EFFECTS OF FOLK MEDICINAL PLANT EXTRACT ANKAFERD BLOOD STOPPER ON BURN WOUND HEALING

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ABSTRACT

Objective: Ankaferd Blood Stopper (ABS), a mixture of five medicinal plant extracts, has been used historically as a haemostatic agent. The aim of this in vivo study was to investigate the effects of ABS on burn healing using a rat burning model.

Materials and methods: A total of 24 male Wistar rats were used in this study. Burns were induced in wistar albino rats divided into two groups as following; Group-I was treated with ABS pad. Group-II (negative control) received no treatment (dressing with salin). Prior to burn injury, the animals were anesthesized. Dorsum of each rat was shaved with an electrical clipper and then the area was burned in order to obtain 20 mm in diameter of second degree burn injury. The method depended on contact burn injury using a metal brass heated in boiling water. Three animals of each group were sacrificed on the 3 rd postburn day, and nine animals of each group were sacrificed on the 14 th postburn day and samples were collected. The efficacy of treatment was evaluated based on the wound diameter, inflammation, granulation, and fibrosis.

Results: There was no significant difference between groups in terms of wound diameter, inflammation, granulation and fibrosis on the third day. Significant differences were found in both groups in terms of wound diameter, wound contraction, inflammation, and fibrosis on the 14 th day. Wound diameter and inflammation were found to be significantly decreased and fibrozis was found to be significantly increased in ABS group. Granulation was found to be no significantly increased in the ABS group than in control one.

Conclusion: It may be concluded that ABS is effective in the treatment of burn wound healing. Further in vitro and in vivo studies are necessary to value the benefits and possible adverse effects of the application of this product on burn wound.

Key words: Ankaferd Blood Stopper® (ABS), burn wound, burn wound healing.

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Introduction

Burns are very common in both developing and developed countries. Burns are extreme thermal injuries that are caused by short or long exposure to a physical, chemical or biological agent. Thermal injuries are very common and burn cause a huge public health problem[1].

The gravity of the injury is associated with the aetiologic agent and the extent and depth to which the lesion affects the body.

Burns are classified according to the depth of the injury. In superficial second-degree burns, the epidermis and the superficial dermis are mainly affected. These kinds of burns are very painful. The main causes of a superficial second-degree burn are hot liquids[2].

Because of burns are one of the most widespread injuries in accidents and remain a global public health issue the need for a local and effective wound healing medication efficient in preventing infection, decreasing fluid imbalance, promoting reepithelialization, reducing the occurrence of scar tissue and is still an important challenge[3,4].

The dynamic process of wound healing involves a series of events including inflammation, granulation tissue formation, epithelisation, collagen synthesis and tissue remodelling[5].
It has been known that the administration of skin substitutes expedites the healing of skin lesions, including burns. Covering burns requires a material that will restore epidermal function and integrate into the process of healing.

Ankaferd blood stopper (ABS) is a folkloric medicinal plant extract product, which has historically been used in Turkish traditional medicine as a haemostatic agent. It is a standardized mixture of the plants T. vulgaris, G. glabra, V. vinifera, A. officinarum and U. dioica. Each ingredient of this mixture has specific characteristics. Several studies have shown that each of these plants has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators.

The aim of this in vivo study was to investigate the effects of Ankaferd Blood Stopper on burn healing using a rat model.

Material and methods

The study was carried out in the Harran University, Faculty of Medicine, Department of Emergency Medicine. Treatment of the experimental animals was approved by the Harran University Animal Research and Ethics Committee in accordance with the European Community Council Directive. Experimental animals were obtained from The Laboratory of Experimental Animals, Gaziantep University, Gaziantep, Turkey.

**Ankaferd Blood Stopper**

Ankaferd Blood Stopper (Trend Teknoloji Ilac AS, Istanbul, Turkey) is a licensed pharmaceutical plant extract that is applied directly to injured skin and mucosa as a liquid (solution) or spray or in a dressing (tampon). ABS contains a standardized mixture of 5 medicinal plant extracts. The active ingredients in the tampon form of ABS (2.5 X 7 cm3 mL) are as follows: 0.18 mg of dried root of Urtica dioica, 0.24 mg of dried leaf of Vitis vinifera, 0.27 mg of dried leaf of Glycyrrhiza glabra, 0.21 mg of dried leaf of Alpinia officinarum, and 0.15 mg of dried leaf of Thymus vulgaris. This study used the tampon form of ABS supplied in 20 mm diameter second degree burn healing in animal model.

**Animals and experimental design**

A total of 24 twenty-week-old male wistar rats weighing 250 to 300 g were used in this study and randomly assigned to two groups of 12 animals each. Prior to burn injury, the animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) intraperitoneally. Dorsum of each rat was shaved with an electrical clipper and then the area was burned in order to obtain 20 mm in diameter of second degree burn injury. The method depended on contact burn injury using a metal brass heated in boiling water. A circle metal (20 mm diameter) was heated in boiling water 60 s and pressed to the shaved and disinfected surface for 20 s in mice.

(Figure 1A)

The rats of each group were housed into separate cages with two or three animals under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) without any restriction of mobilization. All the rats were allowed free to take water and food given ad libitum during study.

The animals of each group were sacrificed with an overdose of pentobarbital (40 mg/kg) on the 3 rd and 14 th day after burn, and the defects together with surrounding tissue were immediately removed for histopathological analysis.

Three animals of each group were sacrificed on the 3 rd postburn day, and the remaining nine animals of each group were sacrificed on the 14 th postburn day and samples were collected. The efficacy of treatment was evaluated based on the wound diameter, inflammation, granulation, and fibrosis.
**Measurement of wound area**

The progressive changes in wound area were measured in mm by tracing the wound boundaries on a scale on 3rd and 14th day. (Figure 1B, 1C, 1D and 1E) The wound areas in all groups were recorded on a graph paper. Wound contraction was expressed as reduction in percentage of the original wound size.

**Tissue Preparation and Histopathological Examination**

The specimens were fixed in 10% neutral buffered formalin overnight at 4°C, for 24 hours. The specimens were embedded in paraffin and cut into 20 semi-serial sections using a microtome, and routine hematoxylin and eosin (HE) staining and Masson Trichrome (MT) staining were performed. The sections were examined with light microscope under 40 and 100x magnification (Olympus BX51 TF, Tokyo, Japan). A histomorphological review was performed by a single blinded oral pathologist to evaluate the presence of inflammation, granulation and fibrosis. The scores for inflammation, granulation and fibrosis were determined by counting the associated cells and their ratio to the total cell count in a standardized area at 40x magnification. The ratio of cells between 0-25% was scored as none, 25-50% as slight, 50-75% as moderate, and 75-100% as advanced.

**Statistical analysis**

Statistical analyses were carried out using the SPSS statistical package, version 11.5 (SPSS, Inc, an IBM Company, Chicago, Illinois) for Windows. The statistical differences between the ABS and control groups were compared by Mann Whitney U test. The mean and standard deviation or median, minimum-maximum data were calculated for each group. All data are expressed as the mean and 95% confidence intervals. The values of p ≤ 0.05 were considered statistically significant.

**Results**

The scores and percentages of wound diameter and wound contraction in both groups are presented in Table 1.

The scores and percentages of inflammation, granulation and fibrosis in both groups are presented in Table 2.

Comparisons between the ABS and control groups indicate a significant variability in the scores of wound diameter, inflammation, and fibrosis (p < 0.001, p = 0.011, p = 0.031 respectively). No significant differences were observed between groups with respect to granulation (p = 0.113).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ABS group (n=9)</th>
<th>Control group (n=9)</th>
<th>Statistical significance (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound diameter (14th day)</td>
<td>0.8 ± 0.3 (0.6-1.0)</td>
<td>1.3 ± 0.3 (1.0-1.6)</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>Wound contraction (14th day)</td>
<td>% 39.4 ± 7.68 (50-70)</td>
<td>40 ± 10.6 (20-50)</td>
<td>p &lt; 0.001*</td>
</tr>
</tbody>
</table>

*From independent samples t-test. Values are median ± IQR.

**Inflammation, granulation and fibrosis scores in the ABS and control groups.**

From independent samples t-test was applied. Values are median ± IQR (min - max). The ratio of cells between 0-25% was scored as none, 25-50% as slight (+), 50-75% as moderate (++), and 75-100% as advanced (+++).

In the ABS group the wound diameter was found 0.8 ± 0.3 (0.6-1.0) mm and in the control group was 1.3 ± 0.30 (1-1.6) mm. Statistically significant differences (p < 0.001) were found between the ABS and control groups. In the ABS group the wound contraction was found 59.44 ± 7.68 (50-70) and in the control group was 40 ± 10.60 (20-50). Statistically significant differences (p < 0.001) were found between the ABS and control groups.

Histopathological changes are seen in figure 2 both ABS and control group on 14th day.

In the ABS group, 55.6% and 44.4% of the specimens showed slight and moderate inflamma-
tion, respectively. In the control group, 11.1% and 33.3% and 55.6% of the specimens showed slight, moderate and advanced inflammation, respectively. Statistically significant differences (p = 0.011) were found between the ABS and control groups.

In the ABS group, 33.3%, 44.4% and 22.2% of the specimens showed slight, moderate and advanced granulation respectively. In the control group, 11.1%, 55.6% and 33.3% of the specimens showed none, slight and moderate granulation respectively. Both groups showed similar range of granulation scores with no statistically significant difference (p = 0.113) between them.

There was statistically significant difference between the groups as for the fibrosis scores (p = 0.031). In the ABS group, 44.4% and 55.6% of the specimens showed slight and moderate fibrosis respectively. In the control group, 11.1% and 88.9% of the specimens showed none and slight fibrosis respectively. Statistically significant differences (p = 0.031) were found between the groups.

Discussion

Due to the high costs of traditional treatments, there has been a growing interest in alternative medicines and natural medicinal products for the local treatment of wounds.

ABS is a standardized mixture of the plants T. vulgaris, G. glabra, V. vinifera, A. officinarum and U. dioica. Each ingredient of ABS has specific characteristics. G. glabra also has antiinflammatory, anti-thrombin, antiplatelet, antioxidant, anti-atherosclerotic, and antitumor activities. T. vulgaris has been shown to exhibit varying levels of anti-oxidant activity, which may help to prevent in vivo oxidative damage, such as lipid peroxidation, associated with atherosclerosis. Inoculation experiments on detached leaves of V. vinifera exhibited enhanced resistance towards pathogens. A. officinarum inhibits nitric oxide production in lipopolysacchride activated mouse peritoneal macrophages. U. dioica can produce hypotensive responses through a vasorelaxation effect mediated by the release of endothelial nitric oxide and the opening of potassium channels, and through a negative inotropic action.

The defects treated with ABS also showed decrease wound diameter and increase wound contraction, which may be related to the increased speed of healing and decreased inflammation which is associated with antioxidant activity of the components of the ABS.

Wound contraction, the process of shrinkage of area of the wound, depends on the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue. The wound contraction was significantly faster and higher in percentage in animals treated with ABS containing pad. Finally, the wound diameter was found to be shorter in animals treated with ABS containing pad (Table 1).

Fig. 2: Photomicrograph of second degree burn wounds in rats at 14 th day (magnification, 100x).

**Control group**: moderate -advanced presence of inflammation stained with H&E (A), slight-moderate granulation and slight fibrosis with MT (B).

**ABS group**: Slight inflammation, moderate-advanced granulation (C) and moderate fibrosis stained with H&E (D).

**Discussion**

Due to the high costs of traditional treatments, there has been a growing interest in alternative medicines and natural medicinal products for the local treatment of wounds.

ABS pads have been applied on the back of rats for 24 hours and pads are changed twice a day in our study. We did not see any adverse effects during the observation and could not make any conclusion.

For now, the effects of ABS on wound healing seem to be limited by the observation of a decreasing effect on microvessel density measurements in the tissue exposed to ABS, which suggest the presence of secondary more sustained mechanism of hemostasis besides the initial protein network.
Collagen production during the healing process (of burns) is essential for epithelial migration and proliferation in the early days post injury\(^{(17)}\). However, at the end of the healing process, high collagen levels may compromise the final result of the injury\(^{(18)}\).

The occurrence of fibrosis was statistically increased in ABS group on 14\(^{th}\) day, the ABS-treated group showed higher fibrosis rate than the non-treated control group, which may be attributed to the increased speed of healing in the ABS group.

Infection is a serious and significant complication of thermal injury due to presenting a suitable moist and nutrient rich environment for bacterial growth, and it is the cause for about 50-75% of hospital deaths\(^{(19,20)}\). ABS would be an advantage in traditional theraphies on several aspects. It has antimicrobial and antifungal effects which was demonstrated by several in vitro studies\(^{(21,22)}\). A novel finding of which ABS was found to be inhibitory for MRSA, Acinetobacter, E. Coli and Pseudomonas species growth was reported by Saribas et al. This is very important, as most of these organisms are responsible for burn wound sepsis\(^{(23)}\).

Burned patients have a consumption coagulopathy which, in combination with haemodilution during operation results in a clinically significant deficiency of coagulation factors II, VII, IX, X, XI, and XIII were not affected by ABS\(^{(25)}\). Any hemo-static agent that does not interfere with coagulation factors would take the advantage over other hemo-statically active products. So the use of ABS in severe burn patients injury may be useful adjunctive measure to prevent fulminant course.

**Conclusion**

As a conclusion, ABS decreases the inflammation and wound diameters and increases wound contraction and tissue fibrosis in rats with burn injury. Therefore, ABS may be a beneficial alternative in complex treatment of burn injury. Further in vitro and in vivo studies are necessary to assess benefits and possible adverse effects of the application of Ankaferd Blood Stopper on burn wound healing.

**References**


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