

Original Research Article

Effect of Ankaferd Blood Stopper on Early Bone Tissue Healing in Extraction Sockets: An Experimental *In vivo* Study

Hatice Balci Yuce¹, Nihat Akbulut^{2*}, Sibel Akbulut³, Kemal Özgür Demiralp⁴, Zafer Karaca⁵ and Kaan Orhan⁶

¹Department of Periodontology, ²Department of Oral and Maxillofacial Surgery, ³Department of Orthodontics, Faculty of Dentistry, Gaziosmanpaşa University, Tokat, ⁴Ministry of Health Public Hospitals Agency of Turkey, Ankara, ⁵Department of Histology, Faculty of Medicine, Gaziosmanpaşa University, Tokat, ⁶Dentomaxillofacial Radiology Department, Dentistry Faculty, Ankara University, Ankara, Turkey

*For correspondence: **Email:** drnihatakbulut@yahoo.com; **Tel:** +905054489263

Received: 19 January 2015

Revised accepted: 29 June 2015

Abstract

Purpose: To investigate the effect of Ankaferd blood stopper (ABS) on early bone healing of extraction sockets in rats.

Method: Twenty-eight male Wistar rats were divided into four groups. The maxillary right first molar tooth of the rats was extracted under general anesthesia. Two groups (C-1, n = 6 and C-2, n = 6) received saline solutions in the extraction sockets immediately and one day after the extraction, respectively, while two groups (A-1, n = 8 and A-2, n = 8) received ABS. The rats in A-1 and C-1 groups were sacrificed after 7 days of post-extraction while the rats in A-2 and C-2 groups were sacrificed after 28 days. Bone samples were taken from the maxillas, and tissues were prepared for histopathological analysis. Osteoid tissue (OT), mineralized bone tissue (MT), remaining area (RA), and inflammatory cell infiltration (ICI) were determined. The histomorphometric results were analyzed statistically using analysis of variance (ANOVA).

Results: Osteoid formation was highest in C-1 group (39.71 ± 9.68 , $p < 0.05$). Differences in OT among other groups were not significant ($p > 0.05$). MT was higher in C-2 group (47.73 ± 12.15) than in other groups ($p < 0.05$). RA was highest in C-1 group (59.95 ± 12.75). ICI was significantly lower in control (C1 and C-2) groups than in ABS (A-1 and A-2) groups ($p < 0.05$).

Conclusion: ABS administered topically to extraction sockets immediately after extraction has no effect on bone healing; in fact, ABS increases inflammation *in vivo*.

Keywords: Ankaferd blood stopper, Wound healing, Mineralized bone tissue, Inflammatory cell infiltration, Osteoid tissue, Tooth extraction

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Extraction socket healing involves the chronic and irreversible destruction of the alveolus and may also cause scar formation during soft-tissue healing [1,2]. To preserve the residual alveolar bone volume and shorten the wound healing

period, biological and synthetic materials capable of enhancing wound healing, inducing new bone formation, and reducing bone destruction are being used topically or systemically [3-5]. Commonly used as a blood stopper, Ankaferd blood stopper (ABS; Ankaferd Health Products Ltd., Istanbul, Turkey) is a traditional folk

medicinal plant extract product that has been approved in the management of external hemorrhage and dental surgery bleedings in Turkey [6].

ABS is a standardized herbal mixture of five plant extracts (*Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*) [6]. The mechanism of the hemostatic action for ABS is the formation of an encapsulated protein network for vital physiological erythrocyte aggregation. This protein network formation with blood cells covers the primary and secondary hemostatic systems without disturbing the individual coagulation factors [6]. The effect of ABS on cell proliferation is not yet well known, but Mihmanli *et al* [7] reported a proliferative effect on human leukocytes. Regarding cell proliferation, it is suggested that lectin protein of *U. dioica* stimulates human lymphocyte proliferation [8]. Contrary to these studies, there is evidence that ABS inhibits cell proliferation [9]. In addition, each of the components of ABS may have effects on endothelium, blood cells, vascular dynamics, mediators, and angiogenesis [10].

Recently, ABS was found to be effective on different groups of multidrug-resistant bacteria, for instance, *Enterococcus* spp, *Enterobacter* spp, and *E. coli* [11]. This effect may be useful in postoperative infection control and the wound-healing process in dentistry. Recently, it was reported that ABS could induce new bone formation and decrease inflammation in rat femur [12]. Along with the antimicrobial effect, inducing new bone formation may be beneficial in treating surgical wounds. The aim of this study was to investigate the effects of ABS on early bone healing after tooth extraction in a rat model.

EXPERIMENTAL

The study was conducted in the Faculty of Dentistry of Gaziosmanpaşa University Turkey. The study protocol and experimental design were approved by the Institutional Review board and Animal Ethics Committee of Gaziosmanpaşa University School of Medicine (no. 2012-168). The guidelines for the care and use of laboratory animals was followed in the handling of the rats [13].

Twenty-eight male Wistar rats, ranging in weight from 270 – 320 g, were used in this study. They were housed in specially designed wire cages and maintained on a 12 h –12 h light – dark cycle at a constant room temperature of 23 °C. Rats

were allowed access to water and standard rodent diet *ad libitum*. All procedures were performed by the same specialist.

The animals were randomly divided into four groups as follows:

1. Control 1 group (C-1 group, n = 6), the rats were sacrificed on the 7th day of the study period.
2. Control 2 group (C-2 group, n = 6), the rats were sacrificed on the 28th day of the study period.
3. Ankaferd 1 group (A-1 group, n = 8), the rats were sacrificed on the 7th day of the study period.
4. Ankaferd 2 group (C-2 group, n = 8), the rats were sacrificed on the 28th day of the study period.

The maxillary right first molars were extracted under general anesthesia (Ketamine, Eczacıbaşı İlaç Sanayi, İstanbul, Turkey) using dental instruments. The extraction comprised of fibrotomy, luxation, and tooth removal. The surgical wounds were left for secondary healing. 1 ml of ABS (Ankaferd Blood Stopper, Ankaferd İlaç Sanayi Kozmetik., İstanbul, Turkey) which is a herbal mixture of five plant extracts (*Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*) was applied to each empty tooth socket by spraying immediately after extraction and one day after extraction. A total of 2 ml of ABS was applied to each of the rats. The control groups (C1, C2) were administered an equivalent volume of saline spray.

Histomorphometric analyses

After euthanasia with an overdose of pentothal sodium (Abbott Laboratories, North Chicago, Illinois, United States), histological analysis was performed by a single examiner who was masked to the identity of samples. The maxilla was fixed in 10 % formalin and demineralized in 10 % formic acid. The specimens were then dehydrated, embedded in paraffin, and sectioned along the molars in an apico-coronal plane for hematoxylin-eosin and modified Masson trichrome staining. Sections of 6-µm thickness, corresponding to the area of the first molars were evaluated using light microscopy (Leica DM 2500, Leica, Wetzlar, Germany).

Using an image analysis system (QImaging, Surrey, BC, Canada) the following parameters

were histomorphometrically evaluated in the molar socket by a blinded examiner: osteoid tissue (OT), remaining area (RA; part of the socket not filled with osteoid tissue or mineralized bone tissue), mineralized tissue (MT), and inflammatory cell infiltration (ICI). All parameters were measured, and the percentage of each parameter to the total socket area was calculated (Figure 1).

Statistical analysis

Data are presented as percentages and were analyzed with SPSS v.15.0. ANOVA followed by Tukey test for pairwise comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

No complications occurred during the study period. The control and test groups showed

similar healing pattern. The data are shown in Table 1.

Osteoid formation was highest in the C-1 group ($p < 0.05$). The differences in OT among other groups were not statistically significant ($p > 0.05$). The MT was higher in the C-2 group than those of the other groups ($p < 0.05$). The differences in MT among other groups were not statistically significant ($p > 0.05$). RA was highest in the C-1 and A-1 groups. The differences between C-1 and A-1 groups were not statistically significant as were C-2 and A-2 ($p > 0.05$). In the control groups, no inflammation was observed in the extraction sites. In the ABS groups, it was observed that ICI primarily consisted of neutrophils, macrophages, plasma cells, and T cells. ICI was significantly lower in the control (C1 and C-2) groups than the ABS (A-1 and A-2) groups ($p < 0.05$), but there was no significant difference between the A-1 and A-2 groups ($p > 0.05$).

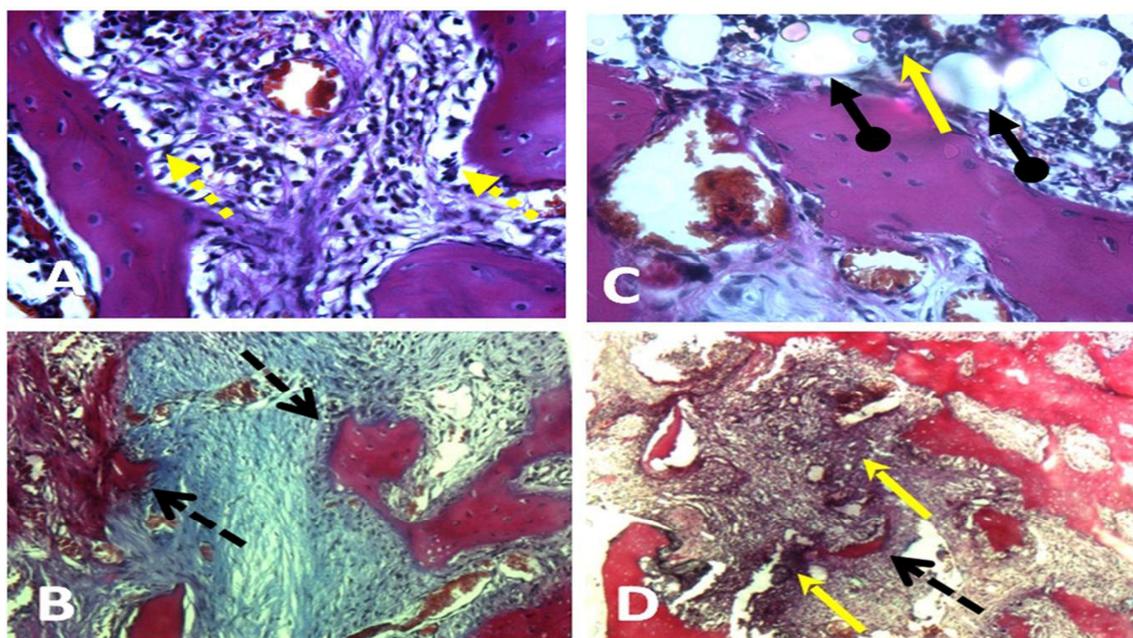


Figure 1: A: A representative image of C-1 group (x100). Dotted yellow arrows point to osteoblast cells. B: A representative image of the C-2 group (x100). Interrupted black arrows point to bone islands, which represent new bone formation in the socket. C: A representative image of the A-1 group (x100). Straight yellow arrow points to inflammatory cells and black arrows with round end point to adipose tissue cells. D: A representative image of the A-2 group (x40). Straight yellow arrows point to inflammatory cells, and interrupted black arrow points to bone island

Table 1: Histological findings for the study groups

Parameter	C-1C-1C-1	C-C-22C-2	A-A-11A-1	AA-2-2A-2
Osteoid	39.71 ± 9.68 ^{a,b}	28.8 ± 10.03 ^b	17.96 ± 10.82	9.05 ± 7.12
Remaining area	59.95 ± 12.75 ^{a,b,c}	13.33 ± 10.32	27.98 ± 11.14	22.28 ± 8.74
Mineralized tissue	3.33 ± 8.16 ^{b,c}	47.73 ± 12.15 ^a	7.56 ± 8.32	21.41 ± 14.9
Inflammatory infiltration	0 ^{a,b}	0 ^{a,b}	43.1 ± 8n93	46.65 ± 9.67

^a $P < 0.05$ vs A-1 group, ^b $p < 0.05$ vs A-2 group, ^c $p < 0.05$ vs C-2 group

DISCUSSION

Tooth extraction is a traumatic dental procedure that may jeopardize the alveolar bone and surrounding soft-tissue healing [1,2]. Following tooth extraction, the healing process of the bone begins with blood coagulum filling the socket, and after 7 days of healing, it is replaced with granulation tissue. At 1 month, woven bone fills the socket and ultimately remodels and restores the defect [1,2]. For this reason, we chose the study duration of 7 and 28 days.

There are many factors that can enhance or delay this process of bone healing [14,15]. These factors include “type of tissue, location and condition of wound, its vascular supply, microbial situation, and local and systemic factors.” To achieve optimal healing of the hard and soft tissues, shorten the healing period, and induce bone repair and regeneration, antibiotics and antimicrobial agents [16,17], antioxidants [5], drugs [4,18] (mood-stabilizing drugs, NSAID, etc.), and traditional medicines [3,12] have been used.

Ankaferd blood stopper is a unique standardized herbal mixture being used as a hemostatic agent in Turkey for many years. The exact mechanism of ABS is yet unknown, but there is a growing body of evidence on the efficacy of ABS on homeostasis. ABS is a strong antimicrobial agent especially affecting multidrug-resistant bacterial species [11]. This particular feature of ABS may be useful in infection control of bone healing. Isler et al [12] reported a positive effect of ABS on bone formation. However, recently, it was found that ABS had no effect on bone healing or oxidative stress in femoral fracture healing [19].

We observed that within the first week of healing, osteoid tissue occurred and, after one month, was replaced with mineralized bone tissue; this is compatible with the literature [1,2]. In ABS groups, the osteoid formation was significantly decreased, but there was no difference regarding the mineralized tissue. Supporting the findings of Amanvermez et al [19], our results show that ABS had no effect on bone healing in extraction sockets. However, ABS increased the inflammatory infiltration, which primarily consisted of neutrophils, macrophages, plasma cells, and T cells. Furthermore, there was a slight decrease in mineralized tissue in ABS-treated rats. Interestingly, we observed an adipocyte cell population in the slides. There is no study reporting the relation between adipocytes and ABS. Adipose tissue and bone tissue differentiate from the same mesenchymal cell

line. ABS may influence the differentiation pattern in favor of the adipose tissue.

The effects of ABS on bone tissue are controversial, but it is quite a possibility that ABS may have cytotoxic effects on various tissues. Alpay et al [20] found that ABS caused irritation on the cornea and conjunctiva. Similarly, Odabas et al [9] reported the cytotoxic effect of ABS on human fibroblast cells, and Mihmanli et al [7] reported a dose-dependent cytotoxicity of ABS on human lymphocytes.

CONCLUSION

Enhancing surgical wound healing and bone regeneration is the primary goal of many studies in dentistry. This study did not find any positive effect of ABS on bone tissue histologically. On the contrary, ABS increases inflammation. The bone is a unique tissue, and ABS is a strong blood stopper. Its use should be restricted to life-threatening conditions or serious complications until the efficacy of ABS on bone is well documented.

REFERENCES

1. Amler MH. The time sequence of tissue regeneration in human extraction wounds. *Oral Surg Oral Med Oral Pathol* 1969; 27: 309-318.
2. Araujo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* 2005; 32: 212-218.
3. Boonyagul S, Banlunara W, Sangvanich P, Thunyakitpisal P. Effect of acemannan, an extracted polysaccharide from aloe vera, on BMSCs proliferation, differentiation, extracellular matrix synthesis, mineralization, and bone formation in a tooth extraction model. *Odontology* 2005; 102: 310-317.
4. Fracon RN, Teofilo JM, Moris IC, Lamano T. Treatment with paracetamol, ketorolac or etoricoxib did not hinder alveolar bone healing: a histometric study in rats. *J Appl Oral Sci* 2010; 18: 630-634.
5. Magro Filho O, de Carvalho AC. Application of propolis to dental sockets and skin wounds. *J Nihon Univ Sch Dent* 1990; 32: 4-13.
6. Goker H, Haznedaroglu IC, Ercetin S, Kirazli S, Akman U, Ozturk Y, Firat HC. Haemostatic actions of the folkloric medicinal plant extract Ankaferd Blood Stopper. *J Int Med Res* 2008; 36: 163-170.
7. Mihmanli A, Ulker Z, Alpsoy L, Ezirganli S. Evaluation of cytotoxicity of a new hemostatic agent Ankaferd Blood Stopper (R) using different assays. *Hum Exp Toxicol* 2012; 31: 780-787.

8. Wagner H, Willer F, Kreher B. Biologically active compounds from the aqueous extract of *Urtica dioica*. *Planta medica* 1989; 55: 452-454.
9. Odabas ME, Erturk M, Cinar C, Tuzuner T, Tulunoglu O. Cytotoxicity of a new hemostatic agent on human pulp fibroblasts in vitro. *Medicina oral, patologia oral y cirugia bucal* 2011; 16: e584-587.
10. Sheela ML, Ramakrishna MK, Salimath BP. Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by *Glycyrrhiza glabra*. *Int Immunopharmacol* 2006; 6: 494-498.
11. Tasdelen Fisgin N, Tanriverdi Cayci Y, Coban AY, Ozatli D, Tanyel E, Durupinar B, Tulek N. Antimicrobial activity of plant extract Ankaferd Blood Stopper. *Fitoterapia* 2009; 80: 48-50.
12. Isler SC, Demircan S, Cakarer S, Cebi Z, Keskin C, Soluk M, Yüzbaşıoğlu E. Effects of folk medicinal plant extract Ankaferd Blood Stopper on early bone healing. *J Appl Oral Sci* 2010; 18: 409-414.
13. Garber JC. *Guide for the care and use of laboratory animals*. Washington: 2011. 246 p.
14. Hsieh YD, Devlin H, Roberts C. Early alveolar ridge osteogenesis following tooth extraction in the rat. *Arch Oral Biol* 1994; 39: 425-428.
15. Devlin H, Sloan P. Early bone healing events in the human extraction socket. *Int J Oral Maxillofac Surg* 2002; 31: 641-645.
16. Antoniadis K, Maggoudi D. The use of prophylactic antibiotics in oral and maxillofacial surgery. *Hell Period Stomat Gnathopathoprosopike Cheir* 1990; 5:53-58.
17. Vezeau PJ. Dental extraction wound management: medicating postextraction sockets. *J Oral Maxillofac Surg* 2000; 58: 531-537.
18. Zeng YT, Fu B, Tang GH, Zhang L, Qian YF. Effects of lithium on extraction socket healing in rats assessed with micro-computed tomography. *Acta Odontol Scand* 2013, 71: 1335-1340.
19. Amanvermez R, Gunay M, Piskin A, Keles G, Tomak L. TNF-alpha, IL-1beta, and oxidative stress during fracture healing with or without ankaferd. *Bratisl Lek Listy* 2013; 114: 621-624.
20. Alpay A, Evren C, Bektas S, Ugurbas SC, Ugurbas SH, Cinar F. Effects of the folk medicinal plant extract Ankaferd Blood Stopper(R) on the ocular surface. *Cutan Ocul Toxicol* 2011; 30: 280-285.