

# Effects of Algan hemostatic agent foam in rat femoral artery injury model: A randomized animal trial

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## Ethics Committee Approval

This study was conducted at Marmara University, Medical School Experimental Animal Implementation and Research Centre and ethical approval for this study was obtained from Local Animal Experiments Ethics Council of the Marmara University (No: 65.2021, Dated: 09.08.2021).

## Conflict of Interest

No conflict of interest was declared by the authors.

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## Abstract

**Background/Aim:** Nowadays, many deaths are related to vessel injury-induced blood loss. Failure to control bleeding also increases the risk of death. This study aimed to investigate the hemostatic effects of the Algan Hemostatic Agent (AHA) foam application in a rat model in which severe femoral artery bleeding was induced.

**Methods:** Fourteen rats were randomly assigned to two groups: (1) control (physiological saline) (n = 7) and (2) AHA foam (n = 7). The left femoral artery of the rats was incised and when the bleeding started, and the area was pressed with another sponge for 10 s in all rats. Afterwards, physiological saline solution impregnated gauze or AHA foam was placed over same area. A chronometer was started and area was checked after 2 min. If no bleeding occurred during the first 2 min of application, it was recorded as “successful”. If bleeding occurred, the same procedure was repeated up to three times. If hemostasis could not be achieved even after the third application, it was considered a failure, and “failed” was recorded. All animals were sacrificed under high anesthesia for least 10 min after the experiment.

**Results:** Application of AHA resulted in complete (100%) control of bleeding in all rats within the first 2 min. In control group, hemostasis was achieved in 1 out of 7 (14.3%) rats by the third application. Failure was recorded for the remaining six rats. The hemostatic success rate of the AHA foam was significantly higher than the rates of control group ( $P = 0.005$ ).

**Conclusion:** AHA foam is a very effective hemostatic agent and can be applied easily on vascular trauma models. Further studies are needed to elucidate hemostatic features of AHA.

**Keywords:** Algan hemostatic agent, Femoral artery, Hemostasis, Bleeding, Rat, Coagulation

## Introduction

Injury of the major vessels is one of the significant reasons of death caused by trauma [1]. Moreover, hemostatic balance can be easily disturbed even after common surgeries, such as cardiopulmonary bypass [2]. Therefore, stopping the bleeding during a surgical process or during emergencies after injury can be a lifesaving process. The need for methods to minimize hemorrhage-derived blood loss problems that are caused by trauma, such as an injury, fracture, cleft, or surgery, are necessary [3]. Direct pressure can be applied to the bleeding area to suspend the circulation after trauma [1]. Besides direct pressure, fast acting and effective procedures and products are needed to expedite hemostasis. Agents that are locally used for this purpose have been reported and include chitosan linear polymer (Celox®), poly-N-acetylglucosamine (Chitin®), fibrin glues, microporous hydrogel-forming polyacrylamide (BioHemostat®), microporous polysaccharide hemosphere (TraumaDEX®), oxidized cellulose (Bloodcare®), and Ankaferd Blood Stopper® (ABS) [4]. However, inflammation and/or infection risks have also been reported in various studies through the use of most of these materials [5–9], and no consensus on which product is the ideal one has been reached. Hence, studies in the literature report a continuous search for an optimal hemostatic agent.

Algan Hemostatic Agent (AHA) Foam is a class III starch-based absorbable hemostatic agent (Certificate number: EC Design-Examination Certificate 1783-MDD-216). It is used to stop all minor and major bleeding, such as internal and external bleeding, bleeding that emerges during surgeries, surgical interventions and operations, rupture, fragmentation, traumatic cuts, and others.

Recently, the hemostatic effects of AHA foam have been reported [10–12]. In addition, the effectiveness and safety of the different forms of AHA have been studied [13–16]. AHA foam generates a polymer network throughout its application area, creating a passive barrier that stops blood from leaking. It is also advantageous in terms of its low cost and ease of handling. Based on recent studies that showed the effects of this new herbal product in various tissues with different surgical models, the study aimed to investigate the effectiveness of AHA foam in a rat femoral artery bleeding model.

## Materials and methods

### Animals

All animal experiments were performed in accordance with the ethical norms approved by the Local Animal Experiments Ethics Council of Marmara University Istanbul, Turkey (Ethics Committee Approval No: 65.2021, Dated: 09.08.2021). Fourteen adult 8–10-week-old, Wistar Albino rats with weights between 230 and 280 g were used, and animals were randomly divided into two groups of seven animals in each group: (1) control (physiological saline solution impregnated gauze) and (2) AHA foam. All animals were housed in an air-conditioned animal room in standard clean polypropylene cages under standard vivarium conditions with 12-h light/dark cycles. All animals were fed with a standard pellet diet and water *ad*

*libitum*. All experiments were performed after one week-long of adaptation period.

### Study design

All rats were sedated with 100 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, İstanbul, Türkiye) and 10 mg/kg xylazine hydrochloride (Rompun, Bayer, İstanbul, Türkiye) intraperitoneally. Anesthetic depth was monitored by examining skin or finger nipping response, palpebra or corneal reflex, heartbeat, respiratory rate, and other physiological parameters. The surgical procedure of our study was performed according to methods described in the literature [4]. After wiping and shaving the left inguinal area of the rats, layers of the skin and subcutaneous tissues were cut open to reveal the left femoral vessels. Femoral arteries were injured with the injector tip to initiate bleeding. Immediately after the bleeding had started, a standard sponge was pressed over the incised area for 10 s in all rats. Immediately after removing the sponge, physiological saline-impregnated gauze and AHA foam were applied to the same injured area in control and AHA foam groups, respectively (Figure 1A, B). Chronometer was started to measure time and the area was checked after two minutes. If there was no bleeding, first 2 minutes application was recorded as “successful”. If the bleeding had not stopped, same application was repeated for 2 additional min, and the bleeding was checked again. If the bleeding had stopped, it was recorded as “second 2-min successful” application and on the contrary, same procedure was repeated for the third time if there was bleeding. If the bleeding had stopped at the third application, it was recorded as “third 2-min successful”. Hemostasis that could not be achieved even after the third application was considered a failure and recorded as “failed” (Table 1). All animals were sacrificed under high anesthesia for at least 10 min after the hemostasis.

Figure 1: Femoral artery in the left inguinal region (A), Algan Hemostatic Agent (AHA) foam application on the injured area (B). Visible hemostasis and adherence of AHA foam to the area after 2 min of pressure (C). Bleeding control after removal of AHA foam in another rat from the same group (D).

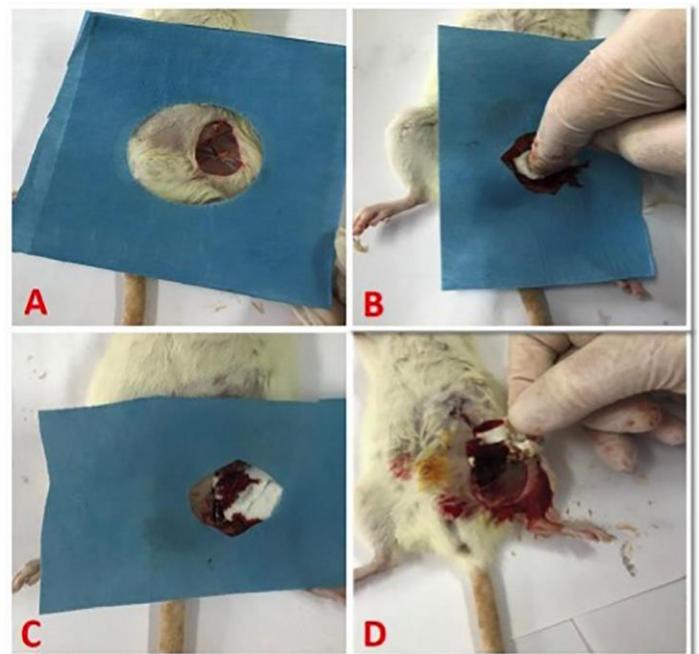


Table 1: Bleeding control interval recordings

	First application success (2 min)	Second application success (2 min)	Third application success (2 min)	Failed
Algan Hemostatic Agent (AHA) foam	7 (100%)	0 (0%)	0 (0%)	0 (0%)
Control (saline impregnated gauze)	0 (0%)	0 (0%)	1 (14.20%)	6 (85.7%)

### Statistical analysis

An exact chi-squared test was used for the comparison between control and AHA results. Numbers and % data were assigned to categorical variables as demonstrative statistics. Statistical analysis is performed with Statistical Package for the Social Sciences (SPSS) software version 24.0 (SPSS Inc., Chicago, IL) and  $P < 0.05$  was considered statistically significant.

### Results

Application of AHA foam to the bleeding area resulted in complete control of bleeding in 7 out of 7 rats after the first application (within the first 2 min) as shown in Figure 1C, D. None of the rats had stopped bleeding after the first or second application in the physiological saline solution group. Nonetheless, hemostasis was received only in 1 out of 7 rats by the third application (within 6 min) in the saline group. For the other 6 out of 7 rats, the bleeding could not be stopped even after 6 min (Table 1). In conclusion, AHA foam resulted in 100% success compared to physiological saline solution (only 14.3%). It has been found that the success of the AHA foam over control physiological saline's success in terms of hemostasis was statistically significant ( $P = 0.005$ ) as shown in Table 2.

Table 2: Bleeding control statistics between study groups

	+	-	Total	P-value
AHA foam	7 (100)	0 (0)	7 (100)	0.005
Control group (saline-impregnated gauze)	1 (14.3)	6 (85.7)	7 (100)	
Total	8 (57.1)	6 (42.9)	14 (100)	

AHA: Algan Hemostatic Agent, +: successful application, -: hemostasis that could not be achieved after third application

### Discussion

This study compared the hemostatic effects of AHA foam and physiological saline solution-impregnated gauze on a rat femoral artery incision model. The results showed that AHA foam was significantly more effective for causing a cessation of bleeding. Although many hemostatic materials were compared in different studies, no agreement on which one of them is the better hemostatic agent [17–20]. Hanks et al. [21] compared the hemostatic effectiveness of oxidized cellulose and fibrin glue and concluded that fibrin glue was more effective. Ersoy et al. [22] studied the hemostatic effects of microporous polysaccharide hemospheres and observed that they accelerated hemostasis. Abacıoğlu et al. [4] studied the hemostatic effects of Ankaferd Blood Stopper (ABS) and showed hemostasis had occurred by 2 min in 40% of all the rats and by 4 min in 60% of rats. In our study, all rats in the AHA foam group had reached hemostasis within the first 2 min, and no need for a second application of AHA foam was required. Wang et al. [23] compared chitosan sponges, chitosan fibers, and standard bandages in a rat femoral artery incision model and found a significant difference in hemostatic effectiveness between chitosan sponge and standard bandage and concluded that the chitosan sponge was more effective. Köksal et al. [24] showed the preeminence of Celox compared to compression in a rat femoral artery injury model in

which Celox caused a significant decrease in the amount of time it took to reach hemostasis. In all groups with hypothermia, normothermia, and warfarin, Celox was found to be more effective than compression. Bertram et al. [25] analyzed the effect of intravenous synthetic platelet injections on rat femoral artery incision models and reported that synthetic platelets led to a reduction in bleeding nearly to half that of the control group. Studies have found no significant contribution from administration of extra amounts of physiological hemostatic substances to hemostasis when they were administered intravenously [26, 27]. In studies conducted to test the effectiveness of AHA in heparinized/non-heparinized rat splenectomy and heparinized/non-heparinized rat hepatectomy models, AHA was found to lead to a decrease in the average time to achieve hemostasis by 97.7% and 98%, respectively [13, 16].

In this study, AHA foam was applied directly to the bleeding area using light pressure. And after 2 minutes, pressure was removed, and the area was observed to detect local bleeding. AHA was found to be quite successful in controlling bleeding due to femoral artery injury, and similar results with AHA have been shown in previous studies. [13–16, 28, 29]. AHA provides hemostasis by creating a local polymer web that binds to the tissue and acts as a mechanical barrier. Its detachment from the application site can create trauma to tissues and vessels; thus, detachment may trigger re-bleeding. Consequently, it is suggested to leave AHA on the application site to prevent rebleeding.

Even though bleeding duration under the effect of compression was found to be 4 minor femoral artery incision [4, 22] and approximately 6 min for tail incision [17], no successful hemostasis could be observed to occur spontaneously. In our study, the bleeding from femoral artery incision was stopped for every rat (100%) in the AHA group within the first 2 min. In the saline group, however, hemostasis was achieved solely in 1 rat (14.3%) within 6 min and for the other 6 rats (85.7%) the bleeding failed to stop. This difference of at least 4 min on bleeding durations supports the significantly positive effect of AHA on severely damaged arteries. Although the supplements were not compared directly, results have shown that AHA is a quick effective agent in vascular damage models.

### Limitations

This study had some limitations. The bleeding area was directly approachable, which is unlikely to be encountered in real life, clinical situations. Thus, this controlled situation can make the application and the effect of the AHA easier to detect compared to real life settings. Second, as a potential source of bias in the study, the time to reach normal hemostasis could have differed independently from AHA due to the genetic structure of the rat genus used in this study, and a 1-sec margin of error in measuring the exact moment of hemostasis in all animals could have been present.

### Conclusions

Treatment with AHA application versus physiological saline solution-soaked gauze was more effective at the bleeding femoral artery incision sites of rats. AHA was more efficient in terms of causing a decrease in bleeding duration and acceleration of hemostasis. Although the application was performed in a controlled environment with limitations, this situation simulated

a real-life situation well enough. The importance of AHA is incontrovertible when considering how bleeding time may change the outcome of a medical emergency. Hence, AHA has great potential among hemostatic agents, and it can be used as a rapid and beneficial tool in the field and on vascular damage models. Moreover, further studies are required to investigate the effects of AHA in animal models of blood-related dysfunctions and to compare its effects with several local hemostatic agents.

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