Efficacy of a Topical Formulation of Henna (Lawsonia Inermis Linnaeus) on the Itch and Wound Healing in Patients With Epidermolysis Bullosa: a Pilot Single-arm Clinical Trial

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Key words: epidermolysis bullosa, Lawsonia plant, wound healing, pruritus, complementary therapies

Introduction: Epidermolysis bullosa (EB) is a rare inherited genetic skin disorder with severe skin itching and recurrent blisters and erosion. There is no effective and specific therapy for all types of EB.

Objectives: The aim of this study was to evaluate a topical formulation of henna (Lawsonia inermis L.) on the itch and wound healing in patients with epidermolysis bullosa: A pilot single-arm clinical trial. Dermatol Pract Concept. 2022;12(3):e2022115. DOI: https://doi.org/10.5826/dpc.1203a115

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Competing interests: The authors declare that there is conflict of interest, in the way that two individuals listed in authors’ list, MMP and ZP, suffer from DEB and they participated in this clinical trial, but they did not have any role in response evaluation of the drug.

Authorship: Conception and design of the work: MN, MMP, MH, NS, MM, ZP; data collection: MN, NS, MM; analysis and interpretation of the data: MN, MH, MM, NS; statistical analysis: MMP, MM; drafting the manuscript: MN, MMP, NS, MM, ZP; critical revision of the manuscript: MMP, MM, MH, NS, MM, ZP; final approval: MN, MMP, MH, NS, MM, ZP.

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Objectives
To the best of our knowledge, there are no studies on the efficacy of henna in the management of wounds and itch in patients with RDEB. The aim of this study was to evaluate the efficacy of henna in the wound healing process and itch-complaints in patients with RDEB.

Methods
Study Design
This study was designed as a single-arm, uncontrolled clinical trial. This study was in compliance with the Declaration of Helsinki (1989 revision) [17], and also approved and monitored by the Ethics Committee of Shiraz University of Medical Sciences (License number: IR.SUMS.REC.1398.761). Moreover, the enrolled patients were informed completely about the protocol of the study and signed the written informed consent. The patients were permitted to withdraw from the study at any time of the study. This clinical trial was registered at the Iranian Registry of Clinical Trials (IRCT) by IRCT20150825023753N14 code (http://www.irct.ir/trial/41647).

Sample Size and Study Population
Patients with RDEB were recruited from Faghihi Dermatology Clinic, Shiraz University of Medical Sciences, Shiraz, Iran. Given the rarity of RDEB, the researchers decided to enroll at least seven patients in this pilot study.

Inclusion criteria were patients with RDEB, who signed the written informed consent (themselves or their parents if less than 15 years old) to participate in the study. The exclusion criteria were a positive history of allergic reaction to henna, or glucose-6-phosphate dehydrogenase (G6PD) deficiency, and any other systemic diseases.

Drug Preparation
Henna (Lawsonia Inermis Linnaeus) leaves were gathered from Shahdad fields (Kerman, Iran) and dried. A botanist

% henna ointment once daily on two erosions and on also two sites with moderate to a severe itching sensation. The total duration of the intervention was 4 weeks with weekly follow-up visits. Patient global impression of improvement, visual analog scale, and clinical global impression of improvement were used for assessing the wound healing process and itching discomfort.

Results: There was a significant improvement in the skin symptoms of epidermolysis bullosa including skin redness, itching, burning, and local warmth (\(P < 0.05\)). Local pain decreased during the study period, but this was not statistically significant (\(P < 0.19\)). One patient reported moderate xerosis of skin after continuous usage.

Conclusions: It seems that the topical formulation of henna may be effective in the management of itching, burning, stringing, and cutaneous warmth sensation in patients with EB. Further controlled studies with larger sample sizes are recommended to better evaluate this formulation.

Introduction
Inherited epidermolysis bullosa (EB) is a rare genetic skin disorder that can affect many extracutaneous organs including the gastrointestinal and genitourinary system, eye and etc [1,2]. There are four major types of inherited epidermolysis bullosa; epidermolytic (EB simplex [EBS]), lucidolytic (junctional EB [JEB]), dermolytic (dystrophic EB [DEB]), and Kindler syndrome [3]. The common characteristics of all subtypes of EB are recurrent blistering and erosions (after even minor trauma or traction) of skin and the organs covered by mucous membrane [4].

The pathogenesis of EB is the mutation of the genes which is caused due to dysfunction of collagen type VII that is the main component of the anchoring fibrils located below the lamina densa layer of the epidermal basement membrane zone [2]. EB patients, especially patients with recessive dystrophic EB (RDEB), suffer from severe skin itching and also recurrent blisters and erosion [2,5]. There is no specific therapy for all types of EB, therefore supportive care including wound care, control of infection and itching are very important [6]. Using topical and systemic antibiotics, analgesics, antihistamines are very popular in these patients.

Henna (Lawsonia Inermis Linnaeus) is one of the most commonly used medicinal plants in traditional Persian medicine as a treatment for dermatological conditions and improving wound healing [7,8]. There are several studies demonstrating the efficacy of henna on skin disorders such as dermatitis including diaper dermatitis, bedsore, itch, and et. [9-12]. It has been shown that henna can improve the wound healing process and also has antipruritic effects [13,14]. In addition, some investigations revealed the antimicrobial and antifungal properties of henna. These effects are considered to be due to high concentrations of some components in this plant including carbohydrates, anthraquinones, naphthoquinone derivatives, flavonoid, and phenolic components. [15,16].
at Kerman University of Medical Sciences authenticated the plant and recorded it with a specified voucher number (No: KF-1408). The maceration method five times was conducted to prepare the hydro-ethanolic extraction (30:70). The gathered extract was purified through filtration and concentrated by a vacuum rotary evaporator and dried in an oven 40°C. The ointment containing 1% henna was prepared by dissolving a one-gram fine powder of dried henna extract in the minimum volume of ethanol 40%, then it was dispersed in 99 grams of Eucerin through geometric dilution. The prepared ointment was packed in 50-gram containers for delivery to the patients.

Pharmaceutical Properties of the Ointment

The quality control of the prepared ointment was performed according to WHO guideline [18]. Pharmaceutical characterizations of henna ointment were evaluated as follows [19-23]:

**Determination of pH**

Some ointment was heated up to the melting point and diluted with a 1:9 dilution ratio (1 unit of drug and 9 units of water) and measured with a pH meter. This procedure was repeated three times and its mean and standard deviation were recorded.

**Homogeneity**

Homogeneity of the herbal ointment was evaluated for any aggregation by the skin test. In this study, 12 healthy volunteers tested some of the product on the back of their hands and were asked to express their satisfaction with the particle being present in the ointment.

**Total Polyphenolic Content**

The total phenolic content of the extract and ointment were measured based on the Folin-Ciocalteu method.

**Rheological Behavior**

Cone and Plate Brookfield rheometer (Brookfield Engineering Laboratories) at 25°C was performed to evaluate the rheological behavior of ointment for triplicate.

**Spreadability Test**

Two horizontal glass plates (10 cm × 10 cm) were conducted to assess the spreadability of the prepared ointment. The spreading diameter of one gram of sample between plates was measured under 25 grams standard weight shear application three times.

**In Vitro Drug Release**

Two grams of henna ointment were poured into a 10-kDa semi-permeable dialysis membrane bag. After dispersing, it was immersed in 50 ml of 25 mM phosphate buffer solution (PBS) at 37 ± 0.5 °C and rotated at 100 rpm. For 24 hours, at a specified interval, 1 ml volume of PBS solution was sampled and replaced with the same volume of PBS. The samples were analyzed by UV spectrophotometer and repeated three times and the amount of active ingredient release was calculated according to the standard curve.

**Microbial Control**

The microbial and fungal control tests of the product were performed by Barij Essence® Pharmaceutical company (serial number:1521M98; batch number: 9805101) for aerobic microorganisms, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, modals, and yeasts enumeration.

**Intervention and Follow-up of the Patients**

The patients were instructed to use a fingertip of henna ointment once a day on two erosions and also on two sites with moderate to severe itching for 4 weeks with first, second, and fourth week follow-up visits. Patient global impression of improvement (PGI-I), visual analog scale (VAS), and clinical global impression of improvement (CGI-I) were used to assess the wound healing process and itching discomfort. Furthermore, photographs were taken of all of the patients.

Furthermore, wound improvement response was defined as excellent (90% improvement), good (50%-90% improvement), mild (20%-50% improvement), and fair (less than 20% or worse) according to general appearance.

At the end of the study, patients or their parents (for children participants) were asked to express their opinions about the efficacy of henna ointment in comparison to other used medications.

**Statistical Analysis**

Statistical Package for Social Sciences, SPSS version 18 (SPSS Inc.), was used for data analysis. According to the low sample size, Freedman test was used for assessment of the effects of henna ointment on the variables of the study. P value equal to or less than 0.05 was considered significant.

**Results**

**Pharmaceutical Characterization**

The measured pH of ointment was 6.4 ± 0.3. This ointment displayed rheological thixotropic behavior. The results of the spreadability test showed that the mean diameter for henna ointment was 9 ± 0.8 cm. Folin-Ciocalteu method was used to determine total phenolic contents in terms of Gallic acid equivalent (GAE) in mg/g of the extract. Based on the equation of the calibration curve ($y = 0.007x+0.006$, $R^2 = 0.999$), the total phenolic content of the extract and ointment were 129.6 ± 1.1 and 0.98 ± 0.18 mg/g of extract, respectively.
The rate of drug substance release is presented in Table 1. As shown in Figure 1, the release of active ingredients of henna ointment follows the Weibull equation \( Q=1-\exp \left(-\frac{t^A}{K}\right) \) with \( R^2=0.97 \) so that \( k \) (time constant) is equal to 6.92 and \( A \) (shape parameter) is equal to 1.03. The prepared product releases half of its active ingredient content up to 4 hours and about 90\% of its active ingredient content releases up to 12 hours after application. This release kinetic is consistent with topical products containing ointment-based hydroalcoholic extracts.

**Participants Enrollment and Basic Characteristics**

A detailed description of the patients’ enrollment and analysis is given in Figure 2. Nine patients were enrolled in the study. Two patients were lost to follow-up. Finally, 7 out of 9 patients including 3 boys and 4 girls completed the study. The age range of the patients was 5-32 years.

**Efficacy Outcomes**

The averaged drug satisfaction rate was reported 74\% (min = 50\%, and max = 90\%) by the patients. For more details, based on PGI-I, 6 patients reported “very much better” and 1 reported “much better” in itching discomfort after using henna ointment. According to CGI-I, the physician concluded that all the patients were “much better” and “very much better” after using henna ointment. There was a significant improvement in the skin symptoms of epidermolysis bullosa including skin redness, itching, burning, and local warmth sensation \( (P < 0.05) \). Local pain decreased during the study period, but this was not statistically significant \( (P < 0.19) \) (Table 2). Moreover, Figure 3 shows the photos of 3 patients with RDEB before the treatment and after four weeks of receiving topical 1\% henna ointment.

**Qualitative Evaluation of Patients Opinion About Topical Henna Ointment**

Five out of 7 patients who participated in the study reported henna as the most effective ointment for their pruritus in comparison to other medications, including corticosteroids, Vaseline, and repair creams. In addition, most patients had a good experience in wound healing effect while using henna ointment, at least as well as conventional medicine such as Mupirocin, MEBO® and BIAFINE® topical emulsion.

**Side Effects Evaluation**

No serious adverse effect was observed. One patient reported moderate xerosis of skin after continuous application of henna after 4 weeks so that he needed to apply larger amounts of emollient medicines.

**Conclusions**

This study showed that the 1\% henna ointment could improve skin redness, itching, burning, and local warmness. But it should be considered that it was a pilot study and further studies with a higher number of cases are necessary to approve these results. Although EB patients suffer from several severe complications related to their disease,
ENROLLMENT
ASSESSED FOR ELIGIBILITY (N=12)
EXCLUDED (3)
- NOT MEETING INCLUSION CRITERIA (N=3)
NOT RANDOMIZED (N=9)
ALLOCATED TO INTERVENTION (N=9)
RECEIVED ALLOCATED INTERVENTION (N=9)
LOST TO FOLLOW-UP (2 LEAVING CITY) (N=2)
DISCONTINUED INTERVENTION (N=0)
ANALYSED (N=7)
EXCLUDED FROM ANALYSIS (N=0)

Figure 2. TRENDS flow chart of efficacy of a topical formulation of henna (Lawsonia inermis Linnaeus) on the itch and wound healing in patients with epidermolysis bullosa.

Table 2. Mean of dermatological complaints scores from baseline to weeks 1, 2 and 4 in patients with epidermolysis bullosa who treated with local henna ointment

| Outcome measures                  | Weeks     |       |       |       | P  |
|-----------------------------------|-----------|-------|-------|-------|----|
|                                   | Week 0    | Week 1| Week 2| Week 4|    |
| Skin redness (mean ± SD)          | 6.28 ± 1.26| 6.14 ± 1.10| 3.57 ± 0.68| 2.42 ± 0.48| 0.003 |
| Itching sensation (mean ± SD)     | 8.57 ± 0.71| 4.71 ± 0.47| 3.42 ± 0.86| 2.00 ± 0.43| 0.001 |
| Skin burning (mean ± SD)         | 3.57 ± 1.21| 2.28 ± 0.77| 1.57 ± 0.61| 1.00 ± 0.436| 0.003 |
| Local warmness sensation (mean ± SD)| 5.14 ± 0.98| 4.00 ± 0.92| 3.14 ± 0.93| 2.00 ± 0.75| 0.001 |
| Local pain (mean ± SD)           | 4.71 ± 1.45| 4.14 ± 1.29| 3.57 ± 1.19| 2.71 ± 0.74| 0.197 |

a. P-value; b. SD = standard deviation

drugs that can manage their complications.

EB is a non-curable hereditary condition with several cutaneous and extracutaneous manifestations. Skin redness (dermatitis), pruritus, burning sensation in the skin, local warmness sensation, repeated ulceration, and pain are the most common cutaneous manifestations and complaints of patients with EB [24]. These manifestations could affect the quality of life in patients with EB and their families [25,26]. Therefore, most of the time patients, their parents, and health care providers try to administer multiple topical medications including natural and herbal remedies to manage or relieve these symptoms [27].

Henna is one of the herbal medications that is used commonly in both traditional and folk medicine for the treatment of skin, hair, and nail diseases, as well as cosmetic problems [7,28,29]. Niazi et al demonstrated that henna ointment could improve the symptoms of contact dermatitis including skin edema, itching, sweating, skin thinning and pain which is consistent with our study [12]. However, unlike our study,
The mechanisms of wound healing of topical henna are unclear till now, but one recent study suggests that these mechanisms may include reduction of tissue inflammation and increasing cellular glucose uptake, which was mediated by up-regulating the expression of glucose transporter-1 and insulin-like growth factor I. Furthermore, this study showed that topical henna could shorten the inflammatory phase of the wound healing process, accelerate cellular proliferation, raise wound contraction ratio, and caused improvement of revascularization, collagen deposition, and re-epithelialization, and promotion of intracytoplasmic carbohydrate storage [34]. According to the knowledge of TPM, “Ghabz”, with nearly meaning of “contraction” in conventional medicine, is the common feature of drugs that are effective in wound healing [35, 36], as well as henna [29, 37], that is in line with the finding of conventional medicine.

The present study showed that 1% of henna ointment had an acceptable effect on skin characteristics in patients with EB. In fact, all the selected wounds of these patients were improved clinically during to first two weeks of the study. Mourad et al demonstrated that the henna gel had a significant effect on wound healing in in-vivo model. The results of this study were confirmed by histological stain assessments [13]. The study of Shiravi et al revealed that henna had anti-inflammatory and anti-bacterial effects in Wistar rats. According to this study, reduction of inflammation, edema, bleeding, and increased collagen formation resulted in acceleration of wound healing, angiogenesis, and vasodilatation in these rats [33].

The mechanisms of wound healing of topical henna are unclear till now, but one recent study suggests that these mechanisms may include reduction of tissue inflammation and increasing cellular glucose uptake, which was mediated by up-regulating the expression of glucose transporter-1 and insulin-like growth factor I. Furthermore, this study showed that topical henna could shorten the inflammatory phase of the wound healing process, accelerate cellular proliferation, raise wound contraction ratio, and caused improvement of revascularization, collagen deposition, and re-epithelialization rate, and promotion of intracytoplasmic carbohydrate storage [34]. According to the knowledge of TPM, “Ghabz”, with nearly meaning of “contraction” in conventional medicine, is the common feature of drugs that are effective in wound healing [35, 36], as well as henna [29, 37], that is in line with the finding of conventional medicine.

The findings of our study showed that topical henna ointment did not relieve pain sensation of the patients with EB. This result may be referred to stimulate pain receptors of the selected sites by pain signals coming from contiguous wounds. This finding was not in line with the other studies. The study of Nesa et al showed remarkable analgesic, anti-inflammatory, and central nervous system (CNS) depressant effects of henna [38]. Hasan Imam et al suggested that the analgesic effect of henna was resulted from alpha
amylase enzyme inhibitory and Anti-inflammatory effects of this herbal remedy [39]. The difference in the results may be due to different doses and routes of administration. Moreover, previous analgesic effects are reported in animal model, but our study was on human EB subjects with potential different in pain pathways.

One out of seven patients reported that the skin of the areas in contact with the drug had become drier and flakier. According to the best of our knowledge, there are some evidences of skin dryness after using topical henna in literature review. However, it is compatible with side effects reported in traditional Persian medicine for henna.

Other reported side effects for topical use of henna are acute allergic contact dermatitis [40], temporary localized hypertrichosis [41], hair and clothing dye allergy [42], vesicular erythema multiforme-like reaction, [43] and hemolysis in patients with G6PD deficiency [44].

Most of the patients were more satisfied with using henna ointment in comparison with conventional medications, especially in the management of pruritus and inflammation, as well as its wound-healing effects. This is the first study investigating the efficacy of herbal medicine in the management of EB complications. Therefore, it was impossible to compare this product with other herbal remedies in EB. But there is a great piece of evidence showing the efficacy of topical usages in dermatological conditions, such as wound, androgenic alopecia, and prevention and treatment of pressure ulcers, dermatitis, and much more [9,11,45-47].

There were several limitations in this study. First, this was a non-controlled single-arm clinical trial; therefore, the results of this study had not been compared with placebo or other medications. We did not administer a second arm for this trial because there is not any standard and defined treatment for EB ulcers and patients usually apply different or multiple medications for controlling the itching sensation and wound healing with different responses. Second, we used a researcher-made checklist for evaluating the patients. Although the face validity and content validity of this questionnaire were acceptable, we could not evaluate the internal validity of the questioner because of a low sample size of the study. Next, because of the rarity of the disease, the sample size of the study was low. Finally, the age range of the patients was wide (5-32 years); therefore, the parents evaluated the efficacy of the drug for children and this can be a probable confounding factor.

According to the results of this pilot study, the topical formulation of henna may be effective in the management of wound, itching, burning, stringing, and cutaneous warmness sensation in patients with EB. In this regard, we suggest further controlled clinical trials with larger sample sizes and longer duration of follow-up to evaluate the efficacy of this herbal medication.

References

1. Murrell DF. Life with epidermolysis bullosa (EB): Etiology, diagnosis, multidisciplinary care and therapy. J Am Acad Dermatol. 2009;61(6):1092–1093. DOI:10.1016/j.jaad.2009.06.011
2. Fine JD. Epidemiology of Inherited Epidermolysis Bullosa. Based on Incidence and Prevalence Estimates From the National Epidermolysis Bullosa Registry. JAMA dermol.2016;152(11):1231–1238. DOI:10.1001/jamadermatol.2016.2473. PMID: 27463098.
3. Fine JD, Bruckner-Tuderman L, Eady RA, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J Am Acad Dermatol. 2014;70(6):1103–1126. DOI: 10.1016/j.jaad.2014.01.903. PMID: 24690439.
4. Fine JD. Inherited epidermolysis bullosa. Orphanet J Rare Dis. 2010;5(1):12. DOI:10.1186/1750-1172-5-12. PMID: 20507631. PMCID: PMC2892432.
5. Rizzo C, Anandasabapathy N, Walters RF, et al. Pretibial epidermolysis bullosa. Dermatology online journal. Oct 15 2008;14(10):26.
6. El Hachem M, Zambruno G, Bourdon-Lanoy E, et al. Multicentre consensus recommendations for skin care in inherited epidermolysis bullosa. Orphanet J Rare Dis.2014;9:76. DOI:10.1186/1750-1172-9-76. PMID: 24884811. PMCID: PMC4110526.
7. Aghili Khorsanani MH, Makhzan al-Advieh. Tebran: Tehran University of Medical Sciences. 2009:341.
8. Arzani MA. Teh-e-Akbari. Qom, Iran: Jalaledin Publication. 2008;
9. Rafiei Z, Mazaheri M, Eghbali-Babadi M, Yazdannik A. The Effect of Henna (Lawsonia inermis) on Preventing the Development of Pressure Ulcer Grade One in Intensive Care Unit Patients. Int J Prev Med. 2019;10:26. DOI: 10.4103/ijpvm.IJPVM_286_17. PMID: 30967912. PMCID: PMC6413520.
10. Keshavarz A, Zainaloo AA, Mahram M, Mohammadi N, Sadeghpour O, Maleki MR. Efficacy of Traditional Medicine Product Henna and Hydrocortisone on Diaper Dermatitis in Infants. Iranian Red Crescent medical journal. May 2016;18(5):e24809. DOI:10.3812/irccmj.24809. PMID: 27478628. PMCID: PMC4948373.
11. Rekik DM, Ben Khedir S, Daoud A, Kouida Moalla K, Rebai T, Sahouni Z. Wound Healing Effect of Lawsonia inermis. Skin Pharmacol Physiol. 2019;32(6):295-306. DOI: 10.1159/0005101730. PMID: 31466077.
12. Niazi M, Mehrabani M, Namazi MR, et al. Efficacy of a topical formulation of henna (Lawsonia inermis L.) in contact dermatitis in patients using prosthesis: A double-blind randomized placebo-controlled clinical trial. Complement Ther Med. 2020;102316. DOI: 10.1016/j.ctim.2020.102316. PMID: 32147071.
13. Jridi M, Sellimi S, Lassoued KR, et al. Wound healing activity of cuttlefish gelatin gels and films enriched by henna (Lawsonia inermis) extract. Colloids Surf A Physicochem Eng Asp. 2017;512:71-79. DOI: 10.1016/j.colsurfa.2016.10.014.
14. Negahdari S, Golehdari H, Kesmati M, Rezaie A, Sharifi G. Wound healing activity of extracts and formulations of aloe vera, henna, adiantum capillus-veneris, and myrrh on mouse dermal fibroblast cells. Int J Prev Med. 2017;8:14. DOI: 10.4103/ijpvm.IJPVM_338_16. PMID: 23832194. PMCID: PMC5364744.
15. Kulkarni S, Kale V, Velankar K. To study the Photodynamic antimicrobial activity of Henna extract and preparation of topical Gel formulation. *The Journal of Phytopharmacology*. 2018;7(3):242-252.

16. Nawasrah A, AlNimr A, Ali A. Antifungal effect of Henna against Candida albicans adhered to acrylic resin as a possible principle for prevention of denture stomatitis. *Int J Environ Res Public Health*. 2016;13(5):520. DOI: 10.3390/ijerph13050520. PMID: 27223294. PMCID: PMC4881145.

17. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *Jama*. 2013;310(20):2191-2194. DOI: 10.1001/jama.2013.281053. MID: 2414171.

18. World Health Organization. *Quality control methods for herbal materials: updated edition of Quality control methods for medicinal plant materials*, 1998; WHO; 2011.

19. Akhtar N, Khan AB, Muhammad S, et al. Formulation and characterization of a cream containing terminalia chebula extract. *Forsch Komplementmed.* 2012;19(1):20-25. DOI:10.1159/000335823. PMID: 22398922.

20. Moldovan M, Lahmar A, Bogdan C, Pârtuana S, Tomuţa I, Crisan M. Formulation and evaluation of a water-in-oil cream containing herbal active ingredients and ferulic acid. *Clin Med.* 2017;90(2):212-219. DOI:10.15386/cim-med.668. PMID: 28559707. PMCID: PMC4335755.

21. Shirwas S, Choukse R, Dwivedi S. Formulation and evaluation of herbal cream containing hydroalcoholic extract of Ipomea cairica Linn. for the treatment of gynecological disorders. *Acta Scientific Medical Sciences*. 2019;3(8):192-196. DOI: 10.31080/ASMS.2019.03.0366

22. Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semisolid formulations: an update. *Pharmaceutical technology North America*. 2002;26(9):84-105.

23. Rower MR, Brown RC. Quantification of total phenols in biooil using the Folin–Ciochette method. *J Anal Appl Pyrolysis*. 2013;104:366–371. DOI: 10.1016/j.jaap.2013.06.011

24. Bruckner-Tuderman L. Dystrophic epidermolysis bullosa: pathogenesis and clinical features. *Dermatol Clin.* 2010;28(1):107–114. DOI: 10.1016/j.det.2009.10.020. PMID: 19945622.

25. Pagliarello C, Tabolli S. Factors affecting quality of life in epidermolysis bullosa. *Expert Rev Pharmacoecon Outcomes Res.* 2010;10(3):329–338. DOI: 10.1586/erp.10.28. PMID: 20545597.

26. Chogani F, Parvizi MM, Murrell DF, Handjani F. Assessing the quality of life in the families of patients with epidermolysis bullosa: The mothers as main caregivers. *Int J Womens Dermatol.* 2021;7(5Part B):721–726. DOI:10.1016/j.iwd.2021.08.007. PMID: 35028371. PMCID: PMC8714583.

27. Shayanegh LH, Levin LE, Galligan ER, et al. Skin cleansing and topical product use in patients with epidermolysis bullosa: Results from a multicenter database. *Pediatr Dermatol.* 2020;37(2):326–332. DOI:10.1111/pedi.14102. PMID: 31944391.

28. Al Saif F. Henna beyond skin arts: Literature reviews. *Journal of Pakistan Association of Dermatology*. 2016;26(1):58–65.

29. Jorjani SE. Zakkireh Khazanshahi. Ehlyae teb-e-tabiei; 2012.

30. Abdlaty R, Hayward J, Farrell T, Fang Q. Skin erythema and pigmentation: a review of optical assessment techniques. *Photodiagnostics and Photodynamic Therapy*. 2021;33:102127.

31. Ansari M, Dehsara F, Omidi­vari A, Ahmad­loo N, Mohammad­an­panah M. Efficacy of topical alpha ­ointment (containing natural henna) compared to topical hydrocortisone (1%) in the healing of radiation-induced dermatitis in patients with breast cancer: a randomized controlled clinical trial. *Iran J Med Sci.* 2013;38(4):293–300. PMID: 24293782. PMCID: PMC3838980.

32. Yucel I, Guzin G. Topical henna for capectabine induced hand–foot syndrome. *J Fuß New Drugs..* 2008;26(2):189–192. DOI: 10.1007/s10637-007-9082-3. PMID: 17857353.

33. Sharri AH, alebooyeh M, Hojati V, Akbari H. The effect of extract of henna leaves (Lawsonia inermis) on skin wound healing in Wistar rats. *Journal of Animal Biology*. 2011;4(4):45–51.

34. Daemi A, Farahpour MR, Oryan A, Karimzadeh S, Tajer E. Topical administration of hydroethanolic extract of Lawsonia inermis (henna) accelerates excisional wound healing process by reducing tissue inflammation and amplifying glucose uptake. *Kaohsiung J Med Sci*. 2019;35(1):24–32. DOI: 10.1002/kjms.12005. PMID: 30844141.

35. Parvizi MM, Handjani F, Moein M, et al. Efficacy of cryotherapy plus topical Juniperus excelsa M. Bieb cream versus cryotherapy plus placebo in the treatment of Old World cutaneous leishmaniasis: A triple-blind randomized controlled clinical trial. *PLoS Negl Trop Dis.* 2017;11(10):e0005957. DOI: 10.1371/journal.pntd.0005957. PMID: 28981503. PMCID: PMC5653599.

36. Joukar F, Godarzi H, Parvizi MM. Can we consider silymarin as a treatment option for vitiligo? A double-blind controlled randomized clinical trial of phototherapy plus oral Silybum marianum product versus phototherapy alone. *J Dermatolog Treat*. 2019;1:5. DOI: 10.1008/9546634.2019.1595506. PMID: 30935260.

37. Qarshi IN. *Al-Muṣaqf al-Tibb*. Islamic Heritage Revival Committee, Supreme Council for Islamic Affairs, Ministry of Endowments; 2004.

38. Nesa L, Munira S, Mollika S, Islam M. Evaluation of analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of Lawsonia inermis barks in mice. *Avicenna J Phytomed*. 2014;4(4):287–296. PMID: 25068143. PMCID: PMC4110786.

39. Imam H, Mahlbub NU, Khan MF, Hana HK, Sarker MMR. Alpha Amylase Enzyme Inhibitory and Anti-inflammatory Effect of extract of Lawsonia inermis. *Pakistan J Biol Sci*. 2010;13(23):1796–1800. PMID: 20506051.

40. Nawaf AM, Joshi A, Nour-Eldin O. Acute allergic contact dermatitis due to para-phenylenediamine after a henna tattoo. *Expert Rev Dermatol*. 2010;5(2):179–184. DOI: 10.1111/j.1525-1470.2008.00654.x. PMID: 18429803.

41. Matsulich J, Sullivan J. A temporary henna tattoo causing hair and clothing dye allergy. *Contact Dermatitis*. 2005;53(1):33–36. doi: 10.1111/j.0105-1873.2005.00626.x. PMID: 15982229.

42. Sidwell RU, Francis ND, Basarab T, Morar N. Vescicular erythema multiforme-like reaction to para-phenylenediamine in a henna tattoo. *Pediatr Dermatol*. 2008;25(2):274–275. DOI: 10.1111/j.1525-1470.2008.00654.x. PMID: 18429803.

43. Lee SWH, Lai NM, Chaiyakunapruk N, Chong DWK. Adverse effects of herbal or dietary supplements in G6PD deficiency: a systematic review. *Br J Clin Pharmacol*. 2017;83(1):172–179. DOI: 10.1111/bcp.12976. PMID: 27081765. PMCID: PMC5338162.
45. Poursadra E, Anvari-Tafti M, Dehghani A, Eghbali-Babadi M, Rafiei Z. Comparing the Effect of Henna Oil and Olive Oil on Pressure Ulcer Grade One in Intensive Care Units Patients. *Adv Biomed Res.* 2019;8:68. DOI: 10.4103/abr.abr_207_19. PMID: 31897406. PMCID: PMC6909547.

46. Vahabi S, Hakemi-Vala M, Gholami S. In vitro Antibacterial Effect of Hydroalcoholic Extract of Lawsonia inermis, Malva sylvestris, and Boswellia serrata on Aggregatibacter actinomycetemcomitans. *Adv Biomed Res.* 2019;8:22. DOI: 10.4103/abr.abr_205_18. PMID: 31016180. PMCID: PMC6446579.

47. Zheng Y, Hu Y, Liu K, Lu Y, Hu Y, Zhou X. [Therapeutic effect of Impatiens balsamina, Lawsonia inermis L. and Henna on androgenetic alopecia in mice]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2019;39(11):1376–1380. DOI: 10.12122/j.issn.1673-4254.2019.11.17. PMID: 31852654. PMCID: PMC6926075.