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Topical Formulation Based on Arnebia euchroma as a Novel Possible Efficient Treatment on Epidermolysis Bullosa Lesions: A Case Series of Fourteen Patients

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Abstract

BACKGROUND: Epidermolysis bullosa (EB) is a skin fragility disorder that results in the disruption of the dermo-epidermal junction and painful blisters on the skin and mucous membranes. Currently, there is almost no definite treatment for EB patients.

AIM: This study aims to assess the effect of topical formulation based on *Arnebia euchroma* (AE) on lesions induced by EB.

MATERIALS AND METHODS: All patients with EB diseases treated with Hochobeh ointment (AE+Beewax+Olive oil) in 2022 at our dermatological clinic were included in the study. EB diseases were diagnosed based on the following criteria: (1) clinical features (2) histopathological findings (3) Linear depositions of IgG, IgA, IgM 4. salt-split skin (SSS) or other immune serological tests compatible with the diagnosis of EB disease. Hochobeh ointment and lotion treatment were initiated. Clinical evaluations were repeated 3 and 7 days after the treatment began, and photographs were taken to document the changes in clinical severity. EB Disease Activity and Scarring Index (EBDASI) and EB Quality of Life (EB-QoL) questionnaires were used to assess the severity of EB and the quality of life, respectively. All statistical analyses were performed using statistical package for the social sciences version 16.

RESULTS: Overall, fourteen patients were included. Improvement in clinical features, including reduction of infection and inflammation and improvement of wound healing, was observed. EBDASI and EB-QoL scores decreased significantly after treatment (91.5 ± 45.7 vs. 68.9 ± 43, p = 0.001; 24.6 ± 6.7 vs. 18 ± 5.2, p = 0.001, respectively).

CONCLUSION: Hochobeh ointment may be effective in treating EB skin and mucosal lesions.

Introduction

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous inherited skin fragility disorder characterized by structural anomalies that disrupt the dermo-epidermal junction or in the basal layer of the epidermis which subsequently increases cutaneous vulnerability to mechanical stress. There are four major EB types based on the target proteins' location and level of the blisters: EB simplex, junctional EB, dystrophic EB, and Kindler EB [1], [2], [3]. The intensity of symptoms varies from mild to severe depending on the level of blistering and the type of mutation involved.

According to the literature, the incidence of EB in the United States was estimated to be approximately 20 per million live births with a prevalence of 11 per million from 1986 to 2002 [4]. Moreover, its prevalence in Australia, the United Kingdom, and Iran have been reported to be 10.3, 34.8, and 6.72 per million, respectively [5], [6], [7].

Due to the impaired wound-healing process, chronic wounds would likely occur over time leading to several life-threatening infections and squamous cell carcinomas if left untreated. EB wounds are commonly colonized by several pathogenic bacteria, including *Staphylococcus* sp., *Streptococcus* sp., and *Pseudomonas aeruginosa* [8].

Currently, there is no specific therapy for most types of EB. Hence, the treatment is mainly supportive, encompassing wound caring, control of infection, nutritional supplements, as well as prevention and treatment of complaints. Therefore, health-care professionals should take into account that care plans are patient-specific and may vary depending on age, severity, symptoms, and complaints.

There are several topical agents routinely used to reduce the bacterial load of infected wounds and boost the healing process. However, in the long run, they have been shown to increase the emergence of antibiotic-resistant bacteria, systemic silver toxicity, and slate gray metallic skin discoloration,

as reported in the case of silver sulfadiazine-based topical agents [9], [10].

That being said, there is still a severe demand for new therapeutical agents with antiseptic and anti-inflammatory effects that can enhance the wound healing process with less severe adverse effects and more efficacy. It has been previously demonstrated that *Arnebia euchroma* (AE) root extract could efficiently alleviate EB symptoms, shortening the course of treatment [11]. Owing to its antibacterial and anti-inflammatory effects, AE root extract has been shown to disinfect wounds and reduce inflammatory cells by enhancing the process of skin epithelial layer formation [12] and vasculogenesis [13], thereby accelerating the wound healing process [14].

In this case study, the influence of a commercial therapeutic formulation (Hochobeh Ointment) containing AE root extract, beeswax, and olive oil on blister improvement was investigated in fourteen patients diagnosed with EB.

Methods

All patients diagnosed with EB and treated with Hochobeh ointment (AE root extract, beeswax, and olive oil) in 2022 at the Bu Ali Hospital Sari were included in the study. The Hochobeh ointment was made by the Sinameh Pharmaceutical Company, Iran. The National Committee approved this study for Ethics in Biomedical Research (ethics approval ID: IR.MAZUMS.REC.1399.8246). All therapeutic interventions were made after obtaining informed written consent from the patients or their guardians in the case of children.

The inclusion criteria were as follow: (1) Being diagnosed with EB, (2) Having no other underlying medical conditions such as hypertension, hyperthyroidism or hypothyroidism, kidney disease, heart disease, lung disease, diabetes, history of allergy, and hypersensitivity.

The diagnosis was made based on the following findings: (1) Clinical features, (2) Histopathological findings [15], (3) Linear depositions of IgG, IgA, IgM, and (4) Salt-split skin (SSS) [16] or other immune serological tests [17] are compatible with the diagnosis of EB disease.

Hochobeh Ointment treatment was initiated. To wash and dress the wounds, water, and shampoo should be used first; we apply a thick layer of ointment (2–3 mm thick) on the wound and close it with sterile gauze and a bandage. After the partial healing of the wounds, the ointment is used as a lotion on the wounds, and to prevent the formation of wounds in the parts that are prone to the formation of wounds, we used the ointment as a lotion on the desired areas. All patients who met the eligibility criteria were included and assessed during a baseline visit. The clinical examination was repeated 3 and 7 days after the beginning of the treatment, and the changes in clinical severity were documented by photography.

EB Disease Activity and Scarring Index (EBDASI) [18] was used to evaluate the severity of EB, which examines the activity of the disease and the destructive effects of the disease separately. The skin, head, mucous membranes, fingernails and toenails, and other epithelial spaces such as the larynx were examined. The score of the activity section is 276, and the damage section is 230. The scores of these two sections were added together and calculated out of 506. The final analysis would be interpreted as follow: Mild; 0–42; Moderate; 43–106; Severe; >107 [18], [19]. Furthermore, the Farsi version of EB-QoL questionnaire, which had been previously translated and evaluated [20], EB-QoL questionnaire was used to measure patients' life satisfaction, EB-QoL is a 17-question questionnaire in which each answer is scored from 0 to 3. The higher the total number, the lower the patient's life satisfaction; this questionnaire measures the patient's psychological and physical status and also their daily activity. Twelve questions are meant to measure patients' daily activities, and the remaining five measure the psychological aspects of patients [21]. A dermatopathologist evaluated variables and scoring was done by a dermatologist and an infectious disease specialist.

Statistical analysis

Mean and standard deviation for continuous and frequency percent for categorical variables we used. The validity of the questionnaires was confirmed by using the content validity index (CVI) and the questions with a CVI <0.7 were removed, and the questions with a CVI between 0.7 and 0.79 items were reviewed. The reliability of the questionnaire was confirmed using Cronbach's alpha coefficient to assess the questions' internal consistency, which showed good reliability (α = 0.90). To check the statistical distribution of the questionnaire data in this study, the one-sample Kolmogorov–Smirnov test was performed, and according to the normal distribution of the data, the Paired-Sample t-test was used to compare the data before and after the intervention. p-values ≤ 0.05 were considered statistically significant. All statistical analyses were performed using statistical package for the social sciences (SPSS) (version 16, SPSS Inc.).

Results

Fourteen patients (7 females and 7 males) diagnosed with EB were analyzed. The mean age was



Figure 1: Clinical outcomes of treatment of Epidermolysis Bullosa patients with Hochobeh ointment (AE + Beeswax+ Olive oil). The process of reducing the infection in the area of the wounds, as well as increasing the repair of the outer layers of the skin, as a result of treatment with Hochobeh ointment can be seen (left figures: before treatment; right figures: after 7 days of treatment)

± SD: 13.8 ± 14.2, and the median was 8 years (range 3–46).

Due to the healing and disinfecting properties of Hochobeh Ointment, as shown in Figure 1, wounds were almost fully disinfected, and also considering reduction in the wound size and the amount of local inflammation took place in all 14 patients.

Based on the (EBDASI) index and EB-QoL questionnaire results, all fourteen patients' skin lesions and blisters were ameliorated, and their quality of life was improved. Both the EBDASI and EB-QoL scores decreased significantly after the initiation of the treatment (91.5 vs. 45.7, p = 0.001; 24.6 vs. 18.2, p = 0.001, respectively) (Table 1 and Figure 1).

Discussion

This study was designed and implemented with the aim of investigating the therapeutic effects of Hochobeh ointment on mucosal and skin lesions of EB patients. It showed that it could play a significant role in improving clinical features, including reduction of infection and inflammation caused by EB, as well as reducing its severity and subsequently increasing the quality of life of individuals suffering from this disease.

Butterfly disease or EB is an inherited disorder of the skin tissues that cause painful bleeding blisters on the skin and mucous membranes. To date, pathogenetic

Table 1: Severity and quality of life of the fourteen epidermolysis bullosa patients who completed the study

Severity before	Severity after	EBDASI before	EBDASI after	QoL before	QoL after	Patients	Sex
Mild	Mild	35	14	27	23	P3	Male
		36	16	38	26	P6	Female
		38	25	17	12	P1	Male
		40	23	21	17	P5	Female
Mean ± SD		37.25 ± 2.21	19.5 ± 5.32	25.75 ± 9.14	19.5 ± 6.24		
Moderate	Moderate	70	50	19	14	P12	Male
		76	52	29	21	P4	Male
		85	61	31	25	P10	Female
		90	60	22	15	P7	Male
		100	85	22	17	P13	Female
Mean ± SD		84.2 ± 11.75	61.6 ± 13.93	24.6 ± 5.12	18.4 ± 4.56		
Severe	Moderate	115	85	28	19	P8	Male
	Severe	135	111	34	23	P9	Female
		142	123	24	20	P2	Female
		150	110	16	11	P14	Female
		170	150	17	10	P11	Male
Mean ± SD		142.4 ± 20.15	115.8 ± 23.59	23.8± 7.56	16.6± 5.77		

SD: Standard deviation, EBDASI: Epidermolysis bullosa disease activity and scarring index, EB-QoL score: EB Quality of Life Questionnaire.

mutations have been implicated in 16 distinct genes in EB that encode proteins that affect cell integrity and adhesion [22].

AE, from the *Boraginaceae* family, grows in Asia and parts of North Africa. It can also readily be found in the mountainous areas of north Iran [23], [24]. Its root contains important chemical components such as naphthoquinones, shikonins, alkanins, and isohexane naphthazarin esters derivatives which have widespread pharmacological features such as wound healing, antibacterial, antifungal, and anti-inflammatory effects [11], [25], [26].

Based on past studies, shikonin and alkaline exert their anti-inflammatory effects by inhibiting tumor necrosis factor-α (TNF-α) and preventing the activation of the nuclear factor-κB (NF-κB) pathway. In addition, increased collagen synthesis, fibroblast proliferation, and neovascularization have also been reported [27], [28], [29]. Moreover, these two compounds are effective on methicillin-resistant *Staphylococcus aureus* strains, which often cause skin and upper respiratory tract infections [30], and also efficient on vancomycin-resistant and semi-resistant pathogens [31]. Furthermore, it has been shown that naphthoquinone derivatives such as α-lapachone and β-lapachone increase the expression of migration-related proteins and VEGF expression, which in turn enhances tissue development and new blood vessel formation [32].

Previously, some results showed that Hochobeh ointment was effective on the pain level of osteoarthritis patients [33]. A prospective, single-blind clinical trial aimed at investigating the effect of AE ointment on second-degree burn wounds showed that the healing time in the AE group was significantly lower than in the silver sulfadiazine cream group (13.9 ± 5.3 vs. 17.5 ± 6.9 days, respectively; $p = 0.001$). Furthermore, the average patient satisfaction score in the AE group was significantly higher than in the other group (7.27 ± 1.8 vs. 5.37 ± 1.7, respectively; $p < 0.001$). During dressing changes, AE wound sites had significantly lower pain and burning scores, while their warming scores were significantly higher. Side effects like re-epithelialization, allergic reactions, transient leukopenia, neutropenia, erythema multiform, crystalluria, and methemoglobinemia were lower in the site treated with AE [34]. In this context, in a 2021 population-based parallel clinical trial performed on 115 hemorrhoidectomy candidate patients, the use of Hochobeh ointment significantly reduced post-hemorrhoidectomy pain at 4, 12, 24 h, and 7 days and the amount of burning sensation at 4 and 12 h after surgery ($p < 0.001$) [35]. This plant's root may contain anti-inflammatory and wound-healing compounds, which make this ointment analgesic and anti-inflammatory. An analgesic or anti-inflammatory properties of the root are probably secondary mechanisms, as there is no evidence that the plant's compounds act on pain receptors. Thus, it reduces patients' need for narcotic drugs by increasing wound healing, reducing inflammation, and improving wound perfusion. It is, therefore, possible that AE can be effective in healing wounds by increasing angiogenesis and improving cell division and migration.

In another single-blinded, single-center clinical study with the aim of comparing the effect of AE ointment with petrolatum on ulcers caused by fractional CO_2 laser, general wound appearance scores were significantly higher on the 2nd and 5th day after treatment in the petrolatum group ($p = 0.001$ and $p = 0.045$, respectively). Furthermore, erythema ($p = 0.035$) and crusting ($p = 0.016$) on the 2nd day and erythema ($p = 0.009$) and edema ($p = 0.034$) on the 5th day were significantly less reported in the petrolatum arm [36]. Despite the therapeutic properties of AE on wound healing, petrolatum appears to be the more effective treatment. In the current study, according to an EBDASI, EB-QoL score, and the opinion of the dermatologist of this study, 14 patients experienced improvements in skin lesions and blisters and quality of life. Among the patients examined in this study, four were at the mild level, five were at the moderate level, and four were at the severe level. All of the patients in this study reported better life satisfaction after using the mentioned ointment.

The major strength of this study is its novelty since no prior study has ever investigated the severity of the disease and quality of life after Hochobeh ointment treatment using EBDASI and EB-QoL questionnaires. The two most important constrictions of this study were the wide range of participants' age (3–46 years old) and the relatively short observation period (7 days). It is worth mentioning that the scarcity of the disease also puts a bigger strain on ongoing studies. Furthermore, due to the pandemic conditions of COVID-19, long-term follow-up was not possible. Well-designed with longer follow-up times, scrutinizing a more comprehensive range of variables is warranted.

Conclusion

This case study demonstrated the beneficial effects of Hochobeh ointment as a potentially novel treatment.

Author's Roles

All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Conflict of Interests

No potential conflict of interest relevant to this article was reported.

Ethical Approval

This study was approved by Mazandaran university medical science (ethics code: IR.MAZUMS.REC.1399.8246). The study was performed by the principles of the Declaration of Helsinki.

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