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Effects of Ankaferd Blood Stopper® on Vascular Response in Rat Carotid Artery

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ABSTRACT

The purpose of this study was to investigate the dose-dependent effect of Ankaferd Blood Stopper® (ABS) on the rat carotid artery model. Carotid arteries were removed and contraction/relaxation of isolated vessel rings were measured for dose-dependent epinephrine, papaverine and (ABS) administrations by a force displacement transducer. The data of each tissue specimen were collected with the use of a computerized system and corresponding software at a sample rate of 1000 kHz, and were expressed as contraction force. ABS is a standardized mixture of five plants, has been used historically as a haemostatic agent. Each plant extracts have different effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators individually but ABS's mechanism of action remains unknown. In study, ABS has a vasoconstriction effect in low concentrations (5 and 10 μL). On the other hand, ABS showed a vasodilatation effect in higher concentration (20-40-80 μL). As a conclusion, haemostatic effect of this solution relies on mechanisms other than those related with vascular contractility, can be said.

Keywords: Blood vessel, Vessel wall tonus, Vasoconstriction, Vasodilatation, Tissue compatibility

ÖZET

Rat karotid Arterinde Damarla İlgili Yanıtta Ankaferd Blood Stopper'in Etkileri

Bu çalışmanın amacı, rat karotis arteri modeli üzerinde Ankaferd Blood Stopper'ın (ABS) etkisinin araştırılmasıdır. Karotis arteri çıkarıldı ve izole damar çeperleri üzerinde epinefrin, papaverin ve ABS'in doza bağımlı kasılma/gevşeme değerleri kuvvet transdüktörü ile ölçüldü. Bütün bilgiler bilgisayar kullanarak 1000 kHz örnek oranında ilgili programda toplandı. Geleneksel olarak hemostatik etki amacıyla kullanılan ABS, beş farklı bitkinin ekstresinden oluşmaktadır. Her bir bitki ekstresinin endotelyum, kan hücreleri, angiogenesis, hücresel çoğalma, damarsal dinamikler ve hücre mediatörleri üzerine farklı etkileri bulunmakta ancak ABS'nin etki mekanizması hala tam olarak bilinmemektedir. Çalışmada, ABS'nin düşük konsantrasyonlarda (5 and 10 μL) vazokonstraksiyon etkisi varken, yüksek konsantrasyonlarda (20-40-80 μL) vazodilatasyon etkisinin olduğunu gösterdi. Sonuç olarak, bu solüsyonun hemostatik etkisinin vasküler kontraksiyonla ilgili olmadığı söylenebilir.

Anahtar Kelimeler: Kan damarı, Damar duvar tonusu, Vazokonstraksiyon, Vazodilatasyon, Doku uyumluluğu

INTRODUCTION

Bleeding can cause significant morbidity and mortality in any clinical setting. In most situations, extractions and other similarly invasive procedures can be managed safely in a community dental practice.[1,2] Careful surgical technique, including an attempt at primary wound closure and local haemostatic measures will usually be sufficent for.[1] Serious problems can be encountered during and after tooth extraction procedures. Especially coagulation problems related to acquired or congenitally diathesis need presurgical preparations.[2] Such circumstances comprise patients with multiple coagulapathies, advanced uraemia without dialysis, platelet count of less than 50000, preliver transplant status, aplastic anaemia and high dose cancer therapy.[1] Oxidised cellulose tranexamic acid rinses, astringents (e.g., aluminum chloride), microfibrillar collagen, thrombin-soaked gauze, fibrin sealant and adhesive, electrocautery[3], absorbable gelatin sponges, and aminocaproic acid (EACA) to prevent clot lysis, have all been suggested as aids to haemostasis. Various types of haemostatic agents can be used for the treatment of patients with haemostatic problems.[4]

Published case reports of serious blood loss after tooth extractions are not uncommon. Nishide et al. reported a case about a 44-year-old female patient on haemodialysis who was scheduled for a renal transplantation within two months. Under general anaesthesia, they extracted 19 teeth and removed the overgrown gingiva. A Total blood loss of 1650 ml was determined, which necessitated trasnfusion of four units of concentrated red blood cells.[5] In a retrospective study, Franchini et al. collected data from 10 years of experience in the oral care of patients with congenital haemorrhagic disorders in three Italian Hemophilia Centers. They used tranexamic acid and/or fibrin glue locally and transfusion of homestatic factors and fresh frozen plasma systemically for homestasis. They recorded 10 bleeding complications (1.9%), most of which occurred in patients with severe/moderate haemophilia A undergoing multiple dental extractions.[6] These reports demonstrate that t here is a current lack of a agent that can be used for all types of disorders efficiently. Ankaferd Blood Stopper® (ABS) is a unique folkloric medicinal plant extract, which has historically been used in Turkish traditional medicine as a agent. Reportedly, the ABS facilitates the formation of an encapsulated protein network, providing foci for vital erythrocytes to aggregate on.[7] There are published successful reports about the management of hemorrhage in life threatening cases.[8-12] Ercetin et al., showed that ABS is an efficient hemostatic agent for dental surgery and has no effect on systemic condition when used topically.[13] However, the basic mechanism of action for the haemostatic effects of ABS is currently unknown. ABS comprises a standardized mixture of the plants Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica (Table 1).[7,14] Each of these plants have selective effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators.[15-20] Among these, Urtica dioica can produce hypotensive response through a vasorelaxation effect mediated by the release of endothelial nitric oxide (NO) and the opening of potassium channels, and through a negative inotropic action.[17] However the cumulative effect of ABS on arterial tissue is unknown.

Consequently, the aim of this study was to investigate the dose-dependent effect of ABS on the rat carotid artery model.

MATERIALS AND METHODS

Locally bred female 6 albino rats (Rattus norvegicus) weighing 300-340 g were used in the present study. The experimental protocol for the use of animals was approved by the Institutional Animal Care Committee. Rats were anesthetized with a mixture of xylazine (Rompun, 2% solution, Bayer, Ger-

Table 1. Plant extracts and amounts composing ABS®

Amount	(g/100 ml)
Urtica dioica	6.0
Vitis vinifera	8.0
Glycyrrhiza glabra	7.0
Alpinia officinarum	7.0
Thymus vulgaris	5.0

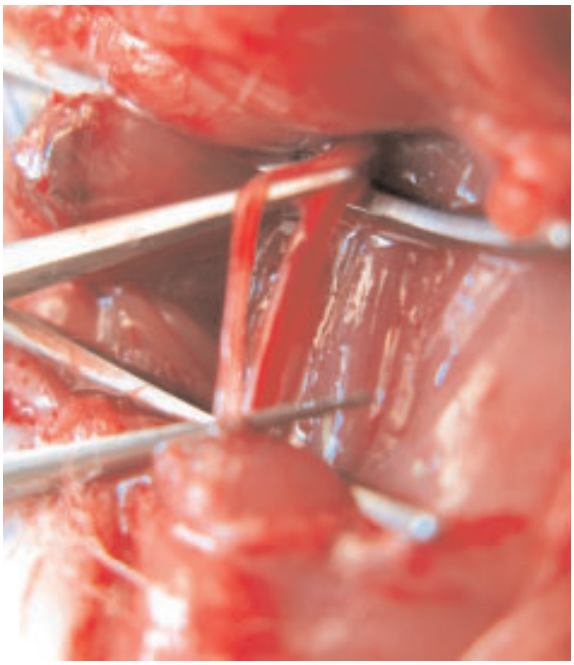

Figure 1. The left carotid arteries were quickly isolated, cleaned of their connective tissue and removed from the animal as described previously by Abebe et al.

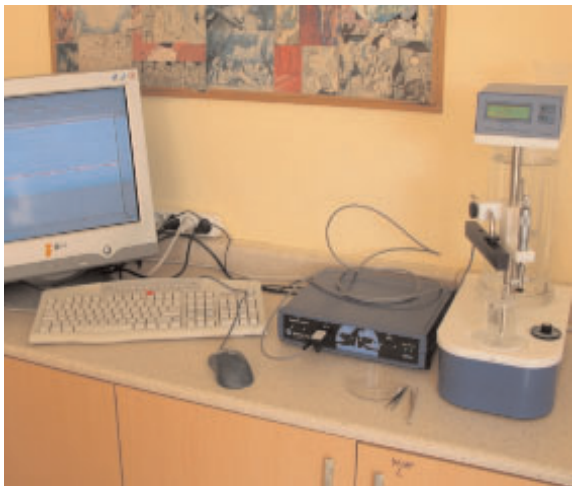

Figure 2. Muscle contractions were recorded to an IBM-compatible PC using a force displacement transducer (SS12LA, Biopac systems Inc., Santa Barbara, CA, USA) at 200 Hz sample rate to which the upper wire was connected. A Biopac MP35 (data acquisition system Biopac systems Inc., Santa Barbara, CA, USA) was used for the recordings and stimulations (Tasman/2000, Cehreli 2002).

many) and ketamine hydrochloride (Ketalar, 30 mg/kg, Parke Davis). The ratio of Rompun and Ketalar in the mixture was 3:1. The left carotid arteries were quickly isolated, cleaned of their connective tissue and removed from the animal as described previously by Abebe et al.[21] (Figure 1). Then, the removed sections were cut into transverse rings of 3 mm length with special care taken not to damage the luminal surface. The rings were fixed vertically between stainless-steel wire hooks and suspended under 1000 mg of resting tension in the tissue bath containing Krebs-bicarbonate solution (pH 7.4) at 37^{0}C, continuously aerated with 95% O_2 + 5% CO_2 mixture. Composition of the Krebs-bicarbonate solution was as follows (in mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 1.3, $NaHCO_3$ 25, $MgCl_2.6H_2O$ 0.5; $NaHPO_4.2H_2O$ 0.9 and glucose 11 (all chemicals were obtained from Sigma St. Louis, MO). Tissues were equilibrated for 30 min. At first epinephrin (2 μL, 4 μL, 6 μL) and papaverine (10 μL, 20 μL, 40 μL) administered and isometric muscle contractions were recorded to an IBM-compatible PC using a force displacement transducer (SS12LA, Biopac systems Inc., Santa Barbara, CA, USA) at 200 Hz sample rate to which the upper wire was connected. A Biopac MP35 (data acquisition system Biopac systems Inc., Santa Barbara, CA, USA) was used for the recordings and stimulations[16,17] (Figure 2). Between and after each measurement interval, the bathing medium was replaced with 15 mL of Krebs solution. Following this measurement, Ankaferd Bloodstopper® was administered in 5 different concentrations (5, 10, 20, 40, 80 μL) and isometric muscle contractions were recorded. By the end of measurements, 4 μL epinephrine and 20 μL papaverine was administered in the same manner as controls, and the isometric force measurements were recorded. Between each group interval, 4 times washing of the rat carotid ring Krebs bicarbonate solution was administered in order to achive the original state of the ring. This experiment repeated with 15 different vascular rings. In each group, recordings were converted to contraction force (in mg). Biopac LabPro V3 6.7 software (Biopac systems Inc., Santa Barbara, CA, USA) was used in recording and analyzing data. Dose-dependent relaxations caused by the test materials were recorded in the same manner in comparison with papaverine (10, 20 and 40 μL, respectively). Differences in the contraction or relaxation forces with regard to the

Table 2. Muscle contraction average values in mg and % and standard deviations (SD).

	Mg	%	SD
Control	899.32	100	107.78
2 μL Epinephrine	907.27	101.028	99.04
4 μL Epinephrine	914.92	101.925	96.50
6 μL Epinephrine	931.85	103.819	97.87
10 μL Papaverine	901.67	100.49	92.33
20 μL Papaverine	891.83	99.281	100.09
40 μL Papaverine	884.35	98.480	96.43
5 μL ABS	948.38	106.048	155.61
10 μL ABS	911.97	101.818	170.08
20 μL ABS	865.83	96.564	190.84
40 μL ABS	816.13	91.138	182.68
80 μL ABS	799.25	89.229	238.17
4 μL Epinephrine-After ABS	914.47	101.876	96.79
20 μL Papaverine-After ABS	891.87	99.230	99.03

different concentrations of the test solutions were determined statistically by repeated measures analysis of variance (ANOVA) overall among all concentrations of individual materials, namely epinephrine, papaverine and ABS. The pairwise differences between control and test solutions were tested by posthoc F test. The statistical significance was assigned to p values less than 0.05.

RESULTS

The contraction and relaxation forces induced by the control and test materials are presented in Table 2. The validity of the test methodology was confirmed on sectioned vessels using different concentrations of epinephrine and papaverine.

Accordingly, the differences of between the control and the contraction forces induced by epinephrine were statistically significant (repeated measures ANOVA, overall, $p< 0.001$; epinephrine 2 μL vs control, $p= 0.076$; epinephrine 4 μL vs control, $p= 0.003$; epinephrine 6 μL, $p< 0.001$).

The differences of between the control and the vasodilatation forces induced by papaverine were statistically significant (repeated measures ANOVA, overall, $p< 0.001$; papaverine 10 μL vs control, $p= 0.658$; papaverine 20 μL vs control, $p= 0.156$; papaverine 40 μL, $p= 0.018$).

5 μL and 10 μL ABS produced epinephrine-like contractions on the rat carotid artery. When the dose was increased, however (20 μL, 40 μL and 80 μL), ABS produced papaverine-like caused dose-dependent, vasodilatation in a dose-dependent manner (Figure 3, 4). Contraction and vasodilatation with ABS was not statistically significant. After the experiment with ABS, 4 μL epinephrine and afterwards 20 μL papaverine administered. Rat carotid artery responds similar with initial ones. Response of the rat carotid artery did not change after the study period with ABS.

Contraction and relaxation effect of the ABS was reversible and the materials did not cause the vessel to collapse.

DISCUSSION

In the present study, the rat carotid artery model was used to mimic the smooth muscle contraction of intraosseous vessels, because behavior of vascular tissues in response to various known vasoactive

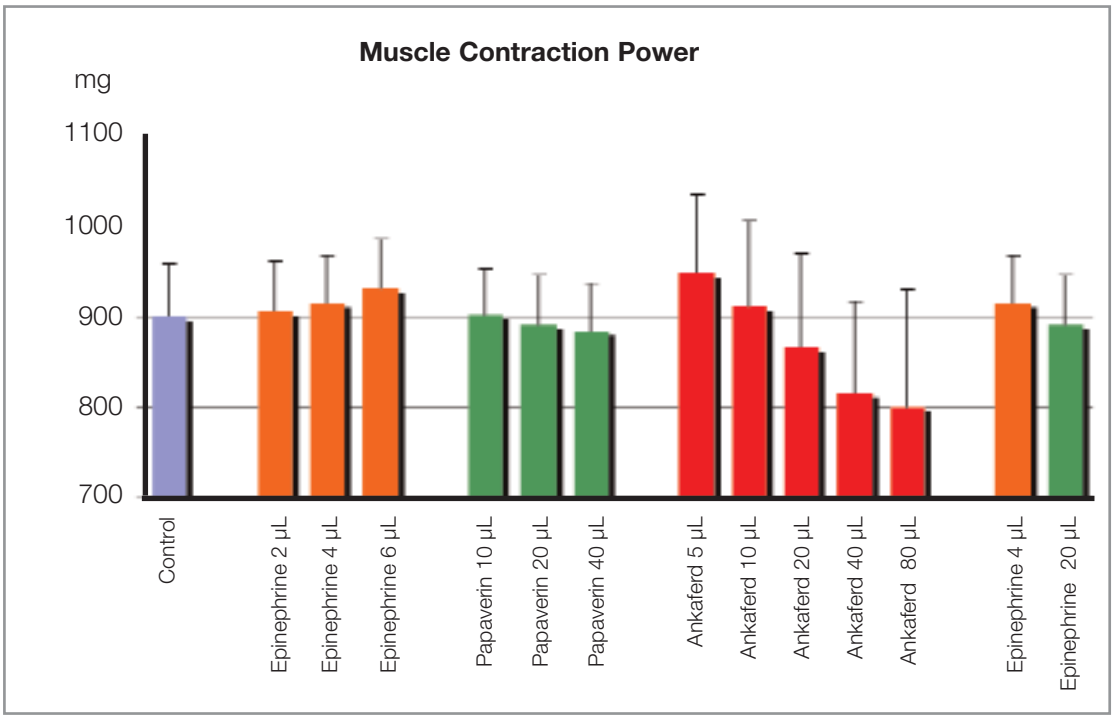

Figure 3. Muscle contraction power increase in low concentration and decrease in high concentration of ABS in mg in order.

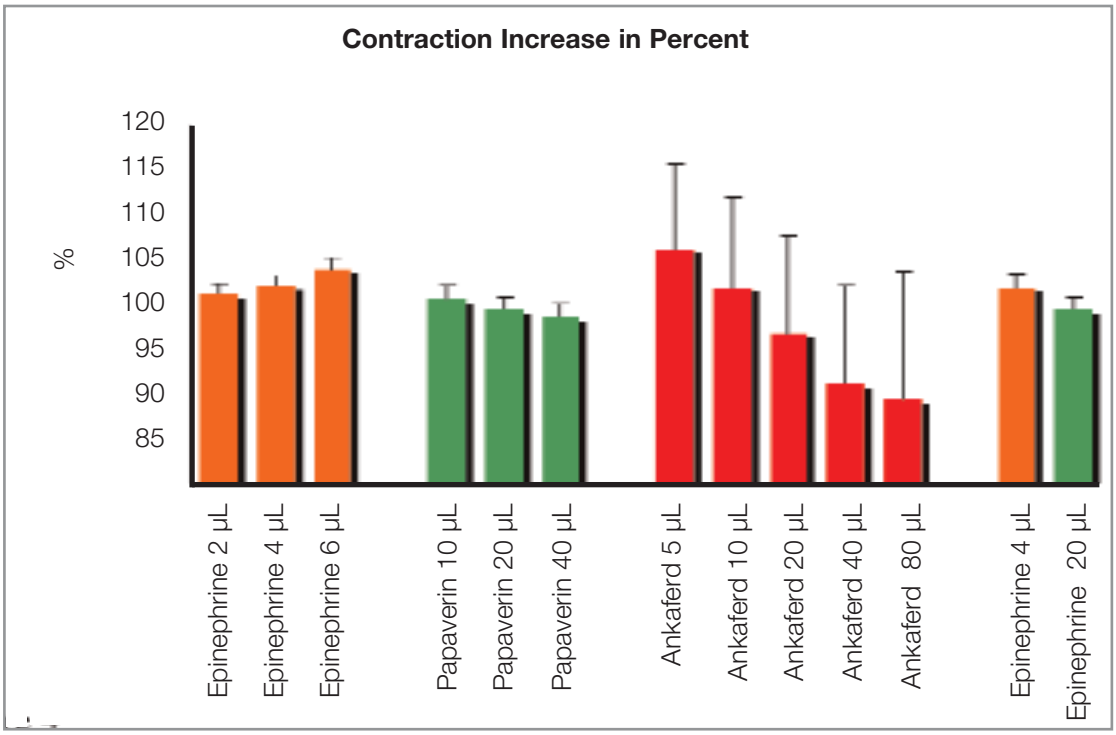

Figure 4. Muscle contraction change in % in order.

agents is not significantly different from this tissue as with other widely studied tissues.[22-25] Rat carotid artery is a well-characterized tissue which facilitates the ability to determine whether or not an agent is vasoactive by making responses more readily quantifiable and reproducible for both smooth muscle and endothelium.[24] Epinephrine was applied to determine the vascular contractibility, as it is known to combine with alpha-adrenergic receptors on arteriolar smooth muscle and cause vasoconstriction.[26] Papaverine was used to evaluate the reversibility of contractions obtained with epinephrine. Papaverine is an opium alkaloid which relaxes many smooth muscles of the body, especially those of blood vessels.[27]

Vascular endothelium plays a pivotal role in the regulation of the homeostasis. Following vascular injury, endothelial cells limit clot formation to the areas where homeostasis is required to restore the vascular integrity.[28] ABS contains plant extracts that have different effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators.[15-20] T. vulgaris has anti-oxidative actions, such as prevention of lipid peroxidation.[16] V. vinifera exerts anti tumor and anti atherosclerotic effects.[19] A. officinarum inhibits nitric oxide production by lipopolysaccharide activated mouse peritoneal macrophages.[15] Finally, U. dioica can produce hypotensive responses through a vasorelaxation effect.[17] In contrast to the findings of Testai et al.[17], our results demonstrate that ABS has a vasoconstriction effect in low concentrations (5 and 10 μL). On the other hand, ABS showed a vasodilation effect in higher concentration (20-40-80 μL), which corroborates the vasorelaxation effect demonstrated by Testai et al.[17] These results strongly suggest that ABS has concentration-dependent effects on vascular contractility. Following exposure to different concentrations of ABS, the same rat carotid artery rings were treated with 4 μL epinephrine and 20 μL papaverine in order to determine vascular condition. The contraction/relaxation forces were almost similar to the readings before treatment of rings with ABS. Thus, the vascular response to ABS was confirmed to be reversible.

The concentration by which ABS was shown to induce vasorelaxation herein is far less than that recommended by the manufacturer (approximately 1.2 ml) for the control of bleeding in a dental extraction socket. Accordingly, one should expect a vasodilatation effect caused by ABS. The impact of this vasorelaxant effect on the overall performance and duration of haemostatic achieved in a tooth extraction socket remains to be substantiated in an animal model, before advocating its potential use in dental surgery. On the other hand, our results indicate that the haemostatic effect of this solution relies on mechanisms other than those related with vascular contractility.

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