Haemostatic Effects of Topical Ankaferd Blood Stopper® On Bleeding Time in A Rat Abdominal Aortic Bleeding Model

Oruç Alper Onk[1], Mehmet Aksüt[2], Fatih Özçelik[3], Didem Onk[4], Erdim Sertoğlu[5], Hüseyin Serkan Erol[6], Ümit Kahraman[1], Osman Nuri Tuncer[1], Ömer Yiğiner[7], Bilgehan Erkut[1]

[1] Erzincan University Faculty of Medicine, Department of Cardiovascular Surgery, Erzincan, Turkey
[2] Kartal Koşuyolu High Specialization Training and Research Hospital, Clinic of Cardiovascular Surgery, İstanbul, Turkey
[3] GATA Haydarpaşa Training Hospital, Clinic of Biochemstry, İstanbul, Turkey
[4] Erzincan University Faculty of Medicine, Department of Anesthesiology and Reanimation, Erzincan, Turkey
[5] Elazığ Military Hospital, Clinic of Biochemistry, Elazığ, Turkey
[6] Atatürk University Faculty of Medicine, Department of Biochemistry, Erzurum, Turkey
[7] GATA Haydarpaşa Training Hospital, Clinic of Cardiology, İstanbul, Turkey

ABSTRACT

Introduction: The use of topical haemostatic agents has been popularised in recent years in many areas of surgery. We aimed to determine whether Ankaferd blood stopper® (ABS) can shorten the bleeding time and decrease the amount of bleeding in a rat abdominal aortic bleeding model.

Patients and Methods: A total of 40 rats were randomly divided into four groups (n= 10 in each group). Saline- and ABS-soaked gauzes were applied to rats that had not received any pre-treatment in Groups 1 and 2, while saline- and ABS-soaked gauzes were applied to rats that had received acetylsalicylic acid plus oral clopidrogel for 5 days in Groups 3 and 4. An incision of about 1 mm was made over the aorta just proximal to the iliac bifurcation. Mean bleeding time (MBT) and mean bleeding amount (MBA) were determined and compared.

Results: There were no significant differences in the age, weight and blood sample laboratory results of rats. Shorter MBT and less MBA were determined in ABS-soaked groups and the differences were statistically significant.

Conclusion: Ankaferd blood stopper® has the potential to reduce the bleeding time and post-operative blood loss after a major aortic surgery.

Key Words: Topical haemostasis; abdominal aorta

Ankaferd® Kanama Durdurucunun Rat Abdominal Aort Kanama Modelinde Kanama Zamanına Etkisi

ÖZET

Giriş: Topikal hemostatik ajanların birçok cerrahi alanda kullanımı son yıllarda popularite kazanmıştır. Ankaferd® kanama durdurucu (ABS) ajanın ratlarda abdominal aort cerrahisinde kanama zamanını kısaltıp, kanama miktarını azaltabildiğini belirtmeye amaçladık.

Hastalar ve Yöntem: Toplam 40 rat random 4 gruba (her grup n= 10) ayrıldı. Grup 1 ve grup 2'deki ön-tedavi almayan ratlara salin ve Ankaferd kanama durdurucu ABS emdirilmiş gaz uygulanırken, 5 gün asetil salisilik asit ve klopidgrel tedavisi alış olan grup 3 ve 4'teki ratlara da salin ve ABS emdirilmiş gaz uygulandı. İliak bifurkasyonun üzerinde abdominal aortaya 1 mm'lik bir kesi yapıldı. Ortalama kanama zamanı ve ortalama kanama miktarı belirlenip karşılaştırıldı.

Bulgular: Ratların yaş, ağırlık ve laboratuvar sonuçları karşılaştırıldığında gruplar arası anlamlı farklılık yoktu. ABS emdirilmiş gaz kullanılan hem tedavi uygulanmış hem de tedavi uygulanmamış gruplarda daha kısa kanama zamanı ve daha az kanama miktarı tespit edildi. Aradaki fark istatistiksel olarak anlamlı bulundu.

Sonuç: Ankaferd® kanama durdurucunun major aort cerrahisi sonrası daha kısa kanama zamanı sağlama ve operasyon sonrası daha az kan kaybı sağlama potansiyeline sahiptir.

Anahtar Kelimeler: Topikal hemostaz; abdominal aort

Correspondence

Oruç Alper Onk

E-mail: alperonk@hotmail.com
Submitted: 21.01.2016
Accepted: 20.02.2016

INTRODUCTION

The use of topical haemostatic agents has been popularised in recent years in many areas of surgery, because these agents facilitate cessation of minor bleeding during surgery. Such topical agents are especially used when bleeding is uncontrollable with common methods, such as suturing, ligation or cauterization. Several topical haemostatic agents such as topical gelatines, collagen preparations, oxidized celluloses, fibrin sealants, synthetic glues and glutaraldehyde-based glues have been suggested to be useful in various clinical like gastrointestinal bleeding and in some surgical bleeding like usage of fibrin glue in vascular surgery. Despite numerous clinical studies, the use of these topical agents has not been introduced in routine clinical practice due to a lack of randomised clinical trials[1,2].

Ankaferd blood stopper® (ABS) is a herbal extract that comprises a standard mixture of various plants, each with beneficial features on cellular proliferation, endothelial functions, angiogenesis or vascular dynamics[3]. ABS achieves haemostasis basically by agglutination and polymerization that modulates plasma protein, especially fibrinogen aggregation, within the endothelium and the vascular wall. These and other important features of ABS, such as having vasoactive functions as reported by an experimental study, make it an ideal haemostatic agent in vascular surgery[4]. Despite its use being tested even in experimental vascular anastomosis models, there is limited knowledge that justifies its routine use in different areas of vascular surgery.

Oozing or ongoing bleeding during aortic surgery is troublesome. In this experimental study, we aimed to determine that ABS can shorten the bleeding time and decrease the amount of bleeding in a rat abdominal aortic bleeding model.

PATIENTS and METHODS

The study was approved by the Committee for Animal Research at Erzincan University, Faculty of Medicine. The experimental protocol was based on a comparison of the bleeding time of rats in whom ABS- (Trend Teknoloji Ilac AS, Istanbul, Turkey) or normal saline-soaked gauzes were used to stop bleeding over incised abdominal aorta. All experimental protocols were in accordance with the guidelines for the Care and 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). All animals were treated in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research.

In this study, Wistar-albino rats, aged between 9 and 18 months (mean 13.8 ± 3.3 months) and weighing between 100 and 150 g (mean 123 ± 23 g) were used. Rats were obtained from Erzincan University Health Sciences Application and Research Center. The animals were housed in standard environmental conditions with a humidity of 45-50%, daylight/dark cycle of 12/12 h and room temperature of 21 ± 2°C. The animals were fed with a consistent normal rat feed and given fresh water each day. Rats were deprived of food but allowed to have water 24 h before surgery. All efforts were made to minimise animal suffering and distress.

Rats were randomly divided into four experimental groups: normal saline-soaked gauzes were applied to rats in Group 1 (n= 10) and ABS-soaked gauzes were applied to rats in Group 2 (n= 10); both Group 1 and Group 2 rats had not undergone any pre-treatment. Normal saline-soaked gauzes were applied to rats receiving oral acetylsalicylic acid at a dose of 30 mg/kg/day plus oral clopidrogel at a dose of 10 mg/kg/day for 5 days in Group 3 (n= 10), and ABS-soaked gauzes were applied to rats in Group 4 (n= 10) with the same pre-treatment given in Group 3.

Before the surgical procedure, rats were anaesthetised with 75 mg/kg of intramuscular ketamine and laid in a supine position on a pad. A 10% povidone-iodine solution was applied over the skin and a midline abdominal incision was made. Abdominal aorta was explored and 3 mL blood samples were taken from the abdominal aorta to measure the levels of red blood cell (RBC), haemoglobin (Hb), hematocrit (Hct) and platelets and coagulation parameters [prothrombin time (PT) and activated partial thromboplastin time (aPTT)]. Subsequently, an incision of about 1 mm was made over the anterior surface of the aorta just proximal to the iliac bifurcation with a fue punch (Ertıp Turkey). Either ABS- or saline-soaked gauze tampons were kept directly compressed over the wound with a 20-g weight. The weight was removed from the wound in every 10 seconds until bleeding stopped. The time taken until the bleeding stopped was recorded. The amount of bleeding was determined by weighing the gauzes on precision scales by subtracting their tare weights. All animals were sacrificed by cervical dislocation on the day of the surgery.

Statistical Analysis

All statistical analyses were performed using SPSS (version 16.0 Inc. Chicago, IL. USA). Histograms and Shapiro-Wilks test were used for determination of normal distribution. Continuous data were defined as mean ± standard deviations. Parametric data were compared using independent sample t-test and non-parametric data using Mann-Whitney test. Categorical data were compared using chi-square test or Fisher's exact test, where appropriate. Non-parametric Kruskal-Wallis test was used to compare the groups for dependent variables due to the low number of the groups (n< 30), and a non-parametric test of correlation (Spearman's) was used for correlation analysis. A p value of < 0.05 was considered to be statistically significant.

RESULTS

The mean RBC, platelet count (Hb, Hct, aPTT, PT levels) and weights of the rats in all the groups are summarised in Table 1, in which there was no significant difference between the groups ($p> 0.05$). A comparison of the bleeding time and amount of bleeding between the groups treated or untreated with ABS is given in Table 2. When Group 1 and 2 rats were compared,

Table 1. Weight and blood test results of all the groups

	Group 1 (n= 10)	Group 2 (n= 10)	Group 3 (n= 10)	Group 4 (n= 10)	*p
Weight, g	126 ± 14	125 ± 14	125 ± 14	124 ± 13	0.9815
RBC, 10^6/uL	6.73 ± 0.32	6.82 ± 0.31	6.90 ± 0.27	6.80 ± 0.25	0.6417
Haemoglobin, mg/dL	14.5 ± 0.7	14.3 ± 0.5	14.2 ± 0.4	14.4 ± 0.6	0.7764
Hematocrit, %	38.3 ± 1.7	39.0 ± 1.7	39.2 ± 1.3	38.9 ± 1.2	0.4560
Platelets, 10^3/uL	702 ± 114	671 ± 97	652 ± 90	693 ± 98	0.5539
PT, s	19.9 ± 1.6	19.2 ± 1.1	20.2 ± 1.4	19.7 ± 0.9	0.3984
aPTT, s	53.9 ± 4.2	52.7 ± 3.8	50.0 ± 4.3	50.5 ± 3.4	0.1318

RBC: Red blood cell, PT: Prothrombine time, aPTT: Activated partial thromboplastin time.
* Comparison of all the groups by the Kruskal–Wallis Test.

Table 2. Comparison of mean bleeding time and mean bleeding amount of all the groups.

	No pre-treatment			ASA + Clopidrogel		
	Group 1 (Saline)	Group 2 (Ankaferd)	p	Group 3 (Saline)	Group 4 (Ankaferd)	p
Bleeding time (min)						
Mean ± SD	1.50 ± 0.40	1.19 ± 0.09		4.70 ± 2.53	2.71 ± 1.11	
Min-Max	1.04-2.37	1.04-1.41	0.025	2.28-10.35	1.38-5.13	0.023
95% CI	1.21-1.79	1.12-1.26		2.89-6.51	1.91-3.50	
Amount of bleeding (mg)						
Mean ± SD	4.06 ± 1.93	1.90 ± 0.60		4.68 ± 1.82	2.75 ± 0.68	
Min–Max	0.79-7.55	1.24-3.00	0.004	2.91-8.36	1.87-3.87	0.003
95% CI	2.68-5.44	1.47 ± 2.33		3.37-5.99	2.26-3.23	

* Mann-Whitney Test, SD: Standard deviation, CI: Confidence interval.

the mean bleeding time (MBT) was significantly shorter in ABS-treated rats (Group 2) than in saline-treated rats (Group 1) (1.50 ± 0:40 and 1:19 ± 0.10 min for Groups 1 and 2, respectively, p= 0.0257). MBT was significantly higher in rats who underwent acetylsalicylic acid and clopidogrel therapy (Groups 3 and 4) in comparison with rats in the untreated groups (Groups 1 and 2) (p< 0.05). In addition, MBT values of Group 3 were considerably longer than those of Group 4 (4.70 ± 2:53 and 2.71 ± 1.11 min for Groups 3 and 4, respectively, p= 0.0232) (Figure 1). Likewise, Group 2 compared to Group 1 and Group 4 compared to Group 3 had significantly lower mean bleeding amount (MBA) (4:06 ± 1.83 versus 1.90 ± 0.60 mg for Groups 1 and 2, respectively, p= 0.0039 and 4.68 ± 1.82 versus 2.75 ± 0.68 mg for Groups 3 and 4, respectively, p= 0.0029) (Figure 2). In the Spearman correlation analysis covering all groups, there was a moderate positive correlation between MBT and MBA values (r= 0.5604, p< 0.0002) (Figure 3).

DISCUSSION

We observed that the topical use of ABS shortened the time required to stop bleeding through an incision on the abdominal

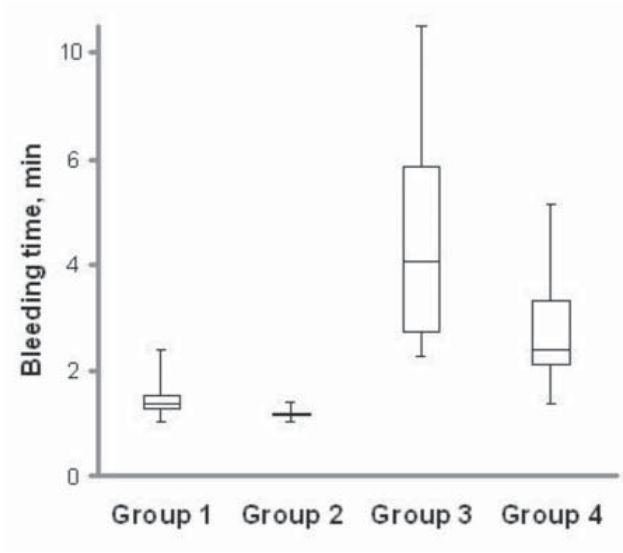

Figure 1. Comparison of mean bleeding time of all groups.

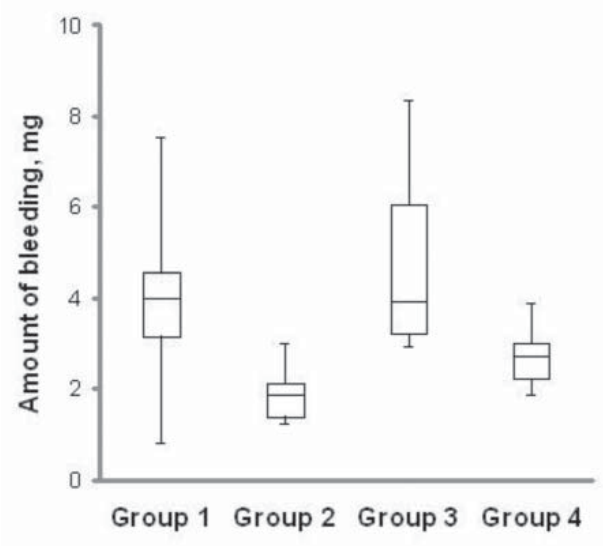

Figure 2. Comparison of mean bleeding amount of all groups.

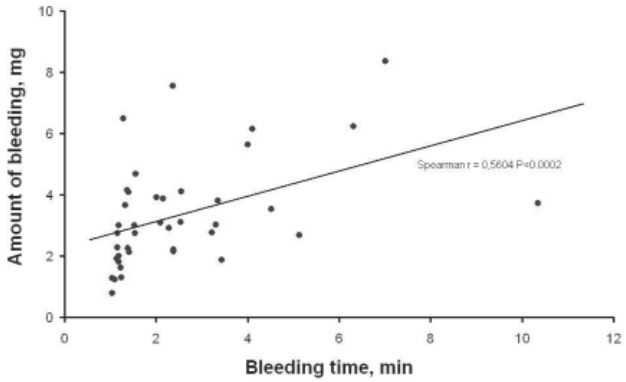

Figure 3. Correlation between mean bleeding time and mean bleeding amount of all groups (p< 0.0002).

aorta even in case of a delayed coagulation due to anticoagulants given before the surgical experiment. The effect size of the difference between the groups that did not receive pre-treatment was only about 30 s, which seems to be of minimal clinical relevance. However, the use of ABS nearly halved the bleeding time in the groups that received pre-treatment, indicating that it has a potential to be used in the vascular surgery as required in patients receiving anticoagulant drugs preoperatively.

There have only been few experimental studies on the use of ABS in the area of vascular surgery although minor oozing bleeding and unpredictable post-operative blood loss are of major concern. Kandemir et al. conducted a study investigating the effect of ABS on the abdominal aortic bleeding model[5]. Similar to us, these authors compared the bleeding times between rats receiving either ABS-soaked or normal gauzes for compression over the bleeding aorta and reported that the ABS-applied group had 36.7% shorter bleeding time as compared to the control group. In addition, in the histopathologic findings recorded on the seventh day after the surgery, they observed that the vascular endothelial cell loss and the inflammatory response did not differ significantly among the groups. In another recent animal study, Abacıoglu et al. randomised 30 rats who would receive either ABS or chitosan linear polymer or serve as controls[6]. By performing an incision over the femoral artery and recording the time until the bleeding stopped, the authors reported that two topical anticoagulants were similar to each other with regard to the bleeding times, but both were provided with shorter times as compared to the controls.

Yesilada et al. created a model where they divided and re-anastomosed the femoral arteries of 14 rats[7]. Half of the subjects were treated with ABS, and the authors reported that its use was associated with a significant decrease in the anastomosis completion time whilst macroscopic and microscopic patency, the existence of micro-aneurysm and inflammatory response was similar to the controls. On the contrary, Sacak et al. similarly divided and then re-anastomosed the femoral arteries of 21 Wistar-albino rats[8]. Further, they were randomly allocated to receive saline or ABS after the surgery. Two of the three groups were also pre-treated with acetylsalicylic acid. The results of that study were intriguing; ABS application provided shorter bleeding times in rats but all the rats that received ABS developed a tunica media degeneration and a contraction of tunica intima leading to an aneurysm formation in the anastomosis sites. Thus, the authors recommended against the use of ABS in the anastomosed vessels.

Besides several experimental studies, there have also been many clinical reports on the use of ABS in various areas of surgery with dental surgical procedures and relatively minor surgical fields being far more common. In a study on 25 patients, it was reported that the use of ABS in patients on antithrombotic therapy eliminated the need for the discontinuation of the antithrombotic drug before dental surgery, because it caused a significant decrease in the bleeding time as compared to controls[9]. In a larger group by Beyazit et al. using the spray form of ABS in 113 dental extractions, it was reported that ABS stopped bleeding less than 10 s in nearly half of the patients[10].

Iynen et al. conducted a prospective randomised study using ABS on patients receiving cold steel adenoidectomy[11]. The authors reported that ABS provided significantly shorter operative time, less blood loss during the surgery and shorter haemostasis. In another study, Guler et al. randomised the patients who underwent total thyroidectomy into ABS or a conventional technique for oozing-type bleeding and reported that the post-operative amount of drained blood was significantly lower in the ABS group[12]. Teker et al. conducted a randomised study on patients with anterior epistaxis and reported interesting results: ABS provided better haemostasis than phenylephrine (79.2% vs. 64.0%, p< 0.05); moreover, 44.0% patients who crossed over from phenylephrine to ABS achieved haemostasis[13]. The

number of re-bleeding rats was also significantly lower in the ABS group.

Although there have been placebo-controlled, prospective randomised studies on the use of ABS even in the setting of cardiac surgery, its safety is still a major concern. Turhan et al. injected one millilitre of ABS into systemic circulation of 12 rabbits[14]. Two rabbits died within 5 min of administration. A post-mortem histopathological examination revealed a hepatic venous outflow obstruction, indicating a potential life-threatening effect of even a small amount of the drug. Nazlı et al. applied ABS-soaked sponges over the abraded epicardium of 16 white rabbits to test whether ABS prevents post-operative pericardial adhesions[15]. The authors reported that ABS did not prevent the adhesions but caused the formation of pericardial adhesions and fibrosis. On the contrary, Mihmanli et al. evaluated its cytotoxicity on human cultured blood cells and concluded that ABS can be safely used as an alternative blood stopping agent[16].

Our study is limited with a lack of adequate data to evaluate the effectiveness of ABS in controlling bleeding from the aortic tissue after anastomosed with native or synthetic tissues, such as Dacron or PTFE grafts. Our data was based on short-term results, and undoubtedly, a later evaluation should provide important knowledge.

CONCLUSION

In conclusion, ABS has the potential to reduce the bleeding time and post-operative blood loss after a major aortic surgery. However, its safety should be well-established before undertaking large-scale clinical studies in the area of vascular surgery.

CONFLICT of INTEREST

The authors reported no conflict of interest related to this article.

AUTHORSHIP CONTRIBUTIONS

Concept/Design: AO, MA
Analysis/Interpretation: OT, BE
Data Acquisition: OT, BE
Writing: OT, AO
Critical Revision: OT, UK
Final Approval: All of authors

REFERENCES

1. Achneck HE, Sileshi B, Jamiolkowski RM, Albala DM, Shapiro ML, Lawson JH. A comprehensive review of topical hemostatic agents: efficacy and recommendations for use. Ann Surg 2010;251:217-28.
2. Sileshi B, Achneck HE, Lawson JH. Management of surgical hemostasis: topical agents. Vascular 2008;16:S22-8.
3. Haznedaroglu BZ, Beyazit Y, Walker SL, Haznedaroglu IC. Pleiotropic cellular, hemostatic, and biological actions of Ankaferd hemostat. Crit Rev Oncol Hematol 2012;83:21-34.
4. Aktas A, Er N, Onur MA. Effects of Ankaferd Blood Stopper (R) on vascular response in rat carotid artery. UHOD 2010;20:156-62.
5. Kandemir O, Buyukates M, Kandemir NO, Aktunc E, Gul AE, Gul S, et al. Demonstration of the histopathological and immunohistochemical effects of a novel hemostatic agent, Ankaferd Blood Stopper, on vascular tissue in a rat aortic bleeding model. J Cardiothorac Surg 2010;5:110.
6. Abacıoğlu S, Aydın K, Büyükcam F, Kaya U, Işık B, Karakılıç ME. Comparison of the efficiencies of buffers containing Ankaferd and chitosan on hemostasis in an experimental rat model with femoral artery bleeding. Turk J Haematol 2015 Apr 27.
7. Karasoy Yeşilada A, Bayraktaroğlu SB, Sevim KZ, Sakız D, Tatlıdede HS. The efficacy of the hemostatic agent Ankaferd blood stopper on end-to-end unilateral sleeve fish-mouth anastomosis. Acta Orthop Traumatol Turc 2014;48:92-7.
8. Saçak B, Akdeniz ZD, Şirinoğlu H, Çilingir ÖT, Çelebiler ÖB, Ercan F, et al. Microvascular anastomosis using Ankaferd blood stopper: demonstration of long-term histopathologic effects on vascular tissue. Blood Coagul Fibrinolysis 2014;25:721-5.
9. Çakarer S, Eyüpoğlu E, Günes ÇÖ, Küseoğlu BG, Berberoğlu HK, Keskin C. Evaluation of the hemostatic effects of Ankaferd blood stopper during dental extractions in patients on antithrombotic therapy. Clin Appl Thromb Hemost 2013;19:96-9.
10. Beyazit Y, Kart T, Kuscu A, Arslan A, Kurt M, Aktas B, et al. Successful management of bleeding after dental procedures with application of blood stopper: a single center prospective trial. J Contemp Dent Pract 2011;12:379-84.
11. Iynen I, Bozkus F, San I, Alatas N. The hemostatic efficacy of Ankaferd Blood Stopper in adenoidectomy. Int J Pediatr Otorhinolaryngol 2011;75:1292-5.
12. Guler M, Maralcan G, Kul S, Baskonus I, Yilmaz M. The efficacy of Ankaferd Blood Stopper for the management of bleeding following total thyroidectomy. J Invest Surg 2011;24:205-10.
13. Meric Teker A, Korkut AY, Kahya V, Gedikli O. Prospective, randomized, controlled clinical trial of Ankaferd Blood Stopper in patients with acute anterior epistaxis. Eur Arch Otorhinolaryngol 2010;267:1377-81.
14. Turhan N, Bilgili H, Captug O, Kurt M, Shorbagi A, Beyazit Y, et al. Evaluation of a haemostatic agent in rabbits. Afr J Tradit Complement Altern Med 2011;8:61-5.
15. Nazli Y, Colak N, Alpay MF, Haltas H, Aksoy ON, Akkaya IO, et al. Assessment of the efficacy of Ankaferd blood stopper on the prevention of postoperative pericardial adhesions. Cardiovasc J Afr 2014;25:100-5.
16. Mihmanli A, Ulker Z, Alpsoy L, Ezirganli S. Evaluation of cytotoxicity of a new hemostatic agent Ankaferd Blood Stopper® using different assays. Hum Exp Toxicol 2012;31:780-