

COMPARATIVE STUDY ON CLINICAL AND HISTOLOGICAL EFFECT OF GRAPE SEED EXTRACT AND GENTAMICIN ON CORNEAL ULCER IN PIGEON

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ABSTRACT

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The present study was showed excellent healing of grape seed extract of eye corneal ulcer of pigeons compare with control and gentamicin groups. Grape seed extract which composite many biochemical components have bioactive to recover and heal ulceration of the cornea. Cornea is a connective tissue to have collagen and fibers and the defect of cornea, therefore, the recovery is slow. The grape seed extract group showed good healing of corneal ulcer that evident the extract has good bioactive component assist in healing compare two groups. Histopathological images reveal to excellent healing. In conclusion the grape seed extract is an excellent agent to treat eye, corneal ulcer in pigeons and other animals.

Key words: Ulcer, grape seed, gentamicin, pigeons

Introduction

Grape seeds are a by-product of making wine from grapes. Grape seed is also known as grape seed extract, grape seed oil, grape seed extract has been used in alternative medicine as a possibly effective aid to treat certain symptoms of chronic venous insufficiency (decreased blood flow back from the feet and legs to the heart). These symptoms include pain or heaviness, tension or tingling, and a feeling of heaviness in the legs (Chedea *et al.*, 2011). Grape seed is a healthy food supplement that comes from the seeds of wine grapes. This dietary supplement is more commonly referred to as grape seed extract or GSE, although it could be confused with grapefruit seed extract, also known as GSE (Furiga *et al.* 2009). Grape seed extract or GSE contains the phenolic compounds of grape seed extract, including oligomeric complexes of proanthocyanidin (OPC), catechin, epicatechin, procyanidins, gallic acid and gallo catechin (Jayaprakasha *et al.*, 2003). The effects of a single 400 mg dose of proanthocyanidin GSE followed immediately by 6 hours of sitting. It has been shown to reduce leg swelling and oedema by 70%, compared to not taking GSE (Kulkarni *et al.*, 2011).

The cornea is clear and appears to be devoid of substance, in fact a highly organized group of cells and proteins. Unlike most tissues in the body, the cornea does not contain blood vessels to nourish it or protect it from infection. Instead, the cornea feeds on tears and watery humor (Shehan,2012). The corneal stroma performs several basic functions in the eye. Optically, this is the primary refractive lens and therefore must combine near perfect transmission of visible light with a precise way of focusing incoming light. In addition, it must be mechanically extremely strong to protect the internal contents of the eye. These functions are governed by their structure at all hierarchical levels. The basic principles of corneal structure and transparency have been known for some time, but in recent years, X-ray scattering and other methods have revealed that the details of this structure are much more complex than we

know previously thought and that the complexity of the arrangement of the collagen lamellae provides the shape and mechanical properties of the tissues (Feizi,2018).

Corneal ulcer is an infection of the cornea. The cornea is the transparent window at the front of the eye. Symptoms and complications vary from case to case. It can also depend on the source of the infection. Some common symptoms include: blurred vision, eye pain, sensitivity to light, redness, and a white patch on the cornea (Dalmon, 2012).

Materials and Methods

Twenty-four mature pigeons have been utilized in the present research. The birds have been clinically healthy and kept in cages at the animal house, Veterinary Medicine College, Univ. of Basrah. Water and food have been freely provided through in the period of the adaptation. The experimentation has been performed on one eye of every one of the animals.

Method for inducing ulcer

A round 5 mm diameter circular filter paper disk that has been produced with the use of the standard paper bunch, this disk has been immersed in 1N NaOH solution for 5 sec; filter paper has been utilized due to the fact that it is molded easily to cornea in the case where it is wet. The eye-lid was secured manually in open position. The filter disk has been placed upon the surface of the central corneal, centered on pupil and gently held in place wit for 30 sec. The eye corneal ulcer induced unilateral in every one of the pigeons (Fig.1, 2, 3). The twenty-four pigeons were divided into two separated groups. Ten birds were treated with watery soluble grape seed twice a day (grape seed treated group) and the other ten birds were treated with Gentamicin eye drops solution 0.3% w/v also twice a day (gentamicin treated group).

Clinical estimation

The external and detailed ophthalmic inspections of both eyes have been performed (Fig.4). The eyes have been

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Induced corneal ulcer unilateral



Fig. 1:a



Fig. 2: b



Fig.3:c



Fig. 4: d

Fig.1: a, b:Filter paper disc saturated with 1N NaOH solution was put on the corneal surface of the pigeon. C: Eye corneal ulceration was obvious with lacrimation and eyelids adhesion. d: ophthalmoscope to examine eye ulceration



Fig. 5: Eye picture after three day of Grape seed extract group



Fig. 6: Eye picture after three day of gentamicin group



Fig. 7: Eye picture after three day of control group



Fig.8: A. Grape seed extract group: Transverse section of the cornea revealed a noticed degree of clean corneal regeneration processes of the epithelial layer which shows a regenerated polygonal layer as well to bowman's layer (black arrow), in addition, there are a normal architecture of the stromal layer of the cornea (blue arrow). H&E stain. 10X

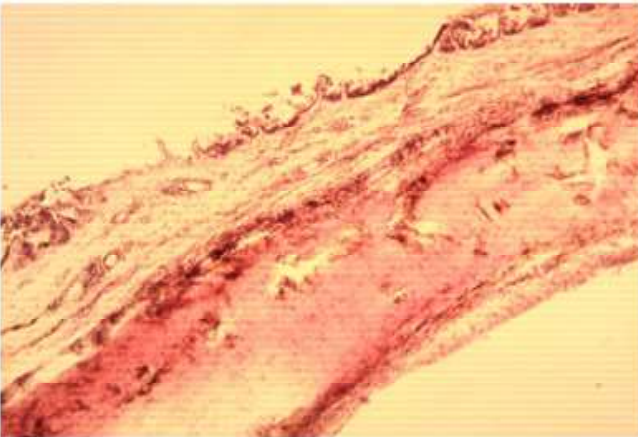


Fig.9: A. Grape seed extract group: Transverse section of the cornea revealed a delayed corneal regeneration of the epithelial layer (black arrow), in addition, there are an inflammatory cells infiltration in the stromal layer of the cornea as well to noticed granulation tissue formation (blue arrow). H&E stain. 10X

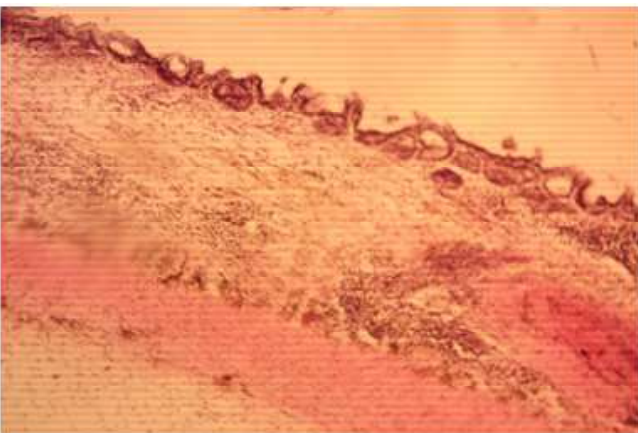


Fig.10: B. Gentamicin group :Transverse section of the cornea revealed a degree of corneal regeneration processes of the epithelial layer (black arrow), in addition, there are an inflammatory cells infiltration in the stromal layer of the cornea (blue arrow). H&E stain. 10X

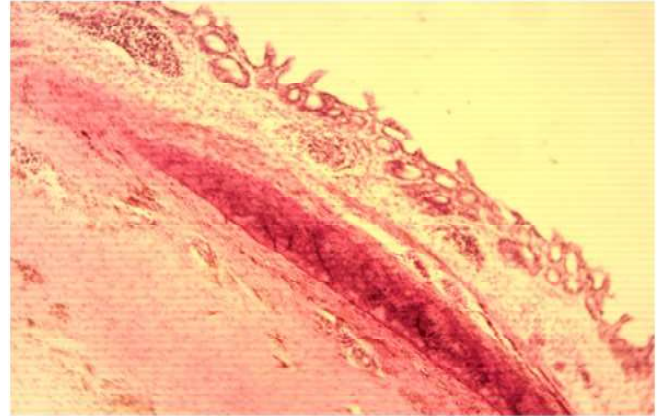


Fig. 11: B. Gentamicin group: Transverse section of the cornea revealed a degree of Fig. 11: Corneal regeneration processes of the epithelial layer which shows a regenerated polygonal layer as well to bowman's layer (black arrow), in addition, there are a complicated granulation tissue formation in the stromal layer of the cornea (blue arrow). H&E stain. 10X

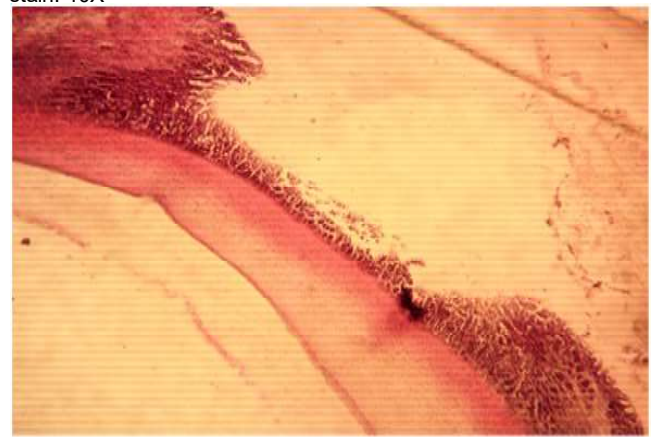


Fig. 12: C. Control group: Transverse section of the cornea revealed a deep desquamation of the epithelial layer which shows an absent of squamous and polygonal layer as well to somewhat of bowman's layer accompanied by the severity of corneal ulceration (black arrow). H&E stain. 10X

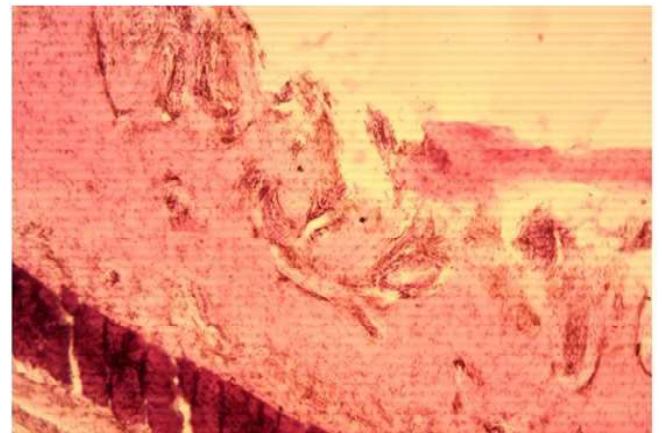


Fig. 13: Control group: Transverse section of the cornea revealed a deep desquamation of the epithelial layer which shows an absent of squamous and polygonal layer as well to somewhat of bowman's layer accompanied by the severity of corneal ulceration (black arrows). H&E stain. 10X

inspected for the presence of corneal ulcer, eyelids adhesion, lacrimation, infection, or pus.

Histopathological examination

On 10th, 20th days following the induced corneal ulcer that sacrifices the birds, the entire eye has been excised from the head with curved surgical scissor. Eyes were immediately placed in 10% formalin. The samples were sent to a private laboratory, routine processing of the tissue, the sections of the tissues have been stained by the Hematoxylin–Eosin stains (H&E) (Luna, 1968).

Results and Discussion

Clinical symptoms

Grape seed extract group were gradually recover after 3rd day while gentamicin group were recover after 7th day as well as control group were showed adhesion and granulation tissue (Fig. 5, 6, 7).

The chemical composition of grape seed plays a major role to treat ulcerative lesion of cornea (Bankova *et al.*, 2000). According to chemical composition and what each one play role to treat and accelerate healing of corneal ulceration. Polyphenols and flavonoids have antimicrobial and antibacterial characteristic against *Pseudomonas*, *Staphylococcus aureus*, and bacteria. Gentamicin is also an effective agent against *Staphylococci* and some species of *Streptococci* other than *S. pneumoniae*. It inhibits bacterial protein synthesis via binding to RNA polymerase (Grange *et al.*, 1990).

On the fifth day after inducing the corneal ulcers, the clinical symptoms of the eyes in both groups that were almost identical due to the same of the lesions showed lacrimation, opacity, but no eyelid adhesion. Caffeic acid phenethyl ester have antiviral and antifungal activity against eye microorganism, Antimicrobial activity is recognized as the most important property of grape seed, particularly activity against bacteria. Several studies have been performed to evaluate this property against a large group of Gram-positive and Gram-negative bacteria; both aerobic and anaerobic types (Inokuchi *et al.*, 2006). Other chemical compositions such as Terpenes, essential oil, and Furfuran lignans also have antimicrobial activity. However, in the gentamicin treated group, clinical results showed notable improvement. Corneal ulcers can range from small epithelial erosions that will heal in less than a week to a descemetocoele that may distract by the end of the day. Treatment of such varied lesions depends on a correct assessment of how deep the ulcer is and whether it is in the process of healing (Williams, 2014).

The antiviral activity of aqueous and ethanol extracts of grape seed and constituents, such as flavonoids caffeic acid, p-coumaric acid, benzoic acid, galangin, pinocembrin and chrysin, was tested against herpes simplex virus type 1 (HSV-1) (Huang *et al.*, 2014). Each antibacterial, antifungal and antiviral the first line to treat the ulceration, and prepare to active other composition to heal the ulceration, the second step are the biological and pharmacological activity of the volatile compound of grape seed such as terpenes and terpenoid, they have pharmacological activity to treat ulceration of the cornea, these compound have hydro carbonic compound such as Alkan, alkenes, monostearate, diesters and enzymes such as succinate dehydrocarbonase, glucose-6-phosphate,

adenosine triphosphatase, and acid phosphatase, all these compound promote collagen and fibroblast activity to accelerate healing of ulceration and comforted the area to against microbes (Raghukumare *et al.* 2010, Amoros *et al.* 1992, Xuan *et al.* 2011, Petrova *et al.* 2010, Melliou *et al.* 2004, Hegazi *et al.* 2002, Arora *et al.* 2013). Gentamycin antibiotic 3% only role against antibacterial and doesn't role in the promotion of collagen and fibroblast activity (Sharma *et al.*, 2014). Histopathological changes in gentamicin group were showed intact corneal epithelium while grape seed group showed inflammatory cells infiltration compare with control group was showed normal epithelium and stroma, other feature of the grape seed of grape seed mononuclear cells and white arrow (recovered whole epithelium), these different change due to pharmacological activity of grape seed, this results agree with most research such as Georgiadis *et al.* (2008), Paris *et al.* (2013), Reddy *et al.* (2013), Meek (2008) and Imayasu *et al.* (2010). After the 20th day, the two groups did not show apparent differences, as the eyes looked close to normal, meaning that both treatments contribute to protecting and improving the healing of the corneal ulcer. Nevertheless, the gentamicin eye drops did better at improving healing clinical signs (Fig. 8-13).

On the day 10th, histopathological sections of grape seed treated corneal tissues showed desquamation of corneal epithelium, edematous stroma with inflammatory cells, and congestion of blood capillaries. But, the antibiotic-treated group showed the intact epithelium and stroma of the cornea with the keratocytes. That is meaning grape seed showed a prolonged inflammatory stage or delayed corneal healing

Conclusion

We can use grape seed extract solution in the treatment of eye cornea in avian and small animal practice can be used.

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