Effects of a new hemostatic agent Ankaferd Blood Stopper® on the intraocular tissues in rat model

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Abstract

Purpose: To investigate the histopathological changes due to administration of Ankaferd Blood Stopper® (ABS) into intraocular tissues by an anterior chamber and intravitreal injections.

Methods: Twenty Wistar albino rats were divided into four equal groups. Group 1 was injected 0.01 mL ABS into anterior chamber. Group 2 was injected intravitreal 0.02 mL ABS. Groups 3 and 4, which were used as controls, were injected into the anterior chamber and intravitreal 0.01 mL and 0.02 mL balanced salt solution (BSS), respectively. At 2, 5, 10, 15 and 20 days after injection, the eyes were examined under an operating microscope and were subsequently enucleated for histopathological examination.

Results: Ophthalmic examination of the rats prior to enucleation revealed ocular complications ranging from conjunctival hyperemia to corneal perforation in group 1 and increased conjunctival hyperemia and discharge in group 2. No physical and histopathological anomalies were detected in groups 3 and 4. All eyes in group 1 showed mixed type inflammatory cell reaction, foreign-body reaction, stromal congestion, disintegration of the collagen fibers and loss of the epithelium of the posterior wall in the iris and ciliary body were observed histopathologically. All eyes in group 2 showed disintegration and separation of the retina, brown pigment accumulation and mixed type inflammatory cell reaction.

Conclusion: Our results indicate that the commercially available form of ABS solution exerts a toxic effect on intraocular tissues. We consider that the intraocular use of different concentrations, rather than multiple time point of ABS should be investigated.

Keywords: Ankaferd Blood Stopper®, intravitreal injection, anterior chamber injection

Introduction

Ankaferd Blood Stopper® (ABS: Ankaferd Drug Cosmetic Co., Istanbul, Turkey) is a unique folkloric medicinal plant extract which has historically been used in Turkish traditional medicine as a hemostatic agent (1). Goker et al. (1) (2008) investigated the hemostatic effects of ABS and reported its therapeutic potential to be used for the management of hemorrhage. Additionally, many studies have demonstrated hemostatic effect of ABS in Animal Models and Humans (2–10).

The use of topical ABS has been approved by the Turkish Ministry of Health for the management of dermal, external post-surgical and dental bleedings (1). It is available in three forms (spray, solution and tampon). Medical tests on ABS have proved its safety, efficacy, sterility, and non-toxicity (11).

Our hypothesis in current study was that ABS might be safe to be administered intracamerally and intravitreally in postoperative hyphema due to ophthalmic surgeries such as neovascular glaucoma or trabeculectomy or intraoperative or postoperative recurrent intravitreal hemorrhage, especially anticoagulated patients. As far as we are aware, there are no intraocular studies with ABS in the literature. This is the first study to investigate intraocular use of ABS.
The aim of this study was to investigate the effect of intracameral and intravitreal injection of ABS.

Methods

The animal protocol of the study adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic Vision and Research, and was approved by the institutional review board and ethics committee. Twenty adult male Wistar albino rats, weighing 390–530 g were used in this prospective, controlled trial. The animals were kept in a room at a constant temperature (22± 4°C) with a 12 h light/12 h night cycle with free access to food and water. The rats were randomly divided into four groups. The right eye of each rat was used for injection. 0.01 mL ABS was injected into the anterior chamber of the rats in group 1, whereas 0.02 mL ABS was injected into the vitreous of the rats in group 2. Groups 3 and 4 were the control groups. 0.01 mL balanced salt solution (BSS) was injected into the anterior chamber of the rats in group 3, whereas 0.02 mL BSS was injected into the vitreous of the rats in group 4.

All rats were anesthetized with intramuscular injections of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, Istanbul, Turkey) and 5.0 mg/kg xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey). Then, ABS or BSS was injected into the anterior chamber from the corneal limbus at the 6 o’clock position with a sterile 30-gauge needle. Intravitreal injection was administered to the approximate middle vitreous cavity of 1.5 mm away from the limbus at the 6 o’clock position with a sterile 30-gauge needle.

At 2, 5, 10, 15 and 20 days after injection, four rats randomly selected from each of the four groups were killed. Then, the eyes were examined under the operating microscope and were enucleated. The enucleated eyes were placed in 10% buffered formalin solution for a minimum of 24 h. Then, the specimens were embedded in paraffin. Sections of 5 µm thickness were obtained using a microtome, and were stained with hematoxylin and eosin, periodic acid–Schiff, and Masson trichrome stain. Histopathological examinations of ocular tissues of rat eyes in all groups (the cornea, iris, lens capsule, ciliary body and retina) were evaluated at 2, 5, 10, 15, and 20 days. Inflammatory cell reaction, cell degeneration, foreign-body giant cell reaction, and necrosis were searched in histological sections.

Results

None of the rats presented injection-induced complications. Ophthalmic examination of the rats prior to enucleation revealed conjunctival hyperemia and discharge on day 2, corneal opacity on day 5, corneal melting and ulceration on day 10 and corneal perforation on days 15 and 20 in group 1. Ophthalmic examination of group 2 rats revealed increased conjunctival hyperemia and ocular discharge. The ophthalmic examination of BSS-administered rats in groups 3 and 4 showed no pathological finding.

Histopathologically, we observed mixed type inflammatory cell reaction, foreign-body reaction, brown pigment accumulation and erythrocyte aggregation in the iris and ciliary body of all eyes in group 1. Additionally, irido-corneal adhesions, stromal congestion, disintegration of collagen fibers and loss of the epithelium of posterior wall were observed in the peripheral cornea (Figure 1B and 1C). The presence of hemosiderin was confirmed by Prussian blue staining of brown pigment accumulation (Figure 1D). In group 2, histopathological findings indicated disintegration of the retina, hemosiderin-laden macrophages as well as mixed type inflammatory cell reaction and multinuclear giant cells with foreign-bodies (Figure 2B and 2C). Prussian blue staining demonstrates hemosiderin in macrophages (Figure 2D). The histopathological examination of the eyes in groups 3 and 4 revealed no abnormal findings (Figures 1A and 2A).

Discussion

ABS comprises a standardized mixture of the plants, *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* in a weight ratio of 6:8:7:7:5, respectively (1,11). Each of these plants has been previously investigated and some effects have been reported. *T. vulgaris* has antioxidant bactericidal and antifungal effect (12–14). *G. glabra* has some effect on the
angiogenesis, cellular proliferation and blood cells (15). *V. vinifera* has anti-inflammatory, antioxidant and bactericidal effect (16,17) *A. officinarum* provides suppression of inducible nitric oxide synthase expression and has some effect on vascular dynamics (18,19). *U. dioica* has some effect on the endothelium, vascular dynamics, and cell mediators (20).

The basic mechanism of action for ABS is the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation (1,2). The ABS-induced protein network formation involves blood cells, particularly erythrocytes without affecting the physiological individual coagulation systems (21). Recently, ultrastructural and morphological analysis of ABS-induced coagulum was illustrated under the scanning electron microscopy (SEM) (22).

Since ABS exerts its hemostatic effect by affecting erythrocytes, bleeding due to low platelet count, warfarin overdose and chronic non-steroidal anti-inflammatory drug use could be controlled more efficiently with ABS. Moreover, *in vivo* hemostatic effect of ABS in defective hemostasis due to enoxaparin and aspirin administration has been studied in rat models and ABS was found to be effective in reducing the duration and amount of bleeding (23). Antibacterial activity of ABS has also been demonstrated (21).

A number of previous clinical and experimental studies used solution and spray forms of ABS and histopathological findings from those studies demonstrated no histopathological deterioration in rat bladder tissue (24), penile cavernosal tissue (25), hepatic tissue (22), liver tissue (26), and partial nephrectomy tissue (27), compared with the control groups. In addition, it has been histopathologically demonstrated that ABS exerts a slight irritant effect on the ocular surface (9) and has no toxic effect on the Tenon’s capsule and scleral tissue (10). Based on these studies, it can be concluded that ABS can be used in surgical interventions or traumas of the eyelids, conjunctiva and sclera in anticoagulated patients.

Our clinical observations and histopathological examinations revealed that ABS has a toxic effect on intraocular structures. An unpublished study conducted in our otorhinolaryngology clinic demonstrated that ABS, when administered into the maxillary sinus, led to increased fibrosis. In previous studies, ABS was directly applied to the bleeding surface after which it is associated with erythrocytes, and the remaining portion of the ABS solution removed from the environment by means of irrigation or surgical compresses. But, in the present study, we applied the agent directly into the eye without bleeding and stayed in this region for a long period of time. From this point of view, we thought that pure ABS solution without coupling erythrocytes with long-term tissue contact might serve as a toxic agent.

Another limitation of the present study is the dosage of the agent administered into the intraocular region. The PH of the agent is 1.8 (11,28). The target of PH for solution for intravitreal injection should be 3–8 (29). In the present study, a commercially available ABS solution has been used. The concentrations and viscosities of the substances used to prevent toxic effect are important parameters.

In this study, we aimed to determine whether ABS has a toxic effect on intraocular tissues and, if not, to test its efficacy in intraocular hemorrhage models. Histopathologically, the presence of erythrocyte aggregation and hemosiderin pigment accumulation in the anterior chamber and vitreous even on day 20 indicates that an encapsulated protein network induced by ABS is not absorbed, or takes a while to be absorbed from the anterior chamber and vitreous.

This study demonstrated that ABS has a toxic effect on intraocular structures in its pure form. A number of previous clinical and experimental studies have reported no observational or histopathological side effects of ABS. However, the effect of ABS on closed cavities remains to be elucidated. Thus, further clinical and experimental studies with different concentrations suitable for intraocular application, rather than multiple time point of ABS are required to examine the efficiency and reliability of ABS in different regions and tissues. Furthermore, improved ultrastructural and morphological analysis by SEM is warranted to fully elucidate the effects of ABS on tissues.

**Declaration of interest**

The authors declare no conflicts of interest.
References


