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Studying the Effect of Dexamethasone and Water Extract Apricot on Experimental Chemical Corneal Ulcer in Rabbit

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Summary

The present study was designed to evaluate the effect of apricot and dexamethasone on rabbit eye ulcer. Bilateral corneal ulcers were induced by a filter paper disk (4mm diameter) immersed with NaOH (1N) on central axis of cornea in 20 rabbits. These animals were divided into two groups as follows; the first group, the corneal ulcer was treated with dexamethasone eye drops 0.1% twice daily while the second group, the corneal ulcer was treated with water extract of apricot 2% twice daily.The clinical signs of corneal ulcer were evaluated by ophthalmoscope and fluorescent technique. After killing the animals, the corneas underwent routine histopathological examination on the 1st, 3rd and 5th day after treatment. Dexamethasone shows improvement in corneal ulcer through increasing the vascularization and infiltration of cells; also it causes an increase in congestion in blood vessels but increases thickness epithelium while the apricot group shows thick corneal epithelium associated with inflammatory cells. These findings indicate that apricot was good in treating for eye ulcer.

Keywords: dexamethasone; apricot; chemical corneal ulcer

دراسه تاثير الديكساميثازون والمسخلص المائي للمشمش في قرحه قرنيه العين المحدثة في الارانب

عبد الباري الفارس اشرف وليد عبد الرزاق رنا خلف عبد الصمد

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الخلاصة

تم تصميم هذه الدراسة لتقييم تأثير المشمش وديكساميثازون على قرحة العين. كان المستحدث لقرحة القرنية الثنائية من خلال قرص ورق الترشيح (MM٤ بقطر) مع هيدروكسيد الصوديوم في المحور المركزي من القرنية في ٢٠ أرنب. تم تقسيم هذه الحيوانات إلى مجموعتين تم علاج قرحة القرنية موضعيا في المجموعة الأولى تعامل مع ديكساميثازون ٠.١٪ والمجموعة الثانية تعامل مع المستخلص المائي للمشمش ٢٪. تم تقييم العلامات السريرية للقرحة القرنية بواسطة منظار العين وتقنية الفلورسنت. بعد قتل الحيوانات خضعت القرنيه للفحص النسيجي للفترات ١، ٣ و ٥ يوم بعد العلاج. ظهر تحسن في قرنية العين بالعلاج بالدكساميثازون من خلال زيادة الاوعية الدموية ووجود خلايا التهابيه كذلك لوحظ زيادة في احتقان الاوعية الدمويه وظهر زيادة في سمك الظهارة. اما المجموعة الثانيه المعالجة بواسطة مستخلص المشمش ظهر سمك في ظهارة القرنية مرتبط مع خلايا التهابيه ادت الى تحسن كامل في ٥ أيام. هذه النتائج تشير إلى ان المشمش وكان حسن المعاملة لقرحة العين.

كلمات البحث: الدكساميثازون، المشمش، قرحة العين الكيميائيه

Introduction

Apricot seeds or kernels (*Prunus armeniaca*) are classified under the *Prunus* species of *Rosaceae* family of the Roseles group. Apricot seed is the small kernel enclosed within the wood-like pit at the center of the apricot fruit. Also, the seed of apricot contain oil; this

oil contains olein, glyceride of linoleic acid and a transparent, crystalline chemical compound, amygdalin, or laetrile. This compound is also known as vitamin B17 (Durmaz and Alpaslan, 2007.; Gomez *et al*, 1998). Apricot kernel is a rich source of oil with up to (54.21%) protein (17.75-22.56%) carbohydrates (21.16-35.26%) crude fiber (0.84-4.71%) and diatory fiber (6.03-22.24%) (Ruiz *et al.* 2006, Beyer and Melton 1990). Antioxidant properties of apricot kernels can it used as antimicrobial, particularly due to its antibacterial activity against Gram-positive, Gram-negative and Candida strains (Yigit *et al*. 2009; Joshi, *et al*., 1993).Kernels are used in the production of oils. Some uses of apricot kernel oil are in cosmetics and for medical purposes (Ibrahim *et al*, 2011; Abd El-Aal *et al*., 1986). Kernels or seeds of apricot grown in central Asia and around the Mediterranean are so sweet that they may be substituted for almonds. Oil pressed from these cultivar kernels, and known as oil of almond, has been used as cooking oil. Kernels contain between 2.05% and 2.40% hydrocyanic acid, but normal consumption is insufficient to produce serious effects. Cyanogenic glycosides are found in high concentration in apricot kernels. Laetrile, a purported alternative treatment for cancer, is extracted from apricot seeds. Apricot seeds were used against tumors. Apricot oil was also used against tumors, swelling and ulcer. Amygdalin (laetrile) may offer a valuable option for the treatment of prostate cancers (Ebtehal, 2011; Nostro *et al*, 2011; Sefer, *et al*., 2006).Corneal wound and inflammation is one of the most common ocular affection in both human beings and animals and can lead to blindness or even cause loss of the eye itself (Oliver, 2003). Because there is a little information about the effect of apricot kernels on corneal wound, the objective of the present study aims to determine the effect of apricot kernels on corneal wound.

Materials and Methods

The Experimental Animals:

20 rabbits of both sexes weighing between 2.5-3.5 kg were used in this study. The rabbits were clinically healthy and kept in cages at animal house, College of Veterinary Medicine, University of Basrah. Food and water were given freely during the adaptation period.

Animal's preparation and anesthesia:

Rabbits were anesthetized by intramuscular injection of 10 mg/kg xylazine and 25 mg/kg ketamine HCl (Hall and Clark 1991). The experiment was carried out on both eyes of each animal.

Materials and Equipments:

1- NaoH 1 N:

NaoH 1N prepared according the following formula (Saadalla, 1980)

$$N\% = \frac{\text{Wt.}}{\text{Eq.wt.}} X \frac{1000}{V}$$

2- Fluorescent strip:

Fluorescein sodium ophthalmic strips each contain 1mg fluorescein sodium USP (Laboratory Chauvin France).

3- Ophthalmoscope:

Welchallyn Company REF 11710 USA (figure 1)

Figure 1: Ophthalmoscope instrument

Surgical Technique for Induced Ulcer:

A round 5-mm diameter circular filter paper disk produced by standard paper bunch was immersed in 1 N NaoH for 5 seconds; the filter paper (Whatman No.3) was used because it is easily molded to the cornea when wet. When the rabbit was anesthetized, the eyelid was secured in the open position using a wire lid speculum. The immersed filter paper disk was placed on the central corneal surface, centered on the pupil and held gently in position with thumb forceps for 30 seconds (Ormerod et. al. 1989) (Figure 2, Figure 3 and Figure 4). Corneal ulcer induced bilateral in each rabbit.

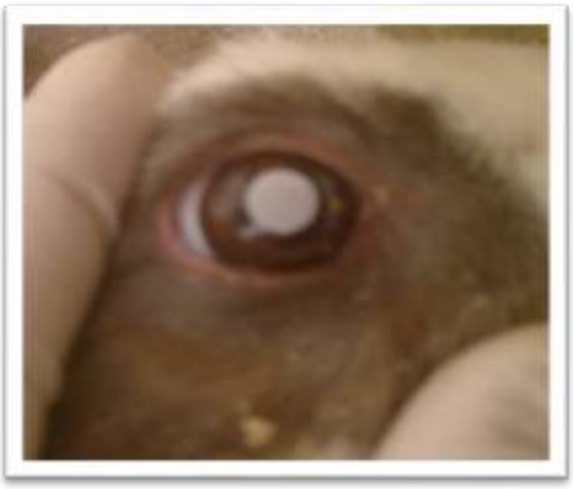

Figure 2: Induced alkali corneal injury

Figure 3: alkali corneal ulcer Figure

Figure 4: fluorescent strip put in eye after injury

Fluorescent technique:

A piece of blotting paper containing fluorescent dye will be touched to the surface of cornea. Blinking spreads the dye around and coats the "tear film" covering the surface of the cornea (Figure 4). By ophthalmoscope a blue light is then directed at rabbit eye. The experimental ulcer on the surface of the cornea will be stained by the dye and appear green under the blue light (Figure 5). This test was used to determine the size, location, and shape of coronial ulcer depending on the spreading of the dye between the damaged tissues of cornea (Feenstra and Tseng, 1992).

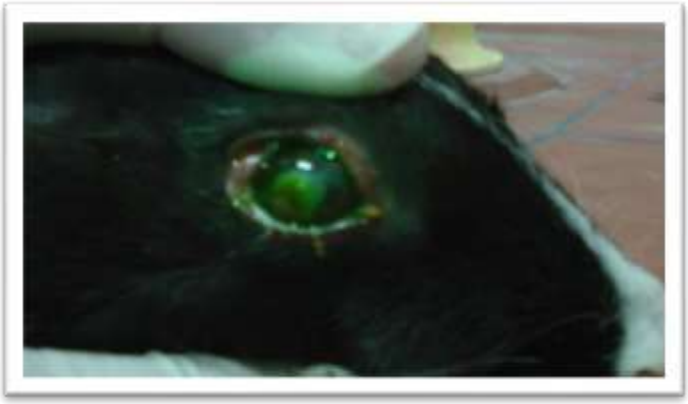

Figure 5: Ulcer is stained with green color

Study parameters:

Clinical evaluation:

External examination of each eye were done once daily over the entire course of the study. Detailed ophthalmic examination of each rabbit was performed every day following the onset of corneal ulceration. Eyes were examined for the presence of perforation, vascularization, lacrimation photophobia eye lids adhesion and pus.

Histopathological examination:

On the day 1^{st}, 3^{rd}, 5^{th}, 10^{th}, post induced corneal ulcer sacrificing the animals, the anterior chamber of eye was entered with a scalpel blade, and the entire cornea was excised from the eye with corneal scissors. Corneas were immediately placed in 10% formalin. Following fixation, routine tissue processing was carried out, the tissues section were stained with Hematoxylin Eosin (H&E) (Luna, 1968). Histopathological section and staining were done at Histology and Anatomy Branch of Veterinary Medicine College / Basrah University.

Preparation of extracts

The fresh fruit samples were transported to the laboratory where the stones were removed, and individual stones were hammered to obtain the seed kernel. Next, the skin was removed, and the kernels were dried on the bench and then ground to powder using an electric blender. Water extracts were also prepared by adding 20 g powder material to 200 ml boiling water in a glass flask, and the solutions were incubated at room temperature for 2 hours on a rotating shaker 200 rpm. The mixture was filtered through filter paper, and the filtrate was lyophilized. All extracts were stored in a freezer at -20C° until use (Femenia, *et al.*, 1998)

Results

Apricot group

On 1 day histopathological section showed a thick corneal epithelium associated with vascularization of sub epithelium, induce ulcer in apricot group (Figure 1).

Figure 1: Histopathological section of induced ulcer in apricot group on 1st day, (A) thick corneal epithelium (B) vascularization of sub epithelium, (H&E 10X)

On 3 day histopathological section showed an increase in proliferation of corneal epithelium and infiltrated of inflammatory cell (Figure 2).

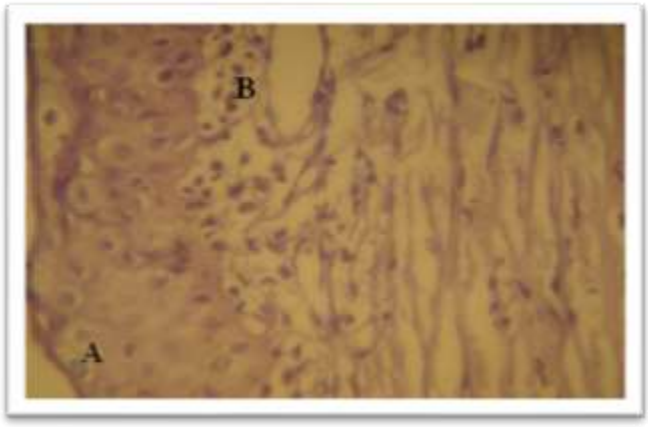

Figure 2: Histopathological section of induced ulcer in apricot group on 3rd day (A) proliferation of corneal epithelium (B) infiltrated of inflammatory cells. .(H&E10X)

On 5 day histopathological section showed that there is no ulcer, it may properly heal (Figure 3).

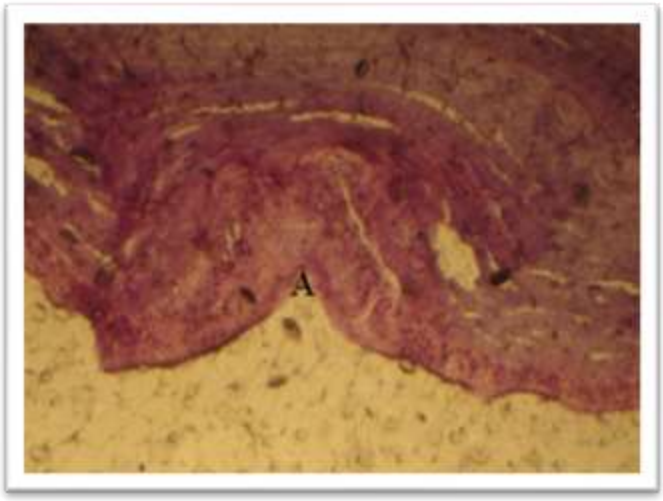

Figure 3: Histopathological section of induced ulcer in apricot group on 5th day there is no ulcer, (A) properly healed, (H&E 10X).

Dexamethasone group

On 1 day histopathological section showing an increase in vascularization with the presence of inflammatory cell in sub epithelium layer (Figure 4).

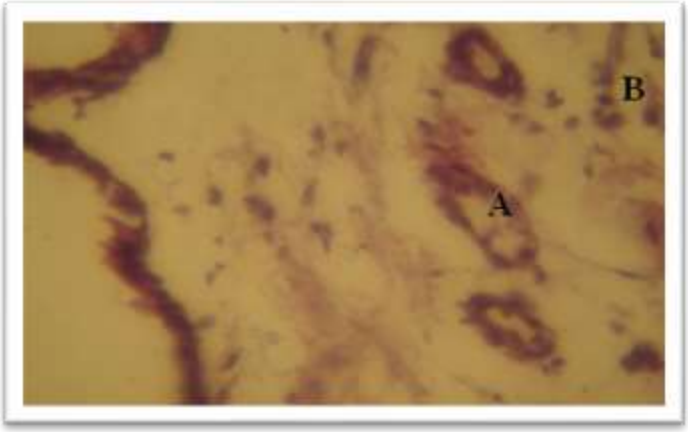

Figure 4: Histopathological section of induced ulcer in dexamethasone group on 1st day (A) vascularization with present of inflammatory cell in sub epithelium layer (B), (H&E 10X)

On 3 day histopathological section showed increase congestion (Figure 5).

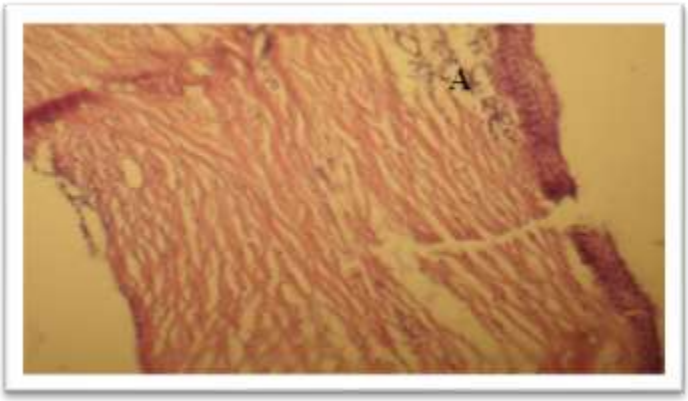

Figure 5: Histopathological section of induced ulcer in dexamethasone group on 3rd day (A) congestion (H&E 10X)

On 5 day histopathological section showed an increase thickness and normal epithelium (Figure 6).

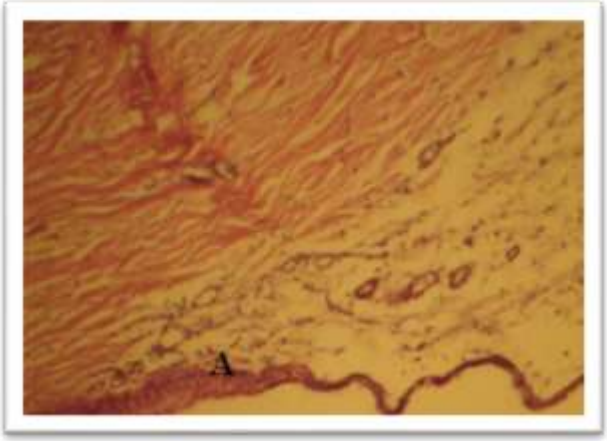

Figure 6: Histopathological section of induced ulcer in dexamethasone group on 5th day (A) thickness and normal epithelium (H&E 10X)

Discussion

Corneal ulcer may change the corneal transparency and affect vision due to the inflammatory reaction involving invasion by inflammatory cells which agrees with Rashid *et al*. (2007). According to our results, dexamethasone showed an improvement corneal ulcer, on 3-5 days after injury but conjunctival edema was present that may be because of inflammatory cells infiltration as shown in histopathological examination of dexamethasone group this result also reported by other researcher (Laria, *et al*. 1997). The early use of corticosteroids reduced initial inflammatory cell infiltration during the acute phase that is effective in preventing corneal ulceration especially during the 3rd day after inducing the corneal ulcer (Davis, *et al*. 1997, Khayyal *et al*.1993).In previous studies, it has been shown that eye ulcer treatment is very difficult, but the apricot extract possesses significant anti-inflammatory properties due to its high vitamin-E content and skin softening properties as aromatherapy, rich in essential fatty acids like oleic acid and high in vitamin A as well as a cooling effect and acts as anti-inflammatory that improved (Yigit, *et al*. 2009, Rashid *et al*. 2007, Nostro, *et al*. 2000).

Reference

1- Abd El-Aal, M. H.; Hamsa, M. A.; and Rahma, E. M. (1986). In vitro digestibility, physicochemical and functional properties of apricot kernel proteins. Food chemistry J. 19: Pp 197-211.

2- Beyer, R.; and Melton, L. D. (1990). Composition of New Zealand apricot kernels. New Zealand J. of Crop Horticulture Science. 18: Pp 39-42.

3- Davis, A. R; Ali, Q. A.; Aclimandos, W. A.; and Hunter, P. A. (1997). Topical steroid use in the treatment of ocular alkali burns. Br. J. Ophthalmol. 81: Pp 732-734.

4- Durmaz, G.;and Alpaslan, M. (2007). Antioxidant properties of roasted apricot (*P. armeniaca* L.) Kernel. J. Food Chem. 100: 1177-1

5- Ebtehal, H. A. (2011). The cytotoxic effect of apricot seed (prunus sarmeniaca) on human and animal tumor cell line. Biotechnology research centre-Al-Nahrin University- Iraq. 6(3): Pp 6-12.

6- Feenstra, R. P. G. and Tseng, S. C. G. (1992). Comarision of Fluorescein and Rose Bengal Staining. Ophthalmology. J. 99: Pp 605-617.

7- Femenia, A.; Rosello, C.; Mulet, A.; Canellas, J. (1998). Chemical composition of bitter and sweet apricot kernels. J. Agric. Food Chem. 43: 356-361.

8- Gomez, E.; Burgos, L.; Soriano, C.; and Marin J. (1998). Amygdalin content in the seeds of several apricot cultivars. J. Sci. Food Agric. 77: 184-186.

9- Hall, L. W.; and Clark, K. W. (1991). Veterinary anesthesia 9th. Ed., Great Britain by press. Avon. Ltd, Filey, North, Your-Shir. Pp 339-350.

10- Ibrahim, G.; Haydar, H.; Mehmet, M.; Derya, A.; Bayram, M.; and Ahmet, U. (2011). Physico-chemical properties of apricot (prunus armenaca L.) kernels. South Western J. of Horticulture, Biology and Environment. 2(1): Pp 1-13.

11- Joshi, D. C.; Das, S. K.; and Mukherji, P. K. (1993). Physical properties of pumpkin seeds. Agriculture and Engineering research J. 54: Pp 219-229.

12- Khayyal, M. T.; El-Ghazaly, M. A.; and El-Khatip, A. S. (1993). Mechanism involved in the anti-inflammatory effect of propolis extract. Drugs Exp. Clin. Res. 19: 197-203.

13- Laria, C.; Alio, J. L.; and Ruiz-Moreno, J. M. (1997). Combained non-steroidal therapy in experimental corneal injury. Ophthalmic Res. 29: 145-153.

14- Luna, L. G. (1968). Manual of histologic staining method of armed forces institute of Pathology. 3rd. McGraw-Hill Book Company. New York.

15- Nostro, A.; Germano, M. P.; D'angelo, V.; Marino, A.; and Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. J. 30: 379-384.

16- Olivier, F. J. (2003). Bacterial corneal diseases in dogs and cats. Clin. Tech. Small Anim. Pract. 18: 193-198.

17- Ormerod, L.; Abelson, M.; and Kenton, K. (1989). Standard models of corneal injury using alkali immersed filter disc. Invest Ophthalmol. Vis. Sci. 30: 2148-2153.

18- Rashid, F.; Ahmed, R.; Mahmood, A.; Ahmad, Z.; Bibi, N.; and Kazmi, S. U. (2007). Flavonoid glycosides from *Prunus armeniaca* and the antibacterial activity of a crude extract. J. Arch. Pharm. Res. 30: 932-937.

19- Ruiz, D.; Egea, J.; Tomas-Barberan F. A.;and Gil, M. I. (2006). Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin color. J. Agric. Food Chem. 53: 6368-6374.

20- Sefer, F.; Misirli, A.; and Gülcan, R. A. (2006). research on phenolic and cyanogenic compounds in sweet and bitter apricot kernels. Acta Horticulturae J. 701: 167-169.

21- Yigit, D.; Yigit, N.; and Mavi, A. (2009). Antioxidant and antimicrobial activities of bitter and sweet apricot kernels. Braz. J. Med. Biol. Res. 42 (4): 346-352.