

Investigation of the effectiveness of algan hemostatic agent in bleeding control using an experimental partial splenectomy model in rats

DeneySEL parsiyel splenektomi modelinde sıçanlarda algan hemostatik ajanın kanama kontrolünde etkinliğinin araştırılması

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ABSTRACT

Objective: Algan hemostatic agent (AHA) is a plant-based hemostatic agent produced in Turkey. Although, there is a great improvement in the hemostatic technologies, more effective hemostatic products are required to be produced. The aim of this study was to demonstrate the efficacy of AHA in a partial splenectomy model in rats. In addition, in this model, postoperative abdominal adhesion was evaluated.

Materials and Methods: In this study 5-7 weeks old 64 rats were used. Rats were randomly divided into 8 groups, each consisting of eight rats (4 groups heparinized and 4 groups non-heparinized). Experimental splenectomy was performed and the gauze impregnated with saline was applied to the control group for the hemorrhage control, the gauze impregnated with liquid AHA, gel and powder form of AHA, was applied to the experimental groups.

Results: The time to reach complete homeostasis was significantly shorter in all AHA groups compared to the control

group. The powder and the gel forms of AHA stopped the bleeding in heparinized and non-heparinized groups in 1 second. The AHA fluid (sponge) form stopped the bleeding in the first application in the control group less than 10 seconds and the second time application was not necessary. The bleeding was able to be controlled in the heparinized control group (saline impregnated sponge) by 55 seconds and in the non-heparinized control group by 38 seconds.

Conclusion: This study showed that AHA is a highly effective hemostatic agent, which would be beneficial in controlling hemorrhage.

Keywords: Algan hemostatic agent, Hemostasis, Rat, Bleeding control, Splenectomy

ÖZ

Amaç: Algan hemostatik ajan (AHA), Türkiye’de üretilen bitki bazlı hemostatik bir maddedir. Hemostatik teknolojilerdeki büyük gelişmelere rağmen, kanamanın kontrolünde kullanılmak üzere daha etkili hemostatik ürünlerin üretilmesi gerekmektedir. Bu çalışmanın amacı, AHA’nın sıçanlarda parsiyel splenektomi modelindeki etkinliğini göstermektir. Ayrıca bu modelde batin yapışıklığı değerlendirilmiştir.

Gereçler ve Yöntemler: Bu çalışmada 5-7 haftalık 64 sıçan kullanıldı. Sıçanların her biri sekiz sıçandan oluşan 8 gruba ayrıldı (heparinize edilmiş 4 grup ve heparinize edilmemiş 4 grup). DeneySEL splenektomi yapıldı ve kontrol grubuna hemoraji kontrolü için serum fizyolojik emdirilmiş gazlı bez uygulandı, deney gruplarına AHA sıvı emdirilmiş gazlı bez, jel ve toz formu uygulandı.

Bulgular: Komple hemostaza ulaşma süresi, tüm AHA gruplarında kontrol grubuna kıyasla anlamlı olarak daha kısa idi. AHA’nın toz ve jel formları heparinize ve heparinize olmayan gruplarda 1 saniyede kanamayı durdurdu. AHA sıvı (sünger) formu, kontrol grubunda ilk uygulamada tüm sıçanlarda kanamayı durdurdu (10 saniyenin altında). Kanama, heparinize edilmiş kontrol grubunda (serum fizyolojik emdirilmiş gazlı bez) ortalama 55 saniyede ve heparinize olmayan kontrol grubunda 38 saniyede kontrol edilebildi.

Sonuç: Bu çalışma, AHA’nın hemorajinin kontrol edilmesinde yararlı olabilecek oldukça etkili bir hemostatik ajan olduğunu göstermiştir.

Anahtar kelimeler: Algan hemostatik ajan, Hemostaz, Sıçan, Kanama kontrolü, Splenektomi.

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Introduction

The reason for many splenectomies nowadays is spleen bleeding caused as a result of elective spleen surgeries applied due to several medical reasons and especially trauma. The spleen is the second most frequently injured organ after abdominal traumas and missed splenic injury is the most frequent preventable cause of death in patients with trauma. For this reason, there are studies on partial spleen protection and in control of spleen hemorrhage following trauma [1-3]. In the early 1900s, the mortality rate for non-surgical treatment of splenic injuries was approximately 100%. For this reason, splenectomy was widely acknowledged treatment option in spleen injuries [4].

Today, for many patients with solid organ damage, laparotomy is not necessary by virtue of imaging techniques. Since, non-surgical treatments in solid organ injuries give better results comparing to the surgical treatments, non-surgical treatment options are highlighted. Besides, the risk of postoperative infection in splenectomy is elevated in spleen injuries, therefore the approaches protecting the spleen come to the forefront. There are many hemostatic agents available such as; bovine collagen, bovine thrombin, autologous plasma, fibrin glue [5-12], and it is necessary to decide which method to use according to the cost of the procedure, bleeding severity and personal experience. However, despite these products and major developments in medicine, an ideal product that can be used to control bleeding is not yet produced and more effective hemostatic products are necessary to be produced.

The algal hemostatic agent (AHA) is the herbal extract derived from the standardized blend of six different plants (Table I) [13,14]. To the best of our knowledge, it is the first and only patented product in the world, made solely of herbs, with no additives. (Patent application no: a2015 / 00018, application publication no. TR2015 0018 A2).

All biocompatibility tests such as sensitization, cytotoxicity and irritation and hemodynamic tests of the AHA had been performed, and the results supported its safety and efficacy as a hemostatic agent [13,14]. AHA can be easily formulated to be applied locally [13,14]. Further, it has low cost and does not require special storage conditions.

The aim of this study is to assess the hemostatic effect of the partial splenectomy model of the AHA. In addition, postoperative abdominal adhesion is evaluated.

Table I. Plants with algal hemostatic agent composition.

The name of the plant	English name	Used part
<i>Achillea millefolium</i>	Yarrow	Flower
<i>Juglans regia</i>	Walnut	Leaf
<i>Lycopodium clavatum</i>	Club moss	Whole plant
<i>Rubus caesius</i> , <i>R. fruticosus</i>	Blackberry	Leaf
<i>Viscum album</i>	European Mistletoe	Whole plant
<i>Vitis vinifera</i>	Vine	Leaf

Materials and Methods

This study was approved by the Institutional Animal Experiments Local Ethics Committee of Kırkkale University (number, 2018/10). All animal studies conformed to the animal experiment guidelines of the Committee for Humane Care. All animals received care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and “Guide for the Care and the Use of Laboratory Animals” prepared by the US National Academy of Sciences and published by the US National Institute of Health (NIH Publications, No:80-23). The experiment was carried out as described in the literature [1].

In the study, 64 rats which are 180-210 grams and 5-7 weeks old were used. Rats were fed as ad libitum and examined under standard laboratory conditions according to a 12-hour dark-light period. Rats were randomly divided into two groups each having 32 rats as; heparinized and non-heparinized groups. Subsequently, the experimental animals were randomly divided into 8 groups each having eight rats. The heparinized group was administered intraperitoneal 640 IU / kg heparin for 3 days once a day. The other group did not receive heparin. Group 1 (Heparinized control group), Group 2 (Heparinized AHA powder group), Group 3 (Heparinized AHA gel group), Group 4 (Heparinized liquid AHA-impregnated sponge group), Group 5 (Non-Heparinized control group), 6th group (Non-Heparinized AHA powder group), 7th group (Non-Heparinized AHA gel group), 8th group (Non-Heparinized AHA liquid impregnated sponge group).

Operation procedure

During the operations, all rats were treated according to the Guide for the Care and Use of Laboratory Animals. Procedures were performed under general anesthesia with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). All efforts were made to minimize animal suffering and the number of animals used.

The furs on the abdominal anterior wall of all rats were removed. After disinfection with povidone-iodine solution, 3 cm median incision was made. The spleen was located and in the lower pole of the spleen 1 cm, partial splenectomy was performed in the rats in all groups. The duration of bleeding was evaluated according to the protocol previously explained [12]. When splenectomy bleeding had started, the bleeding area was pressed with a sponge for 10 seconds, and then the sponge was removed. Liquid AHA-impregnated sponge, and the saline-impregnated sponge were placed in this area and a light pressure was applied to the area. Bleeding was checked 10 seconds after the start of the pressure. If bleeding stopped, it was recorded as 'bleeding stopped'. If not, the procedure was repeated until the bleeding was controlled with the same amount of material. In the AHA gel and AHA powder forms, the bleeding area was left unpressed and was left open after the application. Bleeding was checked. The time at which the bleeding stopped was noted as the time of the bleeding control (Figure 1).

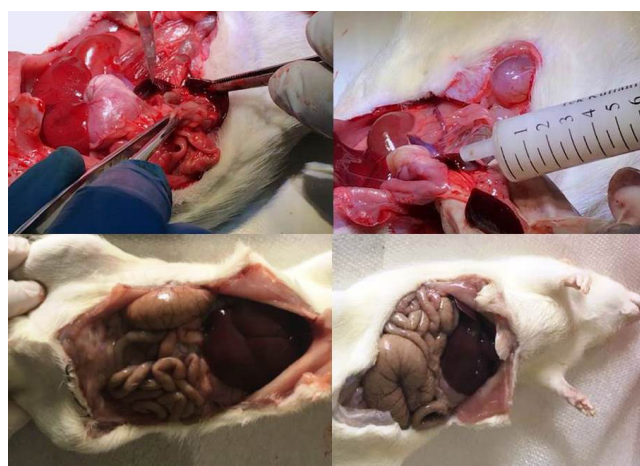


Figure 1. The images of the spleen cut from the center and the application of the AHA gel. On the postoperative 5th day, there is no adherence in the abdomen in the control and AHA liquid groups

On the 5th day of the laparotomy, the rats underwent surgical site disinfection on the anterior wall of the abdomen under anesthesia. The abdomen was opened and the intra-abdominal adhesiveness quantitatively evaluated according to the Bothin scale [12]. Hematoma and fluid collection were examined in the abdomen, if it existed. The rats were sacrificed by cutting inferior vena cava.

Statistical analyses

SPSS software version 22.0 (SPSS Inc., Chicago, IL) was used to analyze the data of this study. Weight, bleeding time and adherence scores were calculated and mean values were compared among the four groups using analysis of variance (ANOVA). When differences were found, any group difference was determined by Duncan's multiple range tests. The results were assessed at a 95% confidence interval and a suggestiveness level of $P < 0.05$.

Results

The shortest duration of bleeding was found in the AHA powder group. This was followed by the gel group and the liquid group. The duration of bleeding in the control group was significantly longer than in the experimental groups (Table II). There was no difference in terms of body weights between the groups. The powder and the gel forms of AHA stopped the bleeding in heparinized and non-heparinized groups in 1 second. The AHA fluid (sponge) form stopped the bleeding in the first time in control group less than 10 seconds and the second time application was not necessary.

The saline-impregnated sponge was able to control bleeding in the heparinized control group at the average of 55 seconds (min: 40-max: 70 sec) and in the non-heparinized

Table II. Mean bleeding time and the body weight distribution of the groups.

	Group 1 (HC)	Group 2 (HP)	Group 3 (HG)	Group 4 (HL)	Group 5 (NHC)	Group 6 (NHP)	Group 7 (NHG)	Group 8 (NHL)	P
Weight (gram)	190.6 ± 5.5	189.5 ± 7.3	183.6 ± 10.6	194.6 ± 5.7	175.6 ± 10.5	182.4 ± 7.5	187.9 ± 7.5	184.3 ± 9.5	$P > 0.05$
Average Bleeding time, second (Min-max)	55 (40-70 sec.) (Min-max)	1 sec.	1 sec.	< 10 sec. (at first control)	38 sec. (30-50 sec.) (Min-max)	1 sec.	1 sec.	< 10 sec. (at first control)	$P < 0.001$

HC: Heparinized control group, HP: Heparinized AHA powder group, HG: Heparinized AHA gel group, HL: Heparinized AHA liquid-impregnated sponge group, NHC: Non-Heparinized control group, NHP: Non-Heparinized AHA powder group, NHG: Non-Heparinized AHA gel group, NHL: Non-Heparinized AHA liquid-impregnated sponge. AHA: Algan Hemostatic Agent.

Table III. Assessment of the adhesion forms of the groups (%)

Adhesion Zone	Group 1 (HC)	Group 2 (HP)	Group 3 (HG)	Group 4 (HL)	Group 5 (NHC)	Group 6 (NHP)	Group 7 (NHG)	Group 8 (NHL)	P
Between omentum and target organ	6	7	6	6	6	7	7	6	>0.05
Omentum abdominal scar	0	0	0	0	0	0	0	0	
Between omentum and other sites	0	0	0	0	0	0	0	0	
From adnexa to target organ	0	0	0	0	0	0	0	0	
Adnexa abdominal scar	0	0	0	0	0	0	0	0	
From adnexa to other places	0	0	0	0	0	0	0	0	
Adhesive tape between any two organs	0	0	0	0	0	0	0	0	
Target organ abdominal scar	0	0	0	0	0	0	0	0	
Target organ abdominal wall	0	0	0	0	0	0	0	0	
Target organ intestine	2	1	1	2	2	1	0	1	
Target organ liver	0	0	0	0	0	0	0	0	
Adhesion in any other organ	0	0	0	0	0	0	0	0	
Total Adhesion Score	8	8	7	8	8	8	7	7	

HC: Heparinized control group, HP: Heparinized AHA powder group, HG: Heparinized AHA gel group, HS: Heparinized AHA liquid impregnated sponge group, NHC: Non-Heparinized control group, NHP: Non-Heparinized AHA powder group, NHG: Non-Heparinized AHA gel group, NHL: Non-Heparinized AHA liquid impregnated sponge. AHA: Algan Hemostatic Agent.

control group at 38 (min: 30-max: 50 sec) seconds (Table II).

There was no hematoma and fluid accumulation in the abdomen on the postoperative 5th day. The lowest adhesion score was 7 and the highest was 8. There was no difference in the adhesion scores between the control and treatment groups (Table III).

All of the applications were performed with the sterile AHA in a sterile environment. All rats in the treatment and control groups were alive on the 5th postoperative day. On the postoperative 5th day, when the abdomen was opened, no surgical infection was observed in neither control nor the AHA groups.

Discussion

All forms of the AHA have stopped bleeding in the first second in the partial splenectomy bleeding model used in this study and we have shown that they are potential candidates as an effective product in the use of hemostasis in splenic injuries or partial splenectomy operations. In heparinized and non-heparinized powder and gel groups, the bleeding was stopped within 1 second. The AHA was significantly different from the control groups in terms of bleeding control efficacy.

Several hemostatic agents have been used in the treatment of solid organ hemorrhages [15-17], and they work by different mechanisms. Tissue adhesives, cyanoacrylates are used in many clinical situations for the hemostatic purposes [18-20].

In one study in the literature, hemostatic efficacy was compared in ankaferd and fibrin glue in a rat model of partial splenectomy [21]. In this study, Fibrin Glue (Tisseel®) was able to stop bleeding for an average of 11 seconds and Ankaferd Blood Stopper for an average of 10 seconds. As we have shown in the current study, AHA managed to stop the bleeding in as fast as 1 second.

In another study, the hemostatic effect of calcium alginate in experimental splenic injury model was investigated. In that study, a spleen laceration model was established and the hemostatic effect of calcium alginate was evaluated. Calcium alginate has been shown to reduce the intraoperative bleeding after spleen injury. When compared to the 0.9% NaCl gauze and sham groups, inflammation, vascularization, and fibrosis have been found statistically higher in calcium alginate group. And the adhesion score has also been found higher in the calcium alginate group [22].

Nowadays, many products used for hemostasis have some difficulties during application such as compression. However, AHA does not require compression compared

to the other products and it has an easy-to-apply feature, as well as an advantage over other products in terms of hemostasis in a much shorter time.

All forms of the AHA and the results of the effectiveness in hemostasis differ greatly from the other available products. Experimental conditions may vary in terms of animal weight, the experience of the practitioner, technical differences, vessel variations, laboratory conditions, and other factors affecting this disparity and the other bleeding arrestors. Therefore, all products need to be compared in the same experiment protocol to evaluate their efficacies in bleeding control.

Prevention of peritoneal adhesions is important issues in surgery. There are many agents such as phospholipase inhibitors, dextran, corticosteroids, phospholipids, and methylene blue used to prevent postoperative abdominal adhesions [23-25].

According to some studies in the literature, some of the haemostatic agents have postoperative abdominal adhesion enhancing effect. Other studies show the opposite of these results [22, 26, 27]. Our study showed that AHA did not have a positive or negative effect on postoperative intra-abdominal adhesion formation.

According to the results of this study and together with the other studies in the literature, AHA is a highly effective hemostatic agent in the partial splenectomy hemorrhage model in the world, but the actual difference can only be demonstrated by comparative studies. The future studies are needed to further this study.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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