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ORIGINAL ARTICLE OPEN ACCESS

The Regenerative Effects of Hemostatic Plant Extract in Second Degree Burn Wound

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Introduction: The effects of Ankaferd Blood Stopper (ABS), a hemostatic herbal agent, on wound healing of experimental second degree thermal burn model were analyzed in this study.

Material and Methods: 24 wistar albino rats were used. All rat subjects were divided into 4 equal groups as control, burn control, Ankaferd Blood Stopper and serum physiologic. In all rats except for those in negative control group, second degree thermal burn was formed with metal plates heated on their backs. Biopsies were taken on 0^{th},7^{th},14^{th} and 21^{st} days. In tissue samples; they were evaluated in terms of fibroblast increase histopathologically, congestion, inflammatory cell increase and epithelization findings. They were also scored. The values were compared statistically.

Results: No significant difference was observed between groups with regard to fibroblast increase ($p>0.05$). On the 7^{th} day, congestion values in burn control group was determined higher than ABS group ($p<0.005$). On 7^{th} day again, inflammatory cell increase in ABS group was identified quite low compared to that of control groups ($p<0.005$). While a distinct increase was determined in epithelization in ABS group on the 7^{th} day, this increase was observed as at medium level in serum physiologic group. There was no increase in epithelization in burn control group. When epithelization findings on the 7^{th} day of burn formation were exained, the values in ABS group were determined significantly high compared to other groups ($p<005$).

Conclusion: Our findings were of supportive quality that ABS has more positive effect on second degree thermal burn healing.

Keywords: Burn, burn healing, epithelization, ankaferd

Introduction

Burn is a trauma continuing to be a cause of morbidity and mortality which is frequently encountered owing to thermal, electricity, radiation and chemical with a developing industry today (1). The damage amount and degree that burn forms in the skin vary with our treatment options. While first degree burns restricted with epidermis recover with a simple medical treatment, there is no treatment apart from surgical option in third degree burns which are full-thickness skin and subcutan tissues. In the second degree burn treatment (burn with a partial thickness) that affects epi-

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dermis together with a part of dermis, no full consensus could be reached in literature (2-5).

Nevertheless, the treatment way generally accepted is to clean the dead tissue firstly, prevent barrier gap formed due to skin loss, protect body against infection and fluid loss and potential secondary pathologies. A vast quantity of chemical and herbal drug was used to ensure burn wound healing that leaves minimum scars in the fastest way, however, no ideal consequence could be reached in these treatment options (6-9).

Ankaferd-5 used as a medical, hemostatic agent is composed of standardised extracts obtained from the so-called plant's dried root and leaves. It contains thymus vulgaris (thymus), glycrrhiza glabra (licorice), vitis vinifera (vitis), alpinia officinarum (galgant) ve Urticadioica (nettle) (10-12). This mixture is utilized in the control of minor and major bleedings (11,13-18).

It has been reported that ankaferdin, proved its efficiency in bleeding control with many studies (19), has a positive and accelerator impact on tissue regeneration and wound healing (8, 12,19-22,) and also antimicrobial (20), antifungal (23), antiinflammatory (24) and anti-carcinogenic (25), antioxidant and antimutagenic features (26). However, when we look at literature, there are no studies examining the effects on thermal wound healing of ABS.

In this study, the effects of herbal Ankaferd Blood Stopper (ABS) have been analysed experimentally in rat model on second degree thermal burn wound.

Material and Methods

Fırat University Animal Experiments Local Ethics Board approval was attained with 2014/2 and 25 numbered decision.

Ankaferd Blood Stopper (ABS)

ABS is a patented product (Turkish Patent No. 2007-0-114485) comprising a standardized mixture of plants G.glabra (90 µg/mL), V. Vinifera (80 µg/mL), U.Dioica (60 µg/mL), T. Vulgaris (50 µg/mL), and A. Officinarum (70 µg/mL) (27). A 2-mL vial of ABS was obtained from the manufacturer (Trend Teknoloji Ilac AS, Turkey).

The Preparation of Subjects

24 Wistar Albino male rats were used in this study. The subjects were randomly divided into 4 groups as control, burn control, Ankaferd and Serum Physiologic. On the first day of the experiment, full-thickness skin biopsies were obtained from the shaved backs of the subjects of control group. After the biopsies attained on the 0th day, the subjects in the control group were sacrificed with carbondioxide gas. In the other groups, however, second degree thermal burn was formed by pressing 2x1 cm 4 metal plates after they had been kept in boiling water for 30 seconds in the way that their body surfaces did not exceed 10% of them under anesthesia (Figure-1).

Figure-1. Situation that rats with burn formation

Following the procedure, the wounds of all 21 subjects were dressed specific to their groups. Closed injury dressing was carried out with only sterile gauze dressing without using

any substance in burn control group. The subjects in Ankaferd group, however, ankaferd was dripped to burn wounds and closed injury dressing was performed with sterile gauze dressing (Figure-2). Burn wounds of subjects in serum physiologic group were made wet with serum physiologic and closed injury dressing was conducted with sterile gauze dressing.

Figure-2. ABS application to burn wound in Ankaferd group on 0th day

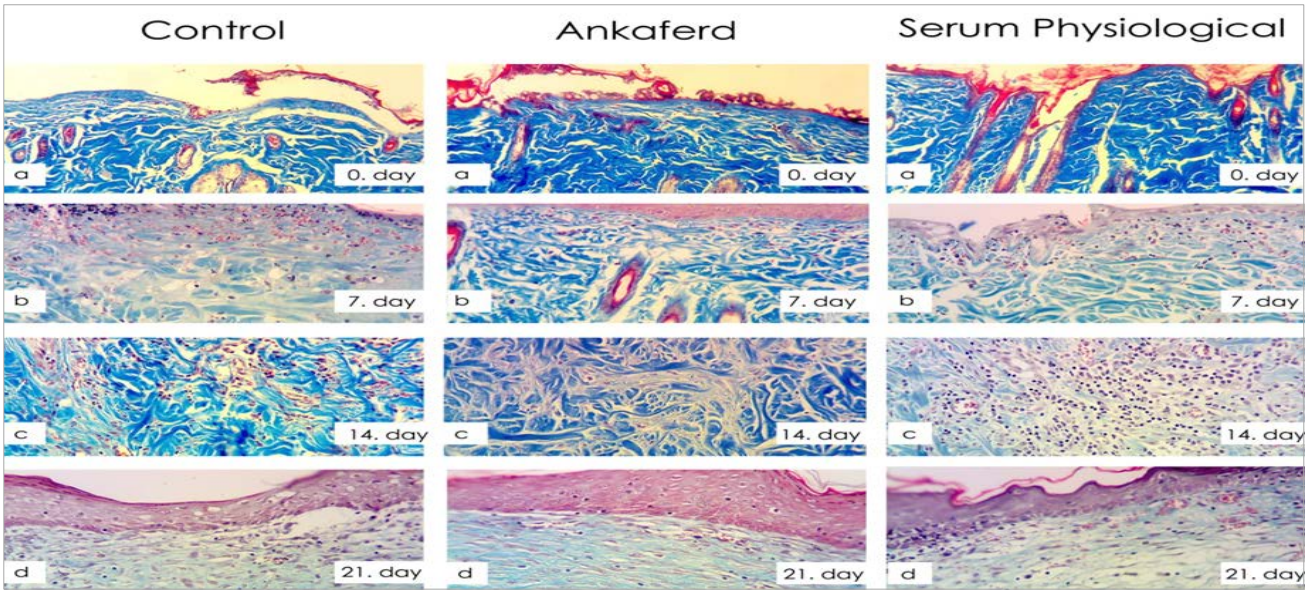
Full-thickness skin biopsies were obtained from burn areas on 0th, 7th, 14th and 21st days of the study for histopathologic evaluation. After the biopsies attained on the 21st day of study subjects were sacrificed with carbondioxide.

The Evaluation of Skin Samples

The obtained tissue samples were fixed in 10% neutral formaldehyde and waited in 5% formic acid. After the routine histopathologic preparations, the materials taken into paraffin blocks were divided into vertical sections as 5µm with help of Leica Rotary microtome. The attained sections were analysed under the light microscope after they had been stained with Masson Trikrom.

Taking the control groups as a reference, the changes (fibroblast increase, congestion, inflammatory cell increase, epithelization) were evaluated as none (0), low (1), medium (2), extreme (3), severe (4) according to their histopathologic situations (Figure 3, 4, 5). Scoring was carried out for every rat and average values were determined for every group.

Figure-3. Control histological images; **Figure-4.** Ankaferd histological images; **Figure-5.** Serum physio-logical group histological images; **Figure 3a,4a,5a**. Severe epidermis damage on 0th in groups; **Figure 3b**. In burn control, there were less fibroblast, severe inflammatory cell increase on 7th day but no epithelization; **Figure 4b**. In Ankaferd group, there were less fibroblast and inflammatory cells on 7th day and too much increase in epithelization. **Figure 5b**. In serum physiologic group, there were numerous fibroblast and inflammatory cells on 7th day and reasonable increase in epithelization. **Figure 3c,4c,5c**. An apparent increase on 14th day in groups; **Figure 3d,4d,5d**. In groups, there were a severe fibroblast increase, less inflammatory cells on 21st day, in Ankaferd group, however, epithelization increased severely and increased really high in burn control and serum physiologic group *(Masson Trikrom x 400)*.



Statistical Analysis

Statistical evaluation of the collected data via subject follow-up forms was performed by using SPSSv21.5 package programme. Statistical significance was accepted as $p<0.05$. Since sample size was composed of 24 subjects, non-parametric tests were utilized in statistical analyses as variations did not fit to normal distribution. Kruskal-Wallis test was used in the comparison of congestion, fibroblast increase, inflammatory cell increase and epithe-lization values of control, burn, isotonic and Ankaferd groups and then Mann Whitney U test was utilized in Post Hoc group comparisons.

Results

A significant difference was not determined in biopsies obtained on 0^{th}, 7^{th}, 14^{th} and 21^{st} days when fibroblast increase was evaluated ($p>0.05$). In the congestion evaluation; a signi-ficant difference was established between An-kaferd and burn control groups when change amounts between histopathologic data on 0^{th} and 7^{th} days were examined. While less increase was seen in congestion in Ankaferd group on 7^{th} day, a severe increase was observed in con-gestion in burn control group (Figure-6). The difference between them was statistically sig-nificant ($p<0.05$).

Figure-6. Change (7-0) Day Congestion

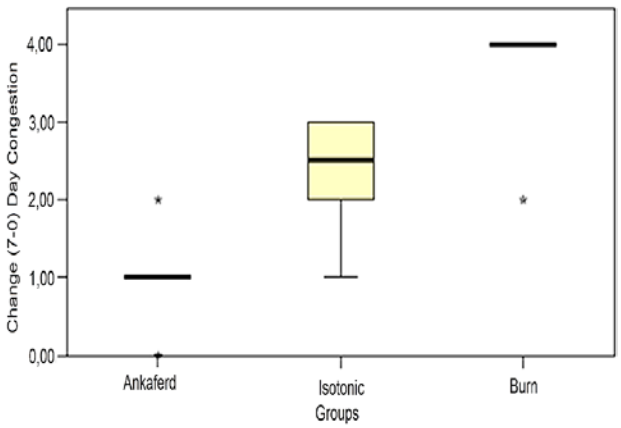
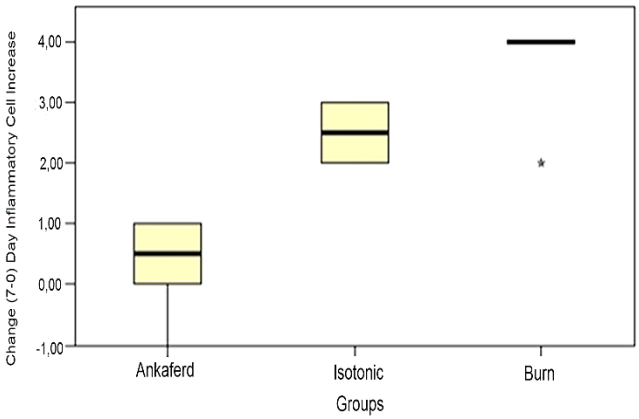
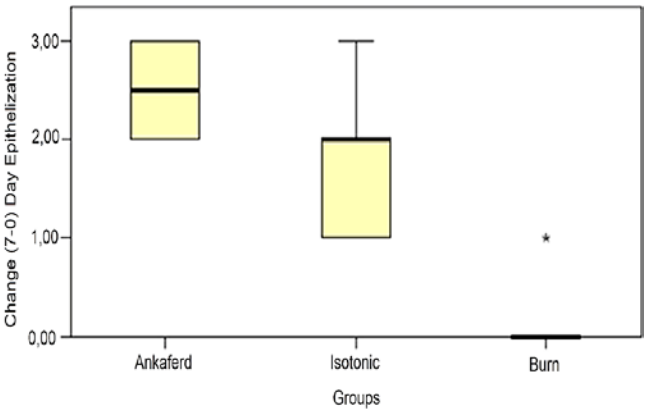


Figure-7. Change (7-0) Day Inflammatory Cell Increase



In the inflammatory cell increase evaluation; a significant difference was identified between Ankaferd and burn control group when change amounts between histopathologic data were analysed on 0^{th}-7^{th} days and 0^{th}-21^{st} days. While less increase was seen in inflammatory cells in Ankaferd group on the 7^{th} day, a severe increase was observed in inflammatory cells in the burn control group (Figure-3b,4b,7). The difference between them was statistically significant ($p<0.05$).

Figure-8. Change (7-0) Day Epithelization



In the epithelization evaluation; a significant difference was established between the burn control group and serum physiologic and the Ankaferd groups when change amounts were observed between histopathologic data on 0^{th}-7^{th} and 0^{th}-21^{st} days. While there was an extreme

increase seen in Ankaferd epithelization on the 7th day, an average increase was seen in the epithelization of serum physiologic group. Yet, there was no increase in the epithelization of burn control group on the 7th day (Figure-3b, 4b,5b,8). The difference between them was statistically significant ($p<0.005$).

Discussion

As long as skin protects its completeness, it can fulfillits duties,on the other hand, a complex recovery period commences that many cells play part including keratinocyte, fibroblast, endothelium cells, macrophage and thrombocytes in case of deterioration of this completeness. New tissue formation initiates with migration, infiltration and proliferation of these cells and wound closes (14, 28, 29). To accelerate the recovery of burn wound, the aim in the treatment methods used was to affect the factors playing a role in wound healing and shorten the periods regarding these phases and ensure an ideal scar formation. Therefore, many topical and systemic agents were used and delay and irregularities were tried to be inhibited in the wound healing. None of these treatment options have a feature that is alternative to others (8). On the other hand, advanced fibroblastic activity and increased inflammatory period cause irregularities in many wound healings including burn wounds and lead to hypertrophic scar or keloid development (15). Therefore, the control of these periods in wound healing and additional agents to be used while recovering are of importance (16).

In line with our results, Arslan et al. applied ABS to a soft tissue laceration developed based on a trauma and with no repair chance with primary closure on a 80-year-old female patient. In the control performed 24 hours later, it was determined that wound opening started to close and was of the opinion that ABS is effective in superficial wound healing (8).

In a study in which the efficiency of Celox and Ankaferd® was analysed as styptic in experiment animals given varfarin by Aktop et al., fibrosis values were found significantly low in Celox® group compared to other two groups. This situation was described to be late in Celox® group but a better improvement. As a result, both agents were effective in bleeding control, it was determined that ABS causes a rapid improvement while Celox® forms a late recovery period (21). In our study, ABS that affects fibrosis in a way equivalent to serum physiologic supports this perspective that it forms a better epithelization.

In a study that full thickness skin defect was formed on the backs of rats by Akalın et al., they evaluated the effects of ABS on a secondary wound healing as histologically and photographically. Similar to our study; fibroblast proliferation, inflammatory cell increase, collagen storage, wound contraction and congestion increase were examined on the 3rd, 7th, 10th and 14th days of skin defects formed in full thickness. Results were obtained in parallel with findings of our study. According to the outcomes of this study, in the experiment group that ABS was applied to wound surface fibroblast proliferation degree, vascularization degree, wound contraction rate and type-I/ type-III collagen rate were statistically and significantly found high compared to those of control group (22).

In a study that Isler et al. created a bone defect in tibias of rats, they studied the effects of ABS on early bone recovery. In the histopathological evaluation of tissues on 7th day, less inflammation and necrosis were seen in the group that ABS was applied compared to control

group and it was reported that there was more new bone formation in the course of early bone amelioration (12). This study complying with our findings showed that ABS which is determined to increase epithelization in an isolated way without forming more activities than serum physiologic in fibroblastic activity affected the other ways of cellular healing.

In the partial nephrectomies performed on 24 rats by Huri et al., they stated that in a group ABS was applied, it reduced partial nephrectomy and hot ischemia and ensured hemostasis. They analysed the effect of the Ankaferd by evaluating glomerular necrosis, acute inflammation, calcification, fibrosis, fibroblast activation and microvascular proliferation histopathologically. They informed that inflammation, fibrosis, fibroblast activation and microvascular proliferation were not significantly affected. It was also supported in this study in a similar way that ABS displayed a regenerative effect beyond its fibroblastic activity. In addition, no symptoms seen such as glomerular necrosis and calcification was evalauted as a positive effect on renal tissue (19).

When the samples were compared in terms of congestion, inflammation, fibroblast proliferation and epithelization in our study, the serum physiologic and ABS groups provided better results compared to burn control group. However, ABS was determined to have superiority over serum physiologic with regard to inflammation control and fibroblast increase ($p>0.005$). Nevertheless, providing an apparent effect of ABS compared to serum physiologic in increasing epithelization ($p<0.005$) was interpreted as Ankaferd has an effect on wound healing and epithelization apart from inflammatory periods.

As a result, Ankaferd seems to contribute to effective wound healing by accelerating epithelization similar to literature data in our study. However, ensuring efficiency without increasing inflammatory period and fibroblast activity makes us think that it can have an effect to cellular regeneration. Moreover, if uncontrolled scar tissue is thought to develop in burn wounds where inflammation and fibroblastic effectivity are high, ABS can be thought to yield better results in burn isolated wound healing. Nonetheless, controlled studies performed in people are required to support this prediction.

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