

# Treatment of injuries in javelin horses with propolis

## Tratamiento de lesiones en caballos lanzadores de jabalina con propóleo

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### ABSTRACT

Debridement and suturing of deep and infected wounds in horses requires sedation and anesthesia due to the limitations of horses. For this reason, it is preferred to perform wound treatment and care with more practical methods. The aim of this study is to evaluate wound healing in 18 horses by utilizing the antimicrobial, anti-inflammatory and local anesthetic effects of propolis. Various wounds resulting from trauma on 18 male, Arabian and British horses aged between 3-8 at Malatya Equestrian Javelin Club were treated by applying liquid propolis in spray form for 5 days. Wound size was measured with a ruler on days 1, 3, 5, 9, 13, 15 and 21 after the application. All wounds started to heal on the 3<sup>rd</sup> day after the application, graze wounds healed on the 9<sup>th</sup> day, and deep wounds healed on the 15<sup>th</sup> day. It was concluded that wound treatment with spray propolis in horses can be preferred because it is easy to apply, economical, creates biofilm on the wound and provides healing in a short time.

**Key words:** Horse; javelin; wound; propolis

### RESUMEN

El desbridamiento y la sutura de heridas profundas e infectadas en caballos requieren de sedación y anestesia, debido a las limitaciones de los caballos. Por esta razón, se prefiere realizar el tratamiento y el cuidado de las heridas con métodos más prácticos. El objetivo de este estudio es evaluar la cicatrización de heridas en 18 caballos utilizando los efectos antimicrobianos, antiinflamatorios y anestésicos locales del propóleo. Se trataron varias heridas resultantes de traumatismos en 18 caballos machos, árabes y británicos de entre 3 y 8 años en el Malatya Equestrian Javelin Club, aplicando para ello, propóleo líquido en forma de aerosol durante 5 días. El tamaño de la herida se midió con una regla los días 1, 3, 5, 9, 13, 15 y 21 después de la aplicación. Todas las heridas comenzaron a sanar el tercer día después de la aplicación, las heridas por raspaduras sanaron el noveno día y las heridas profundas sanaron el decimoquinto día. Se concluyó que el tratamiento de heridas con propóleo en aerosol en caballos puede ser preferible porque es fácil de aplicar, económico, crea biopelícula sobre la herida y proporciona curación en poco tiempo.

**Palabras clave:** Caballo; javelin; herida; propóleo

## INTRODUCTION

Wound is the disruption of soft tissue integrity as a result of surgical, traumatic, pathological, or ischemic events [1, 2]. Wounds, and can occur acutely and chronically [2]. The main purpose of wound care and treatment is to ensure rapid and complete healing, protect the wound from a moist environment and prevent infection. Propolis is a natural substance with antibacterial, anti-inflammatory, antiviral, antifungal, antiulcer, antitumoral, antiseptic, antihepatotoxic, antioxidant, and local anesthetic effects and has been used in traditional medicine for the treatment of various diseases since ancient times [3, 4, 5].

The antibacterial effect of propolis was observed especially on gram (+) cocci and gram (-) bacilli [6]. Owing to these properties, propolis has been used for the treatment of wounds in animals. The removal of foreign bodies and necrotic tissues in wound positively affects wound healing [7].

Skin wounds healing is a complex and well-organized process that involves a series of events, such as inflammation, re-epithelialization and regeneration of the skin, wound shrinkage, and remodeling in living tissue [8, 9].

Wound healing occurs in stages, such as acute inflammation, proliferation, and remodeling. These stages are generally difficult to observe externally. In practice, by observing macroscopic findings, wound healing consists of the stages of inflammation, granulation tissue formation, wound contraction and epithelialization [10].

Wounds in horses (*Equus caballus*), particularly in the distal parts of their legs, may heal slowly. Problems arise due to factors such as the complexity of wound tissues, long healing time of wounds, economic problems, and animal welfare. Many factors delay wound healing in horses. The most common of these is infection [11, 12].

Skin wounds in horses often heal “secondary” and a number of serious complications can develop [13]. Healing of wounds in equine distal limbs can often be impaired, resulting in exuberant granulation tissue and associated aesthetic changes and functional failures [14].

The effect of propolis on the healing process is still a matter of debate and is generally associated with the antimicrobial properties of propolis, free radicals, and stimulation of metabolism, rather than direct tissue regeneration [9].

In wound treatment in horses, the aim is to restore normal function and ensure that it is aesthetically correct [15]. Debridement and suturing applications in horses, especially deep and infected wounds, are very difficult due to the restraint of horses. In such cases, sedation and anesthesia are required. For this reason, it is preferable to perform wound treatment and care with more practical methods. The aim of this study was to use propolis to treat various wounds of 18 horses injured during the traditional equestrian javelin game, by taking advantage of the antimicrobial, anti-inflammatory, and local anesthetic effects of propolis.

## MATERIALS AND METHODS

Eighteen injured Arabian and British horses between the ages of 3-8 from the Malatya Equestrian Javelin Club were used. Of

the horses that were physically examined, open wounds were detected in seven chronic septic wounds in four, chronic wounds in two, graze wounds in two, chronic and graze wounds in one, sharp object wounds in one and open wounds and lacerations in one horse. The breed, age, gender, injury type and region of the javelin horses used in the study are presented in TABLE I.

**TABLE I**  
Race, age, gender, type of injury and region of javelin horses used in the study

Case	Name	Race	Age	Sex	Wound Shape	Wound area
1	Sert dadaş	British horse	3	Male	Open wound	Abdominal and Thoracic region
2	Meriç	Arabian horse	4	Male	Chronic (Septic) open wound	Cidago, Gluteal, Right and left front legs, Right hind leg
3	Baron	Arabian horse	4	Male	Graze wound	Thoracic region
4	Turbo	Arabian horse	4	Male	Graze wound	Cervical region
5	Şakra	Arabian horse	6	Female	Puncture wound	Buccal region
6	Gölge	Arabian horse	4	Male	Chronic wound	Nape
7	Demirkan	British horse	6	Male	Chronic wound, Graze wound	Left front leg, Right front leg
8	Bulut	Arabian horse	4	Male	Open wound	Gluteal region
9	Aşkar	Arabian horse	5	Male	Open wound (Multiple)	Back
10	Atabey	Arabian horse	4	Male	Open wound	Left back and abdominal region, Right shoulder region
11	Aliş	Arabian horse	4	Male	Open wound	Shoulder and spine region
12	Gökbörü	Arabian horse	4	Male	Chronic (Septic) open wound	Right rear Art. phalangea
13	Karakır	Arabian horse	8	Male	Chronic (Septic) open wound	Left lumbar region
14	Aşil	Arabian horse	5	Male	Chronic (Septic) open wound	Left ischii
15	Sadullah	Arabian horse	5	Male	Chronic wound	Abdominal and Thoracic region
16	Poyraz	Arabian horse	5	Male	Open wound	Prepusyum
17	Rüzgar	British horse	7	Male	Open wound, Laceration wound	Left Art. Tarsi, Right Corium coronarium, Metatarsal region
18	Efe	Arabian horse	7	Male	Open wound	Left front Art. interphalangea

## Propolis extract

Propolis extract was purchased from Haşerna Apitherapy Products (Amasya, Türkiye). The total phenolic content of the propolis samples was determined according to the Folin–Ciocalteu method [16, 17]. Gallic acid was used as the standard. The results are expressed as mg gallic acid equivalent/g sample.

The total flavonoid content of the propolis samples was determined as described by Fukumoto and Mazza [18]. Quercetin was used as the standard. The results are expressed as quercetin equivalents (QE) per gram of sample. The antioxidant capacity of the samples was measured using a ferric reducing antioxidant power (FRAP)

assay. The main chemical composition of the propolis extract was determined by gas chromatography (Agilent, USA) coupled with mass spectrometry (Thermo fisher, USA). The extracts were derivatized using N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA).

Biopsy samples were taken from chronic septic wounds to which propolis was applied on the 15<sup>th</sup> days and sent to the pathology laboratory of The Malatya Training and Research Hospital. The biopsy samples were fixed with 10% buffered formaldehyde. After routine tissue monitoring, the tissues were embedded in paraffin blocks. 4–5 µm thick) were obtained from paraffin blocks with a grinding slide. Routine hematoxylin–eosin and Masson's trichrome staining was applied to the sections, examined under a light microscope (Olympus DP26, Germany) and microphotographs were taken. It was examined by hematoxylin–eosin and Masson's trichrome staining.

## RESULTS AND DISCUSSIONS

Various wounds resulting from trauma in 18 male, Arabian, and British horses were treated by spraying liquid propolis simultaneously for 5 days, depending on the size and depth of the wound (FIG. 1).



FIGURE 1. Application of propolis by spraying in a horse injuries

On the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 9<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>, and 21<sup>st</sup> days (d) after application, the size of the surface area of the wounds was measured using the linear measurement technique with a ruler (surface area = length × width × depth) (FIGS. 2 A and B). It is important to determine the depth of deep wounds, and this evaluation provides information on the severity of tissue damage [19].

A significant decrease in wound size and healthy scar formation were observed. All wounds started to heal on the 3<sup>rd</sup> d after the application, abrasion wounds healed on the 9<sup>th</sup> d, and deep wounds healed on the 15<sup>th</sup> d. It was observed that a 4-year-old Arabian horse, which had chronically infected deep wounds in various parts of its body, started to heal on the 3<sup>rd</sup> d after propolis was

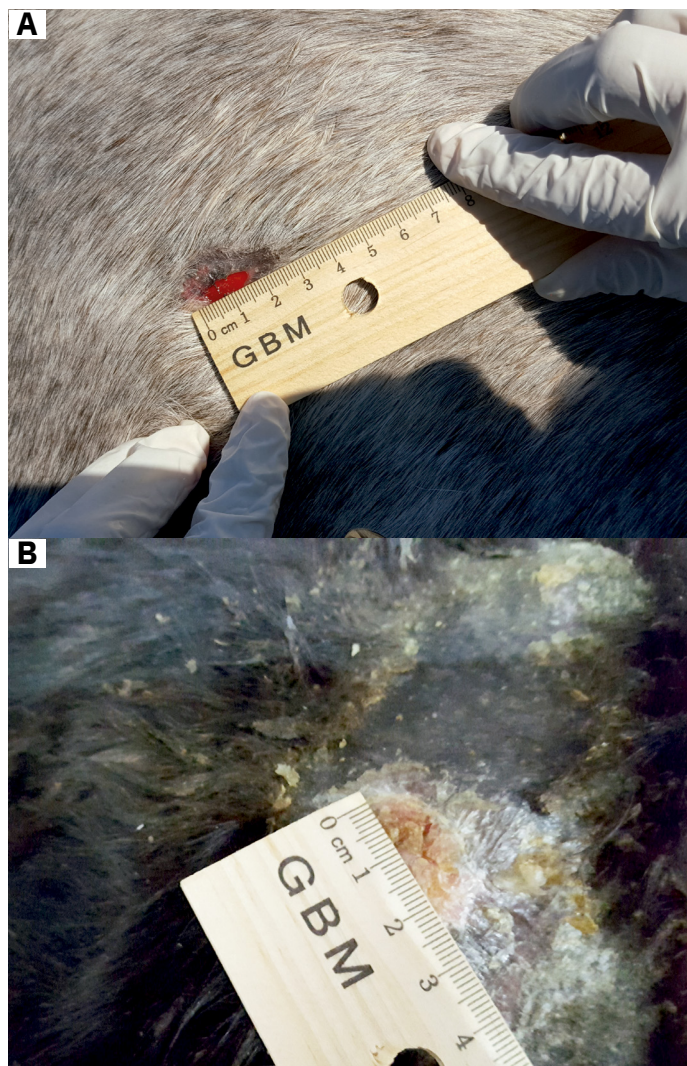


FIGURE 2. Measuring wound sizes with a ruler A and B, respectively. In a horse javelin

applied, its wounds were completely closed on the 15<sup>th</sup> d, and it was completely healed on the 21<sup>st</sup> d without leaving any scars.

## Histopathological findings

The Regeneration and reorganization of the stratified squamous epithelium was completed. Hyperkeratotic and focal parakeratotic areas were observed in most areas of the stratum corneum (FIG. 3A). In addition to the increase in fibroblastic activity in the dermis, it has not completed its development in the form of partial fissures; Neovascularization, partly consisting of capillaries that have completed their development, was observed. Hyperplastic changes were observed in some hair follicles and skin appendages (FIG. 3B). The inflammatory response in the dermis was mild. In some areas, small aggregates consisting of mononuclear cells with a multifocal distribution were observed (FIG. 3C). Regular collagen bundles were observed in the deep dermis (FIG. 3D).

Most soft tissue injuries in horses are wounds resulting from trauma [10]. Almost all our cases consisted of traumatic wounds, which is consistent with the literature. Caring for horse wounds



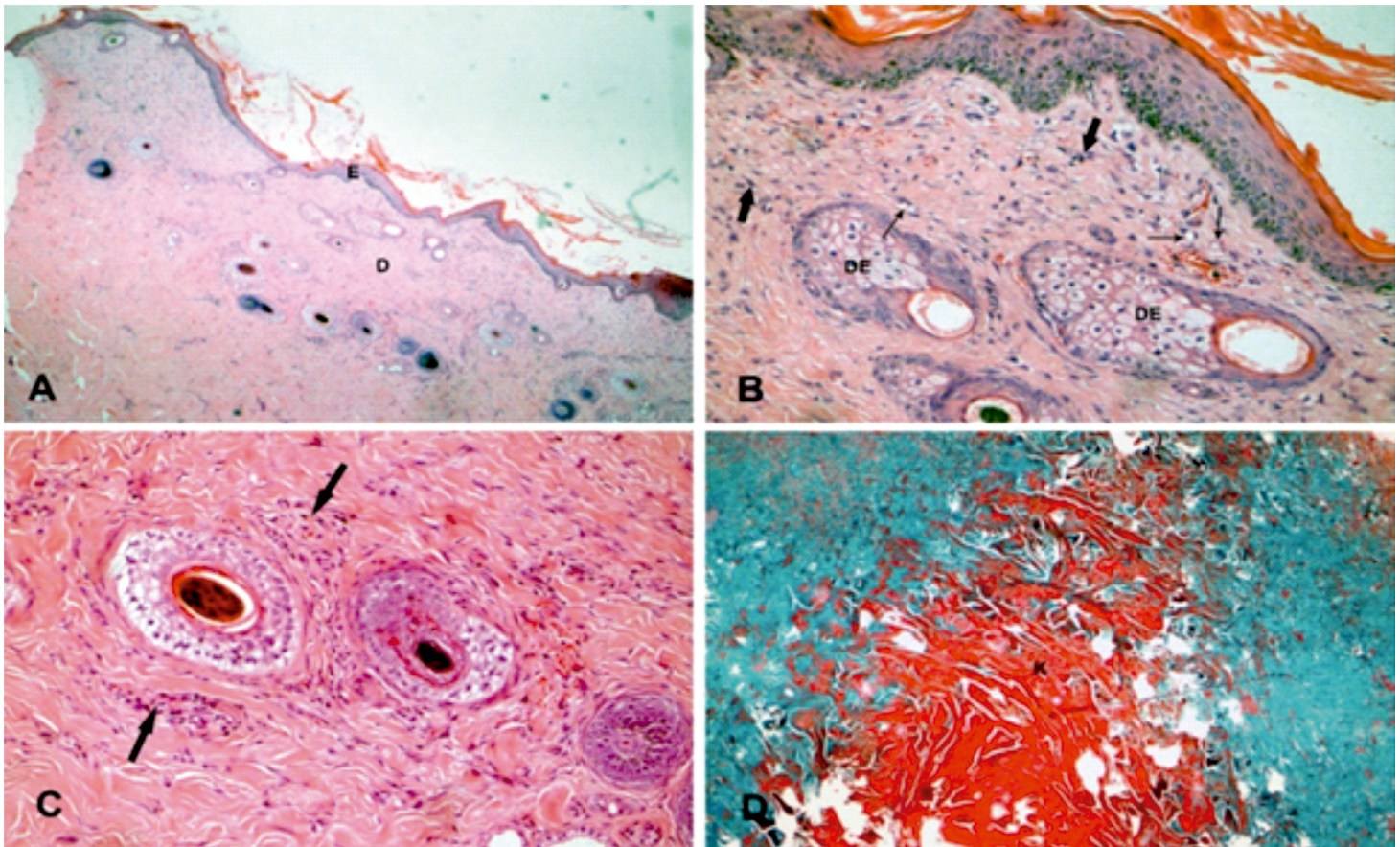


FIGURE 3. Stratified squamous epithelium A) General view of hyperkeratotic and parakeratotic changes in the stratified squamous epithelium. (D: Dermis, E: Epidermis), H&E, cell infiltrates (arrows), H&E, 200 $\times$ . D) Thick collagen bands in the dermis (K), Masson's trichrom, 100 $\times$

in the field is challenging. Often, wound care is complicated by chronicity or improper prior care, in addition to the large degree of tissue trauma that occurs when a horse is injured. Recognition of the involvement of synovial structures, skin loss, and bone damage is critical in the initial examination of wounds [20].

There are many options for wound cleaning, and it is important to avoid further trauma while treating the wound. Therefore, in all cases, physiological saline was used for wound cleaning, which, like other antiseptics, kills bacteria but does not kill healthy cells. The wounds of young horses heal more quickly than those of old horses [10]. Consistent with the literature, the wounds of 13 young horses healed faster.

In clinics, linear measurements are commonly performed using a wound ruler [21]. In this study, wound size measurements were performed directly with a ruler, in accordance with the literature.

Kolaylı *et al.* [22] reported that the total phenolic content of commercially obtained propolis samples varied between  $10.48 \pm 0.12$  and  $41.89 \pm 1.30$  mg GAE·mL<sup>-1</sup>, and the total flavanid content varied between  $1.24 \pm 0.03$  and  $5.77 \pm 0.32$  mg QE·mL<sup>-1</sup>. Keskin and Kolaylı [3], reported that the total phenolic content of different propolis samples varied between  $11.478 \pm 0.03$  and  $75.765 \pm 0.08$  mg·mL<sup>-1</sup>. Oruc *et al.* [23] conducted studies on the standardization of propolis. Bakchiche *et al.* [24] reported that

the amount of cinnamic acid varies by 0.01-1.85 mg per gram of phenolic content. They found that propolis extract contained compounds such as 2-propanone, ferulic acid, butanedioic acid, cinnamic acid and their derivatives. Algerian chemical characterization and antioxidant activity were determined. They reported the highest amount of phenolic content, 2385 mg of gallic acid in every 100 g sample, and the highest level of flavonoid, 379 mg in every 100 g sample. They reported that the antioxidant activity of the propolis samples was  $0.0015 \pm 0.0002$ . Can *et al.* [25] reported the antioxidant activity of propolis samples collected from Azerbaijan to be between 170 and 438  $\mu$ M Trolox·g<sup>-1</sup> and Temizer *et al.* [26] reported that the FRAP value of propolis samples collected from Turkey was  $90.73 \pm 0.24\%$ .

A study was conducted to determine the in vitro antiviral activity of propolis and *Baccharis* sp. on bovine, equine and porcine herpesviruses. In this study, red, green, and jataí propolis obtained from two different bee species (*Apis mellifera* and *Tetragonisca angustula*) were used [27]. *Baccharis* extracts were obtained from four different species (*B. oblongifolia*, *B. burchellii*, *B. dracunculifolia* and *B. uncinella*). While red propolis inhibited three animal herpesviruses, only the extract of the female sample of *B. oblongifolia* inhibited equine herpesvirus.

Healing and protecting wounds in horses is difficult. These challenges include the inability to immobilize sick horses and

providing a clean environment during the critical early stages of recovery. Because of these challenges, equine handlers rely heavily on bandaging and external coaptation techniques to successfully treat and manage wounds. The type of bandage used was determined by the injured body part [28]. In this study, applying propolis as a spray caused the animals to be restrained and there was no need to apply a bandage because it created a protective layer on the applied surface.

It is important to determine the wound depth in deep wounds and this evaluation provides information about the severity of tissue damage [19]. The chemical activity of propolis is affected by factors such as climatic conditions, geographical location, botanical origin, genetic factors of the bee, physical and chemical properties, and the harvest period in which it is harvested [5, 29]. Propolis can be classified according to its geographical and botanical origin, climatic conditions, genetic factors of the bee, and physical and chemical properties [29].

In a study conducted with Mexican propolis, five horses with dermatomycosis were used while the first two cases were treated with ketoconazole ointment, three cases were bathed weekly with propolis-based shampoo, and a significant reduction in lesions was observed at the end of 4 weeks of treatment [30]. Thus, the effectiveness of propolis-based products has been proven, and it has been stated that they are a natural therapeutic alternative for the treatment of cutaneous mycoses in horses and do not have any of the toxic side effects of traditional drugs. It has been stated that this does not have any effects.

In other study the effects of propolis extract on old (chronic) horse wounds was investigated. 35 old wounds on the neck and shoulder areas of 18 horses and 14 donkeys were covered with propolis [9]. Following propolis treatment, clinical wound characteristics improved. A significant reduction in wound size and healthy wound healing were observed. On the other hand, 15 surgically initiated full-thickness skin wounds in the shoulder area of 5 donkeys (3 wounds from each animal) were dressed with propolis, honey, and salt (salina), respectively (5 wound applications in each treatment) for 3 weeks. Clinical wound healing was best in patients treated with propolis. According to the histological parameters, wounds treated with propolis were in better condition than wounds treated with honey and salt, and fibroblast and collagen accumulation was higher. As a result, propolis, compared to honey, was found to be beneficial for the treatment of chronic wounds in horses. In this study, we applied liquid propolis as a spray to 18 javelin horses with different wounds. We found that the healing process varied depending on the nature of the wound, and fibroblast and collagen accumulation peaked on the 15<sup>th</sup> day.

It has been reported that commonly used drugs such as anti-inflammatory and nonsteroidal drugs delay wound healing and the situation should be monitored when using such drugs [15]. However, we observed that wound healing was faster following propolis treatment.

Howaida *et al.* [9], reported good healing parameters in wounds treated with propolis, described as the disappearance of signs of inflammation, leakage, and infection. However, he emphasized that the shape and size of the wound (granulation) and the formation of epithelial tissue were moderately completed.

## CONCLUSION

It was concluded that wound treatment with propolis in the form of spraying in horses can be preferred routinely because it takes a short time and, is both easy to apply and economical.

## Declaration of conflicting interests

The authors declare no potential conflicts of interest concerning the research and authorship.

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