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Evaluating the Combined Effect of Licorice, Coriander, Gallnut, Sagebrush, Withania Coagulans, and Pistacia Atlantica Subsp Kurdica on the Healing and Repair of Wounds in Balb/C Mice

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Abstract

/c ***Background:*** The long and painful process of wound healing, which requires special attention and makes it difficult to carry out daily activities, necessitates a combination of medical interventions which speed up wound healing and prevent injury exacerbation. This study aimed to investigate the combined effect of licorice, coriander, gallnut, sagebrush, Withania coagulans, and Pistacia atlantica subsp. kurdica on healing and repair of burn wounds.

Methods: A total of 48 mature Balb/c mice were used in this experiment, which were divided into four groups of 12. Hydroalcoholic extracts were prepared from mentioned plants. An artificial metal tool with a rod and a flat circular head of 1 cm in diameter was used to create a wound 1 cm in diameter in the dorsal region of the mice. Then, the animals were randomly euthanized and fixed in 10% formalin solution on days 4, 7, 10, and 14. Data was analyzed using ImageJ and SPSS software.

Results: Inflammation and infiltration of neutrophils and lymphocytes were decreased significantly more in the treated group with the plant extract 10% SP than the control groups on days 10, 4, 4 and 14 (p. value < 0.001). The number of fibroblasts followed by collagen production, regeneration of epithelium and new hair follicles at the wound edges significantly increased in the 10% extract and 5% SP treated group on days 10 and 14 (p. value < 0.001). These parameters had a significant increase on day 14 even compared to the group treated with 5% extract and silver sulfadiazine (p. value < 0.001).

Conclusion: According to the results of this study, the used combined extract accelerated the wound healing process in BALBmice.

Key Words: Burn, Coriander, Gallnut, Licorice, Pistacia atlantica subsp. Kurdica, Sagebrush, Silver sulfadiazine, Withania coagulans, Wound.

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1- INTRODUCTION

Burns are a painful injury with the highest estimated reported incidence in Southeast Asia (1, 2). More than 1,000,000 people and about 173,000 children in India and Bangladesh suffer from moderate or severe burns each year (2). In Bangladesh, Colombia, Egypt, and Pakistan, 17% and 18% of children suffer from burns and temporary and permanent disabilities, respectively (2, 3). In rural Nepal, burns are the second most common injury and account for 5% of all disabilities. However, according to the World Health Organization, more than 410,000 burns occurred in the United States in 2008, of which approximately 40,000 required hospitalization. Given the prevalence of burns, rapid healing of burns is critical because it has a significant impact on the rehabilitation of these patients. In addition, wounds remain a major challenge in the care of these patients due to the lengthy healing process; burn injuries, moreover, increase the patient's susceptibility to infection, which is proportional to the total amount of burns (2, 3).

Wounds and burns are caused by a detachment of cells of the skin tissue and a large gap between them, which can affect the underlying tissues and muscles. Burn wounds are generally divided into two types: open and closed. Most wounds are open and can be caused by accidents and cuts with sharp objects that cause tissue damage or even death in vulnerable areas (4, 5). This is because wounds lose the surface layer and protective elements as a result of the horn of the skin and its acidic state, exposing the tissue to microorganisms and other pathogens, potentially increasing the risk of infection and tissue damage. Herbal medicines can accelerate the healing of wounds and contain ingredients that aid in tissue repair (6). Humans and sophisticated animals repair wounds using a sophisticated and advanced mechanism involving coagulation and inflammation, cell migration and proliferation, and finally new tissue development and repair (7, 8). The aim of this study was to investigate the combined effect of licorice, coriander, gall nut, sagebrush, Withania coagulans and Pistacia atlantica subsp. kurdica on the healing and repair of burn wounds.

2- MATERIALS AND METHODS

The dried and rot-free test plants from the ecosystem of Sistan and Baluchestan province were purchased from reliable herbal shops in Zahedan city. To prepare the extract of this combination, the crushed plants were placed in a suitable glass jar and extracted with 70% ethanol in a Soxhlet apparatus, in a place not exposed to direct sunlight to avoid chemical changes due to light interactions with the plant material. The resulting extract was concentrated using a vacuum rotary evaporator (Heidolph, Germany) and the resulting material was incubated at 39 °C for three days. The extract was frozen at -20 °C and then weighed on a suitable balance (Pars-Iran Company, Tehran, Iran) (D-HR300 & A, Japan). Forty-eight adult male BALB/c mice (2.5 months old) weighing 25. 5 g were selected for this experimental study. Mice were kept in clean individual cages with free access to food and water at a 12-h photoperiod, temperature of 23-22 °C, and humidity of 45-50%. Initially, mice were anesthetized with 70 mg/kg ketamine, then hair was cleaned with a razor. The burn site was determined on the dorsal skin of the animals. Using surgical principles, an artificial metal tool with a rod and an apartment circular head of 1 cm diameter were used to create a circular burn wound (grade 2) with the thickness of the epidermis and dermis (deep second-degree burn wound). To do this, the apartment head of the tool was heated on an alcohol lamp for 3 minutes before touching the skin of the mouse for 10 seconds. Mice with deep wounds to the subcutis and

muscles were excluded from the study. Finally, the wound was photographed with a digital camera (11). The mice were divided into four groups of 12: Group I was treated with an ointment containing a hydroalcoholic extract of 10% SP, Group II received standard treatment with a silver sulfadiazine dressing (positive control), Group III was treated with petrolatum (negative control), and Group IV received no treatment (sham control).

Mice were photographed after burns on days 4, 7, 10, and 14. Three mice from each group were randomly selected on days 4, 7, 10, and 14, completely anesthetized, and the burn wound area was sampled with ether. The samples were fixed in 10% formalin and prepared for tissue passage.

The biopsied specimens were fixed in 10% formalin solution for 24 hours. After dehydration with varying degrees of increasing alcohol, the samples were shaped with paraffin and 5-micrometer-thick sections were prepared using a rotary microtome. In addition to hematoxylin and eosin staining, sections were stained with the specific Masson trichrome stain, in which collagen fibers, nucleus, and cytoplasm/muscle fibers were stained blue, black, and red, respectively (12).

In this study, the quantitative data and numerical analysis of collagen fiber density, neutrophil count, lymphocyte count, fibroblast count, epithelialization rate, and hair follicle count at different stages of repair were obtained using ImageJ software. Blood was collected in an EDTA containing tube and was analyzed using an automated cell counter (Bayer Advia 120 Hematology Analyzer).

2-1. Data analysis

The data were analyzed using SPSS ver. 21 software. Mean and standard deviation were used to describe the quantitative data. The quantitative data (neutrophil cells, lymphocyte cells, fibroblasts, collagen fiber density, epithelialization rate, and the number of hair follicles) were compared using the one-way test ANOVA. Then, equality of variances was examined using the Levene's test. In case of equality of variances ($P > 0.05$), the differences and comparisons between groups in relevant parameters were determined by Tukey's post-hoc test. In case of inequality of variances ($P < 0.05$), Dunnett'sT3 post-hoc test was used. A significance level of < 0.05 was considered in all tests.

3- RESULTS

3-1. Examination of the samples on day 4

Analysis of baseline data revealed that neutrophil and lymphocyte counts were significantly altered on days 4, 7, 10, and 14 during the study period. These changes were also observed in other parameters (**Table 1**).

Table-1: Baseline laboratory findings of the study mice in this experimental study

Variable	Day 4		Day 7		Day 10		Day 14	
	item	p-value	item	p-value	item	p-value	item	p-value
Neutrophils count	0.641	0.085	0.618	0.840	0.821	0.511	-	-
Lymphocytes count	0.724	0.670	0.842	0.478	0.879	0.1422	-	-
Fibroblasts	-	-	0.830	0.496	0.750	0.628	0.661	0.775
Collagen fiber density	-	-	-	-	0.788	0.580	0.833	0.492
Hair follicles	-	-	-	-	1.00	0.112	0.866	0.422
Epithelialization	-	-	-	-	0.953	0.788	0.534	0.928

3-2. Examination of the samples on days 4, 7, 10 and 14

Significant differences were observed between the mean values of neutrophils and lymphocytes in different groups of sp 1%, silver, and the other groups (p value < 0.0001) (**Table 2**).

Table-2: Comparison of inflammatory cells in the repair samples between different groups on the 4th day

Group / Variable		Sp%10	Silver	Vaseline	Sham	ANOVA results
Inflammatory cells	Neutrophils	*23.8±1.61	*22±2.9	26.8±2.61	28.3±2.45	F=13.58 P<0.0001
	Lymphocytes	**11±1.24	***9±1.25	12.6±1.26	13±1.63	F=17.92 P<0.0001

*Significant difference between Vaseline and sham groups

**Significant difference between silver and sham groups

***Significant difference between sp10%, Vaseline, and sham groups

The mean number of neutrophils in the sp10% group was significantly different from the vaseline and sham groups (p = 0.001 and 0.045, respectively), but not significantly different from the silver group (p = 0.0677).

On the seventh day. The mean number of lymphocytes decreased significantly in the silver group compared to the vaseline and sham groups (p = 0.004 and 0.008, respectively). Both neutrophils and inflammatory cells did not differ significantly between the sp10% silver groups and the vaseline and sham groups (p = 0.996 and 0.0969, respectively). The mean number of lymphocytes in the sp10% group was not significantly different from those in the silver, vaseline, and sham groups (p = 0.099 and 0.0143, respectively).

The silver group was significantly different from the sp10%, Vaseline, and Sham groups in terms of the mean number of lymphocytes (p values: 0.007, 0.007, and 0.010, respectively). However, the number of lymphocytes in the sp10% group was not significantly different from the vaseline and sham groups (p = 0.999), and the sham and vaseline groups were not significantly different in this regard (p = 1.000). The mean number of neutrophils was not significantly different from the sp10%, silver, vaseline, and sham groups (p value: 0.416 and 0.488, respectively).

The mean number of fibroblasts significantly increased in the sp10% group compared to the Vaseline and sham groups (p value < 0.0001), which was higher in the sham group. A significant increase in the number of fibroblasts was observed in the silver group compared to the vaseline and sham groups (p = 0.001 and 0.007, respectively), but no significant difference was observed between the sp%10, silver, vaseline and sham groups (p value: 0.896 and 0.314, respectively).

The mean number of fibroblasts was significantly higher in the sp10% group than Vaseline and sham groups (p-value < 0.0001), with a higher increase in the sham group. Significantly higher fibroblast cells were recorded in the silver group than Vaseline and sham groups (p-value < 0.001), but there was no significant difference between the mean number of

fibroblast cells in the sp10% group and the silver group (p = 0.143).

Compared with Vaseline and sham groups, the silver group showed a significant increase in the epithelialization rate (p-value < 0.001 and 0.036, respectively), which was higher than the sham group. Epithelialization rates were not significantly different in sham and Vaseline groups (p = 0.084).

Table-3: Comparison of means and standard deviations of factors related to burn repair of samples on the 14th day

Group / Variable	Sp%10	Silver	Vaseline	Sham	ANOVA results
Fibroblasts	*40.2±2.20	*38.3±2.11	34.1±1.66	33±1.69	F=31.11 P<0.001
Collagen fiber density	**0.57±0.051	**0.44±0.039	0.385±0.053	0.32±0.009	F=65.5 P<0.0001
Hair follicles	*6.2±1.47	*5.6±1.07	2.5±1.58	2.3±1.039	F=23.86 P=0.001
Epithelialization	**47.7±3.13	**44.6±1.55	41.9±1.59	39.5±2.058	F=26.78 P<0.001

*Significant difference between Vaseline and sham groups

**Significant difference between silver and sham groups

4- DISCUSSION

In the present study we assessed the efficacy of an herbal mixture in the healing process of the burn caused wound. We found that this mixture significantly accelerates wound healing process in BALB/c mice. Olumi et al., investigated the healing effect of aqueous extract of licorice root on skin wounds in 45 male white Sprague-Dawley rats. A 7 mm skin punch was used to create two uniform wounds on the dorsal portion of each animal on both sides of the spine. An aqueous extract of licorice root was applied to half of the wounds daily for 7 days. The animals were then examined for histopathological and biochemical studies. The aqueous extract of licorice root significantly increased the number of fibroblasts and capillary buds, hydroxyproline content, and tensile strength of the wounds. Thus, they concluded that licorice extract is an effective herbal medicine for wound healing (13).

Abolfazl et al. assessed the effect of coriander oil on the healing of second degree superficial burns with a size of 4 × 2 cm, which had occurred on the dorsal side of 48 adult male Sprague-Dawley rats (approximately 250-300 g). Subsequently, the burn sites of groups 1-4 were treated daily with silver sulfadiazine cream, alpha ointment, coriander cream and petroleum jelly (control) until complete healing. The wound healing process was monitored photographically every three days using ImageJ ver. 1.45 (NIH, Maryland, USA). On days 10 and 17, samples of the burn wounds were sent to pathology to examine the amount of collagen and inflammatory cells. The results showed that the wound healing rates in the coriander and alpha groups were higher than the other two groups from day 14, with a statistically significant difference (P < 0.001). They concluded that the effect of coriander ointment on burn wound healing was better than that of the control and silver sulfadiazine groups and similar to that of alpha ointment (14). Mohammadollah

Tavakoli et al. (2010) studied the effect of hydroalcoholic extract of this plant on wound healing process in 30 male rats. Wounds were inflicted on individual rats and randomly divided into three equal groups which received 10 and 100 mg/ml sagebrush extract while the control group received topical physiological serum. From the first day after wounding, 1.0 ml of each solution was rubbed daily on the wound of each group. Wound area was measured and analyzed on days 1, 5, 9, 13, 17, and 21 after wounding. Significant differences in wound area were found between the control group and both doses of sagebrush extract on days 5 ($p < 0.001$), 9 ($p < 0.001$), 13 ($p < 0.01$) and 17 ($p < 0.05$). The percentage of wound healing differed significantly between the control group and both doses of sagebrush extract on the 5th, 9th ($p < 0.001$), 13th, and 17th ($p < 0.05$) days, and the time required for complete wound healing differed significantly ($p < 0.001$) between the control group and the low and high doses of sagebrush extract. They concluded that the hydroalcoholic extract of sagebrush accelerated the healing process of skin wounds and reduced the time to complete wound healing (15). In another study by Havizi, the healing effect of P. atlantica gum and animal oil together with Wharton jelly mesenchymal stem cells on the treatment of third-degree burns in Wistar rats was investigated. The stem cells were isolated from human Wharton jelly cells from the umbilical cord. For this study, 28 rats were burned with a metal stamp and then randomly divided into a control group (7 mice) and a treatment group (21 mice). The treatment group was divided into three groups (each with 7 mice), which were treated with one envelope, one cell therapy, and one cell therapy plus one envelope. Each rat was subcutaneously injected with 106 cells at the third passage. On the 30th day after treatment, the animals were euthanized with chloroform and tissue sections were prepared with hematoxylin-eosin and Masson trichrome staining for microscopic examination. Microscopic observations showed a faster healing process in the treatment groups than in the control group. After 30 days, the cell therapy plus pack method was significantly more effective than the pack and cell injection alone. Histological examinations showed a significant increase in angiogenesis, collagen synthesis, cell count, thickness of skin layers, and finally accelerated wound healing in the treated samples compared to the control group. They concluded that the simultaneous therapeutic methods used in their study had a significant effect on accelerated healing of skin wounds in animal models (16).

5- CONCLUSION

According to the results of this study, the combined extract of licorice, coriander, gall nut, sagebrush, W. coagulans and P. atlantica subsp. kurdica accelerates the healing process of burn wounds in BALB/c mice. Therefore, this combination can be considered as a potential therapeutic mixture for wound healing. This combined extract is suggested to be studied at different doses of LD50 and LC50 for toxicological evaluation, and then clinical trial studies should be performed for evaluating its effect on humans.

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7- REFERENCE

1. Lateef Z, Stuart G, Jones N, Mercer A, Fleming S, Wise L. The cutaneous inflammatory response to thermal burn

injury in a murine model. Int J Mol Sci. 2019; 20(3):538.

2. Saberi M, Fatemi MJ, Soroush MR, Masoumi M, Niazi M. Burn epidemiology in Iran: a meta-analysis study. 2016.

3. Lotfi N, Shokohi T. A review on fungal infection in burn patients, diagnosis and treatment. J Maz Univ Med Sci. 2014; 23(108):151–65.

4. Baker SP, o'Neill B, Haddon Jr W, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. J Trauma Acute Care Surg. 1974; 14(3):187–96.

5. COPES WS, Champion HR, Sacco WJ, Lawnick MM, KEAST SL, BAIN LW. The injury severity score revisited. J Trauma Acute Care Surg. 1988; 28(1):69–77.

6. Yamasaki S, Kawakami A, Nakashima T, Nakamura H, Kamachi M, Honda S. Importance of NF-kappaB in rheumatoid synovial tissues: in situ NF-kappaB expression and. vitro;

7. Ghaderi R. Novel advancements in wound healing. J Birjand Univ Med Sci. 2014; 21(1):1–19.

8. Nekooie-Marnany N, Jaffary F, Nilforoushzadeh MA. Platelet-rich plasma (PRP) therapy in skin rejuvenation: benefits and adverse influences. J Isfahan Med Sch. 2015; 33(343).

9. Ghaderi R, Afshar M, Akhbar E H, Golalipour MJ, Ghaderi R, Afshar M. Comparison of the efficacy of honey and animal oil in accelerating healing of full thickness wound of mice skin. Int J Morphol. 2010; 28(1):193–8.

10. Afshar M, Ravarian B, Zardast M, Moallem SA, Fard MH, Valavi M. Evaluation of cutaneous wound healing activity of Malva sylvestris aqueous extract in BALB/c mice. Iran J Basic Med Sci. 2015; 18(6):616.

11. Afshar M, Ghaderi R, Zardast M, Delshad P. Effects of topical emu oil on burn wounds in the skin of Balb/c mice. Dermatol Res Pract. 2016; 2016.

12. Nabiuni M, Oryan SH, Ayyobi Por M, Bagheri M. Histochemical study of Verbascum speciocum extract's effects on wound healing in rats. 2011.

13. Abbas MMOADF. Evaluation of healing properties of licorice root extract on skin wounds. J Vet Res [Internet]. 2007; 62(4). Available from: https://jvr.ut.ac.ir/article_18893.html.

14. Abbas Zadeh A, Mahzooni T, Emami S A, Akbari H, Fatemi M J, Saberi M, Bagheri T, Niazi M, Araghi S. The effect of Coriander cream on healing of superficial second degree burn wounds. Tehran Univ Med J. 2015; 73(9):646–52.

15. Âllahtavakoli M, Ârab Bani Âsad F, Mahmoudi M, Jafari Naveh H, Tavakolian V, Kamali M, Mahmoudi M, Mokhtari SS. Effect of Hydro-Alcoholic Extract of Artemisia Aucheri on Healing of Skin Wound in Rat. J Maz Univ Med Sci [Internet]. 2010; 20(77):70–6. Available from: http://jmums.mazums.ac.ir/article-1-702-fa.html.

16. The Effects of Wharton's Jelly Mesenchymal Stem Cells Associated with Gum of Pistacia atlantica for Burn Wound Healing. JAPAD [Internet]. 2020; 13(50):85–96. Available from: http://qjaphd.sinaweb.net/article_674363.html.