Effectiveness of Ankaferd blood stopper in the topical control of active bleeding due to cutaneous-subcutaneous incisions

Behcet Al, MD, Cuma Yildirim, MD, Murat Cavdar, MD, Suat Zengin, MD, Hasan Buyukaslan, MD, Mehmet E. Kalender, MD.

ABSTRACT

الأهداف: تقييم فعالية طريقة إيقاف نزيف الدم باستعمال طريقة انكافيرد (ABS) في التحكم النمطي للنزيف نتيجة للقطع الجلدي تحت الجلد.

الطريقة: قمنا باستعمال كمادة مبللة من (ABS) لدى 37 و إسفنج تعقيم عادية لدى 32 مريض تقرراً توقيف النزيف لديهم الذي ظهر خلال تركيب المعبر الوعائي المشار سريرياً للمرضى المصابين بالسرطان. تم تسجيل معدل النجاح للتحكم في النزيف والوقت المطلوب لإيقاف النزيف ومعاودة النزيف والمضاعفات.

النتائج: شملت هذه الدراسة إجمالي عدد 69 مريض. بلغ معدل الوقت المطلوب لإيقاف النزيف 29.9±32.97 ثانية لمجموعة (ABS) و 47.5±123.75 ثانية للمجموعة التي تم استعمال الإسفنج العادي المعقم. عاود النزيف مرة أخرى في 24% من مجموعة (انكافيرد) و 50.0% من المجموعة التي استعملت لديها الإسفنج المعقم العادي. من بين المرضى الذين طبقت لهم طريقة اناكفريد بشكل نمطي. 13.5% من المرضى تعرضوا لاحمرار موضعي و8.1% أصيبوا بانتفاخ بسيط بينما تعرض 8.1% لألم موضعي في موضع الجرح: لدي 5.4% من المرضى كانت الغرز في موضع الجرح مفتوحة. تم تسجيل نفس القياسات لدى المرضى الذين تلقوا إجراء الإسفنج المعقمة حيث كانت القياسات كالتالي 9.4%، و0.0%، 6.2%، و3.2% على التوالي (p=0.592).

خاتمة: تم إثبات طريقة انكافيرد لإيقاف نزيف الدم في وقت قياسي وبمعدل رجعة ضئيل مقارنة مع الإسفنج المعقم.

Objectives: To assess the effectiveness of Ankaferd blood stopper (ABS) in the topical control of bleeding due to cutaneous/subcutaneous incisions.

Method: We included in this study, 69 patients with cancer that were admitted for port insertion to the Emergency Department of Gaziantep University Hospital, Gaziantep, Turkey, between May and July 2008. We used the wet compress form of ABS in 37 patients (group I), and regular dry sterile sponges in 32 patients (group II), to stop the bleeding that occurs during the clinically indicated vascular port insertion in patients with cancer. The success rate in terms of bleeding control, time needed to stop the bleeding, recurrence of bleeding, and complications were recorded.

Results: A total of 69 patients were included in this study. The average time needed to stop the bleeding was 32.97 ± 29.9 seconds for group I, and 123.75 ± 47.5 seconds for group II. Bleeding restarted in 24% in group I, and in 50% in group II. Among the patients in group I, 13.5% developed localized redness, and 8.1% minor swelling, while 8.1% reported local pain at the wound site; in 5.4% of the patients, the sutures at the wound site opened. The same parameters were recorded for group II; 9.4% for localized redness, 0.0% for minor swelling, 6.2% reported local pain, and the sutures at the wound site opened in 3.2% (p=0.592).

Conclusion: The Ankaferd blood stopper was proven to stop local bleeding in a shorter time, with a lower recurrence rate in comparison with the sterile sponge.

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From the Emergency Department (Al, Yildirim, Cavdar, Buyukaslan), the Oncology Department (Zengin), Department of Medicine Faculty, Gaziantep University, Gaziantep, and the Emergency Department (Kalender), Malatya State Hospital, Malatya, Turkey.

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Address correspondence and reprint request to: Dr. Behcet Al, Emergency Department, Department of Medicine Faculty, Gaziantep University, Gaziantep, Turkey. Tel. +905 (33) 8107690. Fax +903 (42) 3602938. E-mail: behcetal@gmail.com

The medicinal value of plants lies in some chemical substrates that produce a definitive physiological action on the human body.[1] The use of plant extracts and phytochemicals, with established properties, could be of great significance in preventive, or therapeutic approaches.[2-7] Based on the World Health Organization (WHO) reports, more than 80% of the world population relies on traditional medicine for their primary healthcare needs.[3,8,9] Ankaferd is a unique folkloric medicinal plant extract, which has historically been used in Turkish traditional medicine as a hemostatic agent, usually for hemorrhagic skin wounds.[10] Ankaferd comprises a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum,* and *Urtica dioica*.[10] The use of Ankaferd blood stopper (ABS) as a modern medicinal product has only recently gained momentum in Turkey. Ministerial approval was obtained for the management of dermal, external traumatic/post surgical, and dental bleedings, after the results of studies demonstrated its safety and efficacy as a topical agent (www.ankaferd.com). The safety and efficacy reports on the product have indicated its sterility and non-toxicity.[10] The ABS has been shown to promote the formation of an encapsulated protein network, which acts as an anchor for erythrocyte aggregation, without significantly interfering with individual coagulation factors.[10] Exposure to Ankaferd seems to provide tissue oxygenation, as well as a physiological hemostatic process without affecting any individual clotting factor.[10] The aim of this study is to assess the hemostatic efficacy of the wet tampon form of ABS in the control of bleeding associated with subcutaneous surgical dissection.

Methods. In present study, we assessed the effectiveness of ABS in the topical control of bleeding due to cutaneous/subcutaneous incisions. Between May and July 2008, 69 patients (of which 52% were female, and 38% male) with cancer admitted to the Emergency Department of Gaziantep University Hospital, Gaziantep, Turkey for port insertion was included in this study. Prior to the procedure, blood pressure, pulse, and respiratory rates were measured in all patients. The blood workup included a complete blood count, chemistry parameters, and prothrombin time (PT) (INR) measurement. In all patients, the port was inserted from the supraclavicular region, with the right hemithorax being the first choice in most patients. Prior to the procedure, all patients were sedated using midazolam (Demizolam® amp [Dormicom 5 mg/ml] [Roche, Basel, Switzerland]), 5 mg/5 mL) 2 mg intravenous (IV), administered in 30 seconds, and ketamine HCL (Pfizer-Ketalar® vial, [Pfizer, Luleburgaz, Turkey] 50 ml/ml) 2-4 mg/kg IV was used for anesthesia. The site of insertion was cleaned using polyvinylpyrrolidone iodine solution and dried using a sterile sponge, then local anesthesia (40 mg/2 mL lidocaine HCl + 0.025 mg/2 ml adrenaline = Jetokain® amp (ADEKA, Samsun, Turkey) was applied. A 2.5 wide to 0.5 to 1.5-cm-deep cutaneous-subcutaneous incision was made. To stop the bleeding, either ABS, or a regular sterile sponge was used. For this purpose, we used half of the sterile wet compress form of ABS with 2.5 x 7 cm dimensions, which contains 3 ml Ankaferd active ingredient (composition: *Urtica dioica* [dried root extract] 0.18 mg, *Vitis vinifera* [dried leaf extract] 0.24 mg, *Glycyrrhiza glabra* [dried leaf extract] 0.27 mg, *Alpinia officinarum* [dried leaf extract] 0.21 mg, *Thymus vulgaris* [dried grass extract] 0.15 mg). The study samples were manufactured by Ankaferd Drug Inc. Istanbul, Turkey, under the patent number 25.05.2007/32. For the comparison between groups, we used regular sterile sponges with the same dimensions. Thirty-seven patients (group I) were administered ABS and 32 patients (group II) were administered regular sterile sponges. The compresses were placed over the bleeding site and normal pressure was applied. The compresses were briefly removed every 20 seconds to check the bleeding. The compress was applied for a minimum of 20, and a maximum of 240 seconds. The effectiveness of bleeding control and the time needed to stop the bleeding (in seconds), and recurrence of bleeding was recorded. The patients were kept under observation for 2 hours in the emergency department, and discharged after their vital signs became stable. The patients were scheduled for wound care every 2 days, and the sutures were removed on day 10. The patients were followed up for one month, and any complication (redness, swelling, edema, itching at the incision site, and opening of sutures) was documented. The results were compared between the 2 groups. The same individual performed all the procedures. This study was reviewed and approved by the Ethics committee and informed consent was obtained from all the studied subjects.

Statistical analysis. For the statistical analysis, Social Package for Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, IL., USA) was used. All continuous parameters were expressed as mean ± standard deviation, and absolute numbers were indicated as percentages. Student's t-test was used to compare the parameters, and chi-square test was used to compare absolute numbers. The comparison between the variables was made using Pearson's correlation test. A $p<0.05$ was considered statistically significant.

Results. The mean age of patients was 50 ± 13 years (15-70 years) and mean body weight was 69.51 ± 11.07 kg (42-95 kg). The great majority of the patient

population was diagnosed with colon (31.9%) and laryngeal cancer (16%), as well as leukemia (16%). In 91.3% of our patients, the procedure was performed to the right hemithorax, and in 8.7%, to the left hemithorax. The average time needed to stop bleeding in group I was 32.97 ± 29.9 (median 20.00) seconds (20-200 seconds). This result was significantly shorter than the control group (p=0.001, confidence interval [CI]%; 109.589 - 71.965). In group II, the average time needed to stop the bleeding was 123.75 ± 47.5 seconds (60-240 seconds). Recurrence of bleeding was significantly less frequent in group I (24.3%) when compared to group II (p=0.027, CI%; 0.483 - 0.030). Bleeding restarted in 50% of the patients in group II. In all but one group I patient (97.3%), bleeding could be stopped in the first 40 seconds. In 43.7% in group II, bleeding stopped after 120 seconds; in no patient in this group, could bleeding be stopped in the first 60 seconds (**Table 1**). In group I, 13.5% had localized redness, 8.1% had minor swelling, and 8.1% had local pain at the wound site; in 5.4% of the patients, the sutures at the wound site opened. In the regular sterile sponge group, these complications were reported in 9.4% for localized redness, 0.0% for minor swelling, 6.2% reported local pain, and the sutures at the wound site opened in 3.2% of the patients. Although the number of complications was greater in group I, the difference was not statistically significant (p=0.592). In this study, only 5 patients were thrombocytopenic with platelet counts between 20-70 x 10^3/μL; all the remaining patients had laboratory test results that were within the normal interval. No patient was found to have a coagulation disorder.

Discussion. Ankaferd blood stopper as a medicinal product has been approved in the management of external hemorrhage and dental surgery bleedings in Turkey. Safety and efficacy reports indicate its sterility and non-toxicity.[10] Moreover, ABS has had a historical role in the traditional Turkish medicine as a topical hemostatic agent, usually for hemorrhagic infected skin wounds over the centuries.[1,10] There are some hemostatic drugs, including anti-fibrinolytic amino acids (tranexamic acid and aminocaproic acid), aprotinin, and desmopressin. They are used in cardiac surgery, internal bleedings, or congenital bleeding disorders, but they are not intended for local topical use.[11] Topical hemostatic agents, such as fibrin tissue adhesives, collagen, thrombin, and prothrombin is not easily available. Some of them have limited efficacy, and some others (for example, fibrin glue) cannot be produced on an industrial scale because human blood is used as its source. Biologic materials also have the risk of infectious contamination. For these reasons, there is a need for new effective topical agents. Due to the expensive production and the risk for transmission of infectious biological agents, some research has focused on plants. A few plant extracts with proven topical hemostatic effect have been reported.[12-14]

The present clinical study confirmed the efficacy of ABS to achieve homeostasis in external hemorrhage (including skin and sub-dermal injury). Our results show that indications of ABS as a promising surgical hemostatic agent may be expanded for use in other bleeding models, including skin, visceral, arterial, and venous injury in the near future. This agent may also be used in internal bleeding. The ABS has been shown to promote the formation of an encapsulated protein mesh that acts as an anchor for erythrocyte aggregation.[10] Ankaferd does not significantly interfere with individual coagulation factors. That unique function makes this hemostatic agent useful in patients with congenital, or acquired coagulation defects. The ABS comprises a standardized mixture of 5 plants, each with some hematological and vascular actions. *Glycyrrhiza glabra* has anti-inflammatory, anti-thrombin, anti-platelet, anti-oxidant, anti-atherosclerotic, and anti-tumor activities.[15-19] It inhibits angiogenesis, decreases vascular endothelial growth factor production, and cytokine induced revascularization.[20] *Thymus vulgaris* has anti-oxidative actions, such as prevention of lipid peroxidation.[21] *Vitis vinifera* exerts anti-tumor and anti-atherosclerotic effects.[22,23] *Alpinia officinarum* inhibits nitric oxide production by lipopolysaccharide activated mouse peritoneal macrophages.[24] *Urtica dioica* causes vasodilatation by inducing nitric oxide production by the endothelium.[25] The ideal topical hemostatic agent should be easy to use (even in the battlefield), show its effect within minutes, be effective in both arterial and

Table 1 - The comparison of bleeding time using blood stopper and sponge.

Time, seconds	Blood stopper n (%)	(%total)	Sponge n (%)	(%total)	Total n (%)
20	21 (56.8)	(30.4)	0 (0)	(0)	21 (30.4)
40	15 (40.5)	(21.7)	0 (0)	(0)	15 (21.7)
60	0 (0)	(0)	6 (18.8)	(8.7)	6 (8.7)
80	0 (0)	(0)	4 (12.5)	(5.8)	4 (5.8)
120	0 (0)	(0)	8 (25)	(11.6)	8 (11.6)
140	0 (0)	(0)	7 (21.9)	(10.1)	7 (10.1)
160	0 (0)	(0)	1 (3.1)	(1.5)	1 (1.5)
180	0 (0)	(0)	3 (9.4)	(4.3)	3 (4.3)
200	1 (2.7)	(1.5)	2 (6.2)	(2.8)	3 (4.3)
240			1 (3.1)	(1.5)	1 (1.5)
Total	**37 (100)**	**(53.6)**	**32 (100)**	**(46.4)**	**69 (100)**

venous bleeding, and be non-toxic and anaphylactic. Its effect should also be long-term. The current experiment trials and our present study showed that ABS has all those properties. Furthermore, it is economical, has a long shelf life, and does not require a special medium for conservation. The duration of bleeding is known as an indicator of the effectiveness of platelet-thrombus formation. Therefore, a prolonged duration of bleeding may show the presence of severe thrombocytopenia, platelet dysfunction syndromes, vascular defects, or mixed abnormalities such as von Willebrand's disease.

It was reported in different studies that ABS induced very rapid (<1 second) formation of protein network in the plasma and serum samples.[10,26] Coagulation factors (II, V, VII, VIII, IX, X, XI, and XIII) were not affected by the addition of ABS to plasma.[10] Additionally, total protein, albumin, and globulin levels showed significant decreases after the addition of ABS. In these studies, it was observed that this ABS induced protein network was capable of regulating further coagulation and hemostatic reaction. The ABS-induced network formation depended on interactions between ABS and blood proteins, mainly fibrinogen, and indicated that ABS could affect both fibrinogen and other proteins, possibly via agglutination of these molecules. The ABS-induced network formation is related to the functions of blood proteins and red blood cells. The basic mechanism of action for ABS appears to be the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation.[10] Blood cells (erythrocytes and platelets) also aggregated and participated in the network formation, with the erythrocytes forming a mass. Therefore, it is reported that ABS may be effective both in individuals with normal hemostatic parameters, and in patients with deficient primary homeostasis/or secondary homeostasis, including patients with disseminated intravascular coagulation. This unique mechanism of action provides ABS with an advantage over other hemostatically active plant extracts.[27,28]

Ucar Albayrak et al[29] reported that fibrinogen is not a necessary component of network formation, so ABS can be used successfully in patients with afibrinogenemia. They could stop the active bleeding in a patient with afibrinogenemia. Since the patient whittled her finger, the bleeding could not be stopped with suturing and dressing, before ABS application. It was recommended in different studies that ABS could be used in the treatment of Kirim-Kongo hemorrhagic fever. The preliminary results were found to be promising, given its anti-infective and hemostatic efficacy, even with defective platelets, or coagulation factors.[10,26,30-34] The *in vivo* effect of the ABS-induced protein network on the damaged and actively bleeding hepatic tissue in porcine liver-injury modeling comparison with scanning electron microscopy ultrastructural analyses was reported.[35] After the first premolar tooth was extracted, the immediate cessation of bleeding was observed via the topical application of ABS.[35] Cipil et al[36] examined the *in vivo* effects of ABS in anticoagulated rats *in vivo*. First, they separated rats in 2 groups (pretreated with Warfarin and control group), and then amputated the rats' legs to evaluate the duration and amount of bleeding. The ABS shortened the duration of bleeding by 31.9%, and 43.5% in the untreated group and the Warfarin-treated group. The ABS was significantly more effective in shortening the duration of bleeding as compared to the control, where its efficacy was more pronounced in the Warfarin-treated group than the untreated group. Kosar et al[37] administered ABS to the rats treated with acetylsalicylic acid (ASA), enoxaparin, untreated group, and their control subgroups to which saline was administered. The ABS shortened the duration of bleedings by 68.4% (ASA-treated group), 30.6% (enoxaparin-treated group), and 92.9% (untreated group) according to subgroups that saline was administered. Moreover, a very recent clinical case by Kurt et al[26] presented a 52-year-old man who had signs of recent bleedings upon upper gastrointestinal endoscopic examination. The bleeding was controlled by administering topical ABS (15 mL) successfully. An endoscopy during the follow-up did not reveal any stigmata of bleeding. In the present study, we observed that ABS was beneficial as a topical hemostatic agent in skin bleedings. The ABS was found effective in shortening the duration of bleeding, decreasing the amount of bleeding, and reducing the re-bleeding in external hemorrhage

Although ABS was administered in patients with hemophilia A,[38] acquired hemorrhage diathesis,[39] and arteriovenous malformation,[40] endobronchial bleedings,[41] successful results were obtained. Notably, it was observed that ABS had effective antibacterial,[42-44] and antifungal[45] effects. Bleeding can cause significant morbidity and mortality in any clinical setting.

The limitation of the present study was the small number of subjects, and the effectiveness of ABS that was tested only in the topical control of bleeding due to cutaneous/subcutaneous incisions.

Ankaferd blood stopper, a traditional folkloric medicinal plant extract, is a novel effective hemostatic agent that has the therapeutic potential to be used in the management of hemorrhage. Future preclinical and clinical studies are recommended to disclose the long-term hemostatic effect and potential toxicity of ABS. If anti-inflammatory, anti-thrombin, anti-platelet, anti-oxidant, anti-atherosclerotic, and anti-tumor activities, anti-angiogenesis, anti-vascular endothelial growth factor production, anti-oxidative actions, anti-

tumor, anti-atherosclerotic effects, and anti-nitric oxide production of ABS shall be searched in the *in vivo* and *in vitro* studies; this agent can find more fields for itself.

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