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*Original Article*

# Unpredicted effects of Ankaferd® on cartilage tissue

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**Abstract:** Objective: This study aims to investigate the histopathological changes secondary to the administration of Ankaferd Blood Stopper ® (ABS) into the auricular cartilage. Materials and methods: Both of the auricular cartilages of thirty New Zealand rabbits were marked with tattoo ink. A 0.2-cc ABS (study group, n: 30) and 0.2 cc physiological saline (control group, n: 30) were subcutaneously infused into the right auricle and left auricle, respectively. All layers were removed at 14 days. Results: The ABS group had significantly higher level of fibrosis, necrosis, foreign body reaction, inflammation, and cartilage degeneration, compared to the controls. Conclusion: Our study results showed that ABS administration into a closed cavity led to a significantly increased fibrosis and necrosis in the auricular cartilage.

**Keywords:** Ankaferd, hemostatic agent, cartilage

## Introduction

Bleeding is a common problem in head and neck injuries or during or after surgery due to the high level of vascularization of the anatomical site. The management can be complex. Bleeding is often managed by traditional surgical bleeding control. In addition, several hemostatic agents can be effectively used in the management of bleeding today [1-3].

Ankaferd Blood Stopper® (ABS; Ankaferd Drug Cosmetic Co., Istanbul, Turkey) has been traditionally used for hemostasis in Turkey [1]. It is a hemostatic agent composed of plant extracts including Urtica dioica (0.06 mg/ml), Vitis vinifera (0.08 mg/ml), Glycyrrhiza glabra (0.07 mg/ml), Alpinia officinarum (0.07 mg/ml), and Thymus vulgaris (0.05 mg/ml) [4-6].

In recent years, an *in vitro* study showed that addition of ABS to plasma did not affect the individual coagulation factors II, V, VII, VIII, IX, X, XI, and XIII [4-6]. The basic mechanism of action is the formation of an encapsulated protein network which provides focal points for vital erythrocyte aggregation. Ankaferd-induced protein network formation with blood cells, particularly erythrocytes, covers the primary and secondary hemostatic system [4-6].

The ideal topical hemostatic agent has been suggested to be easy to use and effective within minutes in both arterial and venous bleeding, as well as being non-toxic and anaphylactic [7]. In addition, there are many studies demonstrating a hemostatic effect of ABS in animal models and humans. Many studies have suggested that ABS is an ideal topical hemostatic agent [5, 6, 8-11].

Cartilage tissues such as auricle, septum, or larynx can be contacted in otorhinolaryngology surgery. In earlier studies, ABS was used in open cavities. In this study, we investigated the histopathological changes secondary to the administration of Ankaferd Blood Stopper® into the auricular cartilage.

## Materials and methods

We used the liquid form of ABS supplied in 2-mL ampules in this study. ABS was obtained from Trend Teknoloji Ilac AS, Istanbul, Turkey. The study was carried out in accordance with the European Community Council Directive of

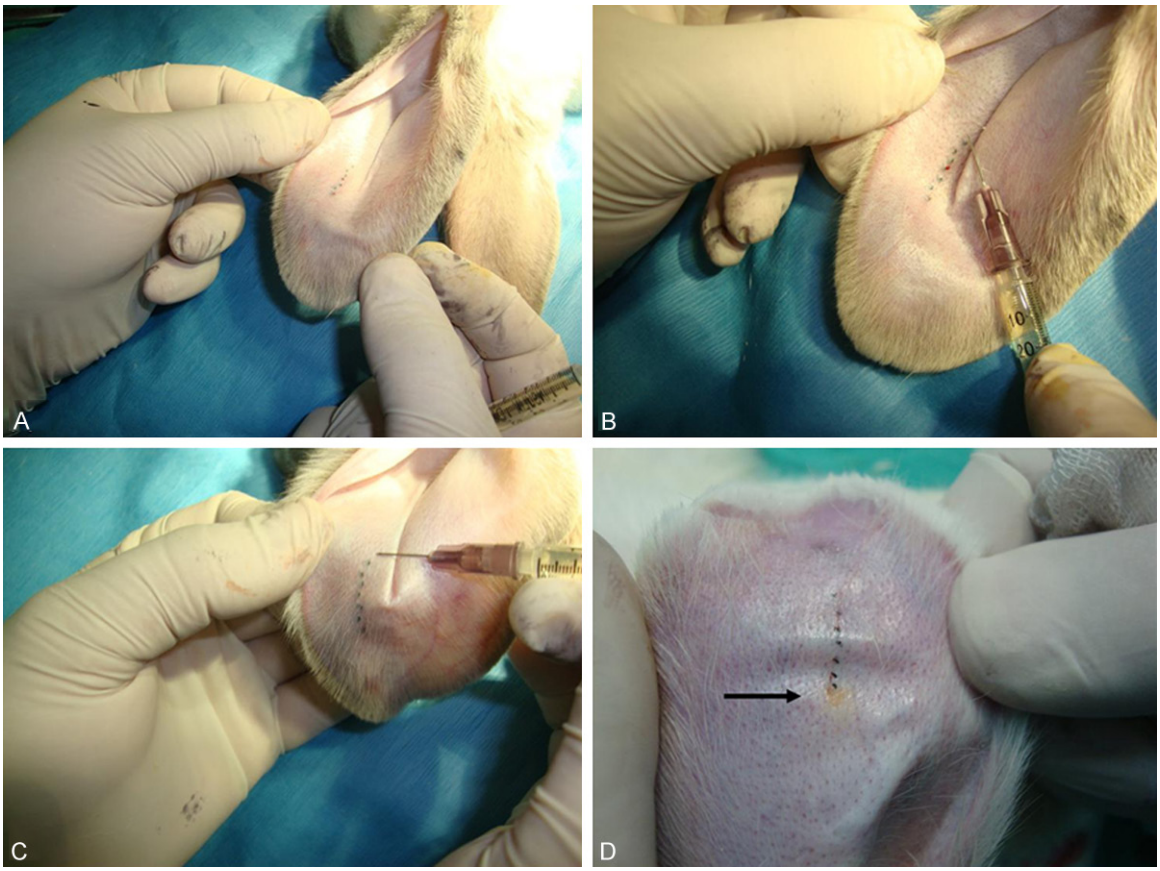


**Figure 1.** A. Tattooed area. B-D. ABS administration.

November 1986 (86/609/EEC). The study was approved by the Bulent Ecevit univecity, Faculty of Medicine, Animal Ethics Committee.

*Animals*

Thirty-two healthy adult female New Zealand rabbits obtained from a commercial vendor and acclimatized for several weeks were used in the study. The animals were kept at constant room temperature with a 12-hour light/dark cycle and had a normal diet consisting of fresh vegetables and free access to tap water. Two animals were lost during the experimental study.

*Surgical procedure*

All surgical interventions were performed under sterile conditions by the same team, at the same operating room. All animals were sedated with intramuscular 5 mg/kg xylocaine hydrochloride (Rompun®, Bayer, Istanbul, Turkey) and 10 mg/kg ketamine chloride (Ketalar, Eczacıbaşı, Istanbul, Turkey). Systemic antibiotherapy (ceftriaxone 5 mg/kg, intramuscularly) was administered one hour before surgery. Standard 1-mL insulin syringes with 27-gauge -0.5-in needles were used for injection. Following sedation, 1-cm area of both auricles was tattooed with Indian ink by using a 22-gauge needle (**Figure 1A**). After 0.2-cc ABS was subcutaneously injected forming a lump under the right auricle skin (ABS group, n: 30) (**Figure 1B-D**). The same procedure was performed in the left auricle with physiological saline (control group, n: 30). On Day 14, the cartilage tissue below the tattooed mark of both ears was incised sharply. A 1-$cm^2$ specimen from each auricle was removed for pathological examination under light microscopy (**Figure 2**). The auricular defect was primarily sutured using 4/0 prolene. The rabbits were then followed for postoperative complications. The wounds were observed throughout the study period by an investigator who was blinded to treatment. None had any hematoma, infection or other wound healing problems. The rabbits

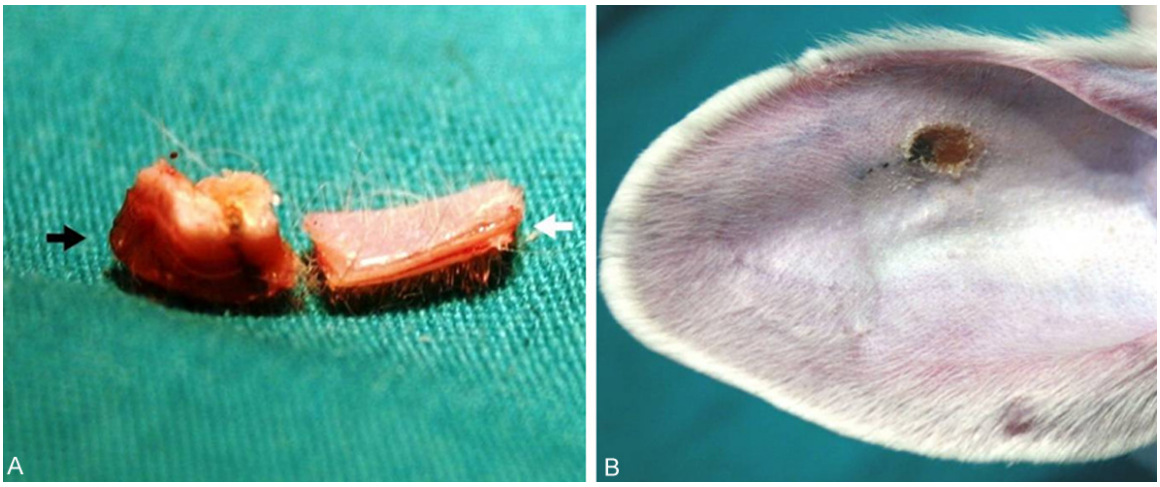


**Figure 2.** A. Black arrow represents the cartilage of the ABS group; white arrow represents the cartilage of the controls. B. Necrotic area.

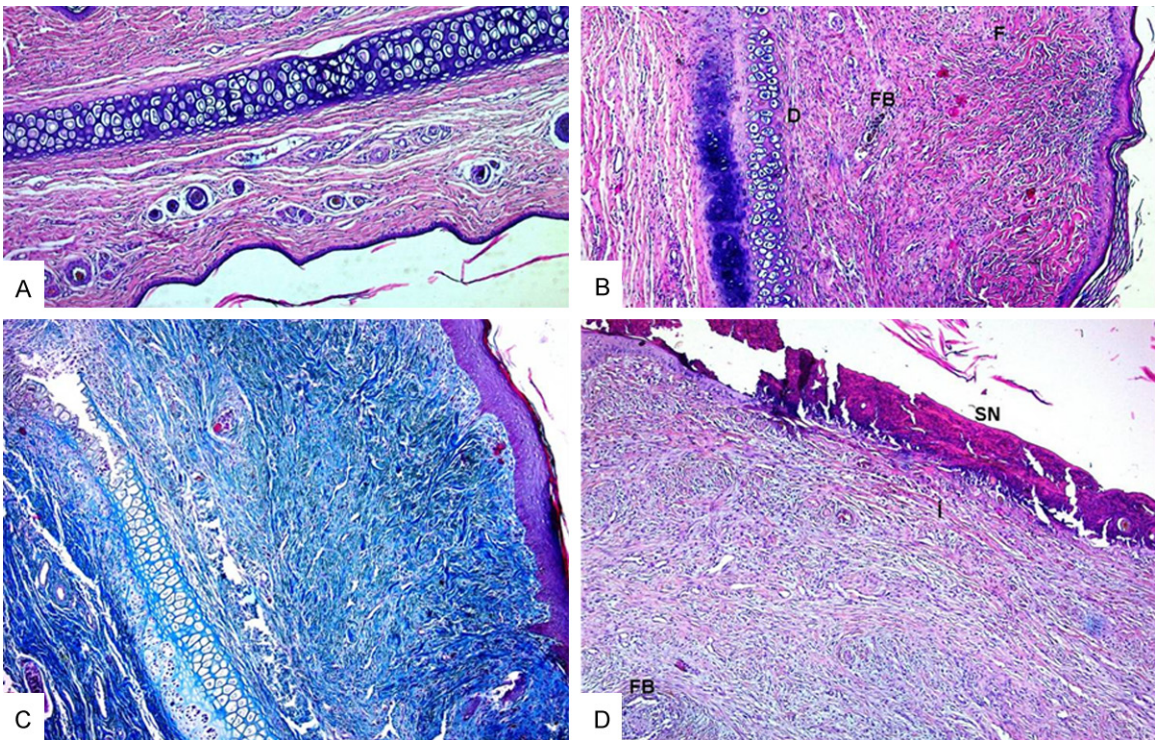


**Figure 3.** A. Normal auricle of the rabbits in the control group (H&E, × 200). B. Fibrosis (F), foreign body reaction (FB), and degeneration (D) of the auricular cartilage in the ABS group (H&E, × 100). C. Fibrous tissue of the auricle was stained with deep blue (Masson trichrome stain, × 100). D. Superficial necrosis (SN), foreign body reaction (FB) and inflammatory cell infiltration (İ) of the rabbit auricle in the ABS group (H&E, × 100).

were not euthanized at the end of the study period (on Day 14).

*Tissue preparation*

Gross examination of the auricles was performed. Initial tissue preparation included immediate fixation in 10% formaldehyde solution for 24 hours, followed by decalcification with 10% formic acid solution for 10 days. The tissue was then embedded into the paraffin wax blocks. Sectioning at 5 μm in the transverse plane enabled visualization of the entire sinus walls in cross-section. Light microscopy

**Table 1.** ABS and control group

| | ABS Group (n: 30) | Control Group (n: 30) | P |
|---|---|---|---|
| Foreign body giant cell reaction | 6 (20%) | 0 (0%) | <0.05 |
| Degeneration | 9 (30%) | 0 (0%) | <0.05 |
| Necrosis | 17 (56%) | 0 (0%) | <0.05 |
| Fibrosis | 20 (66%) | 0 (0%) | <0.05 |
| Inflammatory cell infiltration | 30 (100%) | 0 (0%) | <0.05 |

was performed after Hematoxylin and Eosin (H&E) staining. Sections were evaluated and systematically graded in a random order by a blinded, independent pathologist (the fourth author of this study). The following histologic features were examined: the presence of foreign body reaction, fibrosis, necrosis, degree of inflammation, and cartilage degeneration.

*Statistical analysis*

Statistical analysis was performed using Statistical Package for Social (SPSS) v13.0 (SPSS Inc., Chicago, IL, USA). Pearson's chi-square ($\chi^2$) test and Fisher's exact test were carried out to analyze data. A *p* value of <0.05 was considered statistically significant.

## Results

Gross examination of the specimens revealed a significant difference in the incidence of fibrosis even during the full-layer removal (**Figure 2**).

Histopathological examination showed fibrosis in 20 ears (66.6%) and 0 ears (0%) in the ABS group and controls, respectively. It indicated a statistically significant difference (P<0.05) (**Figure 3B-D**).

In addition, 17 ears (56.6%) had necrosis in the ABS group, while none of the ears (0%) had necrosis finding in the control group, indicating a statistically significant difference between the groups (P<0.05) (**Figure 3D**).

Furthermore, six ears (20%) had foreign body reaction in the ABS group, while none of the ears (0%) suffered from such a complication in the control group. There was a statistically significant difference between the groups (P<0.05). Additionally, 30 ears (100%) had inflammatory cell infiltration. However, none of the ears (0%) had such a reaction in the control group. Therefore, we observed a statistically significant difference between the groups (P<0.05) (**Figure 3B**, **3D**).

Nine ears (30%) had cartilage degeneration in the ABS group, whereas none of the ears (0%) had cartilage degeneration in the control group. Therefore, we observed a statistically significant difference between the groups (P<0.05) (**Table 1**).

## Discussion

Bleeding management has been well-established in many studies and various topical hemostatic agents are used in clinical practice [1-3]. The ideal topical hemostatic agent has been defined as easy-to-use and effective within minutes in both arterial and venous bleeding, as well as non-toxic and anaphylactic nature [7]. Ankaferd Blood Stopper® is a novel topical hemostatic agent which exerts a therapeutic potential in the bleeding management. The basic mechanism of action is the formation of an encapsulated protein network which provides focal points for vital erythrocyte aggregation [4-6].

Previous clinical and experimental studies have shown no significant observational or histopathological side effects of ABS. In a study investigating possible side effects of oral ABS in 12 rabbits at various doses, Bilgili et al. [12] performed biochemistry analysis at one and four days of administration during seven-day follow-up. The authors showed no mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, or biochemical toxicity after ABS administration. Therefore, they concluded that ABS produced no toxicity-related findings in the short-term. In addition, no histopathological deterioration secondary to ABS administration was observed using the partial nephrectomy, penile cavernosal, hepatic, and rat bladder tissues in several studies [13-17]. However, all aforementioned studies primarily investigated the ability to stop bleeding of ABS. Review of the literature showed limited data on the possible effects of ABS administration on the cartilage tissue. Thus, we used cartilage tissue, one of the main objects in otorhinolaryngology practice, in our study.

Based on the gross examination findings, we observed a significantly increased incidence of fibrosis and necrosis in the auricular cartilage following ABS administration. In addition, histopathological analysis showed a statistically significant difference in the rate of fibrosis, necro-

sis, foreign body reaction, and cartilage degeneration between the groups ($P<0.05$). This can be attributed to the fact that ABS remained in the cartilage tissue without draining for a long period of time.

Furthermore, Metin et al. [18] performed wedge-resection and administered ABS spray on the left lower lung lobes in equal sizes in a rabbit model. The control group received no treatment. The rabbits were sacrificed at eight days under high-dose anesthesia. Microscopic and gross examination of tissue specimens were performed for pleural inflammation and fibrosis in the hemithorax and increased fibrosis was observed.

Moreover, Alpay et al. [19] performed a histological analysis of ABS drops in ophthalmologic surgery in rats. The authors reported no inflammation, degeneration, foreign body giant cell reaction, or necrosis in the cornea or conjunctiva. In another study, these authors performed intraocular injection of ABS in rats [20]. Clinical and histological examinations demonstrated retinal rupture, mixed-type inflammatory cell infiltration and multi-nuclear giant cells with foreign bodies. These adverse events were associated with the ABS administration in closed cavities.

Another study was carried out in rats by Simsek et al. [21]. Femoral defects were created and 0.5 mL ABS was administered to the study group, while the control group received no treatment. The subjects were sacrificed at seven, 28, and 42 days following administration. The authors reported increased bone formation in the study group at seven days. At 28 and 42 days, similar results were achieved in both the study and control groups. Clinical observations showed no intraoperative or postoperative allergy or inflammatory reactions in the skin and other tissues. Histomorphometric studies revealed no necrotic area or infectious reactions.

In conclusion, to the best of our knowledge, this study is the first in which ABS was administered in the auricular cartilage. Our study results suggest that ABS has a toxic effect on auricular cartilage with increased fibrosis and necrosis. However, this can be attributed to the fact that ABS was administered in a closed cavity. Therefore, further clinical studies are required to establish the effects of ABS in closed cavities in humans, particularly.

## Disclosure of conflict of interest

None.

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