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ORIGINAL ARTICLE

The Effects of a New Hemostatic Agent on Hearing in Rats*

M. Tayyar Kalcioglu, Tuba Bayindir, Mustafa Iraz, Serdar Kaya, Sermet Can

Department of Otolaryngology Inonu University Faculty of Medicine, Malatya, Turkey, (MTK, TB, SK)
Department of Pharmacology, Inonu University Faculty of Medicine, Malatya, Turkey, (MI)
Medical Student , Inonu University Faculty of Medicine, Malatya, Turkey, (SC)

Objective: Ankaferd Blood Stopper (ABS), a standardized mixture of five plants, has been used as a hemostatic agent. Studies have shown the hemostatic effectivity of this agent that suggests a potential usage in otological surgeries. However,side effects on hearing of this agent are unknown. In this study, hearing affection of local and systemic usage of ABS was investigated by using distortion product otoacoustic emissions (DPOAEs).

Materials and Methods: Thirty-two male Wistar rats were used. The animals were divided into four groups. Baseline DPOAE measurements were performed. Subsequently, intratympanic ABS administration to the firstgroup and intratympanic saline administration to the second group were performed. Intraperitoneal ABS was injected to the third group. The fourth group was not administered any intervention. Side effects of ABS on hearing were evaluated by repeated DPOAE measurements carried out before and at 1st, 7th, and 40th days following the applications.

Results: On days 7 and 40; measurement parameters of DPgrams of intratympanic ABS group were found to have significantly deteriorated in some frequencies (p(0.05). The measurements of the other groups revealed no significant differences (p>0.05). Hearing loss was not observed in systemic absorption group but, determined in intratympanic application group.

Conclusion: Our findings may suggest that hearing loss may be either due to ototoxic side effect of ABS, prolonged mass effect of it, or because of inflammation. Further studies with longer follow up period and histopathological examinations are needed to answer these existing questions.

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Introduction

Although there are certain treatment modalities, bleeding can cause significant morbidity and mortality in some cases. ABS is a standardized mixture of five different plants including Thymus vulgaris, Glycyrrhizaglabra, Vitisvinifera, Alpiniaofficinarum, and Urticadioica, which has been approved and currently used in the management of external hemorrhages and dental surgery hemorrhages in Turkey.[1] The basic mechanism of action for ABS was proposed to be a rapid formation of an encapsulated protein network that provides focal points for erythrocyte aggregation.[1] In the literature, there are some experimental studies or case reports revealing the successful results of this agent on bleeding.[2-13] This agent's effectivity to control bleeding in the ear, nose, or throat area and during the surgery for control surgical bleeding has been shown.[5,9] However, its safety in terms of hearing has to be shown before systemic or topical administration of this agent.

The aim of this study was to investigate the potential side effect of intratympanic or intraperitoneal ABS administration on hearing by using DPOAEs

Materials and Methods

Chemicals: The vials of ABS were obtained from Ankaferd Drug Inc., Istanbul, Turkey (patent number 2007-0-114485). The chemical was of the highest quality commercially available.

Animals: Thirty-two adult male Wistar albino rats, weighing between 250 g and 300 g at age of 3 to 5 months, were used in this study. The animals were fed a standard commercial diet and given water ad libitum during the experimental period. They were housed in a room with 20-22 °C ambient temperature, with a relative humidity of 50∓5 % and 12 hr of light and 12 hr of darkness cycle. Plastic cages containing wood-chip bedding housed three to four rats. The presence of a Preyer's reflex was used as an initial screening of the animals. A normal examination of the outer ear canals

Corresponding address:
M. Tayyar Kalcioglu M.D.
Inonu University, Faculty of Medicine Department of Otorhinolaryngology 44069, Malatya- Turkey
Phone: +90 422 341 06 60 ext. 4607; Fax: +90 422 341 01 28; E-mail: mtkalcioglu@hotmail.com

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and tympanic membranes and normal DPOAEs confirmed the healthy state of hearing of the adult rats. All animals were given one week to acclimatize before the experiment. Animals were used in accordance with a protocol approved by the IU School of Medicine Laboratory Animal Care and Use Committee.

Anesthesia: The rats were anesthetized with a cocktail of ketamine hydrochloride (30 mg/kg) and xylazine (6 mg/kg) administered intraperitoneally (IP) before each DPOAEs recordings and intratympanic or intraperitoneal administration of the chemical or intratympanic administration of isotonic saline.

Experimental design: The animals were randomly divided into four groups of 8 rats each (group A, intratympanic ABS; group P, intraperitoneal ABS; group S; intratympanic isotonic saline; group C, control). Group A was administered intratympanic ABS (0.1ml). Group P was injected with intraperitoneal ABS (1 ml). Group S received intratympanic isotonic saline (0.1 ml). Group C was control group and did not receive any chemicals or saline. All intratympanic administrations were performed through otoscope by the same researcher (TK) using disposable plastic syringes on day 0. In all groups, DPOAEs measurements were performed before and 1, 7, and 40 days after ABS or saline administrations. Experiments were performed between 10.00 AM and 04.00 PM of every study day.

OAE measurements: Only rats with normal replicable DPOAEs before the administration of any substance on day 0 were included in this study. DPOAEs recordings were performed in a quiet room. A quiet cabin was used for all measurements. The DPOAEs were elicited from the right ear of control and experimental animals utilizing a standard commercial GSI Audera DPOAE (Grason Stadler, Madison, USA). Following anesthesia, the primary tones were introduced into the animals' outer ear canal through an inserted earphone, using a plastic adapter that sealed the probe in the outer ear canal.

For DPOAE measurements, the intensities of primary stimuli were set as equilevel (L1=L2) at 65 dB. The frequencies (f1 and f2) were adjusted in such a manner that f2/f1=1.21. DPOAEs were determined as DPgrams. The intensity levels of the primary tones were held constant and DPOAE data were recorded for different frequency regions, ranging from 1418 to 11308 Hz, and plotted as a function of f2. The resolution of the DPgram was obtained at four points per octave. The level of the noise floor was measured at a frequency 50 Hz above the DPOAE frequency, using similar averaging techniques. An emitted response was accepted if the DPOAE at 2f1-f2 amplitude was (3 dB above the magnitude of the noise-floor level at the 2f1-f2 + 50 Hz frequency for the DPgram. Testing method was recorded until the response attained its highest level and then terminated when no increase was noted.

The baseline hearing status of all rats was determined with DPgram. Measurements were made in all rats during experiments on days 0, 1, 7, and 40. The f2 frequencies examined for DPgram ranged from 1418 to 11308 Hz (1418, 1687, 2003, 2378, 3363, 3996, 4757, 5660, 6726, 8003, 9515, 11308 Hz.).

Statistical Analyses: Results were analyzed statistically by non-parametric Friedman test to determine differences in amplitudes of DPOAEs and corresponding noise floor differences for each frequency. Additionally, non-parametric Wilcoxon signed rank test was used to compare the OAE values of each group obtained during the baseline measurements and those obtained during the 1st, 7th, and 40th days of the study.Data were analyzed by using SPSS for Windows 16.0.

Results

DPgram of study groups corresponding to days 0, 1, 7, and 40 are presented in Figures 1-4. On day 0, the initial baseline DPOAEs measurement results presented comparable values in all groups prior to intratympanic or intraperitoneal ABS administration, intratympanic saline administration, or control group ($p>0.05$). On days 7 and 40; measurement parameters of DPgrams of intratympanic ABC group were found to have significantly deteriorated in some frequencies ($p<0.05$). Particularly, statistically significant differences were determined between 0 and 7 days of measurements at frequencies of 5660, 6726, 8003, and 9515 Hz and between 0 and 40 days of measurements at the frequencies of 5660 and 6726 Hz ($p<0.05$). The measurements of the other groups revealed no significant differences between their DPgrams ($p>0.05$).

Discussion

ABS is a patented medicinal plant product that was approved in Turkey by the Ministry of Health as provides active hemostasis in external hemorrhage and bleeding during dental surgeries. Reported tests have

proved its safety, efficacy, sterility, and nontoxicity (www.ankaferd.com). It is a standardized mixture of the plants that appears to provide a composition for physiological hemostatic process without disturbing the levels of any individual clotting factor.[1] It is reported that it may be effective both in individuals with normal hemostatic parameters and in patients with deficient primary or secondary hemostasis such as disseminated intravascular coagulation (DIC).[1] In a study performed by Goker et al[1]., even the coagulation factors II, VII, VIII, IX, X, XI, and XIII were not affected, plasma fibrinogen activity and fibrinogen antigen levels were reported to be decreased in parallel with a prolongation of thrombin time after using ABS. They speculated that ABS stimulates the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation.

In some clinical cases, bleeding may significantly be responsible of morbidity and mortality. Bleeding can also be a problem at ear, nose, or throat such as epistaxis and surgical hemorrhages.[14] Ear surgery is usually performed under microscope and bleeding in this small area complicates the surgery. This problem may be solved with hypotensive anesthesia or by using some chemicals such as epinephrine. In certain cases, hypotensive anesthesia might be inconvenient and epinephrine may remain incapable, and bleeding might go on. In this kind of situations new chemicals would be needed.

There have been some recent case reports dealing with hemostatic effect of ABS, in the literature. Kurt et a[8]. reported a case underwent Billroth II surgery, with unstopped bleeding despite using hemoclips and epinephrine. They observed that bleeding had stopped in 2 seconds, following ABS administration. Uçar Albayrak et al[12]. reported case of an afibrinogenemic patient who had cut her finger with gross hemorrhage despite being sutured. After ABS implication; they

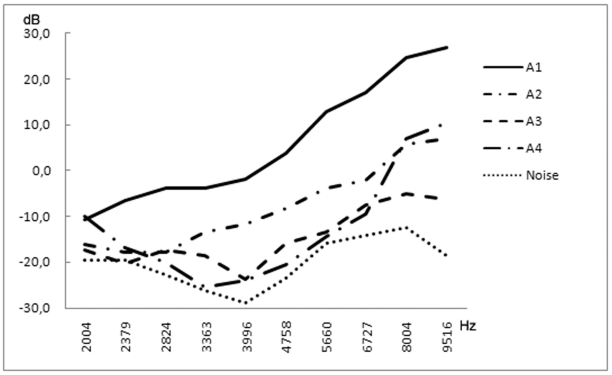

Figure 1. DPgrams measured from intratympanic ABS used group on days 0, 1, 7, and 40. 0 day measurements (A1); 1 day measurements (A2); 7 days measurements (A3); 40 days measurements (A4). The dotted line is noise level.

Figure 2. DPgrams measured from intraperitoneal ABS used group on days 0, 1, 7, and 40. 0 day measurements (P1); 1 day measurements (P2); 7 days measurements (P3); 40 days measurements (P4). The dotted line is noise level.

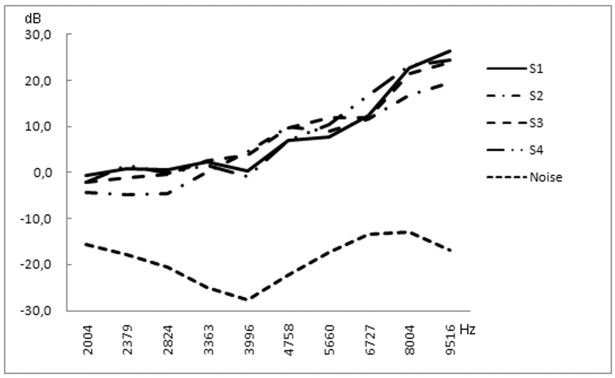

Figure 3. DPgrams measured from intratympanic saline used group on days 0, 1, 7, and 40. 0 day measurements (S1); 1 day measurements (S2); 7 days measurements (S3); 40 days measurements (S4). The dotted line is noise level.

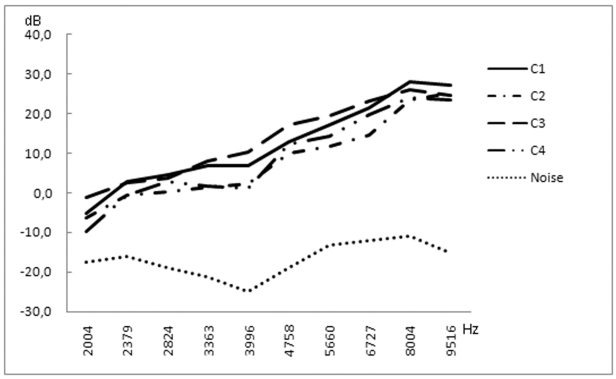

Figure 4. DPgrams measured from control group on days 0, 1, 7, and 40. 0 day measurements (C1); 1 day measurements (C2); 7 days measurements (C3); 40 days measurements (C4). The dotted line is noise level.

noted that hemorrhage was stopped successfully. Similarly, Kurt et al[9]. reported a case with uncontrolled bleeding from multiple areas including epistaxis which could only be controlled by ABS.In a recent study, Teker et al[13]. reported results of 47 consecutive patients underwent cold knife dissection tonsillectomy. They used ABS wet tampon in one side tonsil hemorrhage and knot-tie for the other side. They noted statistically significant differences in hemostasis time and blood loss, in favor of ABS used side. Apatient with hemophilia A,who presented with uncontrolled bleeding and treated successfully by using ABS was also reported.[11] Huri et a[5]. reported another patient with retropubic radical prostatectomy who was treated with ABS following the hemorrhages could not be managed with conventional procedures.

There have been experimental studies concerning administration ofthis agent. Karakaya et al[6]. reported that, ABS is as effective as regenerated oxidized cellulose (Surgicel) in achieving hemostasis following partial liver excision in an experimental rat model.Successful results of ABS were also reported by many authors.[2-4, 7, 10]

These successful results alerted us,as ABS might have practical value in controlling bleeding at ear, nose, and throat regionsuch asin otologic surgeries. But for the patient safety, the possible side effects,especially on hearingshould be investigated when it is applied topically or used systemically. Results of the current study have shown that intraperitoneal usage of the ABS has appeared to be safe in terms of hearing compared to the control group. Significant hearing loss was found in the intratympanic application group of the ABS compared to the control group in some frequencies especially in subacute phase. First day DPOAEs were seemed to be diminished in the intratympanic ABS group in the diagram, however they were not statistically significantly different compared to the control group (Figure 1). Within the 7th day, statistically significant results were found at frequencies of 5660 Hz, 6726 Hz, 8003 Hz, and 9515 Hz ($p<0.05$). The measurements made following 40 days were considered as a relatively long period and improvements at frequencies of 8003 and 9515 Hz were observed, while there were still statistically significant hearing lossremaining at 5660 and 6726 Hz. These results may suggest that the side effect on hearing of the ABS may have been acute and may ameliorate in a long period.

Based on Goker et al.'s study[1], it can be concluded that the hearing loss detected in current study may result from the mass effect of the ABS in the middle ear,which might be resolved partially in a period of time.

In conclusion; in the literature there are studiesregarding successful results of hemostatic effects of ABS including a case report of epistaxis.In this respect, other different fields of application such as otologic surgeryshould be considered. However, before systematic or topical use of this agent during otologic surgeries, one has to be sureas this particular agent has no side effects on hearing. This study was aimed to evaluate hearing result of intratympanic or intraperitoneal administration of ABS in rats. The obtained results showed intraperitoneal application of ABS has no side effect on hearing, while intratympanic usage inferred depriving effect on hearing, partially ameliorated after a longer period. These finding might be secondary to possible ototoxic side effect of ABS, prolonged mass effect of it, or because of inflammation. In our opinion, our study should trigger further studies with longer follow up period and histopathological examinations to elucidate these existing questions.

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Conflict of interest: none

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References

1. Goker H, Haznedaroglu IC, Ercetin S, Kirazli S, Akman U, Ozturk Y, et al. Haemostatic actions of the folkloric medicinal plant extract Ankaferd Blood Stopper. J Int Med Res2008; 36:163-170.

2. Bilgili H, Kosar, A, Kurt M, Onal IK, Goker H, Captug O, et al. Hemostatic efficacy of Ankaferd Blood Stopper in a swine bleeding model. Med Prenc Pract 2009; 18:165-169.

3. Cipil HS, Kosar A, Kaya A, Uz B, Haznedaroglu IC, Goker H, et al. In vivo hemostatic effect of the medicinal plant extract Ankaferd Blood Stopper in rats pretreated with warfarin. ClinApplThrombHemost 2009; 15:270-276.

4. Huri E, Akgul T, Ayyildiz A, Ustun H, Germiyanoglu C. Hemostatic role of a folkloric medicinal plant extract in a rat partial nephrectomy model: a controlled experimental trial. JUrol2009; 181:2349-2354.

5. Huri E, Akgul T, Ayyildiz A, Germiyanoglu C. Hemostasis in retropubic radical prostatectomy with Ankaferd Blood Stopper: a case report. Kaohsiung J Med Sci 2009; 25:445-447.

6. Karakaya K, Ucan HB, Tascilar O, Emre AU, Cakmak GK, Irkorucu O, et al. Evaluation of a new hemostatic agent Ankaferd Blood Stopper in experimental liver laceration. J InvestSurg2009; 22:201-206.

7. Kosar A, Cipil HS, Kaya A, Uz B, Haznedaroglu IC, Goker H, et al. The efficacy of Ankaferd Blood Stopper in antithrombotic drug-induced primary and secondary hemostatic abnormalities of a rat-bleeding model. Blood Coagul Fibrinolysis2009; 20:185-190.

8. Kurt M, Kacar S, Onal IK, Akdogan M, HaznedarogluIC. Ankaferd blood stopper as an effective adjunctive hemostatic agent for the management of life-threatening arterial bleeding of the digestive tract. Endoscopy2008; 40 Suppl 2: E262.

9. Kurt M, Oztas E, Kuran S, Onal IK, Kekilli M, Haznedaroglu IC. Tandem oral, rectal, and nasal administrations of Ankaferd Blood Stopper to control profuse bleeding leading to hemodynamic instability. Am J Emerg Med2009; 27:631. e1-2.

10. Kurt M, Akdogan M, Onal IK, Kekilli M, Arhan M, Shorbagi A, et al. Endoscopic topical application of Ankaferd Blood Stopper for neoplastic gastrointestinal bleeding: a retrospective analysis. Dig Liver Dis2009; Article in Press.

11. Oner A, Dogan M, Kaya A, Sal E, Bektas MS, Yesilmen O, et al.New coagulant agent (Ankaferd Blood Stopper) for open hemorrhages in hemophilia with inhibitor. Clin Appl Thromb Hemost 2009; Article in press.

12. Uçar Albayrak C, Caliskan U, Haznedaroglu IC, Goker H. Haemostatic actions of the folkloric medicinal plant extractAnkaferd Blood Stopper. J Int Med Res2008; 36:1447-1449.

13. 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, Article in press

14. Maniglia AJ. Fetal and major complications secondary to nasal and sinus surgery. Laryngoscope 1989; 99:276-283.