The Efficacy of Ankaferd Blood Stopper in Heparin-Induced Hemostatic Abnormality in a Rat Epistaxis Model

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What is This?
Abstract

Objective. To assess the in vivo hemostatic effect of Ankaferd Blood Stopper (ABS).

Study Design. An experimental study of an animal anterior epistaxis model.

Setting. A tertiary care university hospital.

Subjects and Methods. Wistar rats were randomized into 4 groups of 7 each: group 1, control, no pretreatment, irrigated with saline; group 2, no pretreatment, irrigated with ABS; group 3, control, heparin pretreatment, irrigated with saline; and group 4, heparin pretreatment, irrigated with ABS. In all groups, a standardized rat epistaxis model was obtained by cutting the anterior nasal septal mucosa. To control bleeding, compressive dressings were placed after instilling 1 mL of either ABS or saline to the bleeding area. The hemostasis time and amount of nasal bleeding were measured in all groups to compare the treatments without and with ABS.

Results. Without heparin pretreatment, ABS shortened the hemostasis time by 1.57 minutes \((P = .003)\) and reduced the amount of the bleeding by 0.35 g \((P = .006)\). With heparin pretreatment, ABS shortened the hemostasis time by 2.86 minutes and reduced the amount of the bleeding by 0.49 g \((P = .002)\).

Conclusion. ABS irrigation was more effective than saline irrigation for treating anterior epistaxis hemostasis in animals using a compressive dressing with or without heparin pretreatment.

Keywords

epistaxis, Ankaferd Blood Stopper, hemostasis, heparin sodium, experimental

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Epistaxis is defined as active bleeding arising from the nasal mucosa. It constitutes one of the most common otolaryngology emergencies and can be severe or even fatal. An estimated 5% to 10% of the world’s population experiences an episode of active nasal bleeding annually.\(^1,2\) Spontaneous epistaxis is reported in up to 60% of the population, with peak incidences in those younger than age 10 and older than age 50; it occurs in males more often than in females.\(^2\) Epistaxis is classified as anterior or posterior based on the primary bleeding site. A common source of anterior epistaxis is Little’s area (Kiesselbach plexus), an anastomotic network of vessels on the anterior portion of the nasal septum, which receives its blood supply from the internal and external carotid systems.\(^3\) Epistaxis results from a multitude of local or systemic factors. Common local factors include trauma (nasal fractures or finger manipulation), upper airway infections, breathing cold and dry air, nasal allergies, insertion of foreign bodies in the nasal cavity, septal perforation or deviation, necrosis, and chemical irritants. Common systemic factors are hypertension; coagulopathies, especially due to anticoagulant or antiplatelet adhesion medication; renal failure; alcoholism; and vascular abnormalities.\(^3,4\)

Traditionally, severe epistaxis can be managed effectively in a variety of ways, including pressing the nostrils, applying a cold compress to the bridge of the nose, hot water irrigation, anterior nasal packing, chemical or electrocauterization, topical hemostatic or vasoconstricting agents, and cryotherapy.\(^1,3,4\) For patients on anticoagulant or antiaggregant treatment (ie, patients with clotting disorders), hemostatic agents are used unless the patient requires surgical intervention or develops disseminated hemorrhages in which a single bleeding spot cannot be located and surgical intervention is insufficient.\(^1,5\) Ankaferd Blood Stopper (ABS) is a topical hemostatic agent that contains hemostatic plant extracts and no inorganic or...
hemostasis for the management of external, postsurgical, and traumatic bleeding.\textsuperscript{5,7} ABS facilitates the formation of an encapsulated protein network by interacting with blood proteins in general and fibrinogen in particular. The ABS-induced formation of the protein network provides foci for erythrocyte aggregation in vivo and in vitro. This unique mechanism of action involves the entire physiological hemostatic process.\textsuperscript{7,9} As it does not interfere with the components of the classic coagulation cascade, it might be effective in individuals with normal hemostatic parameters, as well as in those with primary or secondary hemostatic abnormalities.\textsuperscript{10,11}

This study compared the efficiency of a compressive dressing using ABS or saline for achieving hemostasis in an anterior nasal bleeding model in rats. In addition, the hemostatic effect of ABS was evaluated in rats that were pretreated with standard heparin (heparin sodium).

**Methods**

**Ankaferd Blood Stopper**

Ankaferd Blood Stopper (Trend Teknoloji Ilaç 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).\textsuperscript{1} It produces active hemostasis for the management of external, postsurgical, and dental hemorrhage.\textsuperscript{3} ABS contains a standardized mixture of 5 medicinal plant extracts. The active ingredients in the ampoule form of ABS are as follows: 0.12 mg of dried root of *Urtica dioica*, 0.16 mg of dried leaf of *Vitis vinifera*, 0.18 mg of dried leaf of *Glycyrrhiza glabra*, 0.14 mg of dried leaf of *Alpinia officinarum*, and 0.10 mg of dried leaf of *Thymus vulgaris*.\textsuperscript{7}

This study used the liquid form of ABS supplied in 2-mL ampoules to achieve hemostasis in the animal nasal-bleeding model.

**Animals**

Twenty-eight male Wistar albino rats weighing between 180 and 260 g were used in this study. The animals were purchased locally from the Research Institute of Physiology (Gaziantep, Turkey). The rats were maintained in the Physiology Department animal house at a constant temperature of 22 ± 4°C with a 12-hour light/dark cycle and fed standard pellet chow and water ad libitum. The experimental protocol was instituted on the same day by cutting the anterior nasal septal mucosa unilaterally. Standardized, full-thickness mucosal wounds were created in the nasal septum with a 2-mm surgical punch over the anterior part of the nasal septum using slight pressure. The punch was rotated 90° clockwise and 90° counterclockwise to achieve a full-thickness mucosal cut, taking care not to damage the septal cartilage. The circular mucosal injury resulted in anterior nasal bleeding within 1 to 2 seconds in all rats. After the nasal bleeding was established, in groups 2 and 4, the mucosal wound was irrigated with 1 mL of ABS solution and pressed gently with sterile gauze tampons for 1 minute to enable the hemostatic effect of ABS. In groups 1 and 3, the wounds were irrigated with an equivalent volume of saline solution (1 mL) in the same way until hemostasis was achieved. The study groups are summarized in Table 1. Both the ABS and saline solutions were dispensed by a study nurse from similarly taped dark insulin injectors. The personnel who applied the study medication to the mucosal wounds and who examined and evaluated the bleeding were blinded to the medication.

**Bleeding Assay**

The parameters examined were the hemostasis time and amount of nasal bleeding. The interval between the start of bleeding (ie, cutting the anterior nasal septal mucosa) and the achievement of hemostasis was defined as the hemostasis time and was measured using a chronometer. Each tampon used in the experiment was weighed using a precision laboratory scale before and after the procedure by an investigator blinded to the treatment, and the difference in weight was used as a measure of the amount of bleeding. No further bleeding was observed from either nostril during the ensuing 30-minute period.

**Data Analysis**

Statistical analyses were carried out using the SPSS statistical package, version 15.0 (SPSS, Inc, an IBM Company, Chicago, Illinois) for Windows. The hemostasis time and amount of bleeding were compared among groups using the Mann-Whitney *U* test. The mean and standard deviation were calculated for each group. The heparin-pretreated and non-pretreated

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### Table 1. Outline of the Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Heparin Sodium Dose, IU/kg</th>
<th>Duration</th>
<th>Solution Administered for Nasal Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>Saline</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>ABS</td>
</tr>
<tr>
<td>3</td>
<td>640 tid IP</td>
<td>3 days</td>
<td>Saline</td>
</tr>
<tr>
<td>4</td>
<td>640 tid IP</td>
<td>3 days</td>
<td>ABS</td>
</tr>
</tbody>
</table>

Abbreviations: ABS, Ankaferd Blood Stopper; IP, intraperitoneally.
groups were compared separately using the Mann-Whitney U test. All data are expressed as the mean and 95% confidence intervals. A P value <.05 was considered statistically significant.

**Results**

In the non-heparin-pretreated groups (groups 1 and 2), ABS administration shortened the hemostasis time following nasal bleeding by 1.57 minutes or 62.1% (95% confidence interval [CI], 0.84-2.30 minutes) from the original time of 4.14 minutes (95% CI, 3.02-5.27; P = .003). With the heparin pretreatment (groups 3 and 4), ABS shortened the hemostasis time following nasal bleeding by 2.86 minutes or 63.6% (95% CI, 2.03-3.69) from the initial 7.86 minutes (95% CI, 6.61-9.10; P = .002; Table 2 and Figure 1).

Without the heparin pretreatment (groups 1 and 2), ABS administration decreased the amount of bleeding by 0.35 g or 39.7% (95% CI, 0.24-0.46 g) from the original 0.58 g (95% CI, 0.46-0.70; P = .006). With the heparin pretreatment (groups 3 and 4), ABS decreased the amount of nasal bleeding by 0.49 g or 43.7% (95% CI, 0.39-0.58) from the initial 0.67 g (95% CI, 0.57-0.96; P = .002; Table 2 and Figure 2).

**Discussion**

This study evaluated the hemostatic effects of ABS in vivo in a rat anterior epistaxis model with and without heparin pretreatment. Several vasoconstrictors can be used to manage epistaxis, including cocaine, oxymetazoline, xylometazoline, and phenylephrine.3,12-14 In recent years, a wide range of hemostatic agents with different action mechanisms have also been used effectively for the treatment of epistaxis. For example, Surgicel (Johnson & Johnson, Piscataway, New Jersey) and oxidized regenerated cellulose conform to rough surfaces and act to stabilize clots, facilitating hemostasis. FloSeal (Baxter Health Care, Deerfield, Illinois) has been used in controlling epistaxis. However, only 65% of epistaxis cases can be controlled by hemostatic agents alone.3,15,16 In a porcine epistaxis model, Singer et al5 demonstrated that octyl cyanoacrylate (OCA) was significantly effective in achieving hemostasis compared with a control group after full heparinization.

The traditional medicine ABS is an alternative treatment modality used for bleeding, such as external and dental surgical bleeding.6,8 It has also been used successfully in septorhinoplasty patients to minimize postoperative bleeding and to speed

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**Table 2.** Effect of Topical Ankaferd Blood Stopper (1 mL) on the Hemostasis Time and Amount of Nasal Bleeding in Rats Pretreated with Heparin Sodium Standard (640 IU/kg IP for 3 Days) and Non-Pretreated Rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ABS, Mean (95% CI)</th>
<th>Control</th>
<th>ABS vs Control, %</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemostasis time, min Heparin</td>
<td>2.86 (2.03–3.69)</td>
<td>7.86 (6.61–9.10)</td>
<td>63.6</td>
<td>.002</td>
</tr>
<tr>
<td>No heparin</td>
<td>1.57 (0.84–2.30)</td>
<td>4.14 (3.02–5.27)</td>
<td>62.1</td>
<td>.003</td>
</tr>
<tr>
<td>Amount of bleeding, g Heparin</td>
<td>0.49 (0.39–0.58)</td>
<td>0.87 (0.77–0.96)</td>
<td>43.7</td>
<td>.002</td>
</tr>
<tr>
<td>No heparin</td>
<td>0.35 (0.24–0.46)</td>
<td>0.58 (0.46–0.70)</td>
<td>39.7</td>
<td>.006</td>
</tr>
</tbody>
</table>

Abbreviations: ABS, Ankaferd Blood Stopper; CI, confidence interval; IP, intraperitoneal.

*Mann-Whitney U test.

**Figure 1.** The hemostatic effect of Ankaferd Blood Stopper (ABS; 1 mL) on the duration of nasal bleeding (hemostasis time) in rats pretreated with standard heparin sodium (640 IU/kg intraperitoneally for 3 days) and non-pretreated rats.

**Figure 2.** The hemostatic effect of Ankaferd Blood Stopper (ABS; 1 mL) on the amount of nasal bleeding (hemostasis time) in rats pretreated with standard heparin sodium (640 IU/kg intraperitoneally for 3 days) and non-pretreated rats.
A previous in vitro study clearly demonstrated that the addition of ABS to plasma did not affect coagulation factors II, V, VII, VIII, IX, X, XI, or XIII. ABS exerts its unique hemostatic effect by promoting the very rapid (<1 second) formation of a protein network, which acts as an anchor for vital physiological erythrocyte aggregation covering the classical cascade model of the clotting system and makes it an effective hemostatic agent.

ABS-induced protein mesh formation has no effect on the blood cells, coagulation factors, or platelets. It enables effective hemostasis in patients with normal hemostatic parameters, even in those with primary or secondary coagulation defects. However, the use of ABS in patients with hemostatic abnormalities has not been investigated extensively. The present in vivo study demonstrated that ABS has a hemostatic effect on normal and heparin-pretreated rats when applied with a compressive dressing in a rat epistaxis model. Bilgili et al. reported that a 40-second application of an ABS tampon was sufficient to stop the bleeding of skin lacerations, and 1.5- and 3.5-minute applications were used to control hemorrhage from the saphenous vein and artery, respectively, in a porcine model. Kurt et al. found that the topical use of ABS applied endoscopically completely controlled human upper gastrointestinal bleeding within seconds. In our study, the application of ABS solution was sufficient to stop nasal bleeding in normal rats in an average of 1.57 minutes, whereas an average of 2.86 minutes was required to control the epistaxis in heparin-pretreated rats.

The use of anticoagulant agents such as coumadin, enoxaparin, or heparin should be ascertained when evaluating epistaxis. These agents alter the coagulation cascade in the body. Heparin binding to the enzyme inhibitor antithrombin III (AT III) causes a conformational change, resulting in its activation. The activated AT III then activates thrombin and other proteases involved in the blood clotting system, most notably factor X. The in vivo hemostatic effect of ABS has been evaluated in rats as well as in a swine model. Kosar et al. administered ABS topically to the cut tails of rats to determine its effectiveness; in both acetylsalicylic acid– and enoxaparin-treated rats, the application of topical ABS reduced both the duration and amount of bleeding. Cipil et al. reported that the administration of topical ABS to an amputated leg shortened the duration of bleeding markedly in both untreated and warfarin-treated rats. Our data showed that ABS was also effective for treating nasal bleeding in rats pretreated with heparin sodium, implying that ABS irrigation overcomes the actions of this agent given systemically. After confirming continuous bleeding from the anterior nasal septal mucosa in the heparin-pretreated groups, the application of ABS solution resulted in rapid hemostasis (Figures 3 and 4, respectively). The effect of ABS was significant compared with the respective controls in both shortening the hemostasis time and decreasing the bleeding volume. Our study is unique in that it explored the hemostatic effect of ABS in rats with nasal bleeding in both normal animals and animals pretreated with heparin sodium.

**Conclusion**

Ankaferd Blood Stopper was more effective than saline irrigation for obtaining the hemostasis of anterior nasal bleeding with a compressive dressing in both nonheparinized and heparinized animals. ABS, an effective, safe, and rapid-acting hemostatic agent, might be an alternative to the other hemostatic agents for managing epistaxis. Moreover, it has potential as an adjunct for achieving hemostasis during episodes of epistaxis in patients with coagulopathy due to defective platelets or coagulation factors. Future controlled clinical trials should evaluate these effects.

**Limitations**

There are several limitations to this study. First, it is difficult to ascertain the effectiveness of applying a compressive dressing in humans with nasal bleeding for several reasons. The patient may not keep the compress on the nasal mucosa continuously, and the distribution of the gauze may not confer
adequate pressure to the mucosal surface. Therefore, it may have been more realistic to use a gel impregnated with ABS rather than a compressive dressing. Second, in coagulopathic patients, the area of nasal bleeding is completely different from the limited circumscribed region created with the punch biopsy in this study; such patients tend to bleed from a large surface area rather than one limited area. Therefore, our data may be limited to the animal epistaxis model and not fully represent real life.

**Author Contributions**

Ismail Iynen, acquisition of data, analysis and interpretation of data, and final approval of the version to be published; Ozgur Sogut, conception and design, analysis and interpretation of data, and manuscript revision; Rustu Kose, conception and design, and manuscript revision.

**Disclosures**

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**References**


