CMJ Original Research December 2018, Volume: 40, Number: 4
Cumhuriyet Medical Journal 326-333
http://dx.doi.org/10.7197/223.vi.440027

Hemostatic effects of A-Fact

A-Fact'ın hemostatik etkileri

Ceylan HEPOKUR[a], Sema MISIR[a], Mahmut ÖZBEK[b]

[a]Department of Biochemistry, Faculty of Pharmacy, Sivas Cumhuriyet University, 58140 Sivas, Turkey
[b] World Medicine Pharmaceutical Company, Istanbul, Turkey
Corresponding author: Ceylan Hepokur, Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, 58140 Sivas, Turkiye
E-mail: cozsoya@gmail.com
Received/Accepted: July 03, 2018 / December 16, 2018
Conflict of interest: There is not a conflict of interest.

SUMMARY

Objective: A-Fact is a solution contains iron (II) sulfate and forms an iron-ion-protein complex. An ideal hemostatic agent should have been stopping venous bleeding after being applied to the lesion. Our aim in the present study is to investigate the in vitro effects of A-Fact on hemostatic parameters.
Method: Plasma pooling was performed by centrifuging venous blood samples from volunteer people. Total protein, fibrinogen activity, albumin and globulin, coagulation factor (II, VII, IX) levels were analyzed with spectrophotometric method in auto analyzer by applying different concentrations of A-Fact. Microscopic images were obtained by applying 5%, 10%, 15% and 50% A-fact to blood samples for morphological evaluation.
Results: A-Fact ensured the formation of the iron-ion-protein complex. Coagulation tests were performed by 2/20 dilution. A-Fact was not effect on coagulation factors. Plasma fibrinogen activity, total protein, albumin and globulin levels were decreased while prothrombin time was prolonged. Furthermore, it was observed that A-Fact caused an increase in INR values.
Conclusions: In this study, it was showed that the bleeding-stopping effect of A-Fact may be caused by protein formation. It is clear that further studies are needed in this area.
Keywords: A-Fact, Kanama Durdurucu, Iron (II) Sulfate

ÖZET

Amaç: A-Fact demir(II) sülfat içeren bir çözelti olup, demir-iyon-protein kompleksi oluşturur. İdeal bir hemostatik ajan lezyona uygulandıktan sonra venöz kanamayı durdurmalıdır. Bu çalışma A-Fact'ın muhtemel kanama durdurucu etkisini göstermek ve *in vitro* olarak hemostatik parametreler üzerine etkilerini belirlemek amacıyla yapıldı.
Yöntem: Gönüllü insanlardan alınan venöz kan örneklerinden santrifüj edilerek plazma havuzu oluşturuldu. A-Fact'ın farklı konsantrasyonları uygulanılarak total protein, fibrinojen aktivitesi, albümin ve globulin, koagülasyon faktör (II, VII, IX) düzeyleri oto analizöründe spektrofotometrik yöntem ile analiz edildi. Morfolojik değerlendirme için kan örneklerine % 5, % 10, % 15 ve % 50 A-fact uygulanılarak mikroskobik görüntüleri alındı.
Bulgular: A-Fact demir-iyon-protein kompklesinin oluşumunu sağladı. Koagülasyon testleri 2/20 dilüsyon yapılarak gerçekleştirildi. A-Fact'ın koagülasyon faktörleri üzerinde herhangi bir etkisinin olmadığı gözlendi. Plazma fibrinojen aktivitesi, total protein, albümin ve globulin düzeyleri azalırken buna paralel olarak protrombin zamanı uzadı. Ayrıca, A-Fact'ın INR değerlerinde artışa neden olduğu gözlendi.
Sonuç: Bu çalışma ile A-Fact'ın kanama durdurucu etkisinin protein ağı oluşumu üzerinden olabileceği sonucuna varıldı. Bu alanda daha ileri çalışmalara ihtiyaç olduğu açıktır.
Anahtar sözcükler: A-Fact, Kanama Durdurucu, Demir (II) Sülfat

INTRODUCTION

Bleeding is the outflow of blood from the circulatory system [1, 2]. For a healthy human, losing more than 10-15% of total blood volume causes mortality [3-5]. Blood stopping (hemostasis) poses a challenge in medicine. In history, people has been used different plants as a hemostatic agent.

Ferric subsulfate is used as hemostatic agent. For the first time in 1857, it was used in medicine. The agglutination (clustering) of blood proteins is known to occur with the effect of sulfate and ferric ions and acidic pH. Agglutinated proteins occlude the gaps which are the cause of the capillary bleeding. Unlike conventional hemostatic agents, ferric sulfate acts chemically throughout the blood vessels [6].

In this study, A-Fact solution was used as a blood stopper. A-Fact solution contains Iron (II) Sulfate dry active ingredient.

The purpose of the present study was investigated the basic mechanism underlying the hemostatic actions of A-Fact. The effects of A-Fact were studied on the status of the principal primary and secondary hemostatic system components (e.g. coagulation proteins, platelets and blood cells) *in vitro* using routine hemostatic laboratory tests.

MATERIAL AND METHODS

A-Fact Blood Stopper ®

A-Fact was developed by World Medicine İlaç San. ve Tic. A.Ş. (Istanbul, TURKEY) A-Fact Solution contains 69.800 mg Iron (II) Sulfate dry active ingredient (equivalent of 16.548 mg/g Ferric subsulfate) in 1 ml solution. A-Fact solution was sterilized using gamma sterilization method.

Hematologic Tests

A-Fact was diluted different concentrations and treated in human plasma (5% (1/20) 50 µL hemostatic agent / 950 µl plasma, 10% (2/20) 100 µL hemostatic agent / 900 µl plasma, 15% (3/20) 150 µL hemostatic agent / 850 µl plasma, 50% (10/20) 500 µL hemostatic agent / 500 µl plasma). Hematological parameters including coagulation factors (II, VII, IX), prothrombin time (PT, INR), aPTT, fibrinogen, D-dimer, and platelets aggregation tests were performed. The tests took place at 37 ºC, performed within max. 15 min after the preparation of the sample, and each sample was tested twice.

Morphological Evaluation

Morphological evaluation and microscopic examinations of blood cells were carried out in Sivas Numune Hospital Department of Biochemistry Lab. and used an Urised Labumad. Microscopic images of 5%, 10%, 15% and 50% A-Fact solution applied to blood samples were determined.

Biochemical Tests

Total protein, albumin and globulin levels were tested at the Clinic biochemistry Lab. of Sivas Numune hospital using routine biochemical tests. Albumin and globulin were evaluated using an auto-analyzer Cobas 6000 Analyzer Series. Coagulasyon tests were made using an Acltop 3000 Analyzer Series.

Statistical Analysis

It has been determined that research on 32 individuals, adult, healthy and voluntary, will provide a meaningful difference compared to 80% power and $p < 0.05$ values. The paired T test was used in the analysis of the research data.

RESULTS

A-Fact induced formation of a protein network in the plasma in Figure 1.

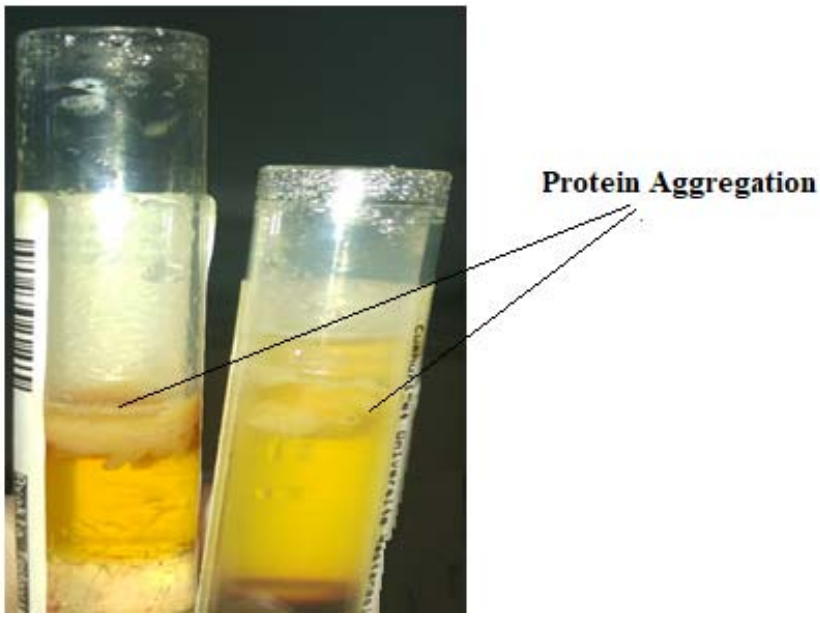

Figure 1. A-fact induced protein aggregation in plasma

Coagulation factors, INR, Fibrinogen, aPTT, D-Dimer, albumin, globulin and total protein, PLT values are shown in Tables 1, 2, and 3.

Table 1. Effect of A-Fact on Coagulation factors, INR, Fibrinogen, aPTT, D-Dimer values

Sample	INR (0.8-1.30)	Fibrinogen (mg/dL)	aPTT (s)	Prothrombin time (s)	D-dimer (ng /mL)	Coagulation Tests (%)		
						2	7	9
1/20	1.21±0.13	84±1,23*	42.3±1.54*	12.8±0.45	144.78±1.54			
2/20	1.80±0.24*	65±1.05*	67.4±1.12*	19.4±0.52*	141.08±1.36*	56.8±1.26*	61.9±2.65*	56±1.25*
3/20	2.40±0.58*	62±1.58*	82.2±2.75*	26.1±1.24*	127.95±1.62*			
10/20	17.49±0.98*	-	-	206.9±2.15*	1880.65±1.36*			
Control	1.05±0.09	172±2.54	30.3±1.54	11.1±0.56	152.15±1.63	86.9±1.56	76.8±1.56	65.3±1.45

*Represent significant results ($p<0.05$) compared to control group

Coagulation tests were performed with 2/20 dilution. Regarding the coagulation factors, Factor II, VII and IX were not affected. A decrease was observed on plasma fibrinogen activities, whereas INR values were increased. As a result of biochemical tests, it was observed that a decreased on total protein, albumin, and globulin values compared to control group.

Table 2. Effect of A-Fact on total protein, Albumin, Globulin values

Sample	Total protein (g/dL)	Albumin	Globulin
2/20	5.3±0,25*	3.2±0,52*	1.1±0,23*
Control	6.8±0,96	4.4±0,62	2.4±0,18

*Represent significant results ($p<0.05$) compared to control group

Table 3. Effect of A-Fact on PLT values

Sample	PLT (440-100)
1/20	$3x10^9$ /L
2/20	$3x10^9$ /L
3/20	0
10/20	-
Control	$343x10^9$ /L

A-Fact induced very rapid formation of a protein network in the plasma samples (Figure 2).

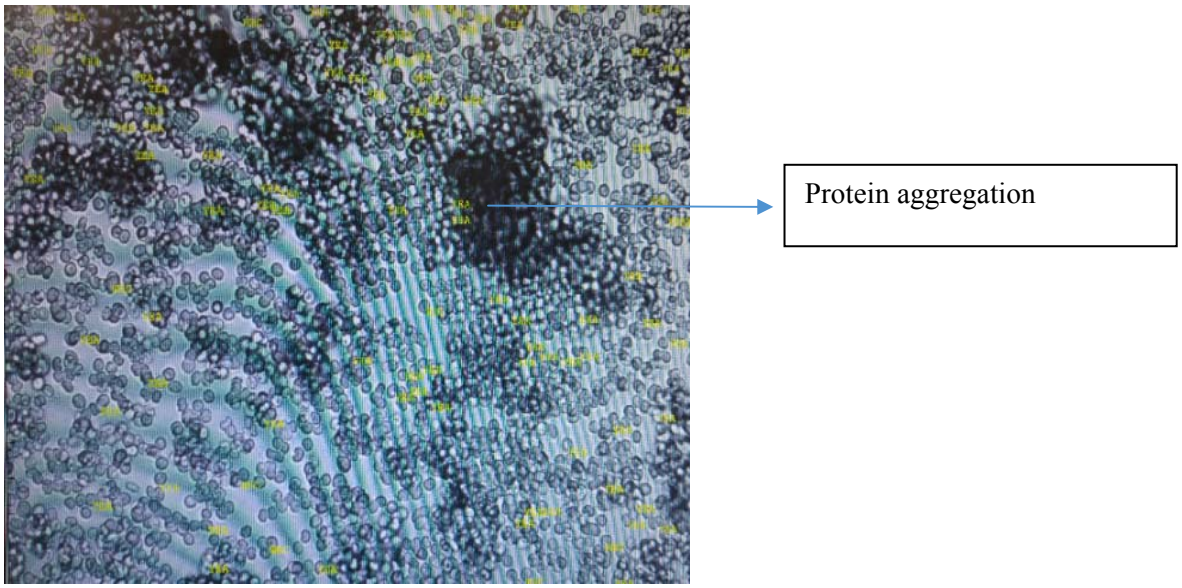

Figure 2a. Microscopic appearance of 1/20 diluted samples (Microscopic appearance of samples were determined using by Urised Labumat Analyser Systems)

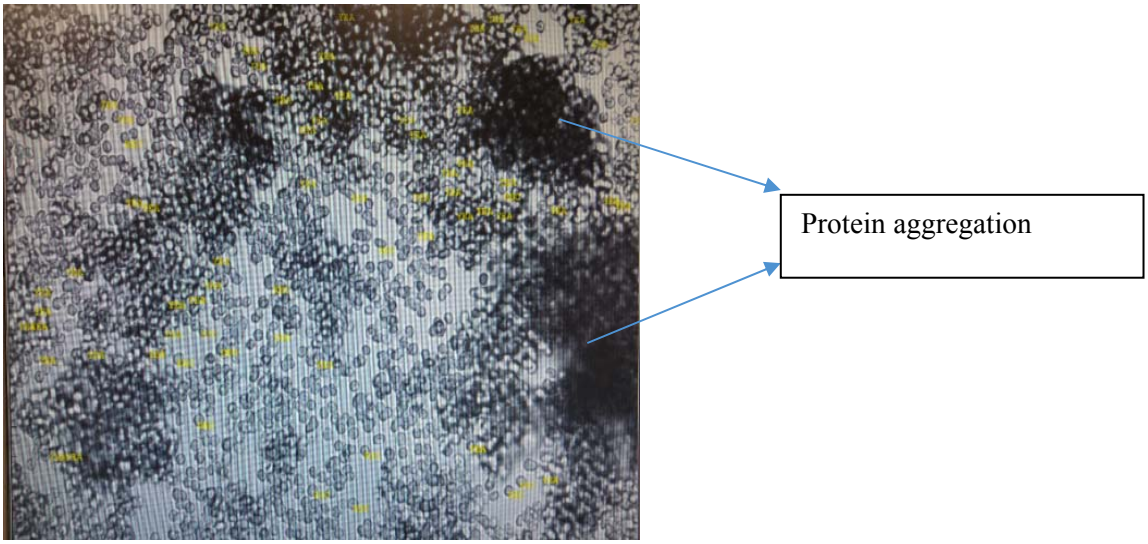

Figure 2b. Microscopic appearance of 2/20 diluted samples (Microscopic appearance of samples were determined using by Urised Labumat Analyser Systems)

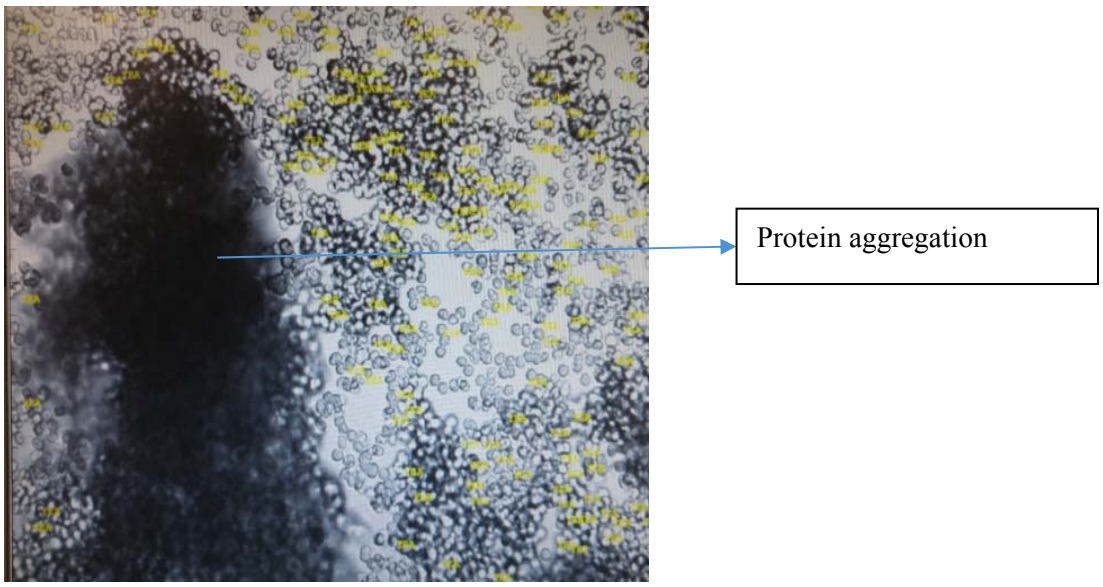

Figure 2c. Microscopic appearance of 3/20 diluted samples (Microscopic appearance of samples were determined using by Urised Labumat Analyser Systems)

Figure 2d. Microscopic appearance of whole blood sample (Microscopic appearance of samples were determined using by Urised Labumat Analyser Systems)

DISCUSSION

Bleeding can one of leading cause of mortality and morbidity after trauma and any clinical setting. If location of the bleeding is unknown, those in the form of a leakage, pressure method or vasoconstrictor agents can be used. Nowadays, hemostatic agents are used in a wide-range of operation and have been reported by many researchers as effective in controlling bleeding [7]. Hemostasis is whole physiological system, that is required for stop the bleeding without blocking blood flow and ensuring the vascular integrity in case of an injury occurs in the vessel wall. Hemostasis can be roughly assessed by bleeding time. It is calculation of time between formation of a small laceration and the complete stop the bleeding by wiping with gauze every 30 sec. typical period endures max. 7.5 min [8]. Vasoconstriction, primary and secondary hemostasis, and fibrinolytic system are played important role in Hemostasis [9, 10].

For centuries, in Anatolia traditional herbal medicine has used as a hemostatic agent [11]. The hemostatic agents used to stop the bleeding caused by different mechanism [10-14]. Some agents have mechanism of action such as the fibrin preparation and vascoconstrictor effect of adrenalin.

A-Fact solution was used as a blood stopper. A-Fact Solution includes Iron (II) Sulfate dry active ingredient. A-Fact is unlike other local hemostatic agents because its mechanism of action is based on forming of the protein network to erythrocyte agglutination. Ferric sulfate interacts with blood proteins and forms ferric-ion-protein complex very quickly after contacting with the blood. The formed ferric-ion-protein complex allows hemostasis by occluding damaged area [15]. In the presence of iron (III) ions, a clotting mechanism different from the clotting cascade is effective. It is known that iron occurs hydroxyl radical in the blood, interacts and binds with fibrinogen proteins. As a result of this iron ion-fibrinogen complex protein, hypercoagulation occurs [16,17]. The reversible binding of iron to fibrinogen is the most important factor of the mechanism initiating hypercoagulation [18]. Many studies showed that iron (III) ions occurs hydroxyl radicals with the blood and allows the polymerization of fibrinogen protein which is effective in blood clotting. This polymerization precipitates the blood cells and proteins via a domino effect in the environment [19, 20]. Ferric sulfate affects via a clotting mechanism independent of the coagulation/ clotting factors that are existing in the composition of the blood. This is a very important advantage, allowing the control of bleeding in patients with hemophilia or using blood thinners. Accordingly, it was shown that iron was not affect the levels of other coagulation/clotting factors (II, V, VII, VIII, X, XI, and XIII) in the blood [21].

In the literature, studies of hemostatic agent are examined; Sönmez et al. demonstrated that APH

(absorbable polysaccharide hemostasis) with warfarin were administered in rats, INR values increased while bleeding time decreased in the APH group according to the control group.

Al et al. were compared ABS and tampons in patients with cancer for hemostasis. They found that ABS stopped bleeding within a shorter time [22].

Goker *et al*. showed that ABS-induced network formation is related to the functions of blood proteins and red blood cells. Plasma fibrinogen activity, antigen levels, total protein, albumin, and globulin levels decreased [23].

ABS was not affected the levels of coagulation factors II, V, VII, VIII, IX, X, XI, and XIII. The activation of plasma fibrinogen and the fibrinogen antigen levels were decreased with an extension of the thrombin time. In addition, the total protein, albumin, and globulin levels were decreased [24-28].

In the current study, the use of A-Fact accelerated the clustering of proteins in plasma samples (Figure 1). Thus, we observed that A-Fact enhances coagulation through the clustering of blood protein [29]. Routine biochemical tests showed that bleeding stopper and blood proteins affect fibrinogen, allowing it to get stacked together with other proteins (Figure 2). Blood cells (erythrocytes and platelets) ensure aggregation formation. Coagulation/clotting factors (II,VII,IX) were not affected by A-Fact. Total protein, albumin and globulin were decreased. Thrombin time, activated partial thromboplastin time (aPTT), INR values were increased however fibrinogen, PLT values were decreased. Our findings are similar to other research. It is hoped that complimentary medicine will lead to the development of a new drug that is active in haemostasis.

CONCLUSION

A-Fact may be a new the potential agent for the management of hemorrhage. A-Fact needs to have further studies.

REFERENCES

1. Ferhanoğlu B. Hemostaz Mekanizması. Hematoloji Ders Kitabı. ISBN: 978-975-404-815-5.Üniversite Yayın No: 4774.Cerrahpaşa Tıp Fak. Yayın No: 269:Istanbul, 2008,p.219- 227.

2. Colman RW, Hırsh J, Moider VJ. Hemostasis and Trombosis, Basic Principles and Clinical Practise.6th ed.USA: Lippincott Williams,2004,p.381-400.

3. Carmona RH, Lim RC, Clark GC. Morbidity and mortality in hepatic trauma. Am J Surg 1982;144:88-94.

4. Kaynaroğlu ZV. Karın travmaları. Ed:Sayeki. Temel cerrahi.3th ed. Ankara: Güneş kitabevi. 2004,466-472.

5. Parks RW, Chrysos E, Diamond T. Management of liver trauma. Br J Surg 1999; 86: 1121-1135.

6. Secer HI, Daneyemez M, Tehli O, Gonul E, Izci Y. The clinical, electrophysiologic, and surgical characteristics of peripheral nerve injuries caused by gunshot wounds in adults: a 40-year experience. SurgNeurol 2008;69:143-52.

7. Sönmez E, Çavuş UY, Civelek C, Dur A, Karayel E, Gülen B, Uysal Ö, İpek G. The efficacy of a hemostatic agent in anticoagulant drug-induced rat bleeding model. Ulus Travma Acil Cerr Derg March 2014; 20: 2.

8. Kurata M, Horii I. Blood coagulation tests in toxicological studies review of methods and their significance for drug safety assesment. J.Toxical Sci 2004;29:13-32.

9. Daniel NG, Goulet J, Bergeron M, Paquin R, Landry Pe. Antitrombosit Drugs:ıs there a surgical risk? J can dent Assoc 2002;68:683-687.

10. Degroot Pg, Sixma Jj. Trombosit Adhesion. Br J haematol 1990; 75:308-312.

11. Goker H, Haznedaroglu IC, Ercetın S, Kırazlı S, Akman U, Ozturk Y, Hc Fırat HC. Haemostatic Actions of the Folkloric Medicinal Plant Extract Ankaferd Blood Stopper. The Journal of International Medical Research 2008; 36:163-170.

12. Seyednejad H, Imani M, Jamieson T, Seifalian AM. Topical haemostatic agents. British Journal of Surgery 2008; 95: 1197-1225.

13. Pogorielov MV, Sikora VZ. Chitosan as a Hemostatic Agent: Current State. European Journal of Medicine. Series B, 2015, Vol.(2), Is. 1.

14. Lapierre F, Houtaud SD, Wager M, Hemostatic Agents in Neurosurgery. Explicative Cases of Controversial Issues in Neurosurger 2011,doi:10.5772/31319.

15. Uzun N, Tanriverdi T, Savrun FK, Kiziltan ME, Sahin R, Hanimoglu H, et al. Traumatic peripheral nerve injuries: demographic and electrophysiologic findings of 802 patients from a developing country. J ClinNeuromuscul Dis 2006;7:97-103.

16. Vance GN and Etheresia P. Iron-enhanced coagulation is attenuated by chelation:a thrombelastographic and ultrastructural analysis. Blood Coagulation and Fibrinolysis 2014;25:845–850.

17. Jankun J, Landeta P, Pretorıus E, Skrzypczak-Jankun E, Lıpınskı B. Unusual clotting dynamics of plasmasupplemented with iron(III). Internatıonal Journal Of Molecular Medıcıne 2014; 33: 367-372.

18. Clark WR, Leather RP, Hemostasis during liver resections. Surgery 1970;67:5556-5576.

19. Evans BE. Local hemostatic agents. N.Y. J. Dent 1977; 47: 109-14.

20. Bleeding Control and Healing Aid Compositions and Methods of Use, Terence Prevendar, US 6,652,840 B1, Nov. 25, 2003.

21. Taylor CA, Braza D, Rice JB, Dillingham T. The incidence of peripheral nerve injury in extremity trauma. Am J Phys Med Rehabil 2008;87:381-5.

22. Al B, Yildirim C, Cavdar M, Zengin S, Buyukaslan H, Kalender ME. Effectiveness of Ankaferd blood stopper in the topical control of active bleeding due to cutaneous-subcutaneous incisions. Saudi Med J 2009; 30: 1520–5.

23. Vance GN, Etheresia P. Iron-enhanced coagulation is attenuated by chelation:a thrombelastographic and ultrastructural analysis. Blood Coagulation and Fibrinolysis 2014;25:845–50.

24. Aysan E, Bektas H, Ersoz F, Sari S, Kaygusuz A, Huq GE. Ability of the ankaferd blood stopper® to prevent parenchymal bleeding in an experimental hepatic trauma model. Int J Clin Exp Med 2010; 3: 186–91. 2.

25. Beyazit Y, Kurt M, Kekilli M, Goker H, Haznedaroglu IC. Evaluation of hemostatic effects of Ankaferd as an alternative medicine. Altern Med Rev 2010; 15: 329–36. 3.

26. Beyazit Y, Kekilli M, Haznedaroglu IC, Kayacetin E, Basaranoglu M. Ankaferd hemostat in the management of gastrointestinal hemorrhages. World J Gastroenterol 2011; 17: 3962–70. 4.

27. Tasdelen Fisgin N, Tanriverdi Cayci Y, Coban AY, Ozatli D, Tanyel E, Durupinar B, Tulek N. Antimicrobial activity of plant extract Ankaferd Blood Stopper. Fitoterapia 2009; 80: 48–50. 5.

28. Kandemir O, Buyukates M, Kandemir NO, Aktunc E, Gul AE, Gul S, Turan SA. 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.

29. Yüce S, Çandirli C, Yenidünya S, Muslu B. New hemostatic agent: the effect of Ankaferd Blood Stopper on healing wounds in experimental skin incision model. Turk J Med Sci 2014; 44: 288-294.