



Effects of ankaferd blood stopper on soft tissue healing in warfarin treated rats

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ABSTRACT

Ankaferd Blood Stopper (ABS) is a new promising local haemostatic agent. Although there are many studies showing its mechanism of haemostasis, histological and biochemical effects of ABS have not been studied in detail. Aim of this study was to evaluate the effects of this new generation local haemostatic agent on warfarin treated rats focusing on short term soft tissue healing. 12 systemically warfarin treated (warfarin group) and 12 none treated Wistar – Albino rats (control group) were selected for the trial. Rats in warfarin group were treated intraperitonally 0,1 mg/kg warfarin and rats in control group were given 1ml/kg saline 3 days earlier to surgical procedure and continued until sacrifice. All rats had incisions on dorsal dermal tissue which was applied ABS or no haemostatic agent (NHAA) before suturing. Six of each group are sacrificed on day 4, and the other 6 were sacrificed on day 8. Prothrombin time (PT) in blood samples, collagen rate and histological evaluation in skin samples were determined. NHAA tissue samples' collagen rate decreased [$p=0,008$] but the collagen rate of ABS administrated tissue samples remained unchanged [$p=0,257$] from day 4 to 8 in the control group. Both ABS administrated tissue samples' [$p=0,026$] and NHAA tissue samples' [$p=0,005$] histologic total damage score values decreased from day 4 to 8 in warfarin group, but values of ABS administrated tissues were significantly higher than NHAA tissues in warfarin group at the eight day [$p=0,006$]. ABS seems to have a negative effect on short term soft tissue healing histologically under warfarin treatment.

Key Words: Warfarin, Ankaferd Blood Stopper, Collagen rate, Healing

INTRODUCTION

The aim of oral anticoagulant therapy is to reduce blood coagulability to an optimal therapeutic range within which the patient is provided some degree of protection from thromboembolic events. This is achieved at the cost of a minor risk of spontaneous bleeding [1]. In the past, it has been suggested that anticoagulant treatment be stopped or reduced for several days before a surgical intervention. Surgical interventions can be performed in patients treated with oral anticoagulants without interruption or diminution of the medication [2].

Warfarin is one of the coumarin group of drugs and is prescribed for various conditions. It blocks the formation of prothrombin and factors II, VII, IX, and X, which are involved in both the extrinsic and common coagulation pathways, and prevents the metabolism of vitamin K to its active form that is needed for the synthesis of these factors. The activity of warfarin is expressed as the International Normalised Ratio (INR), which is the standard introduced by the World Health Organization 20 years ago. It is a prothrombin ratio obtained by dividing the prothrombin time by the laboratory control prothrombin time [3,4].

Ankaferd Blood Stopper® (ABS) (Ankaferd Health Products Ltd., Istanbul, Turkey) is a standardized unique combined medicinal plant extract, which has been approved in the management of postsurgery dental bleeding and

external hemorrhage in Turkey [5]. The basic mechanism of action of ABS is through the formation of encapsulated protein network providing focal points for vital erythrocytes to aggregate on. The ABS induced protein network formation involves blood cells, particularly erythrocytes, without affecting the physiological individual coagulation systems. ABS is a standardized extract from the following plants: *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* [5,6].

The importance of the collagen structure for the strength and function of the skin is widely recognised [7]. Most studies on wound healing and scar formation address attention to qualitative aspects of the structure of this protein [8].

According to our knowledge, there is no study in literature that evaluates collagen rate of ABS treated rat tissues under warfarin regimen in short term soft tissue healing. Our results will help to have an idea about the availability of this local haemostatic agent on short term soft tissue healing in warfarin treated surgery patients.

MATERIALS AND METHODS

Animals and Treatment

We operated totally 24 Wistar-albino rats and 12 of them were systemically warfarin treated as the warfarin group and the other untreated 12 rats were the control group. All 24 experimental animals were male and were in 280-560 g of weight range. Experimental animals were obtained from Department of Experimental Animals Unit, Kocaeli University, (Kocaeli, Turkey) and surgeries and postoperative care have been also performed in this unit. All experimental protocols were approved by Animal Care and Use Ethical Committee of Marmara University (date:15.12.2009; number:16/3-2009).

Control group of rats were injected 1ml/kg saline intraperitoneally for 3 days before surgery, stopped on the day of surgery and continued from postoperatively first day until the day of sacrifice. Six of 12 rats in the control group have been sacrificed 4 days after surgery and other 6 have been sacrificed on the 8th day after surgery. Warfarin group has been injected 0,1 mg/kg warfarin intraperitoneally for 3 days before surgery, stopped on the day of surgery and continued from postoperatively first day until the day of sacrifice. Six of 12 (warfarin group) rats have been sacrificed 4 days after surgery and other 6 have been sacrificed on the 8th day after surgery. Sacrifice procedure has been performed using high dose anesthetics.

After aseptic and antiseptic conditions were achieved, experimental animals were anesthetized with 90 mg/kg Ketamin (Ketalar 500 mg enjektabl 1 flakon, Pfizer İlaçları Ltd.Şti, İstanbul, Türkiye) and 10mg/kg Xylazine (Rompun® Bayer HealthCare, Leverkusen, Germany) combination. One of the rats in control group died due to a complication of the anesthetic and study was completed with a number of 23 rats.

As soon as shaving the dorsal skins, incisions were made on all 23 rats. Incisions were 2 cm long and 2 cm far from each other and perpendicular to the head-tail direction. On the day of surgery 2x20 mm² sized tissue samples were taken from the margin of the wound for further biochemical and histological tests. One of the wounds has been sutured without any haemostatic agent and left to heal naturally. The other wound has been applied 0,25 ml of ABS before suturing. This protocol has been followed both in the control and warfarin groups.

On the day of sacrifice, the whole dorsal skin including the healing wound area has been totally excised. Two different wound healing areas (ABS-nonhaemostatic) have been separated from each other and prepared for the biochemical evaluation. Biochemical tests were performed at Department of Biochemistry, Faculty of Dentistry, Marmara University (Istanbul, Turkey).

Immediately after sacrifice, 2 ml of intracardiac blood sample was taken from all experimental animals and centrifuged at 2000 rpm for 5 minutes to gain blood plasma for PT tests. PT tests were determined in Düzen Norwest Veterinary Laboratories (Ankara,Turkey).

Determination of Collagen Rate

The collagen rate was measured according on the method published by Lopez De Leon and Rojkind [9], which is based on selective binding of the dyes Sirius Red and Fast Green FCF to collagen and noncollagenous components, respectively. Tissue samples were cut with a razor blade, immediately fixed in 10% formalin then samples were embedded in paraffin, and sections, approximately 15 µm thick were obtained. Both dyes used were eluted readily and simultaneously by using 0.1 N NaOH-methanol (1:1, v/v). Finally, the absorbances at 540 and 605 nm were used to determine the amount of collagen and protein, respectively.

Histological evaluation

For histological evaluation, skin tissue samples were fixed in 10% formaldehyde and processed routinely for embedding in paraffin wax. After dehydration in an ascending series of ethyl alcohol, tissue samples were cleared in toluene. Paraffin wax-embedded skin sections 5–6 μm thick were stained with Gomori's one step trichrome histopathological evaluation, and examined under All light microscopic sections were observed and photographed with digital camera (Olympus C-5060, Tokyo, Japan) under a photomicroscope (Olympus BX51, Tokyo, Japan). Microscopic scoring was done by experienced histologists, who were unaware of the treatments received by the animals. Epithelial and hair follicular degeneration, dermal edema and inflammation, collagen density and vasocongestion were evaluated for the skin tissue. Scores for each criterion are given as 0, none; 1, mild; 2, moderate, 3, severe. Maximum score was 12. At least five microscopic areas were examined to score each specimen.

Statistical analysis

All statistical analysis was performed using SPSS 16.0 (Statistical Package for the Social Sciences) Software program. Results were expressed as mean \pm standard deviation. Student t test used for comparison of the quantitative datas that does achieve two independent group's parametric test assumptions. Mann-Whitney U test used for comparison of the quantitative datas that does not achieve two independent group's parametric test assumptions. P values less than 0.05 were considered statistically significant. Kruskal Wallis test used for comparison of the quantitative data that does not achieve three or more independent group's parametric test assumptions.

RESULTS**Biochemical Results****Prothrombin Time Comparison**

PT values were analyzed on the fourth and eighth days. In the control group no change was observed in PT values on days 4 and 8; however, in the warfarin group, the PT values significantly increased on day 8 when compared to day 4 [p=0,005]. The PT values of the warfarin group were significantly longer than the control group both on the fourth and on the eighth days [p=0,014, p=0,0001][Table 1].

	Control group (n:11)		Warfarin group (n:12)		P	
PT(sn)						
Day 4	11,14	\pm 3,1	16,07	\pm 2,4		0,014
Day 8	10,62	\pm 3,3	26,25	\pm 5,7		0,0001
P	0,788		0,005			

Abbreviations: PT: prothrombin time; S.D.: Standard Deviation

^aValues presented as mean \pm SD; values of P < .05 were regarded as significant;
Student t test

Comparison of the Collagen Rate of Tissue Samples Taken During Surgical Procedure Before any Hemostatic Agent Administration in the Control and the Warfarin Groups

No significant difference has been noted between the groups [p>0,05][Table 2].

	Control group (n:11)			Warfarin group (n:12)			P
	Mean	\pm S.D.		Mean	\pm S.D.		
Collagen rate	0,04	\pm 0,01		0,06	\pm 0,021		0,092

Abbreviations: SD: standard deviation

^aValues presented as mean + SD; values of P < .05 were regarded as significant;
Student t test

Collagen rate comparison of tissue samples after heamostatic agent implementation on the 4th day

Collagen rate values of NHAA tissues are significantly higher than ABS administrated tissues in control group at the fourth day [p=0,008].

Collagen rate values of ABS administrated tissues are significantly higher than NHAA tissues in warfarin group [p=0,032],

When the control and warfarin groups are compared on the fourth day, no significant difference has been noted in the collagen rate values of ABS administrated tissues [p=0,819]. Collagen rate values of NHAA tissues are significantly higher in control group than warfarin group [p=0,0001][Table 3].

Table 3: Collagen Rate Comparison of Tissue Samples After Hemostatic Agent Administration on the 4th Day.^a

		Control Group (n=6)			Warfarin Group (n=6)			P
		Mean.	±	S.D.	Mean.	±	S.D.	
Collagen rate	ABS	0,1	±	0,011	0,1	±	0,019	0,819
	NHAA	0,12	±	0,008	0,08	±	0,012	
p		0,008*			0,032*			

Abbreviations: S.D.: standard deviation; ABS: Ankaferd Blood Stopper; NHAA: Non Haemostatic Agent Administered
^aValues presented as mean + SD; values of P < .05 were regarded as significant;
 Student t Test

Collagen rate comparison of tissue samples after hemostatic agent implementation on the 8th day

No significant difference has been noted between the groups on the eighth day [p>0,05][Table 4].

Table 4: Collagen Rate Comparison of Tissue Samples After Hemostatic Agent Administration on the 8th Day.^a

		Control Group (n=5)			Warfarin Group (n=6)			P
		Mean.	±	S.D.	Mean.	±	S.D.	
Collagen rate	ABS	0,08	±	0,026	0,09	±	0,021	0,551
	NHAA	0,09	±	0,017	0,08	±	0,023	
p		0,52			0,216			

Abbreviations: S.D.: standard deviation; ABS: Ankaferd Blood Stopper; NHAA: Non Haemostatic Agent Administered
^aValues presented as mean + SD; values of P < .05 were regarded as significant;
 Student t Test

Comparison of Collagen Rate of Tissue Samples Taken on Days 0, 4, and 8

Both ABS administrated and NHAA tissue samples' collagen rate values increased from day 0 to 4 [p=0,0001 and p=0,0001 respectively], and the collagen rate of NHAA tissue samples decreased [p=0,008] but ABS administrated tissue samples remained unchanged [p=0,257] from day 4 to 8 in the control group. On the other hand, in warfarin group, ABS administrated tissue samples' collagen rate values increased [p=0,001] but NHAA tissue samples' collagen rate remained unchanged [p=0,085] from day 0 to 4. The collagen rates of ABS administrated and NHAA tissue samples did not change [p=0,491, p=1,0] from day 4 to 8[Table 5].

Table 5: The Collagen rate values of tissue samples taken on days 0, 4, and 8. ^{a,b}

Collagen Rate		Day 0			Day 4			Day 8		
		Mean.	±	S.D.	Mean.	±	S.D.	Mean.	±	S.D.
Control Group	ABS				0,1	±	0,011	0,08	±	0,026
	NHAA	0,04	±	0,01 ^{bc}	0,12	±	0,008 ^d	0,09	±	0,017
Warfarin Group	ABS				0,1	±	0,019	0,09	±	0,021
	NHAA	0,06	±	0,021 ^{bc}	0,08	±	0,012	0,08	±	0,023

Abbreviations: S.D.: standard deviation; SOD: Superoxide Dismutase; ABS: Ankaferd Blood Stopper; NHAA: Non Haemostatic Agent Administered

^a Values presented as mean + SD; values of P < .05 were regarded as significant; Kruskal Wallis test, Student t Test, Mann Whitney U test

^b Tissue samples taken in the day 0, has been extracted from the wound zone that would be implemented with no haemostatic agent, before applying ABS were set.

^c In control group, significantly different from NHAA day 4 [p=0,0001]; NHAA day 8 [p=0,0001]; ABS day 4 [p=0,0001]; ABS day 8 [p=0,015]

^d In control group, significantly different from NHAA day 8 [p=0,008]

^e In warfarin group, significantly different from NHAA day 4 [p=0,045]; NHAA day 8 [p=0,041]; ABS day 4 [p=0,001]; ABS day 8 [p=0,009]

Histological Results

The histological evaluation of skin tissue samples taken on control rats at day 0 showed quite regular epidermis and dermis with collagen fibers and hair follicles. After 4 days of wound healing, control rats showed thick epidermis, granulation tissue and severe inflammatory cell infiltration and vascular congestion in dermis region. After 8 days of wound healing, control rats showed thick epidermis, granulation tissue and moderate inflammatory cell infiltration with vascular congestion in dermis region. After 4 days, control rats treated with ABS showed thick epidermis, moderate inflammatory cell infiltration, vascular congestion and collagen fibers in dermis. After 8 days control rats treated with ABS showed mild degeneration in epidermis, moderate vascular congestion, inflammatory cell infiltration, the region filled with newly formed collagen fibers became thinner in dermis. Hair follicles were present near the wound healing region[Figure 1].

The histological evaluation of skin tissue samples taken on warfarin treated control rats at day 0 showed degenerated epidermis and dermis with collagen fibers. After 4 days of wound healing, warfarin treated rats showed thick epidermis, moderate inflammatory cell infiltration, vasocongestion and moderate increase in collagen fibers in dermis. After 8 days of wound healing, warfarin treated rats showed thin epidermis, mild increase collagen fibers, inflammatory cell infiltration and vasocongestion in dermis region. After 4 days, warfarin and ABS treated rats showed moderated degeneration in epidermis, severe vascular congestion, moderate inflammatory cell infiltration and moderately increased collagen fibers in dermis. After 8 days, warfarin and ABS treated rats showed regular

thinner epidermis, moderate vasocongestion, inflammatory cell infiltration and moderated increased in collagen fibers in dermis. Hair follicles were present near the wound healing region[Figure 2].

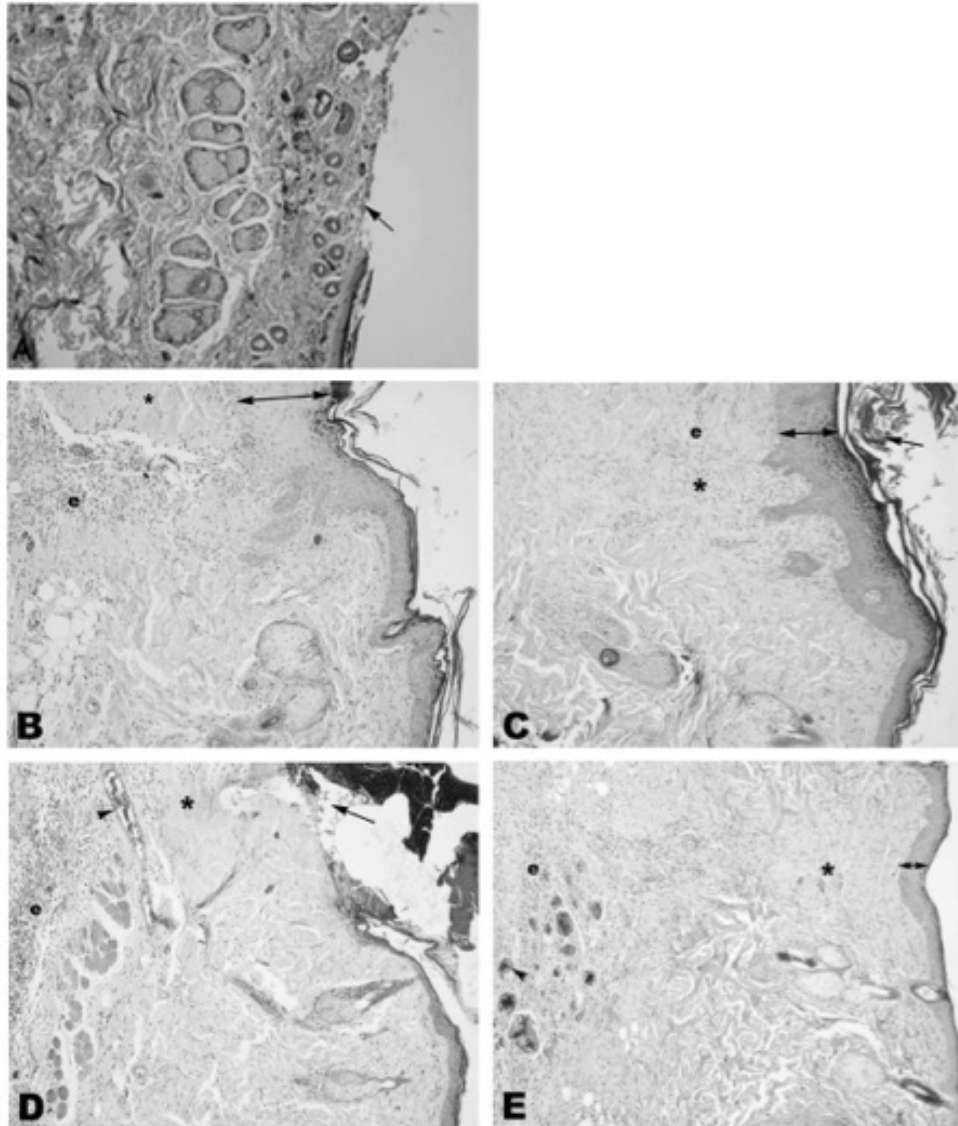


Figure 1. The histological evaluation of skin tissue samples taken on control rats at day 0

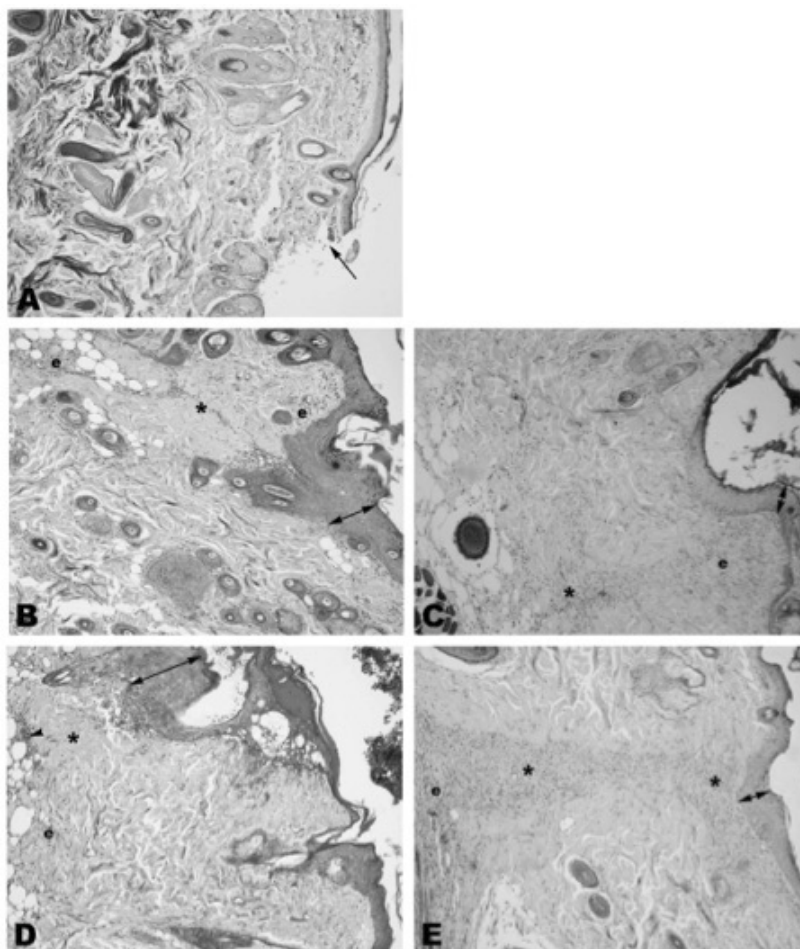


Figure 2. The histological evaluation of skin tissue samples taken on warfarin treated control rats at day 0

Comparison of the Histologic Total Damage Score Values of Tissue Samples Taken During Surgical Procedure Before any Hemostatic Agent Administration in the Control and the Warfarin Groups

No significant difference has been noted between the groups [$p > 0,05$][Table 6].

Table 6: The Histological Total Damage Scores of the Control and the Warfarin Groups' Tissue Samples Taken During Surgical Procedure Before Hemostatic Agent Administration (Day 0).^a

Scores	Control group (n:11)			Warfarin group (n:12)			P
	Mean	±	S.D.	Mean	±	S.D.	
	1,09	±	1,44	0,81	±	1,33	0,655

Histologic Total Damage Score Values comparison of tissue samples after heamostatic agent implementation on the 4th day

Histologic Total Damage Score values of NHAA tissues are significantly higher than ABS administrated tissues in control group at the fourth day [$p = 0,026$][Table 7].

Table 7: Histological Total Damage scores Comparison of Tissue Samples After Heamostatic Agent Administration on the 4th Day.^a

Scores		Control Group (n=6)			Warfarin Group (n=6)			P
		Mean.	±	S.D.	Mean.	±	S.D.	
	ABS	8,5	±	2,14	10,66	±	1,79	0,88
	NHAA	11,16	±	1,46	8,83	±	2,4	0,123
p				0,026*			0,242	
Mann Whitney U								

Histologic Total Damage Score Values comparison of tissue samples after heamostatic agent implementation on the 8th day

Histologic Total Damage Score values of ABS administrated tissues are significantly higher than NHAA tissues in warfarin group at the eight day [$p = 0,006$][Table 8].

When the control and warfarin groups are compared on the eight day, Histologic Total Damage Score values of NHAA tissues are significantly higher in control group than warfarin group [$p=0,001$][Table 8].

Table 8: Histological Total Damage scores Comparison of Tissue Samples After Hemostatic Agent Administration on the 8th Day.^a

		Control Group (n=5)			Warfarin Group (n=6)			
		Mean.	±	S.D.	Mean.	±	S.D.	P
Scores	ABS	10	±	1,78	8	±	1	0,093
	NHAA	9,8	±	1,6	4,5	±	1,25	0,001*
p		0,872			0,006*			

Student t test, Mann Whitney U test

Comparison of Histologic Total Damage Score Values of Tissue Samples Taken on Days 0, 4, and 8

Both ABS administrated and NHAA tissue samples' histologic total damage score values increased from day 0 to 4 [$p=0,001$ and $p=0,001$ respectively], and both NHAA tissue samples [$p=0,146$] and ABS administrated tissue samples [$p=0,289$] remained from day 4 to 8 in the control group. On the other hand, in warfarin group, both ABS administrated tissue samples' and NHAA tissue samples' histologic total damage score values increased from day 0 to 4 [$p=0,001$ and $p=0,001$ respectively], and both ABS administrated tissue samples' [$p=0,026$] and NHAA tissue samples' [$p=0,005$] histologic total damage score values decreased from day 4 to 8 [Table 9].

Table 9: The Histological Total Damage scores of tissue samples taken on days 0, 4, and 8.^{a,b}

		Day0		Day 4			Day 8		
			±	Mean.	±	S.D.	Mean.	±	S.D.
Control Group	ABS			8,5	±	2,14	10	±	1,78
	NHAA	1,09	±	1,44*	±	1,46	9,8	±	1,6
Warfarin Group	ABS			10,66	±	1,79***	8	±	1
	NHAA	0,81	±	1,33**	±	2,4****	4,5	±	1,25

* in control group, significantly different than ABS day 4, ABS day 8, NHAA day 4 and NHAA day 8 [$p=0,001$, $p=0,001$, $p=0,001$, $p=0,001$ respectively].

** in warfarin group, significantly different than ABS day 4, ABS day 8, NHAA day 4 and NHAA day 8 [$p=0,001$, $p=0,001$, $p=0,001$, $p=0,001$ respectively].

*** in warfarin group, significantly higher than ABS day 8 [$p=0,026$]

**** in warfarin group, significantly higher than NHAA day 8 [$p=0,005$]

DISCUSSION

Most often and emergency care needed complication in surgery is bleeding. It becomes difficult to achieve the haemostasis with some patient groups under anticoagulant therapy without any positive pressure to the area, saturation or local haemostatic agents [10-13]. Therefore local haemostatic agents are usually preferred to control bleeding, their affect on healing and the reaction of the healing tissue to these materials should also be investigated.

Tissue repair and wound healing are complex processes that involve inflammation, fibroplasia, neovascularization, wound contraction, and resurfacing of the wound defect with epithelium[14]. The cascade of events starts with activation of the procoagulant pathway and recruitment of inflammatory cells and is followed by a phase of cellular proliferation and tissue repair/resolution of the injury[15].

Collagen, which is beneficial for endoepidermal growth to promote healing, is a major functional extracellular matrix protein of the dermal layer of the skin [16].

Parameters of epithelial and hair follicular degeneration, dermal edema and inflammation, collagen density and vasocongestion have been suggested as evaluative parameters to discuss the healing potential in many studies [16-18]. We scored all these parameters to be the damage causing substances to the wound healing, as it is judged by the evaluation of these substances by presence of them in the timeline of healing cascade. Wound healing comprises four primary stages that occur in a sequential cascade of overlapping process. During the proliferation phase, which is the third phase, fibroblasts migrate from the surrounding connective tissue, proliferate and begin to synthesise a framework of ground substances, fibronectin and extracellular matrices such as collagen, elastin and integrins. In the remodelling phase, which is the fourth phase, macrophages and fibroblasts increase their release of collagenase, resulting in the breakdown of excess collagen. Therefore this was a reason to why we evaluated and attached importance to the collagen change from day 4 to day 8 and we naturally expected a decrease of collagen rate and collagen deposit from day 4 to day 8. We suggested collagen deposit to be a damage causing substance in the histological examinations within this manner [19].

In the proliferation phase, macrophages release numerous growth factors responsible for angiogenesis. Basic fibroblast growth factor and vascular endothelial growth factor are responsible for the synthesis of new vessels [19].

Therefore, vasocongestion of the tissue is expected to decrease from day to day in the period of healing, also counted as a damage causing parameter either.

In the literature most of the studies performed in experimental haemorrhagic diathesis model investigated the haemostatic potential and the healing potential of some haemostatic agents clinically or histologically [20-24].

To our knowledge, the effects of ABS that is used as a local hemostatic agent in surgery, on soft tissue healing has been investigated before, but not ever under anticoagulant therapy. Therefore, the aim of this study was to investigate the effects of ABS on collagen rate values and histological assessment in order to evaluate its potential effects in early stage soft tissue healing in warfarin-treated rats.

In our study, warfarin was administered to animals 0.1 mg/kg once daily intraperitoneally for 3 days, before the surgery, until the day of killing. According to our experience, intraperitoneal way was found to be a more effective way of administering warfarin when compared to oral gavage [25]. The dose of warfarin was confirmed by the PT tests performed on the day of killing in our study [25]. Our study was different from the studies of Cipil *et al* [26] since in our experimental model, warfarin-treated rats, was kept alive after surgical procedures.

In our study, effect of warfarin treatment on collagen rate and histological total damage scores were not significant in the tissue samples taken on day 0. NHAA samples' collagen rate values have shown higher [$p=0,0001$] and; total damage scores have not shown any difference in control group than warfarin group. We think warfarin treatment is an individual negative effect in soft tissue healing.

Huri *et al.* applied haemostatic agents applied to the excision areas and noted no significant difference on the effects of celox and ABS on haemostasis in 40 Wistar rats with partial nephrectomy. They suggested ABS as an effective agent as other haemostatics, showed no fibrosis, adhesion or calcification and better histopathological results[27].

Isler SC *et al.* studied effects of ABS on early bone healing in a seven days of time and concluded that ABS to cause decreased inflammation and necrosis and increased new bone formation [28]. Bulut E *et al.* evaluated the effects of Ankaferd Blood Stopper (ABS) and routine antibiotic prophylaxis (AP) on early healing of bone defects in diabetic rats and they discovered no significant difference in new bone formation was found between AP and ABS treatment in diabetic rats [29].

Kose R *et al.* were the researchers that also studied ABS under an anticoagulant therapy. Their work with heparinized rats showed the positive efficiency of ABS on experimental model of haemorrhagic diathesis [30].

Abacıoğlu *et al.*'s study was about comparison of the ABS and chitosan on hemostasis in an experimental rat model with femoral artery bleeding and ABS and chitosan showed similar results on bleeding control. They mentioned that further research should be continued with systemically unhealthy experimental models [31].

Satar compared ABS and oxidized cellulose in experimental injury in rats. They detected a shorter bleeding time of ABS. In their study, liver sections from ABS group displayed more favorable histopathological changes with minimal signs of inflammation when compared with oxidized cellulose group on day 7 and day 14 [32].

Akalin *et al.* showed higher collagen deposition scores and lesser inflammatory scores in histological examination in ABS groups on the rat skin defect models. The fibroblast proliferation scores were higher in ABS group on 14th day [16]. Unlike our study, Akalin *et al.* have not evaluated the changes of inflammatory or healing promotive parameters of the tissues on consecutive time intervals. We think the effect of ABS could be judged better by evaluating and comparing the resulting data during the following time periods. Akalin *et al.* suggested ABS to be used safely on full thickness wounds.

In our study, an increase in collagen rate and total damage scores from day 0 to 4 in all groups was a naturally expected situation. When the collagen rate values were evaluated on day 4, NHAA samples were higher than ABS administered samples in control group [$p=0,008$]; and ABS administered samples were higher than NHAA samples in warfarin group [$p=0,032$], as just the opposite. On the other hand, collagen rate values of ABS administered samples did not differ in control and warfarin groups either, which means collagen rate values of NHAA samples were higher in control group than warfarin group [$p=0,0001$]. With the help of this data, we think ABS's effect on haemostasis promotes healing indirectly on day 4, while NHAA wounds struggle with haemostatic damage caused by warfarin treatment. In control group, although the collagen rate values of NHAA was significantly higher than ABS administered tissues [$p=0,008$], NHAA histological total damage scores were also higher than ABS administered tissues [$p=0,026$] on day 4. So we think ABS administration might weaken the inflammatory tissue

response and but also collagen deposit on day 4 in control group.

Under warfarin treatment, histological total damage score of NHAA and ABS administrated samples were not significantly different on day 4 but, ABS administrated samples were significantly higher than NHAA samples [$p=0,006$] on day 8. We think ABS administration promoted the tissue with its haemostatic effect and showed an even tissue damage with NHAA samples on day 4 but; on day 8, ABS administrated samples' damage scores were higher than NHAA samples at the end.

Yüce et al.'s investigation results demonstrate that ABS was a safe medicine and did not exert any negative effects on wound healing. Their work on the rabbit skin incision model evaluated the wound healing on days 5, 10 and 30 and they did not noted any fibroblast proliferation and matrix production on day 5, until it was day 10. They also noted the appearance of the fibriller collagen on day 30. According to their exhibited data of their work, collagen deposit of the tissues increased until day 10 and a breakdown and a change to fibriller structure of the collagen tissue was occurred from day 10 to 30 [17]. In our study, differently which was in rats, biochemical collagen rate values of ABS administrated group remained; while NHAA group decreased significantly different from day 4 to 8. We think that this decrease in NHAA group is a sign of the breakdown of excess collagen and a change of collagen to fibriller structures. ABS administrated group's biochemical collagen rate values, which has remained from day 4 to 8, showed us a delay of breakdown of collagen and so a delay in tissue healing. In the warfarin group, both groups' biochemical collagen rate values remained from day 4 to 8, which ensured us to think that warfarin treatment has inhibited the advantage of NHAA group on healing. There were no significant differences in the histological total damage score comparison of groups in the control group, both ABS administered and NHAA groups' scores remained from day 4 to 8. In warfarin group, both ABS administered and NHAA groups' scores decreased significantly from day 4 to 8, but ABS administrated group's scores were higher than NHAA group at the end of day 8. Even though another study of Aktop et al., which evaluates CAT and SOD activities under warfarin treated rats, has given promotive results of ABS on soft tissue healing [33]; in this study we think ABS administration causes more damage to tissue under warfarin treatment within these parameters. And also, Aydin BK et al. have not get favorable results after application of ABS on tendon healing in rats histologically [18].

CONCLUSION

Warfarin treatment has an individual negative effect on soft tissue healing. When the interrelation of collagen rate and histological inflammation changes in the soft tissue healing in tissues are evaluated in healthy or warfarin treated rats, ABS administration might be considered as a negative actor on healing at systemic level in healthy animals, and at inflammatory level in warfarin treated animals.

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