5.63	4.42	12.65	12.74	10.37
ASCs	43.71	41.18	40.64	6.26	5.48	5.52	12.87	12.33	11.32

Table 3: Percentage of density in epidermis, dermis, and complete skin in control, betamethasone, and treatment with ASC extract on days 0, 1, and 7 ($p \leq 0.05$).

Group	Thickness (μm)								
	Epidermal thickness			Dermal thickness			Complete thickness		
	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7	Day 0	Day 1	Day 7
Control	157.3	151	133.8	878	690.1	607	1035.5	841.1	740.8
Betamethasone	164	139.6	124.1	807.75	623.2	598	971.75	762.8	722.1
ASCs	173.5	146.8	122.5	818	718.7	536.8	991.5	865.5	659.3

inflammatory cells and polymorphonuclears infiltration of the dermis increased more than the control group on day 1. The thickness of the dorsal skin dermis and epidermis, exposed to UVB for 7 days before treatment duration, was relatively like the control group in the Betamethasone group on day 1 of treatment duration. Furthermore, perivascular inflammatory cell infiltration was detected.

Additionally, collagen fibers were distorted, broken, and even abnormally accumulated in the dermis of the skin. On day 7 of treatment with betamethasone, improvement of the epidermis layer was observed compared to the control group. Moreover, the thickness of the epidermis layer was decreased considerably. On the other side, inflammatory cell infiltration was significantly dropped compared to the control group on day 7. Collagen bundles were comparatively decreased in the Betamethasone group, although the collagen fibers were arranged better than fibers in the control group on day 7 (Figures 5 and 6).

In the ASC group on day 1, epidermis thickness and width changes were not significant, and also, many collagen bundles could be detected. Similarly, irregularly arranged collagen fibers were observed. Also, infiltration of inflammatory cells in the ASC group was shown and, dermis width and thickness changes were not dramatic and closed to the seventh day of the treatment period in the control group on day 1. The thickness of the dorsal skin epidermis of the ASC group has relatively plummeted after 7 days of UVB irradiation compared to the control group on day 7 of treatment duration. Furthermore, infiltration of inflammatory cells and polymorphonuclear leukocytes decreased in the dermis. The structure and density of dermal collagen fibers were improved, although collagen bundles were observed on day 7 in the ASC group.

3.4. Levels of TGF-β1 Expression in UVB-Induced Skin. Transforming growth factor-β1 (TGF-β1) levels were measured by RT-PCR in UVB-irradiated skin and sham groups. The concentration of TGF-β1 was not changed in the sham group on days 1 and 7. Also, after UVB irradiated to betamethasone and ASC groups, TGF-β1 level was decreased after a day of ASCs, and betamethasone topical administration compared to the TGF-β1 concentration in the control group. This result indicated that TGF-β1 was reduced in the control group, while after using formulations, TGF-β1 contents were increased on day 7 in contrast to day 1 in treated groups (betamethasone and ASC groups). Also, the rise-up of TGF-β1 in the ASC group was lower than the concentration in the betamethasone group ($^{*}p \leq 0.05$) (Figure 7).

4. Discussion

The physiologic response to UV light exposure displayed acute exposure thickens the epidermis, edema, burns, hyperplasia, inflammation, and erythema. In contrast, chronic exposure to UVB irradiation can cause carcinogenesis and the aging process in the skin [7]. UVB can change abundant cell signaling pathways responsible for the skin's inflammation. Furthermore, inflammatory cytokines (i.e., TNFα and IL-6) play pivotal roles in skin inflammation [21]. Several studies have exhibited that product-derived agents from natural sources would show a protective effect on UVB-damaged skin due to their different bioactive compounds. They can also combat human diseases such as carcinogenesis, cardiovascular disease, and inflammatory diseases [22]. Scientists found that NF-κB was caused transfection in human umbilical vein endothelial cells with an NF-κB reporter-driven structure. Pretreatment with polyphenol of apple extracts (24 h) substantially decreased TNF-α-mediated expression of the reporter gene. Also, a decrease of NF-κB was recognized when pretreated MCF-7 cells with apple extract for 2 hours and induced with TNF-α for half an hour [13]. Also, another group of scientists showed that the extract of ASCs affects viability and resistance of the aging process and human stem cell apoptosis. Also, *in vivo* study on 20 women aged 37 to 64 years have used the apple stem cell extract twice a day on the crow's feet

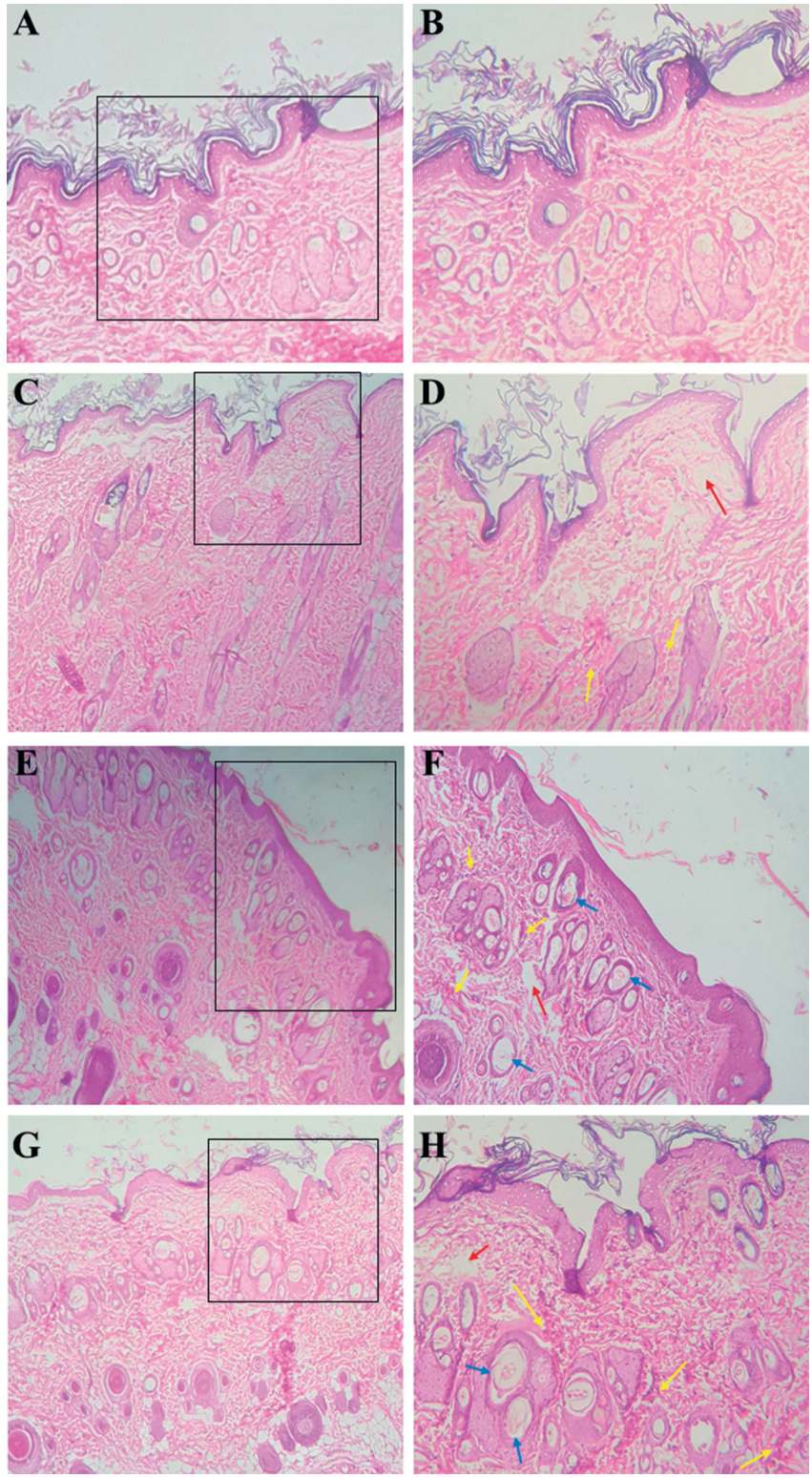

Figure 5: Histological analysis (thickness, inflammatory cell infiltration, collagen fiber regulation conditions) on day 1. Sham group (A; ×40, B; ×100), control (C; ×40, D; ×100), betamethasone (E; ×40, F; ×100), ASC groups (G; ×40, H; ×100) (red arrows: collagen necrosis; blue arrows: collagen bundles; yellow arrows: inflammatory cells infiltration).

area for 4 weeks. Clinical studies showed that 15% of wrinkle depth was reduced after the duration of administration. Furthermore, the extract was presented to be effective in human stem cells protection from UV irradiation, but the mechanism of the extract is not known [14]. Due to the promising properties of apple components in reducing inflammation, ASCs extract was used to investigate the effects of the extract on UVB skin damage. In this study, the topical

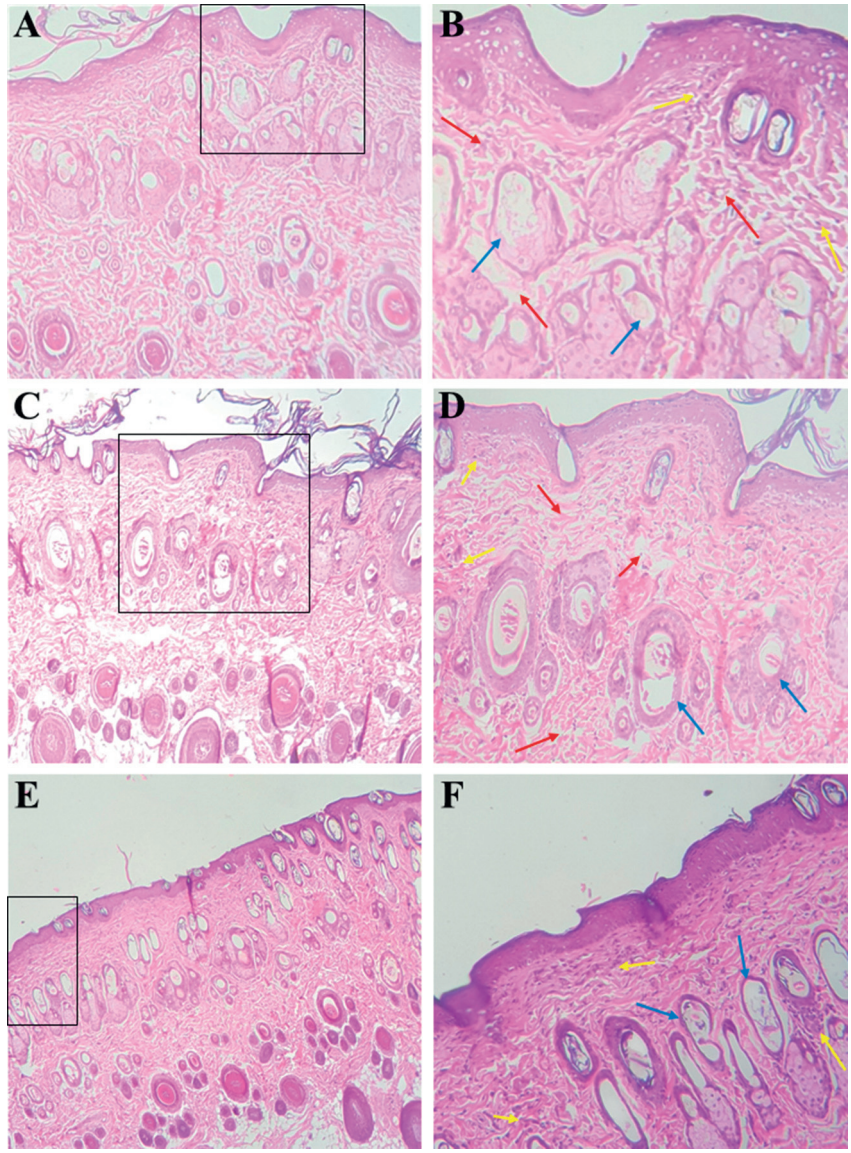

Figure 6: Histological analysis (thickness, inflammatory cells infiltration, collagen fiber regulation conditions) on day 7. Control (A; ×40, B; ×100), betamethasone (C; ×40, D; ×100), ASC groups (E; ×40, F; ×100) (red arrows: collagen necrosis; blue arrows: collagen bundles; yellow arrows: inflammatory cells infiltration).

administration of ASCs extract could ameliorate UVB-induced damages revealed by attenuation of TNF-α expression, improvement of skin structure, and return important contents of the skin. Moreover, animal studies displayed that treatment of ASCs extract could constrict skin photoaging marked by erythema, histologic changes, wrinkles, tanning, thickening, and dryness of the skin. Reich et al. demonstrated that the apoptotic dose of UVB irradiation is close to the minimal erythema dose of UVB, which means that UV-induced erythema might be an inflammatory response to the presence of sunburn and apoptotic cells [23]. The epidermis after the UVB-caused sunburn shows abnormal differentiation and proliferation, which appears to be related to the effect on skin barrier function leading to photo-aging or damage, which seems to be closely related to the effect on the skin barrier function leading to photodamage [24]. In the study of researchers Alves et al., the effect of two topical formulations of *Malus* sp. extract (containing 1.25%) and the equivalent amount of rutin (0.75%) was assessed. Also, the effect of photo-chemopreventive was evaluated on two three-dimensional (3D) skin models and then compared to 3D tissue-engineered skin models. Both formulations could protect skin against the increase of sunburn cell formation induced by UVB, including cyclobutane pyrimidine dimer formation and caspase-3 activation in both skin models. In addition, the formulations prevented the lipid peroxidation and the UVB-induced formation of metalloproteinase [24]. In the present study, a formulation based on ASC extract was prepared to ameliorate the UVB-induced damages of skin irradiated with a dose of 0.3 J/cm^2. The irradiation was continued for an hour to induce photodamages in the rat model. Also, in a study by Huang et al. (2020), the skin was irradiated with UVB a dose of 100 mJ/cm^2 for 12 minutes for evaluating smoothness and antiaging effect of facial masks

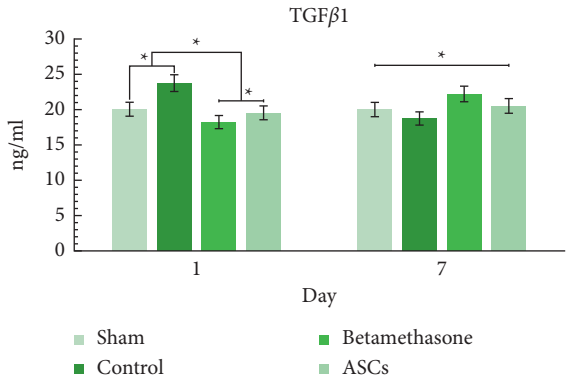

FIGURE 7: TGF-β1 concentration of rat skin in sham, control, betamethasone, and ASC groups on days 1 and 7. Error bar: mean ± SD ($^{*}p \leq 0.05$).

containing farnesol [6]. Mueller et al. found that apple extract including 30% phloridzin and 5% quercetin inhibited the 89% production of TNF-α in a stimulated macrophage model [25]. Moreover, Oresajo et al. demonstrated the protective role of antioxidants including vitamin C, ferulic acid, and phloretin (a potent antioxidant in apple peel extract) on human skin from the UV damages [26], Shoji et al. indicated the inhibitory effect of apple polyphenols (procyanidins and other flavonoids) was more effective than kojic acid or arbutin in melanogenesis [27]. In this study, ASCs extract was used for investigating the positive effects of apple components in UVB-induced damage and inflammation models in the abovementioned studies. The improvement of skin moisture contents was increased by administration of W/O emulsion cream based on a hydroalcoholic extract of apple seeds after 8 months due to TEWL reduction [28]. Additionally, the improvement in mechanical features of the skin was detected, for instance, roughness levels, photoaged wrinkles appearance, and smoothness. Moreover, Khan et al. used a W/O emulsion cream based on a hydroalcoholic extract of apple seeds formulation on the hyperpigmented skin in a clinical study. They found that reducing erythema and melanin amounts but increasing sebum production. In apple extract, these effects on the skin could be associated with quercetin, flavonoid, and hesperetin [29]. TEWL is also used as an indicator of skin barrier changes, consistent with the stratum corneum being the predominant obstruction for water outflow. It has been proposed that UVB disrupts stratum corneum and the rise-up of TEWL level in hairless mice [30]. Also, UVB exposure increased the dehydration and a rise-up in TEWL level of the skin attenuating the skin barrier function and reducing water content, ceramide, and hyaluronan [31, 32]. This study indicated that the ASCs extract reduced the TEWL level by ameliorating the skin function barrier. In Betamethasone and control groups, TEWL was decreased, and the skin structure reformed on day 7. However, the regulation of TEWL and skin hydration level in the ASCs group was more significant than in other groups (Figures 2(a) and 2(b)). Therefore, ASCs compounds may also improve the function of skin barriers and regulate the hydration of the skin with reduction of TEWL. Also, wrinkling may improve because of the enhancement of skin hydration and skin barrier function. Also, ASCs showed the moisturizing effects in this rat model with the regulation of skin hydration. Ultrasound images measure skin echogenicity and skin thickness in different dermis layers. Additionally, cutaneous photodamage is scored clinically. Kim et al. found the ultrasonographic differences between re-epithelialization of skin after normal skin and partial-thickness burns [33]. In addition, UVB exposure to the skin could reveal an enhancement of thickness, density, and wrinkling of the skin. Similarly, it has shown that the reduction in elasticity is crucially correlated with reductions in the level of collagen type I [34]. The present study demonstrated that the thickness of skin and density of dermal and epidermal layer was significantly increased while the elasticity was decreased after UVB exposure duration. In topical application period time, the thickness of rats was declined significantly while the elasticity was increased in the ASCs group. Therefore, amelioration of total thickness and skin elasticity were observed more significantly in the ASCs group. Moreover, erythema was caused by UV irradiation, and it could be characterized by histological (elastic fiber degeneration) and reticular hyperpigmentation. UVB-caused erythema induces wrinkle formation in mice models, which is relevant for assessing wrinkle formation. Erythema is an individual marker for UVB exposure due to the dilation of the dermal vessel and inflammation [34, 35]. In this study, erythema in the ASCs group was remarkably decreased compared to the Betamethasone group. However, the difference between ASCs and the control group was not significant in the dorsal skin of rats. Sumiyoshi et al. showed that oyster protein hydrolysates could prevent skin photodamage with anti-inflammatory effects induced by UVB irradiation by regulating the abnormal expression of MMP-1. The possible molecular mechanism underlying oyster protein hydrolysates antiphotoaging is probably associated with reducing the MAPK/NF-κB signaling pathway regulation. At the same time, the production of TGF-β is increased in the skin [34]. The accumulation of polymorphonuclear and mononuclear cells in the dermis is observed for *in vivo* response of UVB experiment in detecting vascular endothelial adhesion molecules. Cytokines cause the improvement of induced inflammation (UVB) in epidermal production. Strickland et al. studied the protein expression and IL-8 and TNF-α mRNA after UVB irradiation (24 h) in the epidermis. Moreover, the neutrophil accumulation and adhesion molecule expression were evaluated in the dermis. Finally, they found that protein and mRNA expression were formed in the non-irradiated epidermis. After UVB irradiation, protein and TNF-α (mRNA) were insignificantly elevated during 8 h and then achieved the highest level during 24 h. TNF-α was not involved in the early adhesion molecule induction due to the increased E-selectin expression before increasing TNF-α protein during 4 h but IL-8 and TNF-α may increase the inflammatory reaction [36]. The present findings displayed that exposed skin (UVB) could induce damage that initiates inflammation and changes in skin layers and stimulates inflammatory cells and polymorphonuclear leukocytes

infiltration. Also, cytokines such as TNF-α were up-regulated after UVB exposure and photo damages. However, topical application of ASCs could significantly reduce TNF-α concentration and decrease cytokine expression compared to the control group. Additionally, the sebum content was increased in the ASCs group; thus, topical administration of the formulation could improve the sebum content of the skin and reduce aging after photodamage. Hašová et al. investigated hyaluronan's human keratinocyte modulation response to UVB radiation. A single dose of UVB was irradiated to human keratinocytes (HaCaT) and instantly treated with hyaluronan for 6 and 24 hours. The UVB-mediated production of transforming growth factor β was decreased treatment by hyaluronan [37]. *In vivo* study of Xu et al. showed that the expression of collagen types I and III was increased after subcutaneous injection of 10-fold concentrated conditioned medium of dedifferentiated adipocytes, which also decrease the cell proliferation and delayed UVB-induced aging in human dermal fibroblasts. Results suggested that dedifferentiated adipocytes effects were mostly due to the secreted factors, especially TGF-β1, which has a key role in synthesis stimulation of collagen in human dermal fibroblast [38]. TGF-β1 expression in the ASCs group was increased after 7 days of treatment duration while the enhancement the level of TGF-β1 in the group that received Betamethasone, as a common treatment for inflammation, expression of TGF-β1 was significantly increased compared to control and ASCs groups. Also, topical administration with ASCs had a regulatory effect on the TGF-β1 expression that is a pivotal component in the production of elastin and photoaging processes [39, 40]. Becker et al. found that UVB irradiation could increase infiltration of the inflammatory cells in the mice paw tissue. Furthermore, cream based on Copaiba oleoresin inhibited the inflammatory cell infiltration in UVB-induced animal models [41]. Histopathological observation of Huang et al. showed that the facial masks including farnesol were administered before UVB exposure which could improve epithelialization, collagen arrangement, and collagen content [6]. Serafini et al. investigated the benefits of *Morinda citrifolia* topical application on the dorsal skin of mice exposed to UVB. In the UVB-induced model, epidermal thickness was increased and the papillary dermis indicated amplified inflammatory cell infiltration, but the histopathological study in treated groups with *Morinda citrifolia* exhibited a significant reduction of inflammatory contents [42]. Histopathological findings in this study showed that amelioration of dermis and epidermis thickness, and also, collagen arrangement were observed in the ASC group while the improvement process of skin models was detected in the control and betamethasone groups. But also, the changes in inflammatory cells infiltration, thickness, and collagen regulation were more significant on day 7 in the ASC group. ASC group results suggested that anti-inflammatory effects after UVB-induced damages due to the reduction of inflammatory cells and polymorphonuclear leukocytes infiltration. Skin thickness decline was observed in histopathological evaluation. Moreover, the ASC group could increase collagen synthesis and alleviate collagen degradation on day 7, which plays an important role in wrinkle formation and photoaging procedures with reduction of skin thickness.

5. Conclusion

This study demonstrated that topical application based on ASC extract could improve the repair phase in the epidermis and dermis layer with skin barrier functions increment after UVB-induced damage. Also, it could decrease the thickness of the epidermis and dermis in the dorsal skin of animal models. The results indicate that the polymorphonuclear leukocytes and inflammatory cell infiltration were reduced during the 7 days of the treatment period. Therefore, the ASC extract showed that the anti-inflammatory and rejuvenating effects were related to anti-inflammatory mechanisms. Based on these findings, ASC extract is an appropriate candidate for clinical studies and is a part of UVB-induced damage therapy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

D.K. and M.N. contributed equally to this work. The authors declare that the persons named in this article did this work. D.K. and H.A.A. were involved in study design, manuscript preparation, and data acquisition. D.K. wrote the manuscript and performed the data analysis. M.N. and F.H also contributed to data acquisition. H.A.A, F.H., and M.N. critically reviewed the data and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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