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Tavşan Vajinal Yara Modelinde Propolis Uygulamasının Yara İyileştirici Etkisi
Wound Healing Effect of Propolis Application in Rabbit Vaginal Wound Model

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Özet

Amaç: Bu çalışmanın amacı, tavşan vajinal yara modelinde propolis uygulamasının yara iyileştirici etkisini değerlendirmektir.

Gereç ve Yöntemler: Hayvanlar kontrol grubu ve propolis uygulama grubu olarak rastgele iki gruba ayrıldı ve her grupta 5'er adet tavşan kullanıldı. Tüm tavşanlarda vajinal yaralanma introitustan orta vajinal epitel ve stromaya kadar tam kat arka duvar insizyonu ile gerçekleştirildi. Çalışma grubundaki hayvanlara lokal olarak %15 saf Anadolu Propolisi uygulandı ve uygulamaya üç günde bir devam edildi. Kontrol grubuna herhangi bir uygulama yapılmadı. 21 gün sonunda tavşanlar sakrifiye edildi. Diseksiyon ile vajina ve çevresindeki perineal doku çıkarıldı. 3 mikron kalınlığında vajinal kesitler alındı. Bu kesitlerin bir kısmı hematoksilin-eozin (HE) ve Mason's Tricrome (MT) boyaması için normal lamlara, bir kısmı da anti-vasküler endotelyal büyüme faktörü (VEGF-1) immünohistokimya boyaması için pozitif yüklü bir lam üzerine alındı.

Bulgular: Her iki grup fibrozis ve MT boyaması açısından karşılaştırıldı. Her iki grup arasında istatistiksel olarak anlamlı fark bulundu (p=0,039, p=0.039). Her iki grup inflamasyon ve VEGF açısından karşılaştırıldığında iki grup arasında istatistiksel olarak anlamlı fark bulunmadı (p=0,285, p=0,428).

Sonuç: Birinci derece perineal yaraların iyileşme sürecinde yarada aktif kanama yoksa ve yara kenarları düzgün ve birbirine yakın ise propolis uygulaması olumlu katkı sağlayabilir.

Anahtar Kelimeler: Hayvan Modeli, propolis, Yara iyileşmesi

Abstract

Objectives: This study aims to evaluate the wound healing effect of propolis application in a rabbit vaginal wound model.

Material and Methods: Animals were randomly divided into two groups, a control group, and a propolis application group, and five rabbits were used in each group. In all rabbits, the vaginal injury was performed with a full-thickness posterior wall incision from the introitus to the mid-vaginal epithelium and stroma. Locally, 15% pure Anatolian Propolis was applied to the animals in the study group, and the application was continued every three days. No application was made to the control group. At the end of 21 days, the rabbits were sacrificed. By dissection, the vagina and surrounding perineal tissue were removed. Three micron-thick vaginal cross-sections were taken. Some of these sections were taken from standard slides for hematoxylin-eosin (HE) and Mason's Trichrome (MT) staining, and some of them were taken on a positively charged slide for anti-vascular endothelial growth factor (VEGF-1) immunohistochemistry staining.

Results: Both groups were compared in terms of fibrosis and MT staining. A statistically significant difference was found between both groups (p=0.039, p=0.039). When both groups were compared in terms of inflammation and VEGF, no statistically significant difference was found between the two groups (p=0.285, p=0.428).

Conclusion: In the healing process of first-degree perineal wounds, if there is no active bleeding in the wound and the wound edges are smooth and close to each other, propolis application may contribute positively.

Keywords: Animal models, propolis, wound healing.

Introduction

Wound healing is a biological process for the restoration of tissue integrity and functions. In obstetrics, there is a need for applications that are suitable for use during the repair of birth injuries, contribute to the strength of scar tissue, accelerate wound healing, and help mothers return to their everyday lives in a shorter time. A better understanding of wound healing processes in the vagina could pave the way for developing new treatment approaches to improve tissue strength.

It is predicted that a spontaneous perineal tear during delivery may occur at a rate of 53-79%, depending on prenatal care, healthcare professionals following the pregnancy, patient characteristics, and delivery management. It frequently covers first and second-degree perineal lacerations (1). Perineal tears usually occur during the delivery of the fetal head. 30% of women complain of maternal pain and discomfort in the first two weeks after delivery.

Propolis is a resinous mixture that honey bees collect from tree buds and other botanical sources. The chemical composition of propolis varies depending on the geographical region, seasonality, altitude, and the food condition the bee receives during propolis formation (2). However, the overall primary composition of most raw propolis samples is similar. It contains approximately 50% resin, 30% wax, 10% essential oils, and 5% pollen. The remaining 5% consists of other organic components (3,4). The main compounds that make up the content of propolis are flavonoids with antioxidant properties (5). Modern medicine is increasingly using the therapeutic potential of natural substances. This trend can be explained by relatively more accessible access to natural resources, lower toxicity of

natural products, and more attractive prices than pharmaceuticals. Studies have shown that propolis has anticancer, hepatoprotective, anti-inflammatory, and antioxidant activities (6,7).

The aim of this study is to evaluate the wound-healing effect of propolis application in a rabbit vaginal wound model.

Methods

Ethical approval

All experimental procedures applied in this study were examined by Experimental Animal Research Ethics Committee and approved on 27.05.2022 with the number 651. Minimal numbers of animals were used, and every effort was made to minimize their suffering.

Animal experiments

In this project, ten adults, average five months old, unmated, female, 2500-3000gr white New Zealand rabbits, were used as experimental animals. Animals were randomly divided into two groups, the control group and the propolis application group. After the supply of rabbits, they were kept for one week to acclimate to laboratory conditions. Rabbits were kept as single individuals in a cage. The animals were housed in the laboratory's standard animal housing conditions, in a 12-hour light and dark environment, at 21°C, 50-60% humidity, and optionally standard pellet rabbit food and water.

All rabbits have fasted for 12 hours before surgery. Animals were anesthetized by intramuscular injection of 10 mg/kg xylazine (Rompun; Bayer, Turkey) and 50 mg/kg ketamine (Ketalar; Parke Davis, Turkey, Istanbul) and allowed to breathe spontaneously. Rabbits were fixed in the supine position. The skin around the vaginal opening was wiped with 10% povidone-iodine, the vaginal injury was performed with a full-thickness posterior wall incision from the introitus to the mid-vaginal epithelium and stroma. Care was taken to preserve the perineal muscle, while the incision included the skin, subcutaneous tissue, and vaginal mucosa in the perineum. A drop of 15% pure Anatolian Propolis was applied locally to each animal, and the application was continued every three days (7 applications in total). No application was made to the control group. Animals were monitored daily post-surgery for signs of infection. It took 21 days for complete healing and epithelization. At the end of 21 days, the rabbits were sacrificed. By dissection, the vagina and surrounding perineal tissue were removed and stored at -80°C until the study was performed.

Histochemical examination

21 days after the operation, the full-thickness vagina and rectum and around both posterior paravaginal muscles and pubis bones were collected by longitudinal dissection along with the anterior vaginal wall below. These tissues were fixed in a 10% neutral buffered formaldehyde solution at room temperature for 24 hours. In the follow-up process, which lasted for 15 hours with a tissue tracking device (Sacura), dehydration, transparency, and tissue hardening processes were applied by passing through alcohol, xylene, and paraffin stages. In the next step, tissues were made into blocks by embedding paraffin in a tissue embedding device (Thermo Shandon). Formalin-fixed tissues were processed in paraffin block, and 3-micron thick vaginal cross sections were taken on poly L lysine slides (8,9 Alperin et al., 2010; Shveiky et al., 2020). Some of these sections were taken from standard slides for hematoxylin-eosin (HE) and Mason's Trichrome (MT) staining, and some of them were taken on a positively charged slide for anti-vascular endothelial growth factor (VEGF-1) immunohistochemistry staining. Masson's

trichrome (MT) is used in the examination of connective and support tissues by staining and light microscopy. Sections were stained with MT to evaluate collagen and smooth muscle as well as vaginal structures such as epithelial periphery, number of epithelial protrusions (ruga), rugae depth, and vaginal thickness. Sections taken for HE (Facepath) staining were stained in an automatic staining-off device (Sacura) and closed. MT staining (Facepath) was applied to the slides manually with the kit. VEGF plays a role in wound healing, coagulation mechanism, angiogenesis, cytokine synthesis and release, and smooth muscle cell hyperplasia. VEGF-1 antibody (Abcam, Clone: Y103, 1/100 dilution, 32 min incubation time) immunohistochemical staining was performed on the preparations taken on positively loaded slides in an automatic Ventana Benchmark XT device. Then, the slides were closed by dripping entellan. After staining procedures, the samples were assessed in a blinded manner by two pathologists to avoid bias with a light microscope (Leica DM 750). Inflammation and fibrosis were evaluated in HE-stained preparations (Table 1).

Statistical Analysis

After the macroscopic and histopathological evaluation of the control group and propolis group, the statistical phase was started. Count data were given with numbers and percentages, and measurement data with mean, standard deviation, minimum and maximum values. Mann Whitney-U test, which is a non-parametric test, was used to compare both groups. $p < 0.05$ was considered statistically significant.

Table 1: Histopathological Evaluation [5,6,7]

The degree of inflammation	
0	no inflammation
1	rarely presence of giant cells, lymphocytes, and plasma cells
2	presence of giant cells, plasma cells, eosinophils, and neutrophils
3	presence of many inflammatory cells and microabscesses
The degree of fibrosis	
0	no fibrosis
1	mild
2	moderate
3	severe
Percentages of VEGF-1 immunoreactive cells	
0	negative for staining
1	33% positive staining
2	33-66% positive staining
3	66% recorded as positive staining

†:*VEGF: Vascular Endothelial Growth Factor*

Results

When the control group and propolis group were evaluated in terms of inflammation (Figure 1-2), no statistically significant difference was found between the two groups ($p=0.285$). When the control and propolis group were evaluated in terms of MT staining and fibrosis (Figure 3-4), a statistically significant difference was found between both

groups (p=0.039, p=0.039). When both groups were compared in terms of VEGF (Figure 5-6), no statistically significant difference was found between the two groups (p=0.428) (Table 2-3).

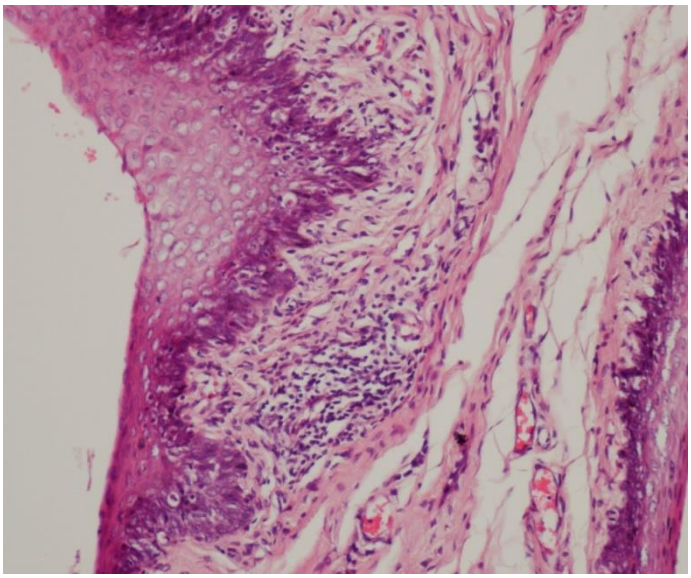

Figure 1. Control group showing moderate inflammation, mild fibrosis (HEX200)

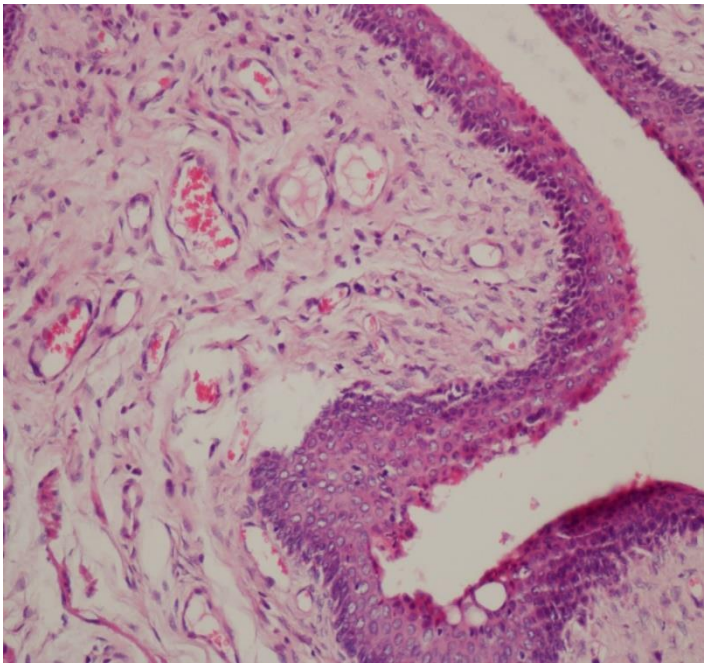

Figure 2. Propolis group without inflammation showing moderate fibrosis (HEX200)

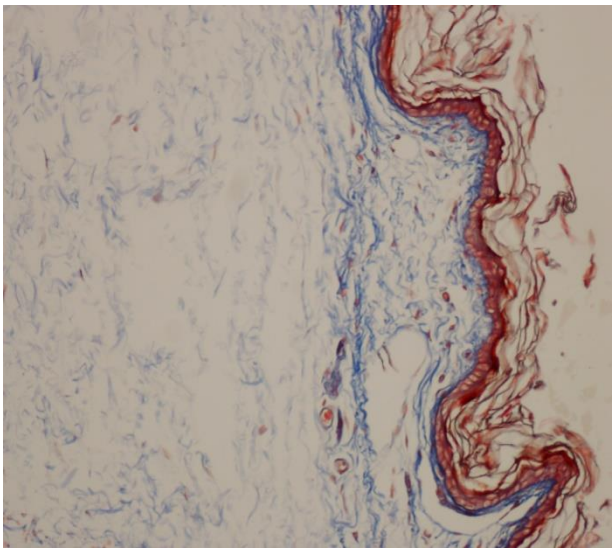

Figure 3. Mild fibrosis, control group (MTx200)

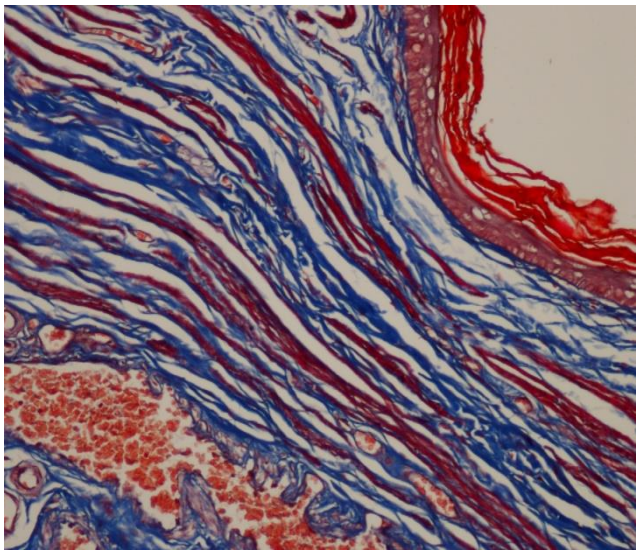

Figure 4. İntense fibrosis, propolis group (MTx200)

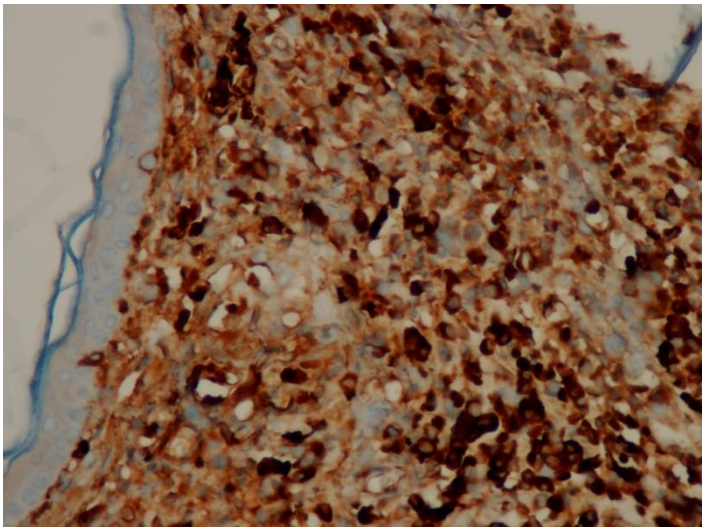

Figure 5. Severe staining with VEGF-1 score 3, control group (HEX400)

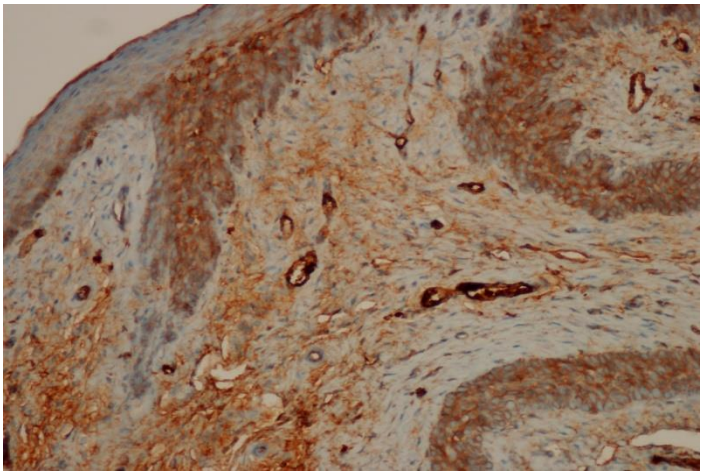

Figure 6. VEGF-1 score 2 moderate staining, propolis group (HEX200)

Table 2: Histopathological Findings

	Control group	Propolis group
Fibrosis	1,1,2,1,1	2,2,2,3,2
Inflammation	0,1,0,2,2	0,0,0,0,1
MT	1,1,2,1,1	2,2,2,3,2
VEGF	1,2,3,3,3	2,2,1,1,3

Table 3: Statistical comparison of histopathological findings

	Control group	Propolis group	p value
Fibrosis	1.20 ± 0.44 (min-1,max-2)	2.20 ± 0.44 (min-2, max-3)	**0.039**
Inflammation	1.00 ± 1.00 (min-0, max-2)	0.20 ± 0.45 (min-0, max-1)	0.285
MT	1.20 ± 0.44 (min-1,max-2)	2.20 ± 0.44 (min-2, max-3)	**0.039**
VEGF	2.40 ± 0.89 (min-1,max-3)	1.80 ± 0.83 (min-1,max-3)	0.428

Discussion

Wound healing is a detailed process in which fibroblasts, keratinocytes, endothelial cells, and inflammatory cells play essential roles, and many enzymes and growth factors are effective (13).

Vaginal delivery can cause up to 80% of injuries to the vagina and surrounding tissues (14,15). These injuries range from small insignificant tears to large tears involving the pelvic floor muscles that lead to prolonged fecal and urinary incontinence and sexual dysfunction (16,17). Vaginal tears usually occur in the midline towards the anal sphincter. According to a systematic review; leaving the skin unsutured or using skin adhesives seems to be preferable to suturing in terms of short-term perineal pain (18). Modern medicine is increasingly using the therapeutic potential of natural substances. This trend is due to more accessible access to natural resources, lower

toxicity, and more attractive prices than pharmaceuticals.

Propolis has been used in the treatment of dermatological disease since ancient times, and its pharmacological potential in healing and repairing various types of wounds is now known (19-21). The first animal experiments on the healing properties of propolis were conducted in the 1990s by scientists from Bulgaria, Brazil, and the USA. They applied propolis topically on skin wounds, burns, and exposed dental pulp and then examined the effect of this compound histologically, and it was observed that propolis accelerated the normal reorganization of damaged tissues (22,23).

It has been reported that flavonoids and phenolic acid compounds are the most critical propolis content responsible for wound healing (24).

The amino acid arginine, which is abundant in the structure of propolis, increases the amount of urea through pyrimidine synthesis. When arginine is converted into argamate by decarboxylation, it accelerates tissue regeneration by increasing mitosis and protein synthesis. Proline, which is the second most abundant amino acid in the structure of propolis, is involved in the synthesis of collagen and elastin (25). The contribution of propolis to wound healing is probably mediated by these amino acids.

Eröksüz et al. compared the effects of propolis and sulfadiazine on wound healing in full-thickness skin wounds in rabbits and found that propolis provided better-wound healing than other groups (19). Similarly, Han et al. investigated the effects of propolis and silver sulfadiazine on wound healing in burn wounds in a study they conducted with 50% propolis cream on rats and reported consistent results (26). Olczyk K et al. investigated the effects of propolis and silver sulfadiazine on the accumulation of chondroitin sulfate and hyaluronic acid in skin burn wounds. They showed that propolis increased changes in the content of certain glycosaminoglycans during recovery more than silver sulfadiazine did. According to these findings, they reported that propolis accelerates the healing of burn tissue by causing the accumulation of glycosaminoglycans necessary for granulation, tissue development, and wound closure (21)

In light of these studies, first-degree perineal laceration was performed in the rabbit vaginal wound model, with the thought that the application of propolis would contribute to wound healing by supporting the suturation of the vaginal injury. According to our results, propolis positively affected wound healing by increasing collagen production and fibrosis in the rabbit vaginal wound model.

In contrast to previous studies that evaluated the 7th or 14th postoperative day in wound healing, in this study 21st post-surgery day was selected to determine the status of the late fibroblastic activity. As a limitation of our study, we could also perform the histological examination on days 7 and 14 and apply higher doses of propolis. In this case, we could find differences in inflammation and angiogenesis between the two groups. Still, there was no previous vaginal administration of propolis, so we chose long intervals and repetitive low doses as the first step.

As a result, we believe that propolis can contribute positively to first-degree perineal tears, which do not have active bleeding during the healing process of perineal wounds, have regular and well-approximated wound edges, and can support treatment if appropriate drug forms and concentrations. To the best of our knowledge, our study is the first propolis study

studied in vaginal tissue. And we hope that it will guide future clinical studies.

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