

## Research Article

# Investigation of Efficacy of the Plant Based Algan Hemostatic Agent, in Hepatectomy Bleeding Model in Rats

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## Abstract

**Objectives:** The purpose of this study is to evaluate the hemostatic efficacy of a novel plant originated blood stopper which is called Algan Hemostatic Agent (AHA) in uncontrolled hepatectomy bleeding model.

**Methods:** The study was performed on 64 rats each of 5-7 weeks old. The rats were randomly divided into 8 groups, each consisting of 8 rats (4 groups: heparinized, 4 groups: non-heparinized). Experimental hepatectomy model was performed and saline-impregnated sponge was applied to control group for managing hemorrhage. Algan Hemostatic Agent (AHA) was applied topically in three different formulation types; AHA-impregnated gauze, gel formulation and lyophilized powder form were applied to study groups to evaluate the effect on hemorrhage control.

**Results:** There is no difference in mean weight and weight of resected liver segment among the groups. The AHA powder form was able to control the bleeding in heparinized and non-heparinized groups in 7 and 4 seconds, respectively. The AHA gel formulation was able to control the bleeding in heparinized and non-heparinized groups in 8 and 5 seconds, respectively. AHA liquid form ceased the bleeding in the heparinized and non-heparinized group in the first 15-second compress application. The bleeding time in heparinized and non-heparinized control groups in 410 and 220 seconds, respectively. The bleeding time was quite shorter in all AHA groups comparing to control group animals.

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

**Keywords:** Algan hemostatic agent, haemorrhage, liver, rat

Controlling internal and external hemorrhages due to any cause is very important in preventing negative consequences due to blood loss. For this purpose, most frequently

used conventional method is compression by a sterile gauze, there are also a number of various products on the market, but there is an urging non-satisfaction about their adequacy

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in stopping bleeding expectancy.

Therefore, there is a tendency about use of antihemorrhagic agents for stopping minor and major bleedings during and after spontaneous or surgical interventions, extracorporeal injuries, dental operations.

When the integrity of the liver is disrupted, bleeding is controlled difficulty because of the sinusoidal structure lacking smooth muscle fibers causing vasoconstriction in normal vessels (Romano F. 2005, Kopelman D. 2000). Liver trauma results in 10-15% mortality, and bleeding is defined as the most important factor (Carmona RH, 1982). Likewise, bleeding itself and complications secondary to bleeding are accepted as one of the most important problems in elective surgical resections for primary or metastatic liver tumors (Laurent C. 2005). Bipolar and monopolar electrocautery, ultrasonic dissectors, radio frequency equipment, cryotherapy and various hemostatic agents are all used to perform liver surgery in a short time and safely (Jamagin WR 2002, Ruitenbeek K. 2014, Gage AA 1998, Ersoy G. 2007, Saiura A. 2006, Davidson BR 2000, Costasis Multi-center Collaborative Writing Committee 2001, Nakajima Y. 2002, Akarsu C. 2011, Jackson MR 2001, Schwartz M. 2004, Fischer L. 2011, Koea JB., Frilling A. 2005, Berrovet F. 2007). Reported alternative therapies include local hemostatic agents that reduce bleeding and complications like infection after surgery (Beyazit Y. 2010, N. Fonouni H. 2018, Miller R. 2010, Gegel B. 2010, Inaba K. 2011, Aktop S. 2014, Kunio NR 2013, Rall JM 2013, Samokhvalov IM 2013, Zhang YJ 2015, Chan LW 2015). These agents are used as topical agents such as collagen, gelatin or cellulose based products, fibrin sealant and synthetic adhesives in addition to traditional surgical techniques, especially in patients with coagulation disorders (Akarsu C. 2011, Beyazit Y. 2010).

The Algan Hemostatic Agent (AHA), the herbal extract derived from the standardized blend of six different plants (Table 1). As we know, it is the first and only patented product made solely of herbs, with no additives in the world. (Patent application no: a2015 / 00018, date of application: 2015/01/05, application publication date: 2016/07/21, application publication no. TR2015 0018 A2, date of issue of pat-

ent document: 2017/11/21).

Each of the plants that form AHA has a content which is effective in hemostasis by alone or in combination. All biocompatibility tests such as sensitization, cytotoxicity and irritation and haemodynamic tests of the AHA were performed, and the results supported its safety and efficacy as a hemostatic agent. It is easily applied locally. Further, it has low cost, and does not require special storage.

Currently, there are products that have clinical trials and have been affected by similar mechanisms that have been granted for internal and external use (Kakaei, F. 2013, Briceño J. 2010, Dąbrowiecki, S. 2012). When AHA used in moist environment, it quickly polymerizes into a thin elastic film which has high tensile strength and firmly adheres to the anatomy of the tissue on which it is applied. Here, we aimed to evaluate the haemostatic effect of AHA in liver surgery in an animal model of hepatectomy.

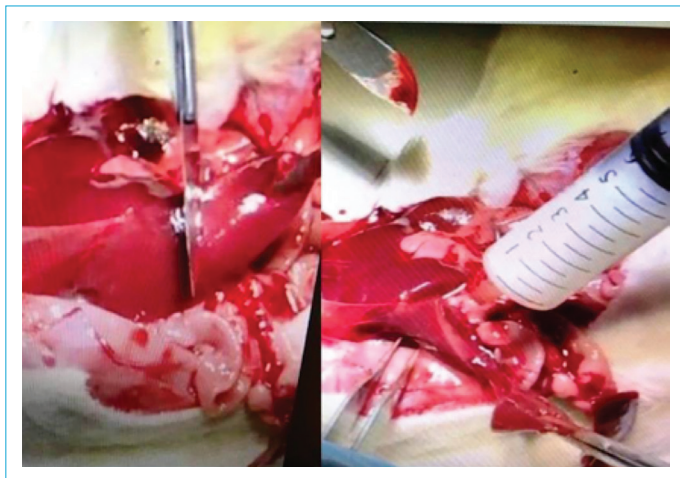
## Methods

For this study, approval was obtained from Kırıkkale University Animal Experiments Local Ethics Committee (Decision no. 2018/04). The experiment was carried out as stated in the literature (Ozdemir, Ibrahim Ali, 2016). In the study, 64 rats which are 180-210 grams, 5-7 week old were used. Rats were fed ad libitum and examined under standard laboratory conditions according to 12-hour dark-light period. The rats were randomly divided into 2 groups as heparinized and non-heparinized; each of the group has 32 rats. After that, rats separated to 8 randomly selected groups of 8. Dose of 640 IU/kg heparin was administered intraperitoneally to heparinized group three times a day for 3 days. The same amount of saline was given to the other group. The groups were formed as follows. 1st group (Heparinized control group), 2nd group (Heparinized AHA powder group), 3rd group (Heparinized AHA gel group), 4th group (Heparinized AHA liquid impregnated sponge group), 5th group (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).

The procedures were performed under general anesthesia with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). To carry out the experiment, laparotomy was performed with abdominal midline incision and the suspension ligaments of liver were released. Non-anatomical surgical resection was performed to the left lobe of the liver (Fig. 1) and the weight of the resected liver tissue was measured. AHA fluid (sponge), AHA powder, AHA gel and SF impregnated sponge in 2 cc volume were applied to the resected liver surface (Fig. 1). AHA powder was directly applied to the bleeding surface by hand, and even any press was

**Table 1.** Algan Hemostatic Agent Product 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



**Figure 1.** In liver lobectomy model; on the left side the beginning of the procedure, on the right side the application of AHA after the partial lobectomy and controlling of bleeding.

not performed. The gel form which was in injector as liquid sprayed directly to the bleeding area, the liquid became gel rapidly after spraying and the pressure was not exerted. The liquid form as a liquid-impregnated sponge was applied directly to the bleeding surface and pressed on surface lightly. In case of continuing bleeding after first application, the procedure was repeated with the same amount of the product. As it was known from previous experiments, AHA usually controlled bleeding within 10 seconds; because of this, the first application lasted 15 seconds, the second application lasted 30 seconds, and the third application lasted one minute. Subsequent applications took one minute. The application was measured chronometrically. After the procedure, hemostasis was observed in each group for 10 minutes. The rats were kept alive for one more week. One week after the first procedure rats were sacrificed and their livers were sacrificed for histopathological investigation of effects of AHA to liver and put in 10% formaldehyde solution for fixation.

## Histopathological Evaluation

Tissue samples were processed routinely at pathology laboratory and examined

under light microscope. These procedures were briefly carried out as follows: during routine follow-up process, tissue fixation in neutral buffered formalin for a period of time proceeded, tissue specimens were dehydrated in graded alcohols and embedded in paraffin, respectively. Five micrometer tissue sections were cut on the rotary microtome and stained with hematoxylin & eosin (H&E).

Histopathological evaluation was performed as described in the literature (Dortler, M. E. 2016). Tissue samples were evaluated by a single pathologist blinded to the groups. Samples were scored from 1 to 3 according to the inflammation status and formation of granulation tissue as follows: 1, mild; 2, moderate; 3, high. A similar scoring system was used for cell necrosis as follows: <25% of the field at 1/40 magnification was scored as 1; 25%–50% of the field as 2; and >50% of the field as 3.

SPSS software version 22.0 (SPSS Inc., Chicago, IL) was used to analyze the data of this study. Weight, bleeding time were calculated and mean values were compared among the four groups by using analysis of variance (ANOVA). The results were assessed at a 95% confidence interval and a significance level of  $p < 0.05$ . The data were expressed as mean (minimum-maximum).

## Results

Average bleeding time, body weight, and resected liver segment weight distribution of the groups were summarized in table 2. The groups were same in terms of body weight and resected segment weight ( $p > 0.05$ ). The shortest bleeding time was measured in the AHA powder group. The AHA powder form was able to control the bleeding in heparinized and non-heparinized groups in 7 and 4 seconds, re-

**Table 2.** Average bleeding time, body weight, and resected liver segment 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 (NHS)	p
MW (gr)	180.4	185.5	179.2	184.9	175.6	183.4	178.9	185.3	$p > 0.05$
MRLS (mg)	0.42	0.44	0.43	0.40	0.41	0.42	0.40	0.39	$p > 0.05$
BT(sn)	410 sec.	7 sec.	8 sec.	< 75 sec.	220 sec.	4 sec.	5 sec.	<45 sec.	$p < 0.001$
(min-max)	(285-645)	(4-9)	(6-9)	(45-115)	(165-405)	(4-7)	(4-7)	(15-45)	
ANR	9	1	1	2.5	6	1	1	1.75	$p < 0.001$
(min-max)	(6-12)			(2-3)	(4-8)			(1-3)	

Abbreviations: 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 group; AHA: Algan Hemostatic Agent; MW: Mean Weight; MRLS: Mean Resected Liver Segment; BT: Bleeding time; ANR: Average number of repetitions.

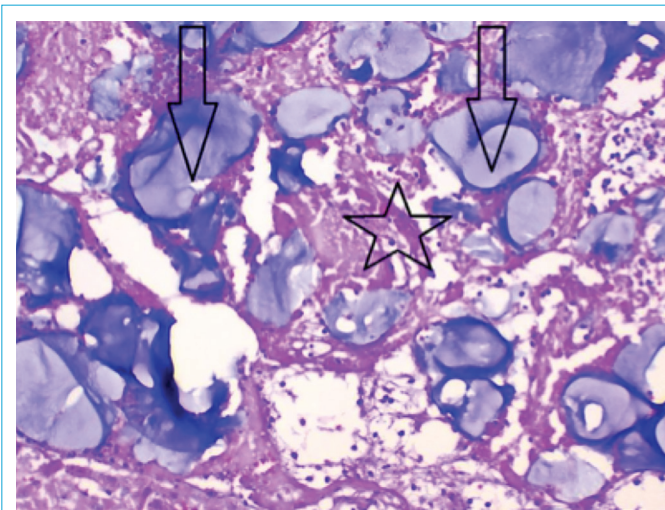


**Table 3.** Hemostasis duration of the control and the AHA liquid groups

	Bleeding stopped in first practice (15 sec)	Bleeding stopped in second practice (30 sec)	Bleeding stopped in third practice (60 sec)	Fourth and after 4 practice (Ineffective)	Average number of repetitions (min-max)	Average bleeding time (min-max)
Control, non-heparinized	0 (0%)	0 (0%)	0 (0%)	8 (100%)	6 (4-8)	220 sec (165-405)
AHA liquid, non-heparinized	4 (50%)	2 (25%)	2 (25%)	0 (0%)	1.75 (1-3)	<45sec
Control, Heparinized	0 (0%)	0 (0%)	0 (0%)	8 (100%)	9 (6-12)	410 sec (285-645)
AHA liquid, Heparinized	0 (0%)	4 (50%)	4 (50%)	0 (0%)	2.5 (2-3)	<75 s

The formula of the number of repetitions of the average process calculation= $(1 \times 1. \text{ number of application}) + (2 \times 2. \text{ number of application}) + (3 \times 3. \text{ number of application}) / \text{total number of application}$ .

The formula of average bleeding control time calculation= $(15 \times 1. \text{ number of application}) + (30 \times 2. \text{ number of application}) + (60 \times 3. \text{ number of application}) / \text{total number of application}$ .

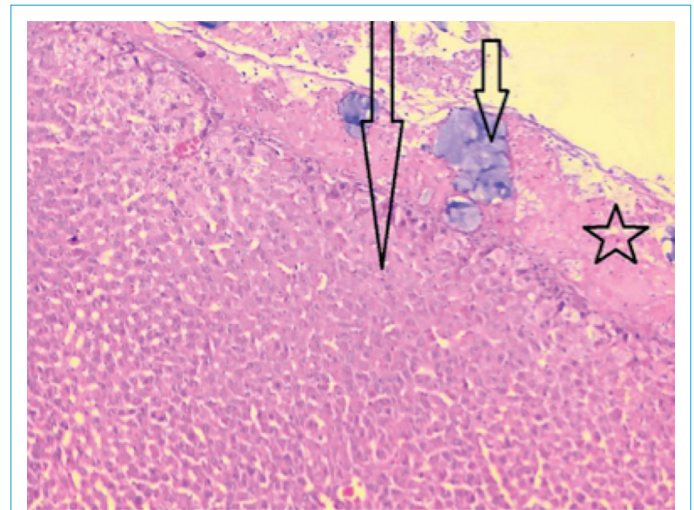


**Figure 2.** The barrier formed on the surface of the liver lobectomy resection line immediately after application of the AHA gel form. It is seen that the gel particles trap the blood between them and form a barrier. Arrow: AHA gel material; Star: fibrin, blood, and blood elements trapped in gel material are visible.

spectively. The AHA gel form was able to control the bleeding in heparinized and non-heparinized groups in 8 and 5 seconds, respectively.

In the AHA non-heparinized liquid group, bleeding control was achieved at 4, 2, and 2 rats in the first (15 sec), second (45 sec) and third (105 sec) applications, respectively.

AHA heparinized liquid group, bleeding control was provided in 4 rats in the 2nd application and in the 4 other rats in the 3rd application (Table 2). The bleeding time in the control group was significantly longer than in the experimental groups (Table 2).

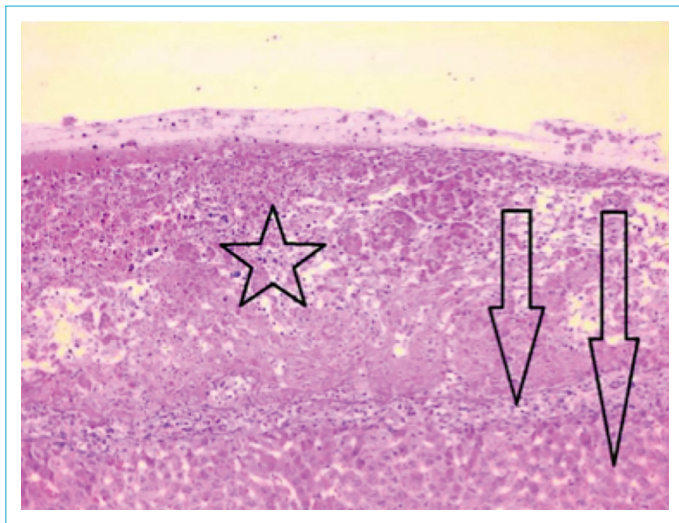


**Figure 3.** Histopathological appearance of AHA gel treated liver a week after procedure.

Star: Early organized image forming barrier of AHA gel form on the surface of the liver lobectomy incision line; Short arrow: AHA gel residue in the mucoid appearance at the site of application; Long arrow: Normal liver image without necrosis and inflammation (Hematoxylin and Eosin stain x40).

Hemostasis duration of the control and the AHA liquid groups were reported in table 3. AHA fluid (sponge) was applied to the bleeding at least 1 to 3 times. The SF impregnated sponge was applied to the resected liver surface at least 6 to 12 times.

The barrier formed on the surface of the liver lobectomy resection line immediately after application of the AHA gel form. It is seen that the gel particles trap the blood between them and form a barrier (Fig. 2).



**Figure 4.** Histopathological appearance in liver treated with AHA liquid a week after procedure.

Star: early organized image forming barrier of AHA gel form on the surface of the liver lobectomy incision line;

Short arrow: macrophages accumulating between the parenchyma of the liver and the AHA barrier,

Long arrow: After 7 days of administration of AHA liquid form, normal liver image without necrosis and inflammation (Hematoxylin and Eosin stain x40).

In histopathological examination mild portal inflammation (score-1) on the contact surface of the liver of rats was observed in the control group and all forms of AHA groups. Although mild fibrosis was observed in the control groups and all forms of AHA groups, necrosis were not detected in any of the control and AHA groups (Fig. 3, 4).

In the histological examination of the first application, hemostatic barrier was observed on the cutted surface of the liver that consist of gel and clot mixture (Fig. 2). One week later, the gel form was nearly absorbed and the macrophage layer was observed between the liver parenchyma and the hemostatic barrier. No necrosis was observed in the liver parenchyma under the macrophage barrier. The hemostatic barrier was seen to be started organizing (Fig. 3 and 4).

## Discussion

Three different forms of AHA were tested in this study and all of them were very effective. Although the powder form controls bleeding a bit quicker than other forms, all of the others are accepted as effective as the powder form, since there is no statistical difference between them in terms of efficacy of bleeding control.

Liver injury, laceration and hepatectomy, hepatic trauma models are used as synonyms in the literature (Satar, N. Y. G. 2013, Matsuoka T. 1995, Ozemir, Karakaya, K. 2009, Ibrahim Ali. 2016, Aysan E. 2010, Holcomb JB. 2000). As stated

in the literature, the average of bleeding time in control group differs among studies. The average bleeding time was measured 223 seconds (Satar, N. Y. G. 2013) and 377 seconds (Ozemir, Ibrahim Ali, 2016) according to studies performed on Sprague Dawley rats. In our study, this time was measured 220 seconds in the non-heparinized control group and 410 seconds in the heparinized control group. In a similar study in the literature, the product Ankaferd Blood Stopper controlled the bleeding at 23 seconds while Surgicel ceased at 47 seconds (Satar, N. Y. G. 2013). In our study, bleeding control was provided at 7, 4, 8 and 5 seconds in AHA powder heparinized group, in non-heparinized group, in AHA gel heparinized group and in non-heparinized group, respectively. According to the results of this study, although the AHA seems to be the most effective hemostatic agent used for this purpose in the liver laceration (lobectomy) model in the literature, the actual differences can only be demonstrated by further comparative studies of different hemostatic agents.

In the literature, liver has been histologically examined after application of hemostatic agent in a small number of studies performed with liver lobectomy. In a study comparing Ankaferd and Surgicel with controls, all rats in the control group died within 12 minutes.

In this study, 3 rats from the ABS group 2 rats from Surgicel group survived less than 30 minutes after the procedure. The other rats were sacrificed after 30 minutes of observation. And the liver was histologically examined. In this study, ABS-treated animals showed no difference compared to the Surgicel group with regard to time to death (Karakaya, K. 2009).

In our study, none of the rats died after the procedure and were alive for another week for histological examination. One week later, the rats were sacrificed and the liver was examined histopathologically. It can be related to the fact that none of the AHA administered rats died within 30 minutes and that all of their lives for one week could have early control of blood loss in the AHA.

In another hepatectomy study, solid carbon dioxide was compared with suture. In this study, solid carbon dioxide was considered as two groups. Suture group and a group of solid carbon dioxide were sampled on the same day, another group of solid carbon dioxide was sacrificed on the 7<sup>th</sup> day and examined histopathologically (Ozemir, I. A. 2016). In this study hemostasis times were 377, 203, 176 seconds for control, suture and solid carbon dioxide, respectively. In our study, the duration of hemostasis was similar in the control group, but the AHA maintained hemostasis for 4 seconds in powder form and 5 seconds in gel form.

The AHA forms that were applied to the liver didn't have any

adverse effect on healing of the liver. We found a decrease in portal inflammation, fibrosis and cell necrosis compared to the control groups. Some of the local hemostatic agents induce thermal injuries, nevertheless AHA does not cause any thermal damage (Wright, J. K., 2004).

In some studies designed as liver haemorrhage model in the literature, the amount of blood lost instead of bleeding time was measured, and tried to show hemostatic efficacy (Karakaya, K. 2009, Holcomb, J. B. 2000). Since in the liver laceration models it is not possible to hold the applied gel, powder and liquid on surface of liver, we consider that measuring the bleeding time is a more decisive method than the amount of blood lost, and designed our study accordingly. We think, the medical literature needs to be rescued from the complexity of concepts about bleeding control, and bleeding models should be standardized.

In this study, we conduct a preclinical animal trial with AHA, and no comparison was made with the other products. Due to the many factors such as animal weight, the experience of the practitioner, technical differences, laboratory conditions, etc., it is necessary to compare the other hemostatic agents with AHA in the same experiment for evaluating bleeding effectiveness. We think that it will be more appropriate to perform future studies comparing AHA with the other hemostatic agents by independent groups.

## Conclusion

AHA, a new herbal hemostatic agent, is effective in controlling local hemostasis in rats liver lobectomy model. Histological studies have shown that the mechanism of action of AHA is physical. Accordingly, when AHA is applied to the liver resection surface, it becomes a gel and forms a barrier by surrounding the fibrin, blood and blood components in the environment. Although this study shows that AHA controls hemorrhage earlier than other hemostats, it is necessary to conduct studies that should be confronted with other products.

## Disclosures

**Ethics Committee Approval:** The study was approved by the Local Ethics Committee.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

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