

Haemostatic and Histopathological Effects of Ankaferd Blood Stopper, on Penile Cavernosal Tissue in Rats

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ABSTRACT

Penile fracture is defined as a traumatic rupture of the tunica albuginea of the corpora cavernosa. The pathologic lesion is a tear of the outer longitudinal layer of the tunica albuginea resulting in hematoma, swelling, and skin discoloration. In this study, we aimed to investigate the effect of Ankaferd Blood Stopper (ABS) on the penile cavernosal tissue histopathologically and possible use as a haemostatic agent in penile cavernosal surgeries.

Experimental penile fracture was formed by incising from the proximal dorsal side of the penis in thirty-two Wistar Albino rats and the rats were randomly assigned into four main groups, each group containing eight rats. In group A, ABS was only applied locally onto the incision region. In group P, the incision was primarily repaired. In group PA; ABS was applied onto the incision region following primary repair. In group C, the incision was not repaired and left to secondary healing. Three weeks later, all rats were sacrificed and penectomy was carried out. In group A, fibrosis was observed in 2 rats and this group seemed to be the best group according to cavernosal healing. Although there was no significant difference between control group and groups A and PA according to inflammation, group A and PA were better than primary repair group ($p= 0.046$). The presence of siderophages and red blood cell aggregation were only observed in group A and group PA.

ABS can be employed to use in cavernosal surgeries due to its haemostatic and anti-inflammatory properties. Erythrocyte aggregation confirmed haemostatic activity of ABS in penile tissue.

Key Words: Ankaferd Blood Stopper, Haemostasis, Penile fracture, Rat penis

ÖZET

Ankaferd BloodStopper'in Rat Penisinde Kavernozaal Dokuya Olan Hemostatik ve Histopatolojik Etkileri

Penil fraktür, korpus kavermozumun tunika albuginea yapısının travmatik rüptürüdür. Patolojik lezyon, tunika albugineanın dıştaki longitudinal tabakasının hematom, şişme ve ciltte renk değişikliğine neden olacak şekilde yırtılmasıdır. Bu çalışmada Ankaferd Blood Stopper'in penil kavernozaal doku üzerine olan histopatolojik etkisini ve bu ajanın penil kavernozaal cerrahilerde hemostatik ajan olarak olası kullanımının araştırılmasını amaçladık.

Çalışmaya alınan 32 Wistar albino sıçanda deneysel penil fraktür modeli penis proksimalinin dorsal tarafına uygulanan insizyon ile oluşturuldu ve sıçanlar her grup 8 sıçan içerecek şekilde 4 gruba ayrıldı. Grup C'deki sıçanların insizyonları onarılmadı ve sekonder iyileşmeye bırakıldı. Grup A'daki sıçanların insizyonları üzerine sadece Ankaferd® damlatıldı. Grup P'de insizyon primer olarak onarıldı ve grup PA'da ise primer onarımı takiben insizyon bölgesine Ankaferd® damlatıldı. Üç hafta sonra sıçanlar sakrifiye edilerek penektomi materyalleri incelendi.

Grup A'da 2 sıçanda fibrozis gözlemlendi ve bu grup kavernoza iyileşme bakımından en iyi grup olarak görünüyordu. Kontrol grubu ile grup A ve grup PA arasında inflamasyon yönünden bir fark izlenirse de grup A ve grup PA'nın primer onarım grubundan daha iyi olduğu gözlemlendi (p= 0.046). Siderofaj ve kırmızı kan hücrelerinin varlığı sadece grup A ve grup PA'da gözlemlendi.

ABS, penil kavernoza cerrahilerde hemostatik ve anti inflamatuvar özellikleri nedeniyle kullanılabilir. Eritrosit agyasyonunun görülmesi ABS'nin hemostatik mekanizmasının peniste de oluştuğunu desteklemektedir.

Anahtar Kelimeler: Ankaferd Blood Stopper, Hemostaz, Penil fraktür, Sıçan penisi

INTRODUCTION

Penile fracture is defined as a traumatic rupture of the tunica albuginea of the corpora cavernosa. The pathologic lesion is a tear of the outer longitudinal layer of the tunica albuginea resulting in hematoma, swelling, and skin discoloration. There are some controversies about the treatment approach for traumatic ruptures of the penis. In earlier studies, the recommended treatment was conservative, consisting mainly of cold compresses with antibiotics and anti-inflammatory drugs.¹ However, 10% to 30% of patients who were treated conservatively experienced a penile deformity, suboptimal erections, and a coitus difficulty.² After the first recommendation of immediate surgical intervention by Fernstrom, the immediate surgery was increasingly used to shorten the hospital stay.³ Today, the immediate surgical repair is widely accepted as the treatment of choice because of the excellent long-term results that have been reported.^{4,5}

Ankaferd Blood Stopper (ABS) comprises a standardized mixture of plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*. ABS as a medicinal product has been approved in the management of external hemorrhage and dental surgery bleedings in Turkey.⁶ The safety and efficacy reports on the product have indicated its sterility and non-toxicity.

In this experimental study we aimed to investigate histopathological effect of a haemostatic agent, ABS, on the penile cavernosal tissue and possible use of ABS as a haemostatic agent in penile fracture and also other penile cavernosal procedures. To our best knowledge this is the first study in the literature involving the penile cavernosal tissue.

MATERIALS AND METHODS

The experimental study was carried out after obtaining the approval of the local Ethics Committee and thirty-two Wistar Albino rats (220-250 g) were enrolled. All surgical interventions were performed under sterile conditions by the same team at the same time and environment. The rats were randomly divided into four equal groups; group C (control), group P (primary repair group), group A (ABS) and group PA (primary repair + ABS).

Single dose ceftriaxon (20 mg/kg) was intramuscularly administered 1 hour before the surgical procedures. The rats were placed in supine position after general anesthesia (ketamine 50 mg/kg, intramuscularly). The genital area was shaved and the penile skin was cleaned with 10% povidon iodine. A 3F urethral catheter was pushed approximately 2 cm up to the midpenile level from external meatus. Experimental penile fracture was formed with a number 15 lancet as described previously.⁷

In group A, ABS was only applied locally onto the incision region. In group P; the incision was primarily repaired with 6-zero polydioxanone. In group PA; ABS was applied onto the incision region following primary repair. In group C, the incision was not repaired but was left to secondary healing.

All procedures were performed with surgical loupes (2.5x). The rats were housed in individual cages after they had recovered from the general anesthesia. They were feed normally during three weeks. Three weeks later, all rats were sacrificed by an excessive dose of pentobarbital sodium (100 mg/kg intraperitoneally) and penectomy was carried out. The penectomy materials of all the groups were placed separately into 10% formol solution.

Table 1. Comparison of the groups according to histopathological features

	Group C (Control) n= 8	Group P (PR) n= 8	Group A (ABS) n= 8	Group PA (PR + ABS) n= 8	p
Cavernosal tissue healing with fibrosis n (%)*	7 (87.5)	3 (37.5)	2 (25)	4 (50)	0.07
Presence of inflammation n (%)**	3 (37.5)	6 (62.5)	2 (25)	2 (25)	0.1
Presence of hyperemia n (%) ***	8 (100)	8 (100)	7 (87.5)	8 (100)	0.4
Presence of siderophages n (%) ϕ	0 (0)	0 (0)	4 (50)	3 (37.5)	0.03
Presence of RBC aggregation n (%) β	0 (0)	0 (0)	6 (75)	8 (100)	0.0001

PR= Primary repair, ABS= Ankaferd Blood Stopper, RBC= Red Blood Cell
According to Pearson's X² test;

* p= 0.039 for group C vs group P and p= 0.012 for group C vs group A

** p= 0.046 for group P vs group A and group PA

*** p= 0.302 for group A vs group C, group P and group PA

ϕ p= 0.021 for group P, C vs group A and p= 0.055 for group P, C vs group PA

β p= 0.002 for group P, C vs group A and p= 0.0001 for group P, C vs group PA

Pathological Evaluation

Approximately 0.5 mm sections, including the repaired region, were prepared from the penectomy materials. Following the application of alcohol fixation for 24 h, these were stained with hematoxylin eosin dye. These sections were examined by a blinded pathologist with regard to the identity of the specimens being examined and the results were compared.

On histopathological evaluation, the groups were compared according to some parameters shown in Table 1. These parameters were cavernosal tissue healing with fibrosis, inflammation, hyperemia, presence of siderophages, presence of red blood cell (RBC) aggregation and total healing.

Statistical Analysis

The statistical data were analyzed using Stastical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 13.0 for Windows. All values were expressed as mean \pm SD. Associations between categorical variables were analyzed by the Pearson's Chi-Square test. P values less than 0.05 were considered to indicate statistical significance.

RESULTS

Mortality was not observed during repair or during 21 days after the operation. Microscopic observations were used as the criteria for evaluating success in our study.

In control group (group C), healing with fibrosis and hyperemia were predominant features (Figure 1a). Hyperemia was observed in all rats (100%) and fibrosis was observed in 7 rats (87.5%).

In primary repair group (group P), inflammation and hyperemia were observed predominantly (Figure 1b). Additionally, fibrosis was observed in 3 rats (37.5%).

In ABS group (group A); a wealthy, gelatinous, and red colored tissue was observed on tunica albuginea macroscopically. Fibrosis and inflammation were observed in 2 rats (25%) (Figure 2a). Hyperemia was present in 7 rats (87.5%). Apart from previous two groups (groups C and P), presence of siderophages (37.5%) and RBC aggregation (75%) were observed.

In primary repair and ABS group (group PA); hyperemia and RBC aggregation were observed in all rats (Figure 2b). Siderophages were also present in 3 rats (37.5%). Fibrosis was another predominant feature and observed in 4 rats (50%).

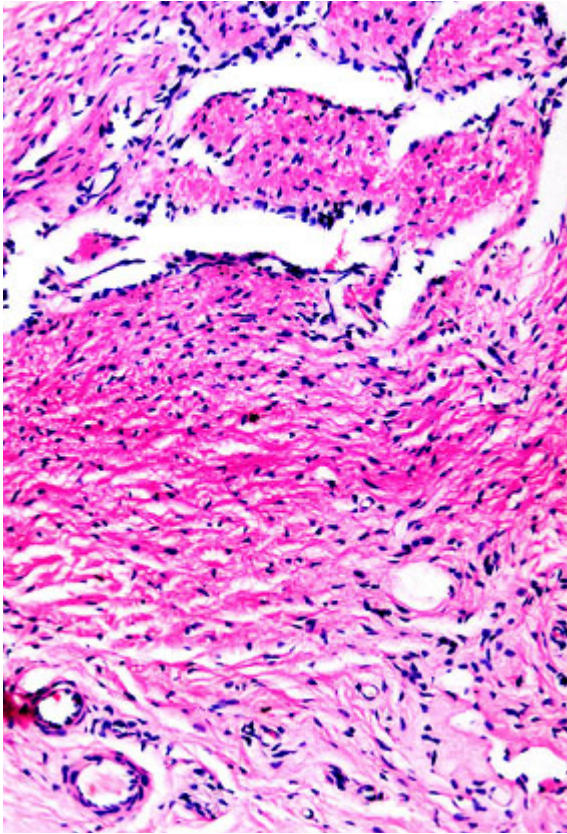


Figure 1a. Control group; fibrosis was apparent and slight hyperemia was seen (H&E x200).

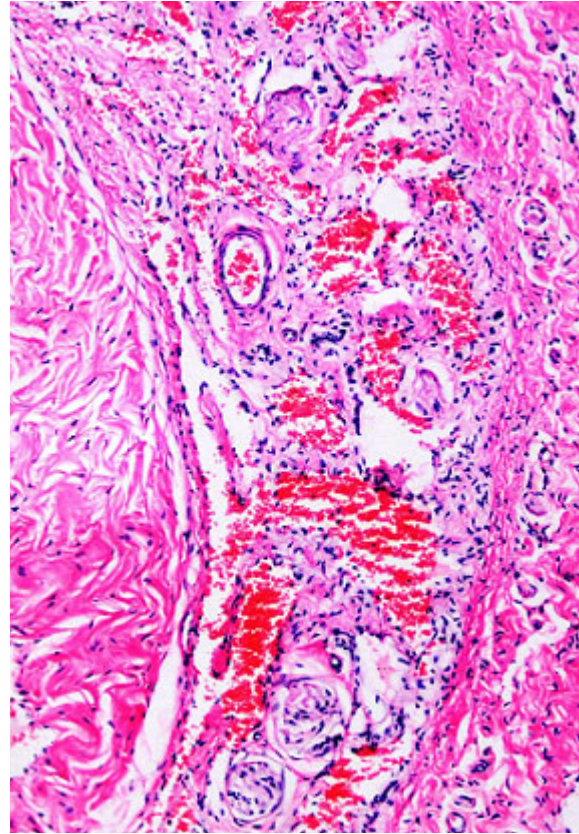


Figure 1b. Primary repair group; inflammation and hyperemia were predominant features (H&E x200).

Fibrosis was significantly increased in control group according to groups P and A ($p < 0.05$). Although there was no significant difference between control group and groups A and PA according to inflammation, group A and PA were better than primary repair group ($p < 0.05$). The presence of siderophages and red blood cell aggregation were only observed in group A and group PA.

DISCUSSION

The tunica albuginea has a high tensile strength requiring a pressure in excess of 1500 mmHg to achieve rupture.⁸ The rupture of the tunica albuginea is usually unilateral and transverse.⁹ The longitudinal tears are rare, occurring as an extension of a transverse tear.^{10,11} Although the fracture occurs more often in the proximal shaft, fractures have also been reported in the distal third of the penis.^{10,12} The aim of immediate restoration of anatomical continuity

in the albuginea is to avoid the formation of fibrous tissue.¹³ The World Health Organization has recommended that all acute injuries to the tunica albuginea should be repaired immediately. The principle of treatments of penile fracture consists of control of hemorrhage, thorough cleansing, debridement, plastic repair, surgical closure and antibiotics.¹³ The penile fracture must be treated surgically as soon as possible after diagnosis.

A previous experimental study by our group showed the contributing effects of cyanoacrylic glue on the healing of penile cavernosal tissue.⁷ There are many other reasons and purposes for operating on penile cavernosal tissue. However, if a penile operation is designed for reconstruction, the urologist should make every effort to preserve erectile function. Hsu et al showed that application of electrocoagulation during cavernosal surgeries has been disadvantageous to erectile tissues of the human pe-

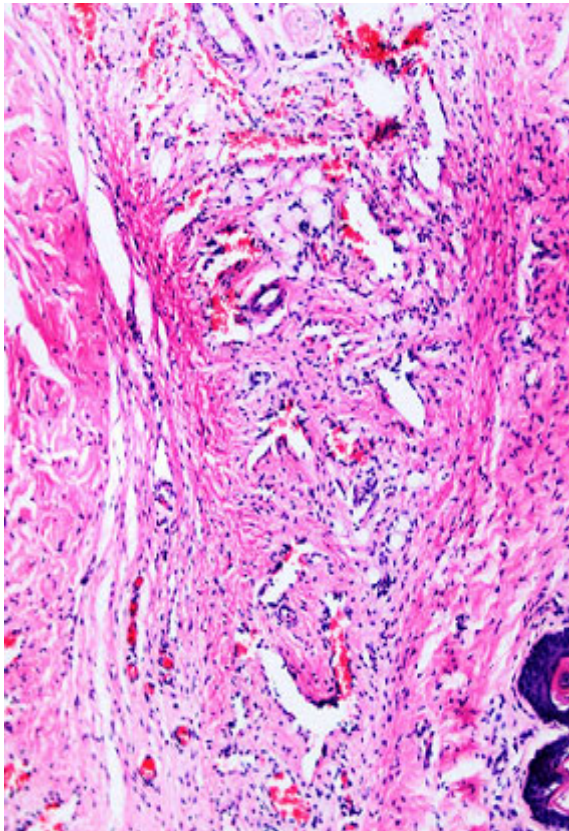


Figure 2a. Ankaferd Blood Stopper group; hyperemia, minimal fibrosis and slight inflammation were observed (H&E x200).

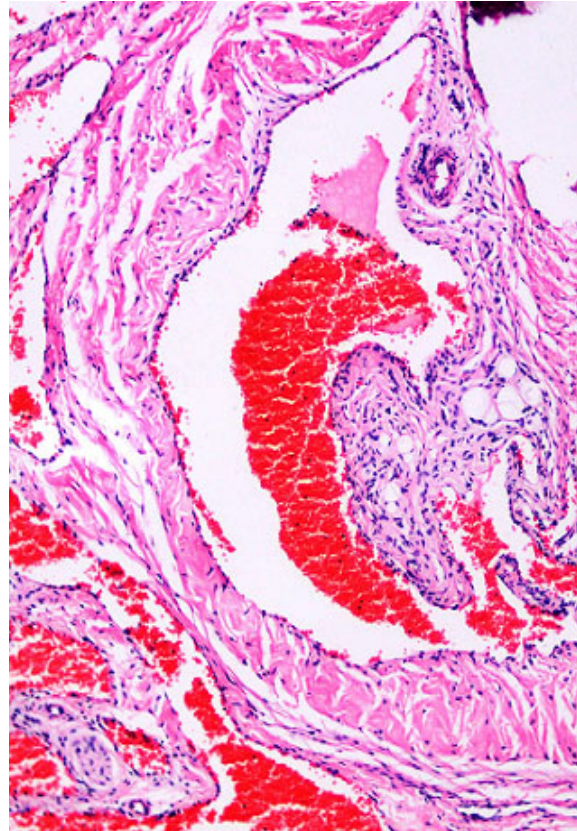


Figure 2b. Primary repair and Ankaferd Blood Stopper group; hyperemia and red blood cell aggregation were predominant features (H&E x200).

nis.¹⁴ The favorable outcome obtained after applying a local treatment model in our previous study and the limitation in haemostasis during cavernosal surgery led us to investigate the effect of a haemostatic agent, ABS, on cavernosal healing and haemostasis.

ABS is a unique medicinal plant extract which has historically been used in Turkish traditional medicine as a haemostatic agent.¹⁵ It is a standardized mixture of the plants 5 mg *Thymus vulgaris*, 9 mg *Glycyrrhiza glabra*, 8 mg *Vitis vinifera*, 7 mg *Alpinia officinarum* and 6 mg *Urtica dioica* in 100 ml Ankaferd solution and each of them has some effect on hematological and vascular parameters and cellular proliferation.¹⁵⁻¹⁹ *Glycyrrhiza glabra* has anti-inflammatory, anti-thrombin, anti-platelet, anti-oxidant, anti-atherosclerotic, and anti-tumor activities.¹⁵ It inhibits angiogenesis, decreases vascular endothelial growth factor production and cytokine in-

duced neovascularization.¹⁵ *Thymus vulgaris* has anti-oxidative actions, such as prevention of lipid peroxidation.¹⁸ *Vitis vinifera* exerts anti-tumor and anti-atherosclerotic effects.^{20,21} *Alpinia officinarum* inhibits nitric oxide production by lipopolysaccharide activated mouse peritoneal macrophages.¹⁷ *Urtica dioica* causes vasodilatation via inducing nitric oxide production by endothelium.¹⁹ This substance has tampon, spray and ampule forms for use. In the present study we used the ampule form and dripped the agent on incised cavernosal region.

In a recent study, the haemostatic mechanism of action of the ABS has been investigated.¹⁵ ABS stimulated the formation of an encapsulated protein network that provides focal points for RBC aggregation.^{15,22-23} We also observed, as a macroscopic finding, that ABS was beneficial as a topical haemostatic agent in bleeding penile cavernosal tissue of rats. ABS was found effective in shortening the

duration of bleeding and decreasing the bleeding volume. The occurrence of RBC aggregation in rat penile cavernosal tissue was a prominent feature in ABS applied groups.

The macroscopic appearance of cavernous tissues in groups A and PA was satisfactory in our study. In group A, inflammation and fibrosis were observed in two rats. In group PA inflammation was observed in four rats. The absence of foreign material reaction in cavernous tissue was in accordance with findings in other studies in the literature. However, slight fibrosis developed in the cavernous tissue of three and two rats in groups A and PG, respectively. This finding showed that ABS has no effect on preventing fibrosis during the healing of penile cavernosal tissue.

There are some limitations in our study. First, we could not measure and compare the bleeding and haemostasis time in rats. Due to anatomical and physiological features of rat penis, it was difficult for us to measure these periods. Second, the small size of the rat penis made us to repair cavernosal incision more difficult than in the human despite using surgical loupes for procedures. The penis was therefore closed in one layer. However, in our opinion, since the aim of the study was to investigate the histopathological features of cavernosal tissue, it seems to be an unimportant limitation for us.

CONCLUSION

ABS can be employed to use in cavernosal surgeries due to its haemostatic property. Demonstrating the erythrocyte aggregation in our ABS groups is compatible with the basic mechanism of action for ABS that appears to be the formation of protein network providing focal points for erythrocyte aggregation. Further experimental and randomized clinical studies with large numbers are required to determine about clinical use.

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