

# Ankaferd Blood Stopper®: Is The Source of Intraperitoneal Adhesion?

## Abstract

**Background:** Ankaferd Blood Stopper (ABS) is a plant extract and it has been used as a haemostatic agent in Turkish traditional medicine. ABS could be used in intraperitoneal bleeding due to any surgery or trauma. However, it is not clear, whether it has any side effect in peritoneum such as adhesions. In our study, we aimed to evaluate the ABS as a possible source of intraperitoneal adhesions. **Materials and methods:** The study was conducted with thirty Wistar albino female rats. The rats were divided into three equal groups. Group C was control group with laparotomy only. Group S was Surgicel® (regenerated oxidized cellulose) group with laparotomy and bleeding on the uterus and application of Surgicel (1 cmx1 cm) to the bleeding site. Group A was the ABS group constituted in uterus bleeding and application of ABS to the site of injury (2 ml). After 21 days, all rats were killed. We scored the adhesions according to their degree of severity and collected specimen for the detection of hydroxyproline levels. **Results:** Macroscopically, there was grade 1 and 2 adhesions in ABS group (median score: 1). In control and Surgicel group, there was no significant adhesion (median score: 0). The mean hydroxyproline level of the control group was significantly higher than Surgical and ABS groups (3.28, 2.11, and 2.48 mg/g wet tissue respectively). **Conclusion:** Application of ABS for intraperitoneal bleeding may cause intraperitoneal adhesion. The relatively low levels of hydroxyproline in ABS group led us to think that ABS causes adhesions by a mechanism that not due to newly organized collagen. Although ABS is a useful haemostatic substance, care must be taken during its intraperitoneal application.

**Keywords:** Ankaferd Blood Stopper (ABS), Intraperitoneal adhesion

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## Introduction

Intraperitoneal adhesion (IA) is a significant clinical problem possibly requiring an emergent surgery and it is a common complication following general abdominal and gynaecological operations ranging between 55% and 95%<sup>(1,2)</sup>. The most encountered sequels of IA are small bowel obstruction, infertility, chronic abdominal and pelvic pain<sup>(3,4)</sup>. IA may lead to the prolonged hospitalization and the rising cost of health care<sup>(5,6)</sup>. Main factors associated with the formation of IA are trauma, foreign bodies, tight suturing, ischemia, and thermal injury<sup>(7,8)</sup>. Organization of the fibrin matrix is the major etiopathological factor responsible for the postoperative IA<sup>(9)</sup>.

Ankaferd Blood Stopper (ABS) is a plant extract and herbal medicine used as an haemostatic agent in some regions of Anatolia. However, the mechanism of its haemostatic effects is not completely understood yet. In our country, ABS is currently applied by many surgeons on bleeding side locally. It has been stated that it is a very effective haemostatic agent<sup>(10,11)</sup>. However, its complications are still unknown, especially when used within the abdominal cavity. We aimed to evaluate the possible effects of ABS for the formation of IA in rats with intraperitoneal use of ABS.

## Materials and methods

Thirty female Wistar Albino rats weighing median 180 g (160-230 g) were used in this study. All rats were kept under standard conditions with free access to oral intake. The study was performed with approval of the animal research committee.

The rats were assigned randomly to three groups (n=10). One of the groups was served as control. Surgicel® (regenerated oxidized cellulose) and ABS were used in the other groups. Surgicel is a blood stopper and no IA was reported after its use in the peritoneal cavity.

Group C (n=10) was the control group. Laparotomy was applied through a vertical incision. No further surgery was added.

Group S (n=10) was the Surgicel group. After laparotomy, the uterus was incised from the superior site towards to bilateral cornus with an approximate length of 1 cm. Afterwards, Surgicel was applied (1x1 cm) onto the incised area.

Group A (n=10) was the ABS group. Laparotomy and uterine injury were made in the same matter as in previous groups. Two ml of ABS was applied onto the injured area.

All rats were killed after 21 days of surgery and bleeding areas were evaluated and graded macroscopically. Adhesions were classified according to a modified adhesion score reported by Zamir et al and Altuntas et al<sup>(12,13)</sup> (table 1). We also collected specimen for defining the hydroxyproline levels from incision area.

### Hydroxyproline assay

Tissue hydroxyproline (HP) levels were determined as previously described by Reddy et al.<sup>(14)</sup> using minor modifications. Briefly, 50 µl 12 N NaOH solution was added to the O-ring screw capped tubes which contains 2 g tissue samples. The samples were hydrolyzed by autoclaving at 120°C for 20 min.

Chloramine T-reagent was added to the hydrolyzate, mixed gently, and the oxidation was allowed to proceed for 25 min at room temperature. Erlich's aldehyde reagent was added to each sample at 65°C for 20 min. Absorbance of each sample was recorded at 550 nm using a spectrophotometer. Acid-soluble collagen and trans-4-hydroxy-2-proline were used as standard. Results are expressed as mg HP/g wet tissue. All spectrophotometric measurements run in duplicate.

## Results

### Macroscopic Evaluation

In the control group, all rats had an adhesion score of zero. In Surgicel group, only two rats had grade 1 adhesions and others had no adhesion at all (median score=0). In ABS group, there were six rats with grade 1 and three rats with grade 2 adhesions (median score=1) (table 2). Significantly lower number of rats had grade 0 adhesions in the ABS group when compared to the control group (table 2).

### Biochemical analysis

Hydroxyproline values are shown on table 3. The control group had higher levels of hydroxyproline than groups S

and A. The difference of hydroxyproline levels between control and Surgicel groups was significant (**p<0.05**).

## Discussion

IAs are abnormal fibrous bands connecting between bowel segments and normal peritoneal surfaces or intraabdominal organs<sup>(2)</sup>. IA after laparotomy is a serious cause of postoperative morbidity and mortality and leads to complications including intestinal obstruction, secondary infertility, enterocutaneous fistula, intraabdominal abscess, and pain<sup>(4,15,16)</sup>. Various causes of adhesion formation exist such as surgery related trauma, inflammation, infection, and foreign body in the peritoneal cavity<sup>(17,18)</sup>. Fibrin-rich exudate becomes organized to form the fibrin gel matrix, producing fibrin bands subsequently and angiogenesis factors initiate the construction of vascular structure within the fibrotic bands. Fibronectin and proteoglycan serve as framework for collagen deposition, and adhesion is a result of collagen deposition. The hydroxyproline levels reflect the collagen synthesis<sup>(1, 9,20)</sup>.

Ankaferd Blood Stoper (ABS) is a plant extract, which has been used in Turkish traditional medicine as a haemostatic agent. It is used for management of external or internal haemorrhage following to trauma and dental surgery. ABS is composed of mixture of five plants including *Thymus vulgaris* (0.1 mg), *Glycyrrhiza glabra* (0.18 mg), *Vitis vinifera* (0.16 mg), *Alpinia officinarum* (0.14 mg) and *Utrica dioica* (0.12 mg)<sup>(21,22)</sup>. ABS produces its haemostatic effects by promoting protein network formation, which acts as an anchor for erythrocyte aggregation and covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets<sup>(10,23)</sup>.

No data is available whether ABS leads to intraabdominal adhesion when used against the intraabdominal organ bleeding including liver, spleen or uterus. Therefore

**Table 1** | Scoring of the adhesions

0	No evidence of any adhesion
1	Fine avascular adhesions. Separated by gentle traction
2	Moderate thick adhesions. Separated by blunt dissection
3	Dense adhesions. Vascularised, separated by sharp dissection

**Table 2** | The incidence of adhesion grade in groups

	Control Group	Surgicel Group	ABS Group
Grade 0	10	8	1
Grade 1	0	2	6
Grade 2	0	0	3
Grade 3	0	0	0

**Table 3** | Hydroxyproline values (mean and SD: standard deviations)

	Mean (mg/g wet tissue) $\pm$ SD
Group C	3.28 $\pm$ 0.21
Group S	2.11 $\pm$ 0.12
Group A	2.48 $\pm$ 0.16

we aimed to evaluate ABS whether it is a causing factor for IA formation when it is used in the intraperitoneal cavity.

Our results showed that ABS leads to mild or moderate adhesions between damaged uterus and intestine. The Surgicel did not lead to any adhesion formation. When hydroxyproline levels were considered, those of ABS group were lower than control group but higher than Surgicel group. Interestingly the highest hydroxyproline levels were in the control group. This paradoxical result made us to think that the mixed plant content in the ABS may cause adhesion without any increase in hydroxyproline levels. We also observed that specimens of the ABS group were dehydrated and shrink than other groups' specimen macroscopically. One or more of these five plants in ABS may be the cause of the adhesion without production of newly organized collagen deposition.

Goker et al.<sup>(11)</sup> suggested that normal haemostatic elements were spared during haemostatic action of

ABS. Protein network formation contains aggregation of blood cells, particularly erythrocytes and interactions between ABS and blood proteins, mainly fibrinogen. Adhesion formation needs the fibrin network. The usage of fibrinogen in haemostatic mechanism results in decrease in hydroxyproline level.

IA related to the ABS might be not the secondary effect of the newly organized collagen.

## Conclusions

As conclusion, ABS may lead to intraabdominal adhesions when used for intraperitoneal bleeding. We suggest that the cause of intraperitoneal adhesion is not due to the de novo synthesis of collagen led by foreign body effect of ABS. The decrease in hydroxyproline levels at ABS may be explained by its haemostatic mechanism. Care must be taken into account when ABS is used in the abdominal surgery. ■

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