The effects of Ankaferd® Blood Stopper on transcription factors in HUVEC and the erythrocyte protein profile

Ankaferd® Kanama Durdurucunun HUVEC'lerde transkripsiyon faktörleri ve eritrosit protein profili üzerine etkisi

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Abstract

Objective: **Ankaferd® Blood Stopper (ABS) is an herbal extract that has historically been used as a hemostatic agent in traditional Turkish medicine. ABS is comprised of a standardized herbal mixture of *T. vulgaris, G. glabra, V. vinifera, A. officinarum*, and *U. dioica*. ABS's basic mechanism of action is the formation of an encapsulated protein web, which represents the focal point for vital erythrocyte masses. The hemostatic effects of ABS have been observed in vitro and in vivo. ABS was registered as a hemostatic agent for external hemorrhages and dental bleeding following phase I randomized, double-blind crossover placebo-controlled clinical research, and safety and efficacy reports. In terms of the potential use of ABS, transcription factors may be novel factors that play a role in the hemostatic and other pleiotropic effects of ABS.**

Materials and Methods: **Hence, the present study aimed to investigate the effects of ABS on endothelium, and possible transcription factor changes in HUVEC (human umbilical vein endothelial cells) and the erythrocyte membrane profile.**

ABS (5 μL and 50 μL) was administered to HUVEC (in 75 cm^2; ~75% fullness) for 5 min and 15 min.

Results: **ABS caused significant increases in the level of activation of the following transcription factors; AP2, AR, CRE/ATF1, CREB, E2F1-5, E2F6, EGR, GATA, HNF-1, ISRE, Myc-Max, NF-1, NFkB, p53, PPAR, SMAD 2/3, SP1, TRE/AP1, and YY1. Following erythrocyte membrane isolation, protein complexes were undissolved, but denatured. The protein complex formed was resistant to heat and detergent. Trypsin and sonication were used in order to break this complex; the complex dissolved and erythrocyte membrane proteins were released in SDS-PAGE.**

Conclusion: **ABS established a very fast and solid protein web, and increased the level of transcription factor activation. Therefore the cellular effects of ABS could be related to different intracellular biological pathways.** *(Turk J Hematol 2011; 28: 276-85)*

Key words: Ankaferd®, endothelium, transcription factors, erythrocyte

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Özet

Amaç: **Ankaferd BloodStopper® (ABS) bir bitkisel karışımıdır ve geleneksel Türk tıbbında uzun yıllardır kullanılmaktadır. ABS, *T. vulgaris, G. glabra, V. vinifera, A. officinarum* ve *U. dioica* bitkilerinin standartlaştırılmış en uygun karışımını ihtiva etmektedir. Temel etki mekanizması vital fizyolojik eritrosit birikimlerinin protein ağı yapısı oluşturmasıdır. Hemostatik etkileri *in vivo* ve *in vitro* çalışmalarda gösterilmiştir. ABS dış kanamalarda ve diş kanamalarında kontrollü klinik çalışmaları yapılmış güvenlik ve etkinlik raporları ile tescillenmiştir. Transkripsiyon faktörleri potansiyel olarak hemostatik ve diğer olası etkilerin merkezinde yer alabilir ve ABS uygulamalarından etkilenebilirler.**

Yöntem ve Gereçler: **Bu çalışmada, ABS'nin endotelde ve olası transkripsiyon faktörleri değişimini HUVEC'lerde (insan umbilikal ven endotelyal hücreleri) ve eritrosit membran profilleri üzerindeki etkilerinin incelenmesi amaçlanmıştır. ABS, HUVE hücrelerine (75cm² yüzeyde; ~%75 dolulukta), 5 μL ve 50 μL hacimlerde 5 ve 15 dakika uygulanmıştır.**

Bulgular: **Transkripsiyon faktörlerinden AP2, AR, CRE/ATF1, CREB, E2F1-5, E2F6, EGR, GATA, HNF-1, ISRE, Myc-Max, NF-1, NFkB, p53, PPAR, SMAD 2/3, SP1, TRE/AP1, YY1'in aktivasyonlarında artış gözlenmiştir. Kandan eritrosit membranı izolasyonundan sonra, protein komplekslerinin denatürasyona rağmen çözünmemiş halde kalmaktadır ve bu kompleksler sıcaklığa ve deterjana dayanıklıdır. Sonikasyon ve tripsin muamelesinden sonra bu kompleksin ayrıştığı ve eritrosit membran proteinlerinin ortaya çıktığı SDS-PAGE'de gözlemlenmiştir.**

Sonuç: **Sonuçlar gözönüne alındığında hemostatik ajan ankaferd'in kanamaları durdururken çok hızlı ve sağlam bir ağ oluşturmaktadır ve uygulandığı bölgedeki hücrelerin içinde de etkili olup transkripsiyon faktörleri seviyelerini de etkileyerek birçok biyolojik mekanizmalar üzerinde etkili olabilir.**

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Anahtar kelimeler: Ankaferd®, endotel, transkripsiyon faktörleri, eritrosit

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Introduction

Ankaferd® Blood Stopper (ABS) is comprised of a standardized herbal mixture of *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*. Each of these herbs has an effect on endothelium, blood cells, angiogenesis, cell proliferation, vascular dynamics, and molecular mediators [1,2]. ABS has been historically used as a hemostatic agent in traditional Turkish medicine [3,4]. ABS's basic mechanism of action is the formation of an encapsulated protein network that provides a focal point for vital erythrocyte aggregation [1,2]. ABS-induced protein network formation with blood cells, particularly erythrocytes, is involved in the physiological primary and secondary hemostatic systems without an unbalanced activation of individual coagulation factors. This unique mechanism is an advantage of ABS, as compared to other hemostatic agents. Exposure to ABS in in the topical endothelial injury area provides a physiological hemostatic effect, together with tissue oxygenation, but without prothrombotic pathological activation of any clotting factor [1,2,5,6]. ABS causes encapsulated protein web formation, which induces erythrocyte aggregation via the interactions of fibrinogen gamma and red blood cells [1].

There are distinct and important molecular components of the ABS-induced hemostatic network. Vital erythroid aggregation occurs with spectrin-ankyrin and actin proteins on the membranes of red blood cells. Essential erythroid proteins (ankyrin recurrent and FYVE bundle-containing protein 1, spectrin alpha, actin depolymerizing factor, LIM bundle and actin-binding subunit 1 isoform a, LIM bundle and actin-binding subunit 1 isoform b, NADP-dependent malic enzyme, NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, mitochondrial NADP (+)-dependent malic enzyme 3, ribulose bisphosphate carboxylase large chain, and maturase K) and the required ATP bioenergy (ATP synthase, ATP synthase beta subunit, ATP synthase alpha subunit, ATP-binding protein C12, TP synthase H+ transporter protein, ADF, and alpha-1, 2-glycosyltransferase ALG10-A) are included in the ABS protein library [7]. The physiological protein profile of red blood cell membranes (Figure 1) and the ABS protein library are similar [7]; therefore, vital erythroid aggregation is crucial to the ABS-induced hemostatic network.

ABS is a hemostatic agent that can be used effectively in clinical practice to control external bleeding, dental and periodontal hemorrhaging, dermal bleed-

ing, and/or superficial mucosal blood leakage [8-11]. The hemostatic effects of ABS have been observed *in vitro* and *in vivo* [12-18]. Use of ABS as a hemostatic agent in external hemorrhages and in dental treatment in humans provided the first data showing that ABS was safe and effective in humans [9]. A phase I randomized, double-blind crossover placebo-controlled clinical study performed with healthy volunteers reported that topical ABS usage was safe [20].

In terms of the potential use of ABS, transcription factors [21-23] may be novel factors that play a role in the hemostatic and other pleiotropic effects of ABS. Hence, the present study aimed to investigate the effects of ABS on endothelium, and possible transcription factor changes in HUVEC (human umbilical vein endothelial cells) and the erythrocyte membrane profile. We also intended to observe the interrelationships between protein profile of ABS and the ABS-induced bond forming structures between red blood cells.

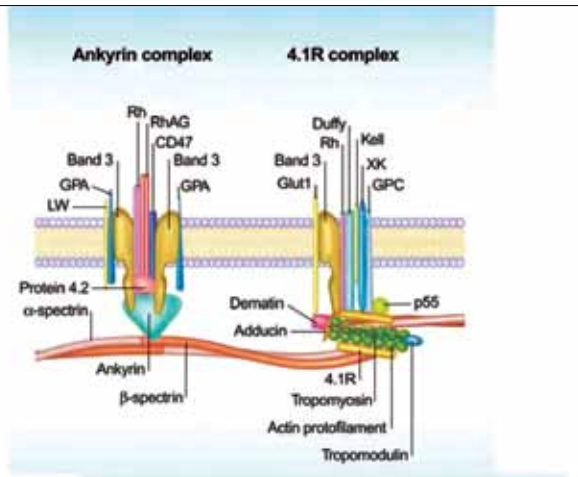

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Figure 1. The red blood cell membrane and and its proteins

Materials and Methods

The production, control, and analyses methods of ABS have been previously described [20]. ABS is available in 3 pharmaceutical forms: ampoule, pad, and spray:

1. Ankaferd® Blood Stopper 2-mL ampoule
2. Ankaferd® Blood Stopper pad
   · 2.5×7 cm (3 mL)
   · 5×7.5 cm (10 mL)
   · 20×20 cm (100 mL)
3. Ankaferd® Blood Stopper spray
   · 5 mL
   · 10 mL
   · 25 mL
   · 50 mL
   · 200 mL

The quantities of the active ingredients in each pharmaceutical form are shown in Tables 1 and 2.

The effects of ABS on transcription factors and the erythrocyte protein profile in HUVEC endothelium were examined. ABS (5 μL and 50 μL) was administered to HUVEC (cellular properties; in 75 cm^2; ~75% fullness) for 5 min and 15 min. Nucleus isolation of HUVEC was performed using a nuclear extraction kit (Marligen Biosciences, USA) and the level of activity of the following transcription

Table 2. Ingredients in the Spray Form of ABS

Active ingredient	Quantity (mg/mL)
Urtica dioica[1]	0.06
Vitis vinifera[2]	0.08
Glycrrhiza glabra[2]	0.09
Alpinia officinarum[2]	0.14
Thymus vulgaris[3]	0.10

[1]Dried root extract, [2]dried leaf extract, [3]dried grass extract

Table 1. Ingredients in the Ampoule and Pad Forms of ABS

Active ingredient	Quantity (mg) Ampoule 2 mL	Pad 2.5×7 cm (3 mL)	5×7.5 cm (10 mL)	20×20 cm (100 mL)
Urtica dioica[1]	0.12	0.18	0.6	6
Vitis vinifera[2]	0.16	0.24	0.8	8
Glycrrhiza glabra[2]	0.18	0.27	0.9	9
Alpinia officinarum[2]	0.14	0.21	0.7	7
Thymus vulgaris[3]	0.10	0.15	0.5	5

[1]Dried root extract, [2]dried leaf extract, [3]dried grass extract

factors was determined using a multiplex transcription factor profiling kit (20-plex) (Marligen Biosciences, USA) and examined (Luminex 100, Marligen Biosciences, USA) according to the manufacturer's instructions; AP2 (activating protein 2), AR (androgen receptor), CRE-ATF1 (cyclic AMP response element or activating transcription factor 1), CREB (cyclic AMP response element-binding protein), E2F1-5, E2F6, EGR (early growth response), GATA (globulin transcription factor), HNF1 (hepatocyte nuclear factor-1), ISRE (interferon (IFN)-stimulated response element), Myc-Max, NF1 (nuclear factor-1), NF-κB (nuclear factor kappa B), p53 (protein 53 or tumor protein 53), PPAR (peroxisome proliferator-activated receptor), SMAD2/3, SP1, TRE/AP1 (TPA response element/activating protein 1), and YY1 (Yin Yang 1). Two independent experiments were performed in duplicate with SD when indicated.

In order to examine the erythrocyte protein web 10 mL of human blood was eluted, according to density gradients (Ficoll analysis solution d=1.077). The erythrocytes were washed 3 times with PBS and ABS was administered in doses of 25 μL/mL, 50 μL/mL, and 150 μL/mL. Erythrocyte membrane isolation was performed and examined in 10% SDS-PAGE.

Results

Interestingly, it is microscopically observed that the endothelial cells arised from the plastic surface and adhered to each other upon the ABS application to the HUVEC. In response to low dose ABS (5 μL) treatment for 15 min, all of the transcription factors reached to the highest level of activity, but at the higher dose (50 μL) the activation level is not further enhanced. The level of activation of the following transcription factors increased significantly in response to ABS; AP2, AR, CRE-ATF1, CREB, E2F1-5, E2F6, EGR, GATA, HNF1, ISRE, Myc-Max, NF1, NF-κB, p53, PPAR, SMAD2/3, SP1, TRE/AP1, and YY1 (Figures 2 and 3 indicate the increments of the transcription factors and the dose and exposure time of ABS).

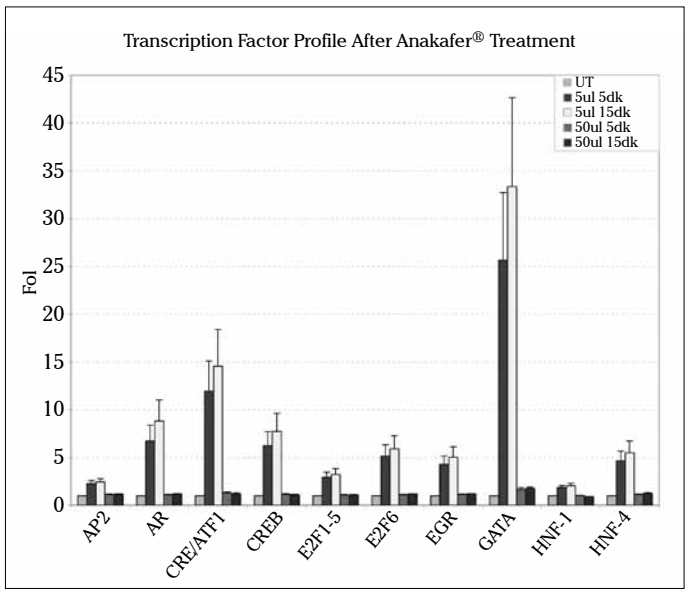

Figure 2. Transcription Factor Profile After Ankaferd® Treatment

During the ABS administration to the erythrocyte suspension, being dose-dependent also, cellular adhesions to each other were observed. Likewise, cellular unifications formed in different dosages were having different sizes of pellets. Following the erythrocyte membrane isolation, protein complexes were undissolved, but denatured. The protein complex that formed was resistant to heat (100°C) and detergent. Trypsin and sonication were used in order to break this complex; the complex dissolved and erythrocyte membrane proteins were released in SDS-PAGE (Figures 4 and 5).

Discussion

The present study investigated the effects of ABS on transcription factors and the erythrocyte protein profile in HUVEC endothelium. ABS effectively stopped bleeding due to rapid formation of a complex between the cells and because the bond formed within the complex was very strong. We think that ABS is very effective at low doses (5 μL) not only outside cells, but inside as well, and can affect many molecular mechanisms in endothelial cells. The level of activity of the transcription factors investigated in the present study (AP2, AR, CRE-ATF1, CREB, E2F1-5, E2F6, EGR, GATA, HNF1, ISRE, Myc-Max, NF1, NF-κB, p53, PPAR, SMAD2/3, SP1, TRE/AP1, and YY1) significantly increased in response to ABS (Figures 2 and 3). These transcription factors regulate a wide variety of biological functions, including hemostasis, infection, cellular proliferation, and inflammation (Table 3).

Numerous studies reported the hemostatic effects of topical ABS in the animals with normal [10,12,14,16-18] and defective hemostasis [15,24],

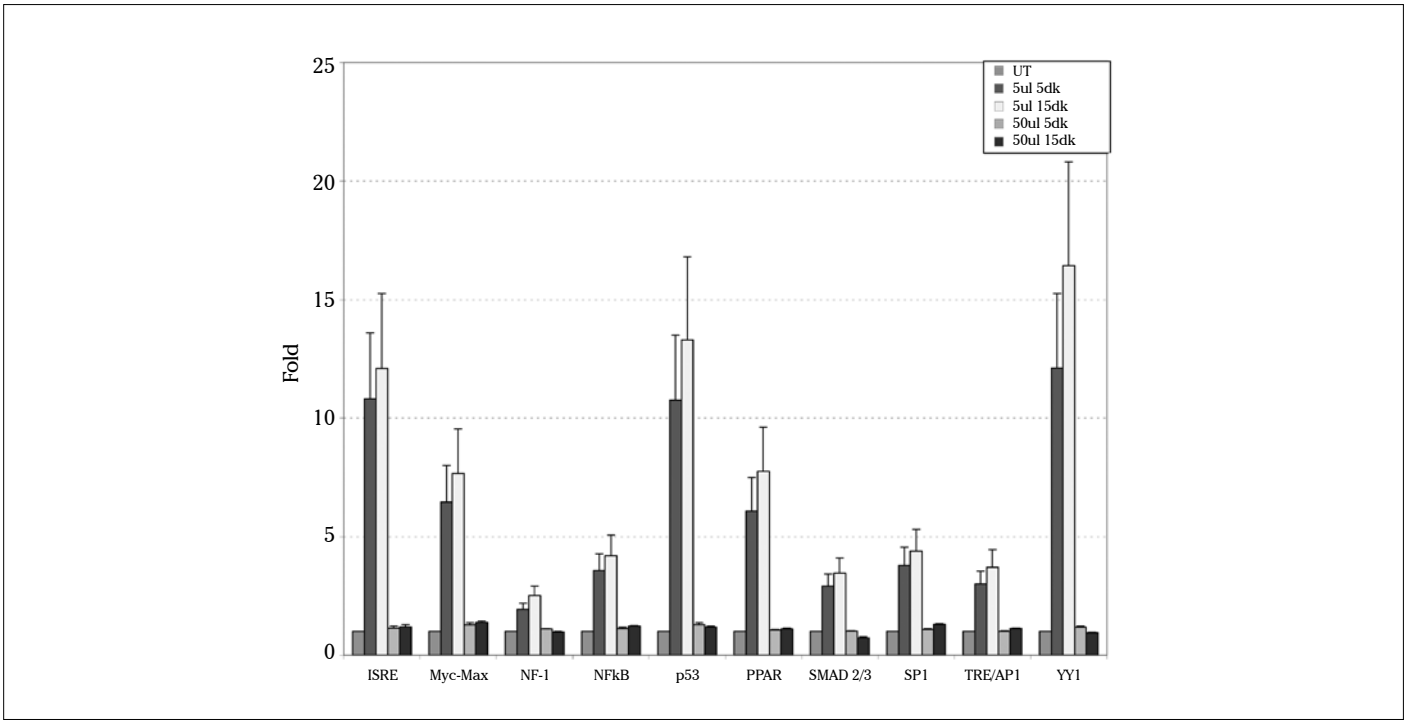

Figure 3. Transcription Factor Profile After Ankaferd® Treatment

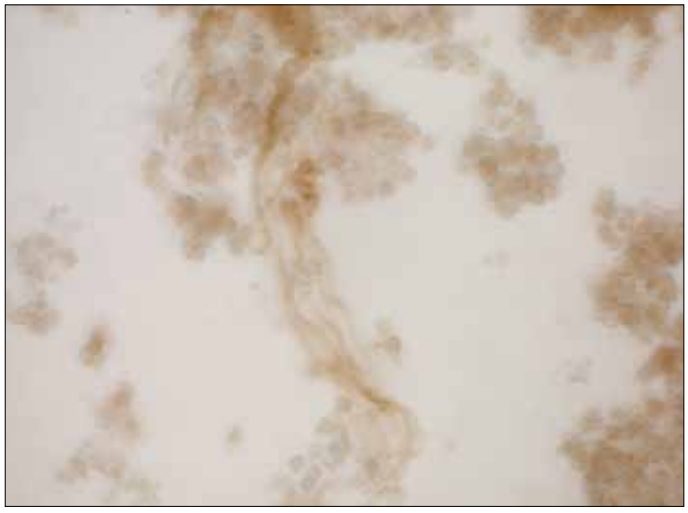

Figure 4. Microscopic View of Erythrocytes Following ABS Treatment

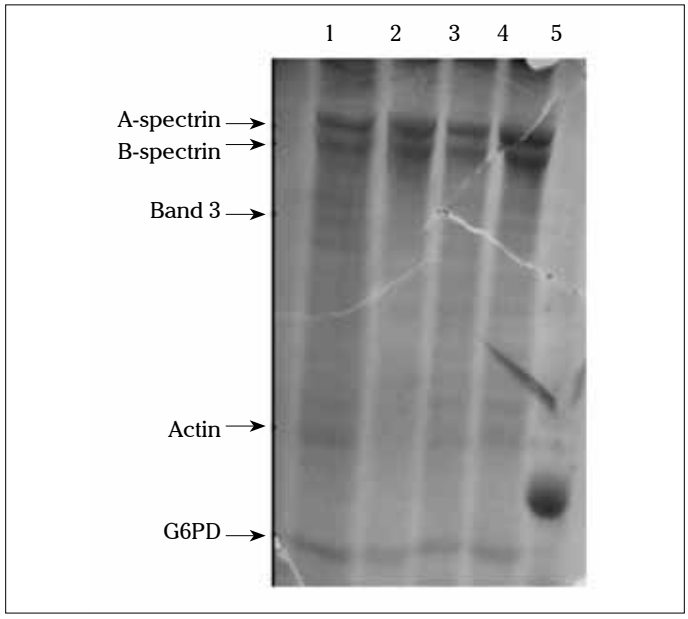

Figure 5. Sds-Page of Abs-Treated Erythrocytes. Lanes 1-4: Trypsin and Sonication After Heat (100°C) and Detergent Treatment. Lane 5: Erythrocyte Membrane Proteins Treated with Heat and Detergent

and have set the preclinical stage for the development of this hemostatic product. The effects of ABS on hemostatic parameters have also been searched [2,6,25]. When added to plasma or serum ABS induced rapid formation of a protein network and erythrocyte aggregation. The levels of coagulation factors II, V, VII, VIII, IX, X, XI, and XIII were not affected by the administration of ABS. Plasma fibrinogen activity and antigen levels decreased following the administration of ABS, along with an increase in thrombin time. Total protein, albumin, and globulin levels decreased after administration of ABS. The researchers suggested that ABS stimulates the formation of an encapsulated protein network that provides a focal point for erythrocyte aggregation [2,6,25].

The short-term hematological and biochemical safety of oral systemic ABS in rabbits have been reported [13]. Acute mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, and biochemical

Table 3. Selected Functions of the Studied Transcription Factors in HUVEC That Were Affected by ABS

Transcription factor	Selected Functions
AP2	AP2 is a critical regulator of gene expression during embryogenesis, and has been implicated in tumorigenesis [19,36,37].
AR	AR is a member of the nuclear receptor superfamily, members of which function as ligand-inducible transcription factors that mediate expression of target genes in response to ligands specific to each receptor, including steroids, retinoids, vitamin D, and thyroid hormone [38].
CRE-ATF1	CRE-ATF1 is a transcriptional activator, and one of many transcription factors that bind a consensus sequence (5'-TGACGTCA) [39].
CREB	The CREB assay detects transcription factors that are responsive to intracellular levels of cyclic AMP. The CREB-binding element is an important indicator of signals propagated by hormones, growth factors, and neurotransmitters. CREB-binding proteins also function in growth factor-dependent cell survival, glucose homeostasis, and in learning and memory [40].
E2F1-5, E2F6	E2F is a group of genes that codifies a family of transcription factors (TF) in higher eukaryotes. Three of them are activators: E2F1, E2F2, and E2F3a. Six others act as suppressors: E2F3b and E2F4-8. All are involved in cell cycle regulation and DNA synthesis in mammalian cells. E2Fs as TFs bind to the TTTCGCGC consensus binding site in the target promoter sequence [41].
EGR	Four members of the EGR family are well known: Egr1 (ZNF225, ZIF268, NGFI-A, Krox-24); Egr2 (Krox-20); Egr3; Egr4 (NGFI-C). EGR-1 and EGR-2 encode nuclear proteins with zinc finger DNA-binding domains and immediate-early genes in T-cell activation, and can regulate transcription synergistically with NF-ATc. A correlation between EGR proteins and Wilms' tumors has been reported [42,43].
GATA	The GATA site 5'-TCAGATAAGA-3' binds GATA transcription factors. GATA transcription factors are transcriptional activators that function via different genes in many different cell and tissue types. There are at least 6 forms of GATA protein. GATA regulates erythroid differentiation, promotes production of erythroid proteins such as spectrin, and is important to the health of red blood cells interacting with urotensin II. [44]
HNF1	Hepatocyte nuclear factors (HNFs) are a group of phylogenetically unrelated transcription factors that regulate the transcription of a diverse group of genes into proteins. These proteins include blood clotting factors, and enzymes and transporters involved in glucose, cholesterol, and fatty acid transport and metabolism [45,46].
ISRE	ISRE is known to induce MHC class I expression in response to IFNs, as well as a region comprising site α/enhancer B, which significantly stimulates constitutive transcription of HLA class I genes. Tumor cells are thought to escape immune surveillance by T cells via suppressing expression of major histocompatibility complex (MHC) class I molecules at their cell surface. ISRE acts as an anti-tumor molecule via acting on this mechanism [47].
Myc-Max	The Myc-Max site 5'-ACCACGTGGT-3' binds c-Myc/Max heterodimers. Different transcription factors from the bHLH-ZIP class of proteins can form homo- or heterodimers and bind to the Myc-Max site to regulate genes associated with the cell cycle. Activation of myc genes are associated with cancer and there is much evidence that myc plays a major role in the pathogenesis of Burkkitt's lymphoma [48].
NF1	The core nuclear factor-1 (NF-1/CTF-1) binding site 5'-TGGNNNNNNGCCAA-3' binds to proteins of the NF-1 family. NF-1 proteins are transcriptional activators that directly interact with TFIIB and facilitate assembly of basal transcription complexes. NF-1 is expressed in a wide variety of cell types, with the exception of B-cells and T-cells. Chick embryo fibroblasts that over-express NF-1 proteins are resistant to transformation by the nuclear oncogenes jun, fos, junD, myc, and qin, but are readily transformed by cytoplasmic oncogenes such as src, raf, ras, and fps [49].
NF-κB	Complexes comprising homo- or heterodimers of proteins from the NFκB or rel family bind to the NFκB site (5'-AGGGGACTTTCCCA-3'). Transcription factors of the Rel NFκB family are ubiquitous in cells and are activated in response to signals that lead to cell growth, differentiation, inflammation, and apoptosis. NFκB proteins are connected to various signaling pathways that affect important biological responses, which has made them a high priority as pharmaceutical targets [50].
p53 (protein 53 or tumor protein 53)	p53 is important in multi-cellular organisms, regulating the cell cycle and thus such functions as a tumor suppression. As such, p53 has been described as the guardian of the genome, the guardian angel gene, and the master watchman, referring to its role in conserving stability by preventing genome mutation [51].

PPAR	The PPAR site, also known as the peroxisome proliferation response element (PPRE), 5'-TGACCTTGACCT-3' binds transcription factors from the PPAR family as homo- and heterodimers. Three PPAR isotypes-PPAR-alpha, PPAR-beta/delta, and PPAR-gamma-have been identified. PPARs have a DNA-binding domain and a ligand-binding domain with specificity for prostanoids, fatty acids, fibrates, and thiazolidinediones. Once activated by ligand binding, PPARs bind to DNA at the peroxisome proliferator response elements (PPREs) within genes and modulate transcription. PPARs display differential tissue distribution with PPAR-alpha and PPAR-gamma, playing a role in the pathogenesis of chronic diseases such as diabetes, obesity, and atherosclerosis. There is substantial evidence that different ligands may determine the specificity of PPARs interaction with particular co-activators, and drugs such as hypolipidemic fibrates and insulin-sensitizing thiazolidinediones (pioglitazone and rosiglitazone) have been developed to modulate their activity [52].
SMAD2/3	SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signal of transforming growth factor (TGF)-beta, and thus regulates multiple cellular processes, such as cell proliferation, apoptosis, and differentiation [53,54].
SP1	Sp1 is a human transcription factor involved in gene expression in the early development of an organism. SP1 contains a zinc finger protein motif, with which it binds directly to DNA and enhances gene transcription. Sp1 interacts with ATF7IP, ATF7IP2, POGZ, HCFC1, AATF, and PHC2 [55,56].
TRE/AP1	AP1 mediates gene regulation in response to many different physiological and pathological stimuli, including cytokines, growth factors, stress signals, and bacterial and viral infections, as well as oncogenic stimuli. The most widely studied AP1 complex is a c-Jun/c-Fos heterodimer. Studies on genetically modified mice and cells have highlighted the crucial role AP1 plays in a variety of cellular events involved in normal development and neoplastic transformation causing cancer. AP1-binding proteins are targets being considered by the pharmaceutical industry [57,58].
YY1	YY1 plays a fundamental role in normal biologic processes, such as embryogenesis, differentiation, replication, and cellular proliferation. As YY1 expression and function are known to be intimately associated with progression through phases of the cell cycle, the physiologic significance of YY1 activity has recently been applied to models of tumor biology [59].

toxicity were not observed during the short-term (7 days) follow-up period [13]. Use of ABS as a hemostatic agent in external hemorrhages and in dental treatment in humans provided the first data showing that ABS was safe and effective in humans [9]. A phase I double-blind, randomized crossover, placebo-controlled clinical study with a 5-d wash-out period between the crossover periods that included healthy volunteers reported that ABS was safe [20]. Physiological cell-based coagulation was clinically managed using topical ABS to prevent and treat bleeding associated with many clinico-pathological states [10,18,26-28]. Erythrocyte masses are critical in the ABS-induced hemostatic protein network (Figures 4 and 5). Proteins on the cytoplasmic surface of erythrocyte membranes, including spectrin and actin, are thought to comprise the red cell cytoskeleton. Actin added to erythrocyte ghosts selectively associated with a component at the cytoplasmic surface of the membrane, and actin binding occurred via stimulation of actin polymerization (Figure 1). Haznedaroglu et al. [29] proposed that, platelets are not directly affected, but leukocyte activation is evident following the ABS exposure to whole blood according to the ultrastructural scanning electron microscopic (SEM) morphological analyses [29]. Further investigations are needed to elucidate that vital issue since cellular hemostasis is a critical component of ABS hemostatic effects.

In the present study, GATA activity significantly increased following the administration of ABS. GATA regulates erythroid differentiation, promotes production of erythroid proteins such as spectrin, and is important to the health of red blood cells (Table 1).

The *in vitro* antibacterial activity of ABS was evaluated against 26 indicator strains, including gram-positive and gram-negative bacteria, using the agar diffusion method, and was observed to be effective against all strains [30,31]. Nisin, a food preservative bacteriocin used as a control, was inactive against gram-negative strains. In addition to its high inhibitor activity against various pathogens, and gram-positive and gram-negative bacteria, ABS was more stable than nisin at various temperatures and in the presence of enzymes. The antimicrobial activity of ABS was tested against many pathogens, including *A. baumannii, E. coli, K. pneumonia, P. aeruginosa, Enterobacter spp., Stenotrophomonas maltophilia,* MRSA, methicillin-resistant coagulase

negative *Staphylococcus,* vancomycin-susceptible Enterococcus, and VRE, and was noted to be active against all the isolates, with zones of inhibition between 10 and 18 mm in diameter [32]. In addition to ABS's hemostatic effects in hemorrhagic wound healing, its antimicrobial property can also be beneficial, and ABS has the potential to protect against various types bacterial pathogens [31,32]. In the present study, ABS upregulated numerous transcription factors (Figures 2 and 3) in endothelial cells that play a role in the process of infection and inflammation in response to a wide variety of pathogens and in wound healing, including AR, ISRE, EGR, HNF1, NF-κB, PPAR, SMAD2/3, and YY1 (Table 3). There may be a relationship between transcription factor activation and anti-infective activities of ABS. therefore, those data in our study represent true basis to further search the 'mechanism of action' of Ankaferd against distinct pathogens and wound healing as described in previous *in vitro* studies [30-32].

There are several hypotheses such as decreased angiogenesis, increased apoptosis, and interactions with tumor hemostasis that attempt to account for ABS's mechanism of action on tumor tissue [8,33-35]. The hemostatic action of ABS is correlated with a reduction in tumor neo-angiogenesis. Topical ABS administration to gastrointestinal neoplastic tissue resulted in the control of bleeding and decreased tumor vascularization in rectal and gastric cancers [26]. Moreover, there is a close relationship between coagulation factor expression and solid tumor progression, via mechanisms other than angiogenesis. In the present study ABS significantly increased the level of activity of the following transcription factors; AP2, AR, CRE-ATF1, CREB, E2F1-5, E2F6, EGR, ISRE, Myc-Max, NF1, NF-κB, p53, PPAR, SMAD2/3, SP1, TRE/AP1, and YY1. These regulator molecules affect distinct steps of cellular proliferation, such as cell cycle regulation, angiogenesis, signal transduction, apoptosis, inflammation, acute phase reaction, and immunity, and several metabolic molecular pathways (Table 3). *In vivo* preclinical models will be designed to elucidate the effects of ABS on neoplastic tissue, as described in previous *in vitro* studies [33,34] and *in vivo* studies on bleeding tumors [8,35].

The pleiotropic effects of ABS on vascular endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and cellular mediators are being investigated to determine the potential role of ABS in many pathological states, including neoplastic disorders, infectious diseases, and inflammation. Our observations in this report about the cellular effects of Ankaferd may shed further light on that perspective.

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Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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