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Oral Systemic Administration of Ankaferd Blood Stopper Has No Short-Term Toxicity in an In Vivo Rabbit Experimental Model

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Abstract

Background: Ankaferd blood stopper (ABS) is a standardized herbal extract obtained from 5 different plants. In Turkey, it has been approved for local topical applications in external postsurgical and postdental surgery bleedings. Ankaferd blood stopper, besides its hemostatic activity, has in vitro anti-infectious and antineoplastic actions. **Objective:** The aim of this study was to assess short-term hematological and biochemical safety following the oral systemic administration of ABS to rabbits. *Methods:* Twelve rabbits (aged 6-12 months) were included to test the safety of oral ABS. Animals were divided into 4 groups, which had ABS administered orally at doses of 1, 3, 6, and 9 mL, irrespective of their weight. The general well-being and feeding patterns of the animals were observed for a period of 7 days. Blood samples (5.5 mL) were obtained just before oral administration, on days 1 and 4. **Results:** During the observation period of 7 days, none of the animals showed any abnormal behavior or deviation from the normal. Acute mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, and biochemical toxicity were not observed during the short-term follow-up of the animals. **Conclusions:** No signs of toxicity were observed in rabbits during short-term study with oral ABS administration.

Keywords

ankaferd blood stopper, rabbit, toxicity, oral administration

Introduction

Ankaferd blood stopper (ABS) is a standardized herbal extract obtained from 5 different plants *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum*, and *Urtica dioica*.[1] In Turkey, ABS has been approved for local topical applications in external postsurgical and postdental surgery bleedings. Topical safety of ABS in normal healthy volunteers was established in a randomized double-blind cross-over phase I clinical trial. Ankaferd blood stopper also has been used topically for the management of hemorrhages uncontrolled by standard measures in a wide variety of difficult clinical conditions.[2-7] Ankaferd blood stopper represents its unique local hemostatic effect by promoting the very rapid (<1 second) formation of a protein network that acts as an anchor for vital physiological erythrocyte aggregation, covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets (Figure 1).[1,7] Proteomic analysis, unique effects on critical transcription factors, in vitro anti-infectious and anticancer effects, suggested that ABS may affect pathobiological courses of tissues as well as its unique action on hemostasis.[8-13]

With the introduction of ABS as a topical hemostatic agent into clinical practice, data on the distinct in vitro pleiotropic

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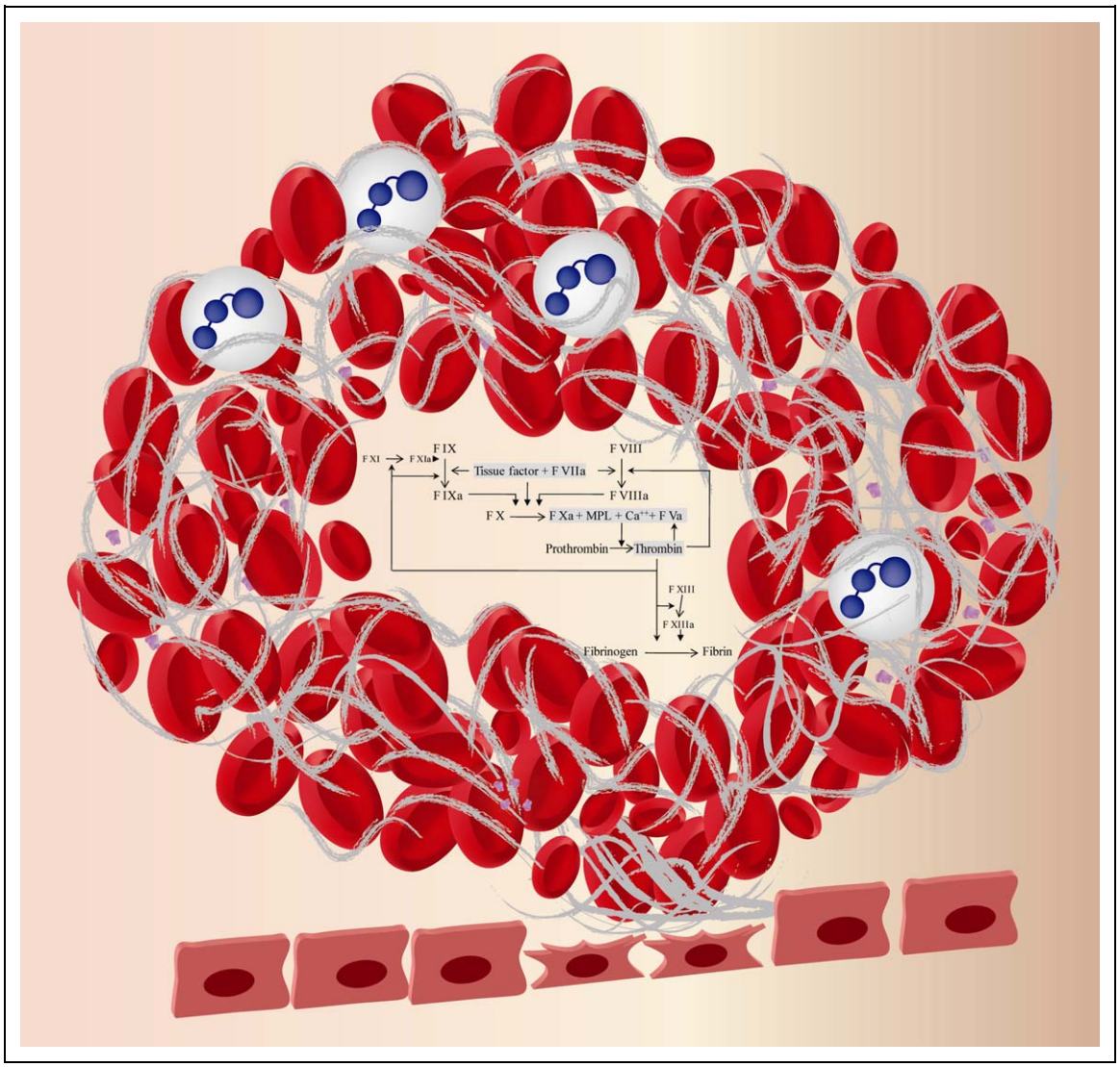

Figure 1. The basic mechanism of action for ABS is the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation. ABS-induced formation of the unique protein network within the vital erythroid aggregation covers the entire physiological hemostatic process. RBC elements (such as spectrin and ankrin surface receptors, and internal ferrochelatase enzyme), related transcription factors (such as GATA-1) and RBC-related proteins (such as urotensin II) are the main targets of ABS. The production of spectrin is promoted by the transcription factor GATA-1. Those proteins and the required ATP bioenergy are included in the protein library of ABS.[1,8,9] ABS indicates ankaferd blood stopper; ATP = adenosine triphosphate; RBC = red blood cell; GATA-1 = globin transcription factor-1; MPL = membrane phospholipid.

effects of ABS have raised the need for research on the systemic effects of ABS administration. Although ABS is currently solely used topically to control bleedings, local oral and gastrointestinal ABS application may result in systemic absorption of the agent. Furthermore, demonstrated in vitro anti-infectious and antineoplastic actions of ABS represent the basis to investigate the in vivo efficacy and safety of this herbal product for fitoterapia in upcoming researches. Therefore, the systemic toxicity of ABS should be studied in animal models to proceed with those investigations. The aim of this study was to assess short-term hematological and biochemical safety following oral systemic administration of ABS to rabbits.

Materials and Methods

In this study, 12 rabbits (aged 6-12 months) were included to test the safety of oral ABS. All animals were housed in metal cages with a wire netting bottom and maintained at a temperature of 23°C ($\pm$5°C). The animals were then allowed free access to solid diet and tap water. They were allowed to roam freely for an hour in a small garden, twice daily as well. The experimental study was conducted with the approval of the Fatih University Medical School Ethics Committee. All procedures were in full compliance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26 and with the Helsinki Declaration of World Medical Association recommendations on animal studies.

Each rabbit was randomly assigned a number from 1 to 12. Animals were divided into 4 groups, which had ABS administered orally at doses of 1, 3 6, and 9 mL, irrespective of their weight. The general well-being and feeding patterns of the animals were observed for a period of 7 days. Blood samples (5.5 mL) were obtained just before oral administration, on days 1 and 4, from which complete blood count as well as serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), Alkaline phosphatase (ALP), total and direct bilirubin, blood urea nitrogen, and creatinine were measured. Alterations in the blood specimen workup before and after the administration were evaluated for statistical significance using the SPSS 16.0 for Windows.

Results

The median weight of the animals was 3325 mg (2300-4550), and the median amount of ABS administered was 1.39 mL/kg (0.26-2.61). There was no difference between the groups with regards to weight, biochemical, and complete blood count parameters before and after the oral administration of ABS. During the observation period of 7 days, none of the animals showed any abnormal behavior or deviation from the normal. The results are depicted in Table 1.

Table 1. Baseline, the First and Fourth Day Biochemical and Hematological Parameters of the Studied Rabbits Following the Oral Administration of ABS[a]

		Group				
Lab Values	Day	1 mL	3 mL	6 mL	9 mL	P^b
AST	0	66	86	93	32	.31
	1	38	37	63	28	.18
	4	55	35	42	26	.08
ALT	0	78	89	79	87	.10
	1	93	88	70	90	.28
	4	84	73	67	82	.60
GGT	0	22	21	18	25	.20
	1	22	20	18	24	.20
	4	22	20	18	25	.36
ALP	0	144	107	94	145	.46
	1	122	103	84	126	.60
	4	122	83	93	100	.37
Total bil	0	0.36	0.41	0.61	0.43	.91
	1	0.42	0.33	0.37	0.34	.59
	4	0.37	0.42	0.27	0.44	.06
BUN	0	20	18	22	21	.48
	1	19	16	19	18	.12
	4	21	19	22	18	.37
Cr	0	0.7	0.8	0.7	1.0	.70
	1	0.9	0.8	0.8	1.0	.49
	4	0.9	0.9	0.6	0.9	.76
WBC	0	7.4	9.4	9.9	9.3	.96
	1	5.9	6.9	7.6	8.4	.09
	4	5.6	6.3	6.7	11.25	.19
Erythrocyte	0	6.53	6.37	5.97	6.79	.26
	1	6.03	6.37	6.39	6.37	.87
	4	5.96	6.36	5.79	5.66	.69
Hb	0	13.9	12.9	12.4	14.0	.26
	1	12.7	12.6	12.7	13.2	.98
	4	12.1	12.5	11.4	11.5	.78
Hct	0	41.4	37.8	33.6	41.0	.28
	1	38.0	37.3	36.9	39.2	.96
	4	36.1	37.4	34.4	33.8	.59
MCV	0	63.5	59.4	56.2	60.4	.18
	1	63.0	58.0	58.0	61.0	.21
	4	62.0	59.0	58.0	60.0	.09
MCH	0	21.4	20.3	20.8	20.6	.17
	1	20.9	19.7	19.8	20.4	.18
	4	20.7	19.5	19.3	20.3	.21
Plt	0	402	503	544	449	.73
	1	495	447	535	513	.87
	4	492	541	476	463	.62
MPV	0	6.2	5.6	4.8	5.5	.30
	1	8.5	9.2	8.8	9.2	.82
	4	8.2	8.3	7.6	7.6	.26

NOTES: ABS = ankaferd blood stopper; Hb = hemoglobin; WBC = white blood cell; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = γ-glutamyl transferase; ALP = alkaline phosphatase; BUN = blood urea nitrogen; Cr = creatinine; Hct = hematocrit; MCV = Mean corpuscular volume; MCH = mean corpuscular hemoglobin; Plt = platelet; MPV = mean platelet volume.

[a] The "Group" Refer to the Dose of Oral ABS Administered to a Given Rabbit Subgroup.

[b] Kruskal-Wallis analyses.

Discussion

In this study, short-term hematological and biochemical safety of the oral systemic administration of ABS to rabbits have been shown. Acute mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, and biochemical toxicity were not observed during the short-term follow-up of the animals. These results provide a starting point for further research on possible systemic confounding effect of ABS, when applied to internal topical surfaces. Topical hemostatic efficacy of ABS has been previously tested in animals with normal and defective hemostasis.[14-16] Physiological cell-based coagulation could be clinically managed via topical ABS application to prevent and treat bleeding in many distinct clinicopathological states.[2-6] Neither local nor systemic adverse effect and/or toxicity have been observed in association with experimental and anecdotal topical application of ABS.

Besides its hemostatic activity, ABS may also inhibit the growth of bacteria. Anti-infectious activity of ABS may represent an advantage suggest to its current clinical use, because it inhibits the growth of bacteria in the area used mainly for its hemostatic activity such as traumatic-infected wounds. The antimicrobial activity of ABS was tested against 102 clinical isolates in a previous study.[13] The isolates included *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter* species, *Stenotrophomonas maltophilia*, methicillin-resistant *Staphylococcus aureus*, methicillin-resistant coagulase-negative *Staphylococcus*, vancomycin-susceptible *Enterococcus* and vancomycin-resistant *Enterococcus*. They have reported that ABS was active against all these isolates, with zones of inhibition within the 10- to 18-mm-diameter range.

Antibacterial activities of ABS against several gram-positive and gram-negative food and human pathogens were also reported in another study.[12] If in vivo studies support the hypothesis that ABS may be useful for the management of infected hemorrhagic wounds, possible concerns regarding the potential systemic absorption of the topical hemostatic agent may be raised. Therefore, future research focusing on any possible systemic adverse effect of ABS when applied to internal topical surfaces is warranted.

ABS-induced formation of the protein network with vital erythroid aggregation covers the entire physiological hemostatic process.[1,7] Mainly, there are distinct important components of the ABS-induced hemostatic network. Vital erythroid aggregation takes place with the spectrin and ankrin receptors on the surface of red blood cells. Those proteins and the required adenosine triphosphate (ATP) bioenergy are included in the protein library of ABS.[8] Ankaferd blood stopper also upregulates globin transcription factor (GATA)/Friend of GATA (FOG) transcription system affecting erythroid functions.[9] Urotensin II is also an essential component of ABS and represents the link between injured vascular endothelium, adhesive proteins, and active erythroid cells.[8] These concepts have been developed via Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) proteomic molecular analyses, cytometric arrays, transcription analysis, and scanning electron microscopic (SEM) ultrastructural examinations as well as numerous investigations interacting with basic and clinical research facilities.[1,2,7-11,13-16] Therefore, ABS could be effectively used both in individuals with normal haemostatic parameters and in patients with deficient primary and/or secondary hemostasis. In vitro data on the anti-infectivity profile of ABS and bleeding control in the settings of gastrointestinal disorders and mediastinal bleedings shed further light on the upcoming controlled trials.[2-6] More investigations on the biological features of ABS as an efficient hemostatic agent are still in progress. If further observations suggest the systemic safety of ABS, new avenues on the upcoming perspectives of ABS may be opened.

Declaration of Conflicting Interest

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