Original Article

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Investigation of The Effectiveness of Plant Based Algan Hemostatic Agent in a Rat Model of Femoral Arterial Bleeding

Ratlarda Femoral Arter Kanama Modelinde Bitki Bazlı Algan Hemostatik Ajanın Etkinliğinin Araştırılması

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ABSTRACT

Objective: The aim of this study is to evaluate the efficacy of the Algan Hemostatic Agent (AHA) available in three different physical form (liquid, powder and sponge absorbed) in the femoral artery incision model in rats.

Methods: A total of sixty-four 5-7 weeks old rats were used in the study. Rats were randomly divided into 8 groups each consisting of eight rats (4 groups heparinized and 4 groups non-heparinized). An experimental femoral artery incision was created. As a control, physiological saline absorbed sponge was applied. AHA liquid, AHA powder and AHA sponge absorbed forms were applied to the experimental groups.

Results: Upon application to the bleeding sites, all the AHA forms stopped bleeding in a significantly shorter time compared to the control group ($p<0.05$). In contrast, bleeding in control group could not be controlled within 4 minutes. The best result was in AHA powder form and it was able to control bleeding in the non-heparinized group at 87.5% in the first minute and 12.5% in the second minute. In the heparinize group, the AHA powder form was able to control the bleeding at 62.5% in the first minute and 37.5% in the second minute.

Conclusion: This study shows that AHA is a highly effective and promising hemostatic agent in bleeding control.

Keywords: Algan, hemostasis, femoral artery, rat

ÖZ

Amaç: Bu çalışmanın amacı, sıçanlarda femoral arter insizyon modelinde üç farklı fiziksel formda (sıvı, toz ve spançа emdirilmiş) bulunan Algan Hemostatik Ajanın (AHA) etkinliğini değerlendirmektir.

Yöntemler: Çalışmada toplam 64 adet 5-7 haftalık rat kullanıldı. Ratlar rastgele olarak her biri sekiz rattan oluşan 4 gruba (4 grup heparinize ve 4 grup nonheparinize) ayrıldı. Deneysel femoral arter insizyonu oluşturuldu. Kontrol olarak serum fizyolojik emdirilmiş spanç uygulandı. Deney gruplarına AHA sıvısı, AHA tozu ve AHA sıvısı emdirilmiş spanç formları uygulandı.

Bulgular: Kanama bölgelerine uygulanan tüm AHA formları kontrol grubuna göre anlamlı olarak daha kısa sürede kanamayı durdurdu ($p<0.05$). Buna karşılık, kontrol grubunda kanama 4 dakika içinde kontrol edilemedi. En iyi sonuç AHA toz formundaydı ve heparinize olmayan grupta kanamayı birinci dakikada %87,5 ve ikinci dakikada %12,5 oranında kontrol edebildi. Heparinize grupta AHA toz formu birinci dakikada %62,5, ikinci dakikada ise %37,5 oranında kanamayı kontrol edebildi.

Sonuç: Bu çalışma, AHA'nın kanama kontrolünde oldukça etkili ve umut verici bir hemostatik ajan olduğunu göstermektedir.

Anahtar Szöcükler: Algan, hemostaz, femoral arter, rat

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Introduction

Acute excessive bleeding is still considered the leading preventable cause of death in modern medical practice. Recent efforts in preoperative car- and trauma-related hemorrhages have provided some improvements in minimizing undesired incidences. Uncontrolled hemorrhage due to large vascular damage is the most commonly known cause of death in patients with serious trauma. Additionally, the incidence of death as a consequence of uncontrolled bleeding is preventable with appropriate intervention measures in a short period before an excessive blood loss occurs, as trauma-related death incidences occur within the first 6 h of trauma (1). Therefore, quick but effective bleeding control is critically important to minimize the mortality rate. In addition to conventional techniques, such as cauterization, pressure application, and ligation to stop bleeding, the use of fast-acting local hemostatic agents provides additional therapeutic advantages. Many studies have been conducted to reveal the effect and safety of locally acting hemostatic agents over the last decades (2-7).

Nowadays, several hemostats in different physical forms, e.g., powder, liquid, and gel, are commercially available in the market worldwide. Active hemostatic agents (Active HAs) contain a drug active substance, such as thrombin, which is available either as a sole agent or in a combination with a medical device, a mechanically acting hemostat. A gelatin sponge carrying thrombin is an example of a combination product that can be directly applied to bleeding sites (8-10). Thrombin containing active HA releases a high concentration of thrombin to the bleeding site. Thrombin converts fibrinogen to fibrin that facilitated the deposition of a fibrin clot at the bleeding site (1). Due to thrombin's role in the coagulation cascade, thrombin containing active HAs can be ideal treatment options for patients with coagulopathies including clotting factor deficiencies other than those with hypofibrinogenemia, platelet dysfunction, or antithrombotic medications (11). Thrombin source is critically important. For instance, bovine thrombin has been associated with immunologic reactions. Additionally, the use of human or animal-sourced thrombin carries out the risk of contamination with infectious disease agents such as bovine spongiform encephalopathy (11,12). Immunological reactions due to human and bovine-derived topical thrombin products have been reported in several studies that focus on cardiothoracic surgery (13,14).

Mechanical HAs (MHAs) exert their function by forming a physical barrier matrix at bleeding sites. Collagen, oxidized cellulose, polysaccharide spheres, and gelatin products are among the well-known MHAs available in the market (1,11). The matrix formed by MHAs over the bleeding site activates the extrinsic clotting pathway and facilitates additional setting for platelet aggregation, which in turn accelerates clot formation (13). Additionally, polysaccharide beads absorb free water that facilitates the accumulation of proteins and platelets in wound edges vicinity (13,15). Therefore, MHAs should be kept in place until clot formation occurs and gently removed to avoid clotting disturbance and bleeding recurrence (11,16). Due to the absence of an active substance as a coagulation factor, MHAs were considered the most effective first-line treatment option for minimal bleeding. The use of MHAs is only appropriate in patients with an adequately functioning coagulation system (6,16).

Despite the major advances in medicine and many hemostatic products available in the market, an ideal hemostatic product has not been introduced into the market yet, thus developing an effective hemostatic product is necessary. An ideal hemostatic product should be effective with a fast-acting mechanism, safe, cost-effective, and easy to use.

Algan hemostatic agent (AHA) is a polysaccharide-based herbal extract. Several studies tested the efficacy of hemostatic products in a model of femoral artery bleeding in animals (17). This study aimed to evaluate the effectiveness of AHA in a rat model of femoral artery bleeding.

Methods

Animals

This study was conducted following the Local Ethics Committee of Animal Experiments as specified in the literature (Kırıkkale University Animal Experiments Local Ethics Committee, protocol number: 2018/16). This study used 64 180-210 grams and 5-7 weeks old rats. Rats were fed ad libitum and were housed under standard laboratory conditions with a 12-12-hour dark-light cycle.

The rats were first divided into two groups of random heparinized and non-heparinized, each include 32 animals. The subjects were then divided into eight groups each consisting of 8 randomly selected subjects (Table 1). The heparinized group received heparin intraperitoneally at 640 IU/kg daily for 3 days. No other procedure has been performed.

The procedures were performed under general anesthesia that is induced with a combination of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). At the end of the study, the rats were killed with 100 mg/kg intravenous sodium thiopental (Pental Sodium®, I.E. Ulagay).

Bleeding Test

The right inguinal region of the rats was shaved and wiped with Batticon, and the femoral vein and artery were exposed by cutting

Table 1. Animal groups

Groups	Explanation
Group 1	Non-heparinized control
Group 2	Heparinized control
Group 3	Non-heparinized AHA powder
Group 4	Heparinized AHA powder
Group 5	Non-heparinized AHA liquid
Group 6	Heparinized AHA liquid
Group 7	Non-heparinized AHA liquid impregnated sponge
Group 8	Heparinized AHA liquid impregnated sponge

AHA: Algan Hemostatic Agent

the skin and subcutaneous tissues. The bleeding duration was evaluated according to the literature protocol (18). The femoral artery was damaged by a green-colored injector tip. As soon as the bleeding began, another person pressured the area with a sponge for 10 s. Once the sponge is removed, the AHA powder, liquid, and AHA-impregnated or saline-impregnated sponges were applied to this area and light pressure was applied to the region. Bleeding was checked after 1 min of time initiation. If bleeding stopped, it was recorded as "first minute-controlled bleeding."

If the bleeding continues in the first application, the same amount of material was added, and the compression was continued for another minute. The bleeding was checked and was recorded as "controlled bleeding at the second minute" if the bleeding stopped. If bleeding continues, the same procedure was performed for the third time and waited for 2 min. The applications are shown in Figures 1-4. Finally, if the bleeding stopped, it was recorded as controlled bleeding at the fourth minute. If the bleeding persists after the fourth minute, it was recorded as unsuccessful. After the application in the form of AHA liquid, the bleeding area was not pressed and was left open. In addition to the results of the second and fourth minutes, the time of bleeding was measured with the stopwatch.

Statistical Analysis

The Statistical Package for the Social Sciences software version 22.0 (SPSS Inc., Chicago, IL) was used to analyze the data. The bodyweight and bleeding time were calculated, and the mean values were compared among the four groups using variance analysis. The Duncan test was used for multiple comparisons in case of differences. The results were evaluated at a 95% confidence interval. A p-value of <0.05 was considered significant.

Results

No differences were found among the groups in terms of body weight. The AHA powder form gave the best result in the heparin-free group in achieving hemostasis at 87.5% and 12.5% success in the first and second minutes, respectively. This rate was 62.5% and 37.5% in the heparinized group in the first and second minutes, respectively. AHA powder, liquid, and sponge forms were found to be more effective in bleeding control than the control group (P<0.05). Additionally, the AHA powder form was more effective in bleeding control than the AHA fluid and AHA sponge form (P<0.05). Results were given in Table 2 and Figures 1, 2, 3, 4.

Discussion

AHA is a plant-based hemostat that is produced in various forms and used in case of many types of bleeding. The AHA powder, sponge absorbed, and liquid forms used in this study were very effective in hemostasis in a rat model of femoral artery bleeding.

A variety of hemostatic agents with varying contents and mechanisms of action are available (19). An active substance, e.g., thrombin

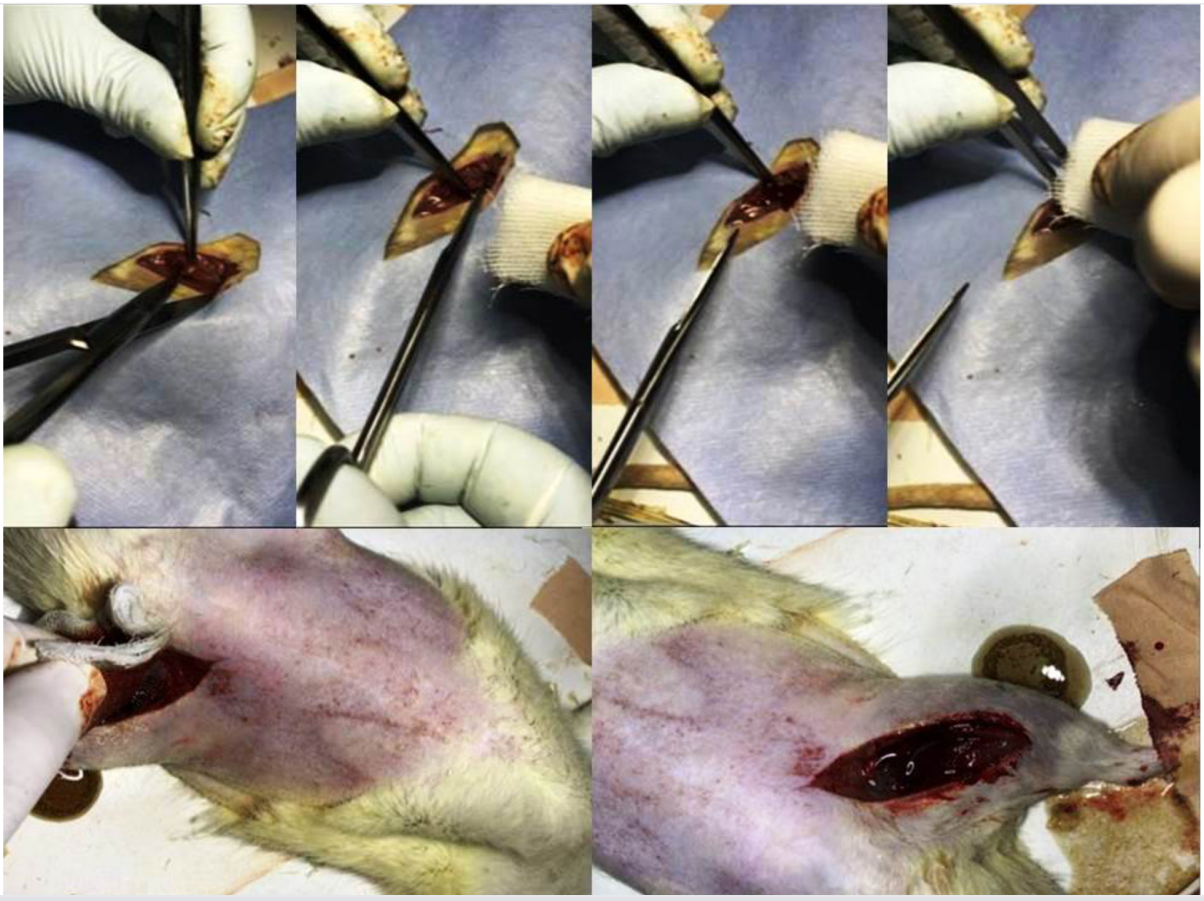

Figure 1. Dry sponge application after bleeding in the control group. Bleeding continues with removal of sponge

Table 2. Homeostasis times of groups

		Bleeding controlled at 1 minutes.	Bleeding controlled at 2 minutes.	Bleeding controlled at 4 minutes.	Unsuccessful
Non-heparinized	Control	0 (0%)	0 (0%)	0 (0%)	8 (100%)
	AHA powder	7 (87.5%)a	1 (12.5%) a	0 (0%) a	0 (0%)
	AHA liquid	3 (37.5%)b	4 (50%) b	2 (12.5%) b	0 (0%)
	AHA sponge	2 (25%) b	4 (50%) b	2 (25%) b	0 (0%)
Heparinized	Control	0 (0%)	0 (0%)	0 (0%)	8 (100%)
	AHA powder	5 (62,5%)d	3 (37,5%) d	0 (0%)d	0 (0%)
	AHA liquid	1 (12.5%)e	5 (62.5%) e	2 (25%)e	0 (0%)
	AHA sponge	2 (25%)e	4 (50%) d	2 (25%)e	0 (0%)

a, b, c, d, e, f Mean within in the same column with different letters are statistically significant (P<0.05)

containing HAs, releases thrombin to the bleeding sites in a high concentration that directly converts fibrinogen into a fibrin clot (11,20). Active HAs are probably the best option to intervene in bleeding in patients with coagulation factor deficiencies (13). However, thrombin has side effects, such as fostering infection and facilitating immunological reactions (11,12). Several immune reaction developments are reported as a consequence of hemostats produced of human or bovine thrombin (13,14). A prospective observational study investigating the immunogenicity of topical use of bovine thrombin has reported the presence of antibodies in patients who have undergone coronary artery bypass graft and valvar cardiac surgery (13). Patients who had cardiothoracic surgery have postoperatively increased anticardiolipin antibodies within 4-8 weeks of operation in 56% of patients who receive bovine thrombin, but anticardiolipin antibodies postoperatively increased only in 9% of patients without topical thrombin (14). Observing the establishment of successful hemostasis within 3-10 min in cardiothoracic surgery, a randomized clinical trial reported a similar success rate of hemostasis for topical human-derived (Evithrom) and bovine-derived thrombin (Thrombin-JMI). However, the number of patients who used Thrombin-JMI (13%) was significantly higher compared to those who used Evithrom (3%) using antibody development, which remains a major concern in bovine-derived thrombin products despite clear achievement in hemostasis. Clinically notable AHA side effects are undetermined immunological reactions.

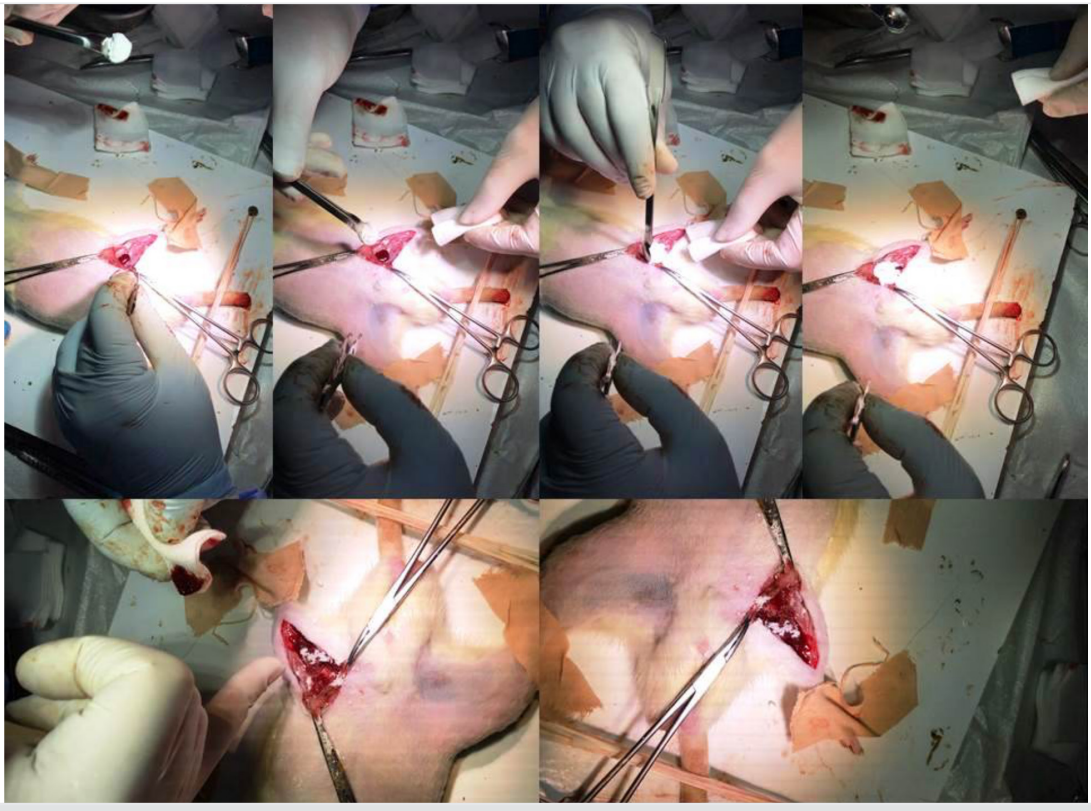

Figure 2. Femoral arterial hemorrhage in the bleeding area before AHA powder application. Controlled appearance of femoral arterial hemorrhage following AHA powder administration

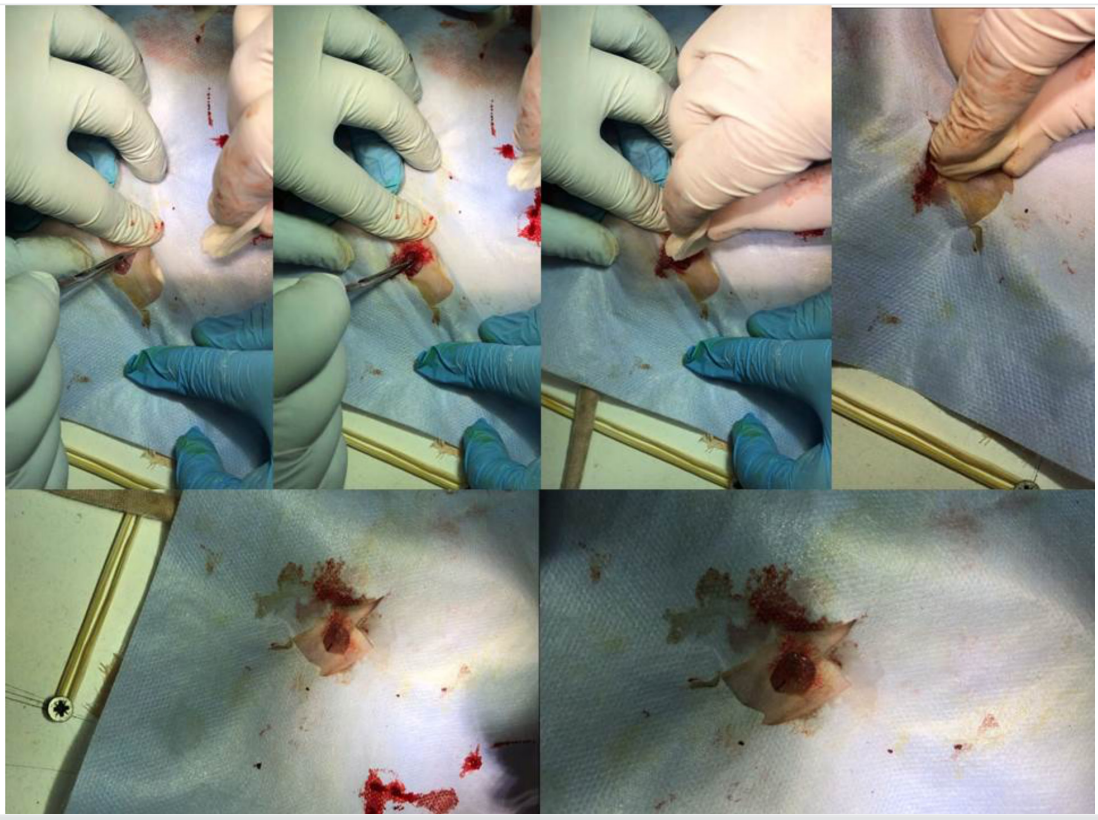

Figure 3. Femoral arterial hemorrhage in the bleeding area before AHA sponge application. Controlled appearance of femoral arterial hemorrhage following AHA sponge administration

Mechanical HA agents do not contain an active drug substance and form a physical barrier over the bleeding site. Several MHA, which is produced from various chemicals, including oxidized cellulose, gelatin, polysaccharide spheres, and collagen, are available in the market (11,12). The matrix barrier activates the extrinsic clotting pathway and facilitates the formation of additional settings for platelet aggregation, which in turn expedites clotting plug formation (13). Additionally, polysaccharide beads absorb free water that facilitates protein and platelet accumulation at the wound edges (13). MHAs should therefore be kept in place until clotting plug formation and gently removed to avoid clotting disorder and bleeding recurrence. Since mechanical HAs do not contain a specific active drug substance for blood coagulation, they are most effective as a first-line treatment option for minimal bleeding. Importantly, mechanical HAs are considered appropriate to use in patients with an adequately functioning coagulation system (12,16).

According to the hemostatic report, AHA primarily provides hemostasis by forming a mechanical barrier at the bleeding site as a hemostatic agent. Additionally, AHA activates both intrinsic and extrinsic clotting pathways to facilitate a strong hemostatic effect.

Among the types of hemostatic agents are synthetic sealants that are commonly used to prevent suture hole bleeding in cardiac and vascular surgeries. They form a watertight barrier at bleeding sites and are used as tissue adhesives (12).

This study used three different physical forms of AHA, which were in powder, liquid, and sponge. A significant statistical difference was found compared to the control group, thus all AHA forms were very effective. However, the powder form controlled the bleeding more quickly than AHA liquid and sponge forms. However, no compressions were made to the bleeding area in the liquid group. The bleeding duration in the control group was very long compared to the AHA experimental groups. In internal hemorrhages that cannot be compressed, the liquid form gave hope as an effective hemostat.

A limited number of studies were found in the literature that compared local hemostatic agents with similar femoral artery methods (2,18). Similar studies by Ankaferd and Chitosan were unable to control the bleeding in the first minute, but Ankaferd was able to provide 40% and Chitosan 30% hemostasis in the second minute. In the fourth minute, Ankaferd and Chitosan were able to provide 60% homeostasis. With Chitosan, bleeding was not controlled at 10% in the fourth minute (18). This study excised the femoral artery and vein. Our study damaged the femoral artery using the needle tip. In a study in the literature, similar to our study, the femoral artery has been damaged by the incision method (2). Our study used a 24-gage needle to puncture the femoral artery. Once the excessive bleeding had been observed, the HAs were poured directly onto the bleeding site, and a 200-g scale weight was placed on for 30 s. The bleeding was evaluated at 30 s intervals. Upon the scale weight removal, hemostasis was assessed. If hemostasis was not established, the

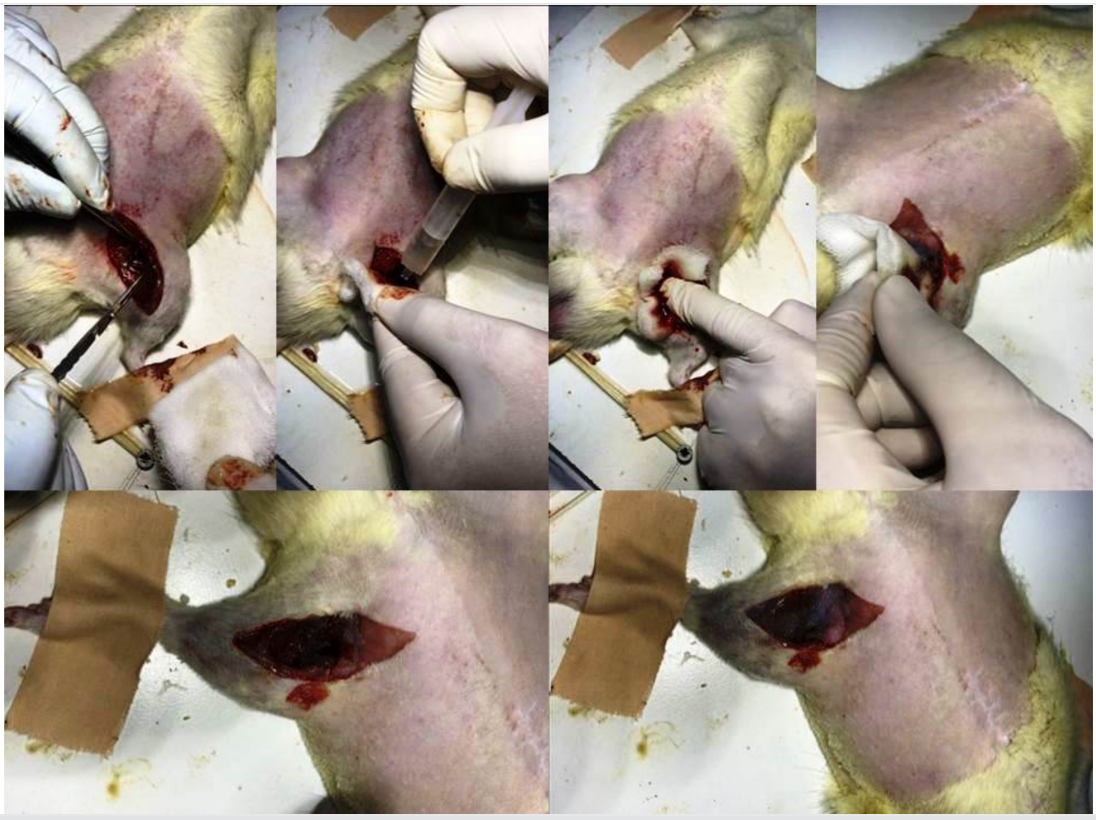

Figure 4. Femoral arterial hemorrhage model with no sponge applied to the bleeding area after AHA liquid application and the appearance of the bleeding under local control

weight was reapplied. If hemostasis did not occur after the third application, the test was scored as failed.

In our study, unlike in the use of the AHA powder and sponge forms, no pressure was applied to the bleeding site when the AHA liquid form is used that might have prolonged the bleeding period. However, one should not question the effectiveness of the AHA liquid, as there is no similar product in the literature. Compared to the AHA powder and sponge, the AHA liquid was the least effective hemostat in bleeding control; however, it seems to be more effective than many local hemostats available in the literature made by the same method (18).

A study by Karahaliloglu et al. (21) revealed that a standard sponge stopped the bleeding after 245 s. The hemostasis time of native and coagulative agents-doped hemostatic dressings ranged from 80 to 180 s. Compared with the standard sponge, hemostatic dressings had significantly better performance. According to this study, the native and active agents-doped hemostatic dressings quickly stopped the bleeding in a femoral artery bleeding rat model with low mortality, more platelet activation, reduced tissue reaction, and improved biological tissue compatibility compared to a standard sponge. Additionally, native or active agent-doped hemostatic dressings would provide impressive and safe hemostasis for the femoral artery bleeding model.

An additional important aspect is that the prepared hemostatic dressings can become a unique tool for surgeons with ease of handling and low cost as HAs (21). AHA products are easy to apply, inexpensive, and can be applied with or without compressions. AHA products do not need the removal of any kind of cleaning procedure because of biodegradability; however, they are easy to clean from the field of application.

Few guidelines are available for the use of HAs (16,19). AHA products are available in various forms, e.g., liquid, powder, and sponge that provide a wide range of indications in controlling various types of bleeding (22,23,24,25).

Our study applied the AHA liquid form to the area in the femoral artery bleeding model in the absence of compressions. Its hemostatic activity was almost similar to the sponge and powder forms. Liquid hemostats come into prominence to control internal bleeding, where the application of other forms, such as sponge, powder, and fiber and nonwoven type hemostats, is impossible. AHA liquid form immediately turns into gel after application, thus it can be effectively used in the military area, in cases where physical access to the bleeding area is difficult or impossible in gunshot wounds.

The literature reported various rates of mortality and exothermic reaction (26,27). For instance, the femoral artery injury had resulted in 75% mortality in the standard dressing group. However, active component coated dressings had dramatically decreased the mortality to 37.5% (approximately two-fold). The addition of kaolin to hemostatic dressing had increased the mortality rate compared with other fabricated experimental groups. Literature

reports that the mortality rate for commercial products, such as Celox Gauze and ChitoGauze, are 90% and 70%, respectively. Combat Gauze showed a mortality rate of 60% (26).

Letourneau et al. (27) studied the hemostatic potential of the active dressings group and revealed that the overall survival rate was 54%. Many studies in the literature with similar test methods have different results in control groups among effective local hemostatic agents (28,29). Compared to other products, further studies are required to evaluate the bleeding control of other bleeding arrestors due to many factors, such as animal weight, experience, technical differences, vessel variations, and laboratory conditions that affect this difference.

The results of this study should be supported with larger studies. Our study used healthy rats. Additional studies are needed to evaluate the results of already hypovolemic and hypotensive subjects due to major artery bleedings. The limitations of this study include the absence of data on the mean arterial pressure, blood gases analysis, and histological examinations.

Conclusion

The AHA effectively controlled the bleeding in a short period in a well-characterized animal model: the rat femoral artery bleeding. The AHA offers some advantages due to its availability in three different forms since it can be used in various types of bleeding.

Ethics

Ethics Committee Approval: This study was conducted following the Local Ethics Committee of Animal Experiments as specified in the literature (Kırıkkale University Animal Experiments Local Ethics Committee, protocol number: 2018/16).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: H.E., A.K., A.M., E.Y, Design: H.E., A.K., A.C.O., H.D., A.M., M.S.B., E.Y., Data Collection or Processing: H.E., A.K., A.C.O., H.D., A.M., Analysis or Interpretation: H.E., A.K., A.C.O., H.D., A.M., Literature Search: H.E., A.C.O., H.D., A.M., M.S.B., Writing: H.E., S.S.D., A.C.O., H.D., A.M., E.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Abacıoğlu S, Aydın K, Büyükcam F, Abacıoğlu S, Aydın K, Büyükcam F, Kaya U, Işık B, Karakılıç ME. Comparison of the efficiencies of buffers containing Ankaferd and Chitosan on hemostasis in an experimental rat model with femoral artery bleeding. Turk J Hematol 2016;33:48-52.
2. Aktop S, Emekli-Alturfan E, Ozer C, Gonul O, Garip H, et al. Effects of Ankaferd Blood Stopper and Celox on the tissue factor activities of warfarin-treated rats. Clin Appl Thromb Hemost 2014;20:16-21.
3. Bennett BL, Littlejohn LF, Kheirabadi BS, Butler FK, Kotwal RS, Dubick MA, et al. Management of External Hemorrhage in Tactical Combat Casualty Care: Chitosan-Based Hemostatic Gauze Dressings. JSOM 2014;14:12-29.
4. Bracey A, Shander A, Aronson S, Boucher BA, Calcaterra D, Chu MWA, et al. The use of topical hemostatic agents in cardiothoracic surgery. Ann Thorac Surg 2017;104:353-60.
5. Brunett PH, Cameron PA. Trauma in adults. 1669-1676. In: Tintinalli JE, Stapczynski JS, Ma OJ, Cline DM, Cydulka RK, Meckler GD, Editors. Tintinalli's Emergency Medicine: A Comprehensive Study Guide. McGraw Hill, New York. 2010.
6. Ersoy G, Kaynak MF, Yilmaz O, Rodoplu U, Maltepe F, Gokmen N. Hemostatic effects of microporous polysaccharide hemosphere in a rat model with severe femoral artery bleeding. Adv Ther 2007;24:485-92.
7. Glickman M, Gheissari A, Money S, Martin J, Ballard JL; CoSeal Multicenter Vascular Surgery Study Group. A polymeric sealant inhibits anastomotic suture hole bleeding more rapidly than gelfoam/thrombin: results of a randomized controlled trial. Arch Surg 2002;137:326-31.
8. Hanks JB, Kjaergard HK, Hollingsbee DA. A comparison of the hemostatic effect of Vivostat patient-derived fibrin sealant with oxidised cellulose (Surgicel) in multiple surgical procedures. Eur Surg Res 2003;35:439-44.
9. Huri E, Akgül KT, Yücel MÖ, Astarcı HM, Üstün H, Germiuanoğlu RC. The second step in vitro trial of Ankaferd® Bloodstopper®: comparison with other hemostatic agents. Turk J Med Sci 2011;41:7-15.
10. Jackson MR. New and potential uses of fibrin sealants as an adjunct to surgical hemostasis. Am J Surg 2001;182:36-9.
11. Karahaliloğlu Z, Demirbilek M, Ulusoy İ, Gümüşkaya B, Denkbaş EB. Hemostatic activities of nano/microporous bilayer dressings in a femoral artery bleeding rat model. J Appl Polym Sci 2016;133:43657.
12. Letourneau PA, McManus M, Sowards K, Wang W, Wang YW, Matijevic N, et al. Aged plasma transfusion increases mortality in a rat model of uncontrolled hemorrhage. J Trauma 2011;71:1115-9.
13. Lew WK, Weaver FA. Clinical use of topical thrombin as a surgical hemostat. Biologics 2008;2:593-9.
14. Lih E, Lee JS, Park KM, Park KD. Rapidly curable chitosan-PEG hydrogels as tissue adhesives for hemostasis and wound healing. Acta biomater 2012;8:3261-9.
15. Midi A, Kumandaş A, Ekici H, Arda S, Karahan S, Şimşek AK, et al. Investigation of the effectiveness of algan hemostatic agent in renal venous bleeding model in rats. EJMI 2018;2:129-32.
16. Midi A, Ozyurek HE, Karahan S, Ekici H, Kumandas A, Turkmen I, et al. Investigation of efficacy of the plant based algan hemostatic agent in hepatectomy bleeding model in rats. EJMI 2018;2:195-201.
17. Midi A, Ekici H, Kumandas A, Durmus O, Bodıc B, Tıryakı M, et al. Investigation of the effectiveness of algan hemostatic agent in bleeding control using an experimental partial splenectomy model in rats. Marmara Med J 2019;32:27-32.

18. Midi A, Kumandas A, Ekici H, Bayraktar F, Karapirli K, Karahan S, et al. Investigation of the Efficacy of Algan Hemostatic Agent in Liver Laceration Model in Rats. EJMO 2019;3:37-42.
19. Ohta S, Nishiyama T, Sakoda M, Machioka K. Development of carboxymethyl cellulose nonwoven sheet as a novel hemostatic agent. J Biosci Bioeng 2015;119:718-23.
20. Ortel TL, Mercer MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. Ann Surg 2001;233:88-96.
21. Schreiber MA, Neveleff DJ. Achieving hemostasis with topical hemostats: making clinically and economically appropriate decisions in the surgical and trauma settings. AORN J 2011;94:1-20.
22. Spotnitz WD, Burks S. Hemostats, sealants, and adhesives: components of the surgical toolbox. Transfusion 2008;48:1502-16.
23. Spotnitz WD, Burks S. State-of-the-art review: hemostats, sealants, and adhesives II. Update as well as how and when to use the components of the surgical toolbox. Clin Appl Thomb Hemost 2010;16:497-514.
24. Spotnitz WD. Hemostats, sealants, and adhesives: a practical guide for the surgeon. Am Surg 2012;78:1305-21.
25. Srivastava CM, Purwar R. Chitosan-finished Antheraea mylitta silk fibroin nonwoven composite films for wound dressing. J Appl Polym Sci 2017;134:44341.
26. Su Z, Izumi T, Thames EH, Lawson JH, Ortel TL. Antiphospholipid antibodies after surgical exposure to topical bovine thrombin. J Lab Clin Med 2002;139:349-56.
27. Ward KR, Tiba MH, Holbert WH, Blocher CR, Draucker GT, Proffitt EK, et al. Comparison of a new hemostatic agent to current combat hemostatic agents in a swine model of lethal extremity arterial hemorrhage. J Trauma 2007;63:276-83.
28. Xu HX, Yan YU, Wang XY, Ma K, Chao Y, Sun PJ, et al. Hemostatic effect of a medical polysaccharide hemostatic healing sponge on hemostasis of bleeding from femoral artery in pigs. Med J Chin PLA 2015;40:245-7.
29. Yan D, Hu S, Zhou Z, Zeenat S, Cheng F, Li Y, et al. Different chemical groups modification on the surface of chitosan nonwoven dressing and the hemostatic properties. Int J Biol Macromol 2018;107:463-9.