Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 143 (2020) 110150

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Ankaferd hemostat (ABS) as a potential mucosal topical agent for the management of COVID-19 syndrome based on its PAR-1 inhibitory effect and oestrogen content

Fatma Beyazit[a], Yavuz Beyazit[b,*], Alpaslan Tanoglu[c], Ibrahim C. Haznedaroglu[d]

[a] Department of Obstetrics and Gynecology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[b] Department of Gastroenterology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[c] Department of Gastroenterology, Sultan Abdulhamid Han Training and Research Hospital, Istanbul, Turkey
[d] Department of Hematology, Hacettepe University Faculty of Medicine, Ankara, Turkey

ARTICLE INFO

Keywords:
Ankaferd hemostat
Oestrogen
PAR-1
SARS-CoV-2
COVID-19

ABSTRACT

COVID-19 due to the SARS-CoV-2 infection is a multi-systemic immune syndrome affecting mainly the lungs, oropharyngeal region, and other vascular endothelial beds. There are tremendous ongoing efforts for the aim of developing drugs against the COVID-19 syndrome-associated inflammation. However, currently no specific medicine is present for the absolute pharmacological cure of COVID-19 mucositis. The re-purposing/re-positioning of already existing drugs is a very important strategy for the management of ongoing pandemy since the development of a new drug needs decades. Apart from altering angiotensin signaling pathways, novel drug candidates for re-purposing comprise medications shall target COVID-19 pathobiology, including pharmaceutical formulations that antagonize proteinase-activated receptors (PARs), mainly PAR-1. Activation of the PAR-1, mediators and hormones impact on the hemostasis, endothelial activation, alveolar epithelial cells and mucosal inflammatory responses which are the essentials of the COVID-19 pathophysiology. In this context, Ankaferd hemostat (Ankaferd Blood Stopper, ABS) which is an already approved hemostatic agent affecting via vital erythroid aggregation and fibrinogen gamma could be a potential topical remedy for the mucosal management of COVID-19. ABS is a clinically safe and effective topical hemostatic agent of plant origin capable of exerting pleiotropic effects on the endothelial cells, angiogenesis, cell proliferation and vascular dynamics. ABS had been approved as a topically applied hemostatic agent for the management of post-surgical/dental bleedings and healing of infected inflammatory mucosal wounds. The anti-inflammatory and proteinase-activated receptor axis properties of ABS with a considerable amount of oestrogenic hormone presence highlight this unique topical hemostatic drug regarding the clinical re-positioning for COVID-19-associated mucositis. Topical ABS as a biological response modifier may lessen SARS-CoV-2 associated microthrombosis, endothelial dysfunction, oropharyngeal inflammation and mucosal lung damage. Moreover, PAR-1 inhibition ability of ABS might be helpful for reducing the initial virus propagation and mocasal spread of COVID-19.

Background

COVID-19 is a multi-systemic immune syndrome following the SARS-CoV-2 infection [1]. There are tremendous ongoing efforts for the aim of developing drugs against the COVID-19 syndrome-associated inflammation. However, currently no specific medicine is present for the absolute pharmacological cure of COVID-19 mucositis. [2]. The major limitations of the drug development against COVID-19 are the slow pace/substantial costs of drug discovery process and the urgency of the currently ongoing pandemy. Those challenges have lead many researchers to drug repurposing strategies for identifying anti-COVID-19 potentials of the already approved or investigational drugs that are outside the scope of the available original medical indication [3,4].

Ankaferd hemostat (Ankaferd Blood Stopper, ABS) is a traditional medicine of plant origin comprising a standardized mixture of T. vulgaris, G. glabra, V. vinifera, A. officinarum, and U. dioica. The mechanism of action regarding the hemostatic and wound healing properties of ABS is dependent upon vital erytroid aggregation and fibrinogen gamma [5,6]. ABS is approved as a topically applied hemostatic agent for the management of post-surgical/dental bleedings

* Corresponding author.
E-mail address: yavuzbeyazit@comu.edu.tr (Y. Beyazit).

https://doi.org/10.1016/j.mehy.2020.110150
Received 12 July 2020; Accepted 28 July 2020

and healing of the infected inflammatory mucosal wounds [7,8]. Although initally presented as a hemostatic agent, compelling body of evidence suggest that ABS has anti-inflammatory, anti-infective and anti-oxidative properties in distinct disease states [9–14]. Moreover, Urtica dioica which is the major component of ABS possesses broad-spectrum antiviral properties including SARS-CoV strains [15]. There are preliminary evidence that ABS has in vitro anti-viral actions on the Bovine Herpes virus type 1 (BHV-1) [13] as well as other established anti-infective properties in a wide variety of pathogens [11–13,16,17]. Most importantly, topical ABS applications for severe oral mucositis in patients with pediatric and adult cancer receiving chemotherapy/radiotherapy [18,19] and mucosal healing of the pulmonary tissue in lung-bleeding patients [14] demonstrated the efficacy and safety of the drug for mucosal disease control in clinical backgrounds. Therefore, we herein hypothesize that ABS could be a possible topical drug candidate for the management of COVID-19-induced mucositis based on its pharmacobiology [5–7] and the nature of the SARS-CoV-2 [20,21] induced widespread oropharyngeal and pulmonary mucosal damages. The pathogenetic nature of COVID-19 is found to be related with red blood cells and ABS is pharmacologically located at the crossroads of many functional erythroid elements [7,9,22–26].

SARS-CoV2 shares a highly similar behavior pattern and gene sequence with the SARS-CoV [27]. Haznedaroglu research team recently disclosed immunoinflammatory genomic pathways with regard to the SARS-CoV, which represent a basis for the understanding of SARS-CoV-2 associated genesis of the COVID-19 [21]. Sex hormones, particularly estrogen, modulate the immune response to respiratory viral pathogens including SARS-CoV-2 [28]. The anti-inflammatory and proteinase-activated receptor axis properties of ABS [25] with a considerable amount of oestrogenic hormone constituent [29] presence highlight this unique topical hemostatic drug regarding the clinical re-positioning for COVID-19-associated mucositis. Topical ABS may lessen SARS-CoV-2 associated microthrombosis, endothelial dysfunction, oropharyngeal inflammation and mucosal lung damage. Moreover, proteinase-activated receptor-1 (PAR-1) inhibition ability of ABS might be helpful for reducing the initial virus propagation and mucosal spread of the COVID-19.

COVID-19 related inflammation and the role of proteinase-activated receptor-1 (PAR-1)

Appropriate inflammatory response is crucial for combatting against SARS-CoV-2 [21,30]. Thus, excessive or uncontrolled inflammatory response in cellular level is the major cause of disease severity and mortality in the patients with COVID-19 [31]. The pathogenicity of SARS-CoV-2 depens upon severe inflammation accompanied by the exaggregated host immune response in disease course supports the important role of inflammation in the progression of COVID-19 mucosal lesions [32]. Furthermore, the excess production of the pro-inflammatory cytokines such as interleukin-6, tumour necrosis factor, and interleukin-1β results in a cytokine release syndrome, causing to detrimental effects on vascular hyperpermeability, multiple organ failure, and finally death when the cytokine storm is unabated over the time [33].

Overproduction of the pro-inflammatory cytokines due to coagulation system activation during the COVID-19 is another pathophysiologic contributor to the negative outcome. In a study by Tang et al. [34] it has been demonstrated that non-survivor COVID-19 patients revealed considerably higher fibrin degradation product (FDP) and D-dimer levels compared to the survivors on their hospital admission. By the late courses of the hospitalization, the fibrinogen and antithrombin activity levels were found to be decreased in non-survivors, suggesting that conventional coagulation parameters during the course of COVID-19 were significantly associated with prognosis. Being a major component of coagulation cascade, thrombin is generated in abundance at sites of vascular injury and promotes clot formation by activating platelets and by converting fibrinogen to fibrin. It could specifically and efficiently activate inflammation via proteinase-activated receptors (PARs), mainly PAR-1 [35]. PAR-1 is the major functional thrombin receptor and mediates thrombin-induced platelet aggregation as well as the interplay between inflammation, coagulation, and fibrotic responses, all of which are significant aspects of the pathophysiology of fibroproliferative lung disease [36] such as seen in COVID-19. Therefore, it is reasonable to suggest that by targeting the thrombin and/or PAR-1, SARS-CoV-2 associated microthrombosis, lung injury, and poor outcomes can be prevented or treated before becoming severe enough to result in life-threatening organ dysfunction or death. Within this context, ABS raise as a potential drug candidate of COVID-19 with is antithrombin and PAR-1 down-regulating hemostatic actions [25,37].

The role of estrogens on immune cell functions: The association with COVID-19

Similar to other body systems, the immune system displays enormous diversity on gender dynamics potentially related to sex hormones, microbiome, X-chromosomes, and epigenetics [38]. Women tend to have highly responsive and robust immune system in comparison to their male counterparts. Being the primary female sex hormone, estrogen can potentially alter cellular subsets of the immune system through estrogen receptor-dependent and -independent pathways, thereby affecting the outcome of autoimmune and inflammatory immune responses [38,39]. Although, females respond more aggressively to self-antigens and are more susceptible to autoimmune diseases, accumulating evidence suggests that COVID-19 related hospital admissions and mortality were higher among males than females. Currently, male dominance mortality in COVID-19 is reported in 37 countries that have provided sex-disaggregated data suggesting plausible effect of gender differences in adaptive and immune responses to infections [28]. The impact of estrogen on sex-biased differences in SARS-CoV-2 infection seems to be associated with virus entry receptors such as angiotensin- converting enzyme 2 (ACE2), and Toll-like receptor 7 (TLR7). ACE2 is an X linked gene that is identified as a functional receptor for the SARS-CoV-2 and is down-regulated by oestrogens [40,41]. TLR7 is also a X-linked gene which acts as an initial sensor for endosomal or extracellular nucleic acid patterns of viral origin [42]. The levels of activation of the immune cells are higher in women than in men, and it is correlated with the trigger of TLR7 and the production of interferons. Compared with males, adult females have a greater production of interferon-α from plasmacytoid dendritic cells which is an effect modulated by estrogens [43,44]. Within this context, ABS raise as a potential drug candidate of COVID-19 with is high estrogen content and related physiological actions on vascular endothelial hemostatic kinetics [22,26,29].

Hypothesis

We hypothesize that small dosage topical formulation of ABS could be diluted as 1:10 v/v, 1:20 v/v, 1:40 v/v with water and could be topically applied as a gargling solution to the COVID-19 patients with oro-pharyngeal mucositis. Re-purposing of the topical oral usage of ABS against COVID-19 may be clinically followed up and further clinical investigations could be performed within near future. Non-toxic and non-irritable properties of ABS and with its antimicrobial, virucidal and anti-inflammatory features could make it a pharmacologically ready-to-use plant-based medical mixture in clinical trials. Furthermore, the nebulized solution and/or endobronchial application of diluted ABS can be an effective, safe and inexpensive therapeutic option for the SARS-CoV-2 pulmnary virus infection at the initial steps of the COVID-19 syndrome.

Evaluation and discussion of the hypothesis

Anti-inflammatory effect of Ankaferd hemostat

ABS is a unique hemostatic agent that has pleiotropic effects on the hematologic and immune systems including anti-microbial, anti-tumoral, anti-mutagenic, and anti-oxidant effects as well as tissue-healing properties [8]. The induces the formation of an encapsulated complex protein web with vital erythroid aggregation and covers the entire physiological hemostatic process. This protein complex mainly depends on the interplay between ABS and blood proteins, particularly with fibrinogen-gamma [7]. In addition to these diverse biological activities, ABS also have substantial anti-inflammatory and wound-healing properties [13,14,18,19,45]. Although the exact pathophysiology underlying the unique effect of ABS remains an area of active investigation, the anti-inflammatory effect of ABS is reported to be based on the effect of proinflammatory cytokines including IL-6, IL-1β and TNF-α. Moreover, Dynactin, EGR-1, Midkine, NF-1, Twinfilin, V-Myc, and YY1 (Yin Yang 1) can contribute both the anti-inflammatory and tissue-healing effects of ABS with various mechanisms [46]. Studies also highlight the potential benefits of ABS in numerous distinct inflammatory conditions related to the oral mucosa, intestinal mucosa, cartilage tissue, liver, pericardium, cervix and uterus [19,47–51].

Ankaferd hemostat, its oestrogen content and Proteinase-activated receptor-1

The indisputable effect of ABS on the endothelium, blood cells, angiogenesis, cell proliferation, vascular dynamics, and/or cell mediators has been established by a range of in vitro and in vivo studies [7,8]. Moreover, ABS has dual diverse dynamic reversible actions on endothelial protein C receptor (EPCR) and plasminogen activator inhibitor-1 (PAI-1) inside human umbilical vein endothelial cells (HUVECs). Both of these molecules are important biological mediators of fibrinolysis, infection, neoplasia, obesity, and wound healing and play key roles in numerous hemostatic, vascular, and immunological pathways [25,52–54]. EPCR and PAI-1 molecules are also considered as the associates of the PAR-1 in several pathobiological conditions. Furthermore, endothelial PARs have several pleiotropic characteristics contributing to the body hemostasis, inflammation and coagulation and participates in the regulation of vascular dynamics, angiogenesis, contraction, cellular proliferation, and tumorigenesis [25,55,56]. Based on these similar pleiotropic actions of ABS and PAR-1 on inflammation and coagulation, it is rational to find a close interaction between ABS and PAR-1 in numerous pathophysiologic occasions. In this context, Karabiyik et al. [37] demonstrated a dose-dependent reversible PAR-1 down-regulation, which is mediated by ABS inside the HUVECs. Therefore, ABS could be regarded as a topical biological response modifier by acting on PAR-1 at the vascular endothelial and cellular level.

The pivotal role of estrogen in the regulation of PAR-1 gene expression has been a vast topic of investigation for the last ten years [57,58]. Salah et al [57] were the first that demonstrated estrogen induced tumor development in breast carcinoma by eliciting PAR-1 gene expression. The interrelationships between cellular migration, estrogen and PAR-1 had been disclosed [59]. Estrogen receptor and PAR-1 biology is closely related to cellular proliferation [57,58,60,61]. Among a number of its ingredients, estradiol is one of the most important molecule inside the content of ABS drug. Although, HUVECs express estrogen receptor beta and rapid HUVEC cellular responses to estrogen can be mediated by estrogen binding to estrogen receptor, Ardıçoğlu et al [29] investigated the estradiol content of ABS in a recent study. The authors demonstrated a very high estradiol concentration in ABS (1452.6 pg/mL), whereas progesterone level was found to be very low (6.06 ng/mL) indicating a tight relationship between vascular endothelial cells, hemostasis, and estradiol inside ABS.

Ankaferd hemostat inhibiting PAR-1 and COVID-19

Ankaferd hemostat has distinct pleiotropic properties with a non-selective PAR-1 antagonistic effect. ABS acts as a topical biological response modifier by affecting PAR-1 at the vascular endothelial and cellular level [55]. In this context, PAR-1 is known to be widely expressed in a number of cell types relevant to COVID-19 pathobiology, including endothelial cells, pneumocytes, fibroblasts and thrombocytes [62]. Therefore PAR-1 might contribute to the pathophysiology of COVID-19 especially complications associated with thrombosis and inflammation. The increased detection rates of endothelial cell inflammation and thrombosis in patients with COVID-19 provides a vigorous incentive to determine the potential utility of PAR-1 inhibitors to improve the clinical outcome of such patients. Based on these considerations PAR-1 may represent a potential therapeutic target in the treatment of COVID-19 and ABS might be a good candidate for COVID-19 treatment based on its PAR-1 antagonistic effect.

Topical Ankaferd hemostat in clinical backgrounds for mucosal healing

Topical ABS has been already administered for the management of cancer-associated chemotherapy/ radiotherapy-induced severe oral mucositis in the patients with pediatric age groups and adults [18,19]. Likewise, pulmonar mucosal hemorrhages and resistant severe hemoptysis due to lung lesions had been controlled via the endobronchial administration of ABS [14]. The amount of used topical oral ABS was 3–4 ml four times a day [19] reaching to a total of 30 ml in four adult patients, 50 ml in three adult patients, 60 ml in one patient and 100 ml in nine patients. Median extract amount was calculated as 74.50 ml (30–100 ml) and median healing time was 6,6 days (3–10 days). Except for the temporary metalic taste, no adverse effect was observed in those patients [18]. Therefore, we herein hypothesize that ABS could be a possible topical drug candidate for the management of COVID-19-induced mucositis. Phase I safety and clinical activity study of ABS among healthy volunteers demonstrated no significant side effects fo ABS compared with placebo in respect to local skin findings and systemic laboratory tests [63]. Moreover, controlled clinical studies performed to evaluate the effectiveness of ABS in distinct states of bleeding disorders demonstrated that ABS is safe, effective, and has minimal side effects compared to other conventional anti-hemorrhagic medications [7,64]. Based on the available clinical experience that ABS is effective and safe in the prophylaxis and treatment of oral mucositis secondary to chemotherapy in childhood and adult cancers [18,19]; the only expected side effect of even the “highest” dose of topically used ABS as a gargling solution in COVID-19 can be a bitter taste in the throat and mouth irritation, which could be treated and, in almost all circumstances, would not lead to permanent harm.

Conclusion and perspectives

Given the limited therapeutic potential of current drug therapy in the management of COVID-19, ABS with its relatively benign side effect profile and favorable pharmacodynamic properties would be a promising adjunctive treatment for COVID-19 patients. At the initial steps of the disease ABS could be topically used as a gargling solution for the management of SARS-CoV-2 induced oropharyngeal mucositis. In the late stages of COVID-19, ABS could be topically used via inhalation-based methods for the management of virus-induced pneumonitis as a possible way to mitigate systemic adverse effects. Further research is warranted to define the efficacy and safety of ABS in patients with COVID-19 with respect to the oropharangyeal mucositis and broncopneumonia presentations of the pandemic syndrome.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed 2020;91:157–60.
[2] Jin Z, Liu JY, Feng R, Ji L, Jin ZL, Li HB. Drug treatment of coronavirus disease 2019 (COVID-19) in China. Eur J Pharmacol 2020;883:173326.
[3] Fan S, Xiao D, Wang Y, Liu L, Zhou X, Zhong W. Research progress on repositioning drugs and specific therapeutic drugs for SARS-CoV-2. Future Med Chem 2020. https://doi.org/10.4155/fmc-2020-0158.
[4] Haggag YA, El-Ashmawy NE, Okasha KM. Is hesperidin essential for prophylaxis and treatment of COVID-19 Infection? Med Hypotheses 2020;144:109957.
[5] Ciftciler R, Ciftciler AE, Malkan UY, Haznedaroglu IC. Pharmacobiological management of hemostasis within clinical backgrounds via Ankaferd hemostat (Ankaferd blood stopper). SAGE Open Med 2020;8. 2050312120907811.
[6] Çiftçiler R, Haznedaroglu IC. Ankaferd hemostat: From molecules to medicine. Turk J Med Sci 2020. https://doi.org/10.3906/sag-1908-161.
[7] 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.
[8] 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(4):329–36.
[9] Çiftçiler R, Aksu S, Dikmenoğlu Falkmarken N, Haznedaroğlu İC. Effects of Ankaferd Hemostat on red blood cell aggregation: a hemorheological study. Turk J Med Sci 2019;49:356–60.
[10] Çiftçiler R, Haznedaroğlu İC. On Being a "Physician Patient" with His Own Experimental Therapeutic Drug. Turk J Haematol 2018;35:302–3.
[11] Çiftçiler R, Koluman A, Haznedaroğlu İC, Akar N. Effects of Ankaferd Hemostat on Helicobacter pylori strains and antibiotic resistance. Turk J Med Sci 2019;49:347–55.
[12] Deveci A, Coban AY, Tanriverdi Cayci Y, Acicbe O, Tasdelen Fisgin N, Akgunes A, et al. In Vitro Effect of Ankaferd Blood Stopper (R), a Plant Extract Against Mycobacterium tuberculosis Isolates. Mikrobiyoloji Bulteni 2013;47:71–8.
[13] Haznedaroğlu IC, Çelebier M. Anti-infective and wound-healing pleiotropic actions of Ankaferd hemostat. Turk J Med Sci 2020. https://doi.org/10.3906/sag-2004-94.
[14] Uzun O, Erkan L, Haznedaroglu IC. Effective management of hemoptysis via endobronchial application of Ankaferd hemostat. Arch Bronconeumol 2014;50:407–9.
[15] Kumaki Y, Wandersee MK, Smith AJ, et al. Inhibition of severe acute respiratory syndrome coronavirus replication in a lethal SARS-CoV BALB/c mouse model by stinging nettle lectin, Urtica dioica agglutinin. Antiviral Res 2011;90:22–32.
[16] Koluman A, Akar N, Haznedaroğlu İC. Antibacterial activities of ankaferd hemostat (ABS) on shiga toxin-producing escherichia coli and other pathogens significant in foodborne diseases. Turk J Haematol 2017;34:93–8.
[17] Saribas Z, Sener B, Haznedaroglu IC, Hascelik G, Kirazli S, Goker H. Antimicrobial activity of Ankaferd Blood Stopper® against nosocomial bacterial pathogens. Cent Eur J Med 2010;5:198–202.
[18] Atay MH, Arslan NA, Aktimur S, et al. Safety and Efficacy of Ankaferd Hemostat (ABS) in the Chemotherapy-Induced Oral Mucositis. Int J Hematol Oncol 2015;25:166–71.
[19] Patıroğlu T, Erdoğ Şahin N, Ünal E, Kendirci M, Karakükcü M, Özdemir MA. Effectiveness of Ankaferd BloodStopper in Prophylaxis and Treatment of Oral Mucositis in Childhood Cancers Evaluated with Plasma Citrulline Levels. Turk J Haematol 2018;35(1):85–6.
[20] Göker H, Aladağ Karakulak E, Demiroğlu H, et al. The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. Turk J Med Sci 2020. https://doi.org/10.3906/sag-2005-395.
[21] Turk C, Turk S, Temirci ES, Malkan UY, Haznedaroglu İC. In vitro analysis of the renin-angiotensin system and inflammatory gene transcripts in human bronchial epithelial cells after infection with severe acute respiratory syndrome coronavirus. J Renin Angiotensin Aldosterone Syst. 2020;21. 1470320320928872.
[22] Özel Demiralp D, Haznedaroglu İC, Akar N. Functional proteomic analysis of Ankaferd® Blood Stopper. Kanama durdurucu Ankaferd® ve etki mekanizmasının proteomik analizi. Turk J Haematol 2010;27:70–7.
[23] Gulec A, Gulec S. Ankaferd Influences mRNA Expression of Iron-Regulated Genes During Iron-Deficiency Anemia. Clin Appl Thromb Hemost 2018;24:960–4.
[24] Haznedaroglu BZ, Haznedaroglu IC, Walker SL, Bilgili H, Goker H, Kosar A, et al. Ultrastructural and morphological analyses of the in vitro and in vivo hemostatic effects of Ankaferd Blood Stopper. Clin Appl Thromb Hemost 2010;16:446–53.
[25] Karabıyık A, Yılmaz E, Güleç S, Haznedaroğlu I, Akar N. The Dual Diverse Dynamic Reversible Effects of Ankaferd Blood Stopper on EPCR and PAI-1 Inside Vascular Endothelial Cells With and Without LPS Challenge. Turk J Haematol 2012;29:361–6.
[26] Yılmaz E, Güleç Ş, Torun D, Haznedaroğlu İC, Akar N. The effects of Ankaferd Blood Stopper on transcription factors in HUVEC and the erythrocyte protein profile. Turk J Haematol 2011;28:276–85.
[27] Chan JF, Kok KH, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 2020;9:221–36.
[28] Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol 2020;20:442–7.
[29] Ardıçoğlu Y, Akar N, Haznedaroğlu I. Endothelial cells, ankaferd hemostat, and estradiol. endotel hücreleri, ankaferd ve estradiol. Turk J Haematol 2016;33:261–2.
[30] Ciftciler R, Ciftciler AE, Haznedaroglu IC. Local Bone Marrow Renin-Angiotensin System and COVID-19. Int J Hematol Oncol 2020;30:113–20.
[31] Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033–4.
[32] Feng X, Li S, Sun Q, et al. Immune-Inflammatory Parameters in COVID-19 Cases: A Systematic Review and Meta-Analysis. Front Med (Lausanne) 2020;7:301.
[33] Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. Lancet Respir Med 2020;8:e46–7.
[34] Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18:844–7.
[35] Posma JJ, Grover SP, Hisada Y, et al. Roles of Coagulation Proteases and PARs (Protease-Activated Receptors) in Mouse Models of Inflammatory Diseases. Arterioscler Thromb Vasc Biol 2019;39:13–24.
[36] José RJ, Williams AE, Chambers RC. Proteinase-activated receptors in fibroproliferative lung disease. Thorax 2014;69:190–2.
[37] Karabiyik A, Güleç S, Yilmaz E, Haznedaroglu I, Akar N. Reversible protease-activated receptor 1 downregulation mediated by Ankaferd blood stopper inducible with lipopolysaccharides inside the human umbilical vein endothelial cells. Clin Appl Thromb Hemost 2011;17:E165–70.
[38] Khan D, Ansar AS. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. Front Immunol 2016;6:635.
[39] Zhu C, Xie Q, Zhao B. The role of AhR in autoimmune regulation and its potential as a therapeutic target against CD4 T cell mediated inflammatory disorder. Int J Mol Sci 2014;15:10116–35.
[40] Liu J, Ji H, Zheng W, et al. Sex differences in renal angiotensin converting enzyme 2 (ACE2) activity are 17β-oestradiol-dependent and sex chromosome-independent. Biol Sex Differ 2010;1:6.
[41] Tukiainen T, Villani AC, Yen A, et al. Landscape of X chromosome inactivation across human tissues. Nature 2017;550:244–8.
[42] Mihm S. COVID-19: Possible Impact of the Genetic Background in IFNL Genes on Disease Outcomes. J Innate Immun. 2020;12:273–4.
[43] Seillet C, Laffont S, Trémollières F, Rouquie N, Ribot C, Arnal JF, et al. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. Blood 2012;119:454–64.
[44] Berghöfer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H. TLR7 ligands induce higher IFN-alpha production in females. J Immunol 2006;177:2088–96.
[45] Ozturk O, Koklu S, Basar O, Haznedaroglu IC. Severe radiation esophagitis successfully treated with Ankaferd hemostat. Gastrointest Endosc. 2015;81:1048–9.
[46] Simsek C, Selek S, Koca M, Haznedaroglu IC. Proteomic and transcriptomic analyses to explain the pleiotropic effects of Ankaferd blood stopper. SAGE Open Med. 2017;5. 2050312117722569.
[47] Beyazit F, Buyuk B. An immunohistochemistry and histopathological study of ankaferd blood stopper in a rat model of cervical inflammation. Rev Assoc Med Bras 1992;2019(65):183–90.
[48] Büyük B, Beyazıt F. The efficacy of Ankaferd Blood Stopper® in an experimental Asherman syndrome model created in rats. Turk J Obstet Gynecol 2019;16:7–14.
[49] Taş A, Köklü S, Beyazit Y, et al. Percutaneous ankaferd injection to in vivo liver tissue in comparison to ethanol in an experimental rat model. Clin Res Hepatol Gastroenterol 2011;35:549–53.
[50] 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.
[51] Koçak E, Akbal E, Taş A, et al. Anti-inflammatory efficiency of Ankaferd blood stopper in experimental distal colitis model. Saudi J Gastroenterol 2013;19:126–30.
[52] Pontecorvi P, Banki MA, Zampieri C, et al. Fibrinolysis protease receptors promote activation of astrocytes to express pro-inflammatory cytokines. J Neuroinflammation 2019;16:257.
[53] Che SPY, Park JY, Stokol T. Tissue Factor-Expressing Tumor-Derived Extracellular Vesicles Activate Quiescent Endothelial Cells via Protease-Activated Receptor-1. Front Oncol 2017;7:261.
[54] Yürürer D, Teber S, Deda G, Egin Y, Akar N. The relation between cytokines, soluble endothelial protein C receptor, and factor VIII levels in Turkish pediatric stroke patients. Clin Appl Thromb Hemost 2009;15:545–51.
[55] Kim S, Han JH, Nam DH, Kim GY, Lim JH, Kim JR, et al. PAR-1 is a novel mechanosensor transducing laminar flow-mediated endothelial signaling. Sci Rep 2018;8:15172.
[56] McEachron TA, Pawlinski R, Richards KL, Church FC, Mackman N. Protease-activated receptors mediate crosstalk between coagulation and fibrinolysis. Blood 2010;116:5037–44.
[57] Salah Z, Uziely B, Jaber M, et al. Regulation of human protease-activated receptor 1 (hPar1) gene expression in breast cancer by estrogen. FASEB J 2012;26:2031–42.
[58] Yang E, Cisowski J, Nguyen N, et al. Dysregulated protease activated receptor 1 (PAR1) promotes metastatic phenotype in breast cancer through HMGA2. Oncogene 2016;35:1529–40.
[59] Chu HW, Cheng CW, Chou WC, et al. A novel estrogen receptor-microRNA 190a-PAR-1-pathway regulates breast cancer progression, a finding initially suggested by genome-wide analysis of loci associated with lymph-node metastasis. Hum Mol Genet 2014;23:355–67.

[60] Eroğlu A, Karabıyık A, Akar N. The association of protease activated receptor 1 gene -506 I/D polymorphism with disease-free survival in breast cancer patients. Ann Surg Oncol 2012;19:1365–9.
[61] Nag JK, Bar-Shavit R. Transcriptional Landscape of PARs in Epithelial Malignancies. Int J Mol Sci 2018;19:3451.
[62] Sriram K, Insel PA. Proteinase-activated receptor 1 (PAR1): A target for repurposing in the treatment of COVID-19? Br J Pharmacol. 2020. https://doi.org/10.1111/bph.15194.
[63] Balcik O, Koroglu M, Cipil H, et al. A Placebo-Controlled, Randomized, Double-Blinded, Cross-Over Phase I Clinical Study To Demonstrate Safety of Ankaferd Blood Stopper Topical Usage In Healthy Volunteers. Int J Lab Hematol 2012;32:126–7.
[64] Teker AM, Korkut AY, Gedikli O, Kahya V. Prospective, controlled clinical trial of Ankaferd Blood Stopper in children undergoing tonsillectomy. Int J Pediatr Otorhinolaryngol 2009;73:1742–5.