1. Introduction

Hydrofluoric (HF) acid is widely used in petrochemical production, glass etching, glass making, semiconductor industry and mineral processing and electropolishing of metals. [2, 6, 11, 15]. In spite of relatively high incidence of ocular burns caused by hydrofluoric acid there is little information regarding the optimal treatment of these burns [1, 2, 7, 10]. The extent of the HF acid injury depends mostly on concentration and amount of it [1].

Progressive vascularization of the corneal stroma, white calcific plaque formation in the anterior corneal stroma, conjunctival hemorrhage and erosion of the corneal epithelium are manifestations of experimental ocular HF acid burns in the rabbit [7]. HF acid causes more extensive injury unlike other acids due to the ability of the fluoride ion to penetrate deep tissues where it forms complexes with calcium and magnesium [1, 5].

Systemic use of Shikonin, soluble extract of the root of Lithospermum erythrorhizon Siebb. et Zucc., and alkannin, soluble extract of the root of Alkanna tinctoria TAUSCH (A. tinctoria) have been reported [8, 13]. Shikonin has antibacterial [14], anti-inflammatory [14], antitumor [12] and wound healing effects [9, 14]. TANAKA et al. [14] reported that shi-
konin and alkannin are equally effective in their anti-inflammatory activity and inhibition of oedema formation. Shikonin and alkannin are found as a mixture in the ratio of 2 : 98 in the dried root of A. tinctoria. A. tinctoria was used for burning and ulcers [9].

Confusing and contradictory results have been reported on the treatment of HF acid eye injuries in the literature. To our knowledge, there is no information, regarding the use of A. tinctoria on HF acid injured eyes clinically or experimentally. The aim of this study was to evaluate the effects of A. tinctoria, Acular and Blephamide on experimental HF acid induced corneal burns in rabbits.

Abbreviations : A. tinctoria : Alkanna tinctoria TAUSCH.

2. Materials and methods

This experimental protocol was approved by the Animal Use Committe, and all the animals were treated in accordance with national and local animal welfare legislation based on European Council Directive. This study was carried out in rabbit eyes. A total of 54 rabbits were used. The animals were divided into 3 groups. Group 1 received Acular\(a\) nonsteroidal anti-inflammatory agent (0.5 % ketorolac tromethamine), group 2 was given Blephamide\(b\) (0.2 % prednisolone acetate, 10 % sulphacetamide sodium, 0.12 % phenylephrine hydrochlorure), group 3 was treated with A. tinctoria. Each group was treated for 2, 7 and 14 days. Six animals were used for each 2, 7 and 14 days of treatment periods totaling of 18 animals in each group. The eyes of rabbits were examined and found to be healthy before the experiment. Rabbits were anesthetized with 0.5 ml xylazine HCl 10 %, 100 mg ketamine HCl and 0.2 ml acepromazine, followed immediately by 14 mg/kg dipyrrone intramuscularly prior to corneal burn injury. Thereafter all the animals received 7 mg/kg dipyrrone intramuscularly for reducing pain every 6 hours for 2 days based on the protocol given by BEIRAN et al. [1]. Corneal burns of both eyes were induced by instillation of 0.05 ml 2 % HF acid for 60 seconds by the method described by BEIRAN et al. [1]. Following this, the eyes were irrigated with 500 ml normal saline. After that, one drop of Acular, Blephamide and A. tinctoria were instilled to the left eyes 3 times a day for 2, 7 and 14 days. Right eyes were kept as controls and treated with 0.9 % normal saline 3 times a day for 2 days in all groups. Injured eyes were examined clinically after fluorescein staining by ophthalmoscope immediately and at days 1, 2, 7 and 14. Since A. tinctoria is not water soluble, it was prepared for 8 % in Huile de Ricin in weight basis. It was heated for 10 minutes in low temperature heat, waited for a day and then the mixture was filtered through a 0.22 µm filter and dispensed in a dropper bottle.

Treatment results were determined for corneal clarity as severe (total haziness - not able to see details beyond the cornea), slight (slight haziness - enabling examination of anterior chamber details), mild (mild haziness - unable to see pupil and iris details), and normal (clear cornea) by the clinical evaluations.

3. Results

A) CLINICAL FINDINGS

Corneal erosion was not present immediately after HF acid exposure and it was formed after day 1 in all groups. Corneal erosion was smaller at day 7 (Fig. 2A) than at day 2 (Fig. 1A) and turned to be worse with marked vascularization at day 14 (Fig.3A) than at day 7 (Fig. 2A) in the controls. While Acular treatment group had almost identical levels of corneal erosion with slight decrease at days 2, 7 and 14 (Figs. 1B, 2B, 3B, respectively), Blephamide\(c\) (Figs. 1C, 2C, 3C, respectively) and A. tinctoria (Figs. 1D, 2D, 3D, respectively) groups showed gradual improvement through days 2, 7 and 14 with complete disappearance of corneal erosion at the end of 14 days (Figs. 3C and 3D). Corneal erosion was smaller in the A. tinctoria treatment group (Fig. 2D) than in the Blephamide\(c\) treatment group (Fig. 2C) at day 7.

Corneal haziness was maximal immediately following HF acid exposure. Partial clearing was observed at day 2 in all groups and improved up to 14 days in the Blephamide\(c\) and A. tinctoria treatment groups with marked deterioration in the controls and Acular\(c\) treatment group at days 7 and 14.

Conjunctival damage increase was maximal at day 2 in all groups and thereafter there was a gradual improvement in the Blephamide\(c\) and A. tinctoria treatment groups. It was nearly stable at days 7 and 14 in the Acular\(c\) treatment group, but it was worse in the controls at day 14 than at day 7. Marked proliferation of capillaries were observed in the A. tinctoria treated group at days 7 and 14.

Marked chemosis was present immediately after exposure. Its decrease was considered as moderate at days 2 and 7, and as mild through 14 days with complete disappearance at day 14 in the Blephamide\(c\) and A. tinctoria treatment groups. On the other hand, Acular\(c\) treatment group showed mild chemosis through day 4, thereafter, no chemosis was observed. Moderate levels of chemosis were observed in the controls after 7 days throughout the remaining days.

Pannus (Fig. 4) was found in 2 rabbits at days 7 and 14 in all groups, being more severe in the controls and Acular\(c\) treatment group.
FIGURE 1. — Clinical appearance of the lesions at day 2 following treatment. Control (A), Acular® (B), Blephamide® (C), A. tinctoria (D).

FIGURE 2. — Clinical appearance of the lesions at day 7 following treatment. Control (A), Acular® (B), Blephamide® (C), A. tinctoria (D).

FIGURE 3. — Clinical appearance of the lesions at day 14 following treatment. Control (A), Acular® (B), Blephamide® (C), A. tinctoria (D).

FIGURE 4. — Pannus formation in the Acular® treatment group at day 7.

FIGURE 5. — Appearance of normal cornea, H&E X50.
FIGURE 6. — Day 2 of treatment. Loss of epithelium, necrosis and edema in the stroma. A. Control, B. Acular®, C. Blephamide®. Loss of epithelial layer with inflammatory cell infiltration and edema formation can be noted in the A. tinctoria treatment group (D), H&E X50.

FIGURE 7. — Day 7 of treatment. Loss of epithelial layer with necrosis and edema in the upper part of the stroma in the control group (A), Acular® (B), and Blephamide® (C) treatment groups. Reepithelialization (arrow) along with presence of edema can be noted in the A. tinctoria treated eye (D), H&E X50.

FIGURE 8. — Day 14 of treatment. Inflammatory cell infiltration, necrosis and edema in the control (A), Acular® (B) treatment groups. New blood vessels (arrows) in the control group (A). Incomplete reepithelialization occurred in the Blephamide® treatment group (B) whereas complete reepithelialization with disappearance of edema was noted in the A. tinctoria (D) treatment group, H&E X50.

FIGURE 9. — Appearance of normal cornea (A), and following treatment with A. tinctoria at day 7 (B) and 14 (C), H&E X400.
B) HISTOPATHOLOGIC FINDINGS

Day 2 following treatment:

Normal appearance of healthy cornea is shown in Figure 5. Corneal epithelial necrosis along with oedema in the stroma were predominant findings in the control, Acular® and Blephamide® groups (Figs. 6A, B, C). Although epithelial necrosis was also evident in the A. tinctoria treated group, slight stromal necrosis was observed just beneath the denuded epithelial region in the A. tinctoria treated group as compared to the control and the other 2 treatment groups. In addition to these, hyperemia was observed in the corneoscleral junction in all groups. Inflammatory cell infiltration was noted and was confined to the upper part of the stroma in the A. tinctoria treated group (Fig. 6D).

Day 7 following treatment:

Stromal oedema and corneal epithelial necrosis were found in the control (Fig. 7A), Acular® (Fig. 7B) and Blephamide® (Fig. 7C) treatment groups. Necrotic changes were more marked in the upper part of the stroma in the control, Acular® and Blephamide® treatment groups. Although degenerative changes were still present in the epithelial layer in the A. tinctoria treatment group, reepithelialization started by day 7. Inflammatory cell infiltration was lower at day 7 than at day 2. Hyperemia was observed in the corneoscleral junction in all groups.

Day 14 following treatment:

Corneal epithelial and stromal necrosis were observed in the control (Fig. 8A) and Acular® (Fig. 8B) treatment groups. In these groups, slight corneal reepithelialization started at the periphery of the cornea where marked stromal oedema and inflammatory cell infiltration were observed. Incomplete epithelial regeneration was present in Blephamide group (Fig. 8C). A. tinctoria treated group had complete reepithelialization (Fig. 8D). No stromal oedema and inflammatory cell infiltration were observed in both A. tinctoria and Blephamide® treatment groups. New blood vessels, stromal oedema and increase of fibroblasts (keratocytes) in the connective tissue were typical findings in the control group.

Figure 9A shows the physiologic appearance of normal cornea. At day 7 in the A. tinctoria treatment group epithelial cells were found to be located mainly parallel to the surface of the outer cornea (Fig. 9B). On the other hand, physiologic reconstruction of the burned cornea took place at day 14 in the same group (Fig. 9C), resembling that of normal cornea (Fig. 9A). Identical results were also observed at the same periods with Blephamide® treatment group, but they were not as remarkable as in the A. tinctoria treatment group.

4. Discussion

Treatment of HF acid eye injuries is confusing, and contradictory results have been reported. Optimal mode of therapy has not been established clinically and experimentally. Irrigation of the eye with normal saline followed by instillation of 1 % calcium gluconate [3, 6] or administration of calcium gluconate as eye drops after lavage [16] have been recommended. Topical application of ophthalmic steroids, antibiotics, scopalamine cyclopentolate and 1 % calcium gluconate in different modes of delivery are presently accepted methods of treatment for ocular HF acid burns [1, 4, 10]. Although 1 % calcium gluconate was advocated in the treatment of HF acid eye injuries [2], in a controlled study, the use of 1 % calcium gluconate showed no advantage in clinical outcome of HF acid eye injuries [1].

In the current study the efficacy of three different agents Acular®, Blephamide® and 8 % solution of A. tinctoria in Huile de Ricin (w/w) on the HF acid eye injuries have been tested. Other than irrigation with normal sterile saline, no any other adjuvant therapy was used. Soluble extract of A. tinctoria have been used on the treatment of burns, inflammations, wounds and ulcers for centuries in Europe and Far East. Shikonin and alkannin are found as a mixture in the ratio of 2 : 98 in the dried root of A. tinctoria [14]. It was reported that shikonin and alkannin are equally effective in their anti-inflammatory activity and inhibition of oedema. In addition to these, shikonin has antibacterial effect [14].

Clinical and histopathological results revealed that Blephamide® or A. tinctoria applications were equally effective in the treatment of HF acid eye injuries in the rabbit. On the other hand, Acular® treatment had no accelerating effect on the healing of corneal erosion as far as conjunctival and corneal haziness is concerned throughout experimental procedure. Phenylephrine (α1 receptor stimulant - vasoconstriction) included in Blephamide is thought to result in relieving conjunctival damage with resultant effect of improvement on the healing of corneal erosion in this group. There was no healing observed in the controls treated with saline irrigation. Although some improvement was observed by day 7, clinical and histopathological signs were deteriorated after day 7. The area of corneal erosion was smaller in the A. tinctoria treated group than in the Blephamide® treated group at day 7. This was attributed to the proliferation of conjunctival capillary vessels seen in the A. tinctoria treated group. This is in accordance with the results reported by SEKINE et al. [13] on the proliferation of capillaries due to the shikonin effect in cotton pellet induced granulation tissue formation. Complete reepithelialization of the cornea in the A. tinctoria treated group were also superior to that in the Blephamide® treated group at days 7 and 14. Although Blephamide® treated group reached the healing level of A. tinctoria group at day 14, reepithelialization was still incomplete. Placement of epithelial cells only parallel without perpendicular placements suggests the ongoing process of epithelialization. Proof of this was seen at day 14 with both perpendicular and parallel placement of epithelial cells as in normal healthy cornea with complete reepithelialization. Stromal oedema was present in the controls and Acular® treatment group at days 2, 7 and 14. It was also present in the Blephamide® and A. tinctoria treatment groups at days 2 and 7, being higher at day 2 and completely disappeared by day 14, in both groups.

Based on our results, the use of Blephamide® and especially of A. tinctoria should be introduced to the armamentarium of HF acid eye injuries.
5. References