

Effects of Chitosan and Hyaluronic Acid in Healing of Chemically Induced Oral Ulcer in Rabbits

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Abstract

Twelve healthy, local bread male rabbits were used in this study, weighting (1.5-2) kg , divided equally into three groups: in First group (Control group), the ulcers were induced by local application of Sulfuric Acid in concentration 38% in rabbit cheek mucosa ,animals were left without treatment. In second group, same procedure was performed then 0.03 gm of chitosan raw was applied locally on ulcer site twice daily at 1st, 2nd, and 3rd day post induce ulcer while animal under general anesthesia. In third group, same procedure was performed, then saturated gauze with 5 drop of hyfresh drug contain hyaluronic acid 2.0 mg/ml were applied locally on ulcer site twice daily at 1st, 2nd, and 3rd day post induces ulcer while animal under general anesthesia. Grossly finding at 3rd day shows limited area of necrotic mucus membrane (m.m) with presence of sluphing area in the second group, while in the control and third groups at same period showed presence of necrotic area of (m.m) surrounded by elevated congestion (m.m), as well as at day 7 evidence regression of necrotic area with definite re-epithelization area in the second group, while in the third group showed advance in regression of necrotic area when compared with earlier period and beginning of re-epithelization, in control group record decreased in necrotized area surrounded by congested area and also small ulcer area was found. Histopathological results in control group at 3rd day showed inflammatory cells particularly neutrophils infiltration between necrotic muscle fibers with hemorrhage in subepithelial layer while in the second group showed granulation tissue consisting from congested blood vessels and marked fibroblast proliferation with collagen fiber deposition, but in the third group showed presence of inflammatory cells particularly neutrophils and macrophages between necrotic muscle fiber. At day 7 in control group showed severe inflammatory cells particularly neutrophils infiltration between marked necrotic muscle fibers, while In the second group at the same period showed thickness collagen fibers with macrophages and lymphocytes infiltration with congested blood vessels, in third group showed inflammatory cells with congested blood vessels, edema, necrotic debris with severe neutrophils infiltration between marked necrotic muscle fiber.

تأثير الكيتوسان و حامض الهالورونك في التئام قروح الفم المحدثه كيميائياً في الارانب

1 اريج كامل مهدي 1نادية حميد رجه الفلاحي 2حسين هادي ناهي

1كلية الطب البيطري- قسم الجراحة والتوليد - جامعة بغداد - بغداد - العراق

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الخلاصة

أجريت الدراسة على (18) أرنب محلي بالغ سليم ظاهرياً من الأمراض تراوحت أوزانها (1.5-2 كغم) قسمت إلى ثلاث مجاميع متساوية:

المجموعة الاولى (مجموعة السيطرة): تم تخدير جميع الحيوانات تخدير عام باستخدام مزيج الكيتامين والزايلازين وبجرعة (35 ملغ كغم كيتامين و 5 ملغم كغم زايلازين) حقنا بالعضلة وبعدها احدثت تقرحات في الغشاء المخاطي لخد الارنب الايمن بوضع 5 قطرات من حامض الكبريتيك بتركيز 38 %.

المجموعة الثانية (مجموعة الكيتوسان): تم تعريض حيوانات هذه المجموعة الى مثل المجموعة الاولى واستخدم في هذه المجموعة مسحوق الكيتوسان 0.03 غم وضعت على منطقة التقرح مرتين يومياً عند اليوم 1, 2, 3 بعد العملية بعد تخدير الحيوان.

المجموعة الثالثة (مجموعة حامض الهالورونك): تم تعريض حيوانات هذه المجموعة الى مثل المجموعة الاولى واستخدم في هذه المجموعة قطنه مشبعة ب 5 قطرات من عقار (هاي فريش) يحتوي على حامض الهالورونك بتركيز 0.2 % وضعت على منطقة التقرح مرتين يومياً بعد تخدير الحيوان عند اليوم 1, 2, 3 بعد العملية.

اظهرت النتائج العيانية عند اليوم الثالث بعد العملية في المجموعة الثانية وجود منطقة متخثرة من الغشاء المخاطي مع وجود منطقة منسلخة، بينما لوحظ في كلا من مجموعة السيطرة والمجموعة الثالثة عند نفس الفترة وجود منطقة متخثرة واحاطة منطقة التقرح بمنطقة محتقة مرتفعة، وعند اليوم السابع في المجموعة الثانية شوهد قلة المنطقة المتخثرة مع وجود منطقة نمو جديدة من الظهارة لكن لوحظ في المجموعة الثالثة قلة المنطقة المتخثرة عند مقارنتها بالفترة السابقة وبدأ نمو الظهارة، اما في مجموعة السيطرة سجل قلة في منطقة التخر مترافقة بمنطقة محتقة ولم تزل منطقة صغيرة من التقرح موجودة. وقد شوهد عياناً وكانت النتائج اكتمال الالتئام في المجموعة الثانية عند اليوم التاسع بينما لوحظ في المجموعة الثالثة عند اليوم الثالث عشر وفي مجموعة السيطرة عند اليوم الخامس عشر. نتائج الفحص النسيجي المرضي لمجموعة السيطرة والمجموعة الثالثة عند اليوم الثالث ارتشاح الخلايا الالتهابية ما بين العضلات المتخثرة لوحظ في المجموعة الثانية تكاثر واضح للارومات الليفية والنسيج الليفى الرابط. لوحظ عند اليوم السابع في مجموعة السيطرة ارتشاح حاد للخلايا الالتهابية خاصة خلايا العدلات ما بين الالياف العضلية المتخثرة بينما لوحظ في المجموعة الثانية أستبدال المنطقة المتخثرة جزئياً بالنسيج الليفى الرابط اما في المجموعة الثالثة فشوهد ارتشاح للخلايا الالتهابية مع تكاثر في النسيج الليفى الرابط.

نستنتج من هذه الدراسة ان مسحوق الكيتوسان (0.03 غم) قام بتسريع التئام قرحة الفم المحدثه في الارانب عند مقارنته بحامض الهالورونك (0.2 ملغم/مل)، بينما احتاجت مجموعة السيطرة وقت اطول لحدوث الالتئام بشكل كامل.

Introduction

Oral mucous membranes are specialized frail membranes and are susceptible to erosion for that it is a frequently occurred and producing painful “aphthae,” (1), the term aphthae means ulcer; it has been used for many years to describe areas of ulceration on mucous membranes this condition is also known as aphthous stomatitis (2). This may appear anywhere in the mouth, but are frequently found on the inside of the lower lip or cheeks, or on the sides or base of the tongue ,it painful and may be single or multiple, symmetric or irregular in shape (17), ulcer floor is initially yellowish but assumes a grayish as healing and epithelialisation proceeds. They are surrounded by an erythematous halo and some edema (3). Trauma is one of the most common causes of recurrent oral ulcers, results from mechanical, chemical, or thermal irritation of the mucosa. These are generally acute short-lived events producing painful ulcers, which heal readily within a few weeks without scarring (4).

Recurrent aphthous stomatitis (RAS), commonly known as canker sores, in most cases are limited to the oral mucosa and tend to be multifactorial in etiology rather than attributable to a single factor ,trauma, stress, hormonal influences, genetics, food allergies, infections, and immunologic factors are all suggested causes (5,6), a third category ,known as herpetiform ulcers, while Behcet’s disease is a multisystem chronic relapsing inflammatory disease of unknown cause, which is characterized by recurrent oral (aphthous) ulcers, genital ulcers(4).

The ideal treatment of aphthous ulcers would improve ulcer healing by stimulating mucosal cell growth and removing bacterial cells that otherwise retard the healing process (7,8).

Chitosan is a de-acetylated derivative from the bio- polysaccharide chitin which is present in insects’ exoskeletons, crustaceans’ shells and fungi cell wall, chitosan has shown high bioactivity; biodegradability; reactivity of the deacetylated amino group; selective permeability; polyelectrolyte action; antimicrobial activity; ability to form gel and film (9). Properly Chitosan is an excellent applicant for the treatment of oral mucositis. Its bio adhesive and antimicrobial properties offer the palliative effects of an occlusive dressing and the potential for delivering drugs (10) In additional recently hyaluronic gel 0.2% which used for treatment of oral stomatitis which evaluated the efficacy of topical use in patients with recurrent aphthous ulceration (11). Hyaluronic acid (HA) is a linear polymer of glucuronic acid N- acetylglucosamine saccharide. Most cells have the capacity to synthesis (HA) during some point of their cell cycle, the main function of (HA) appears to be in tissue healing, is implicated in a range of activities including activation and moderation of the inflammatory responses, promoting cell proliferation, migration, angiogenesis, and promoting re-epithelization via proliferation of basal keratinocytes and reducing collagen disposition and scarring (12).

Current study propose to study the potential therapeutic benefits of use chitosan powder and hyaluronic acid drops applies locally on accelerate healing of induced buccal ulcer by Sulfuric

Acid in rabbits.

Material and Methods

Twelve healthy, local bread rabbit were used in this study weighting

(1.5-2 kg), animals were divided into three equal groups.

Control group: Animals were initially weighted and later anaesthetized by intramuscular injection of mixture Xylazine- ketamine hydrochloride (Xylazine :5mg/kg B.w and Ketamine: 35mg/kg B.w), ulcer was induced in mucus membrane of left twice daily at 1st, 2nd, 3rd day after ulceration (Fig.1(2)) while the animal under general anesthesia.

Third group: Ulcers were induced in the same manner, then saturated gauze with 5 drops of Hyfresh drug each ml contain sodium hyaluronate 2.0 mg sodium chlorite (stabilized) as preservative applied locally on ulcer site twice daily (Fig.1(3)) at 1st, 2nd, 3rd day after ulceration while animal under general anesthesia.

cheek of all animals by local application of 5 drops of Sulfuric Acid in concentration (38%) (Fig.1(1)) ,animals were left without any treatment .

Second group: Ulcers were induced in the same manner, then 0.03 gm of chitosan powder was applied locally on ulcer site

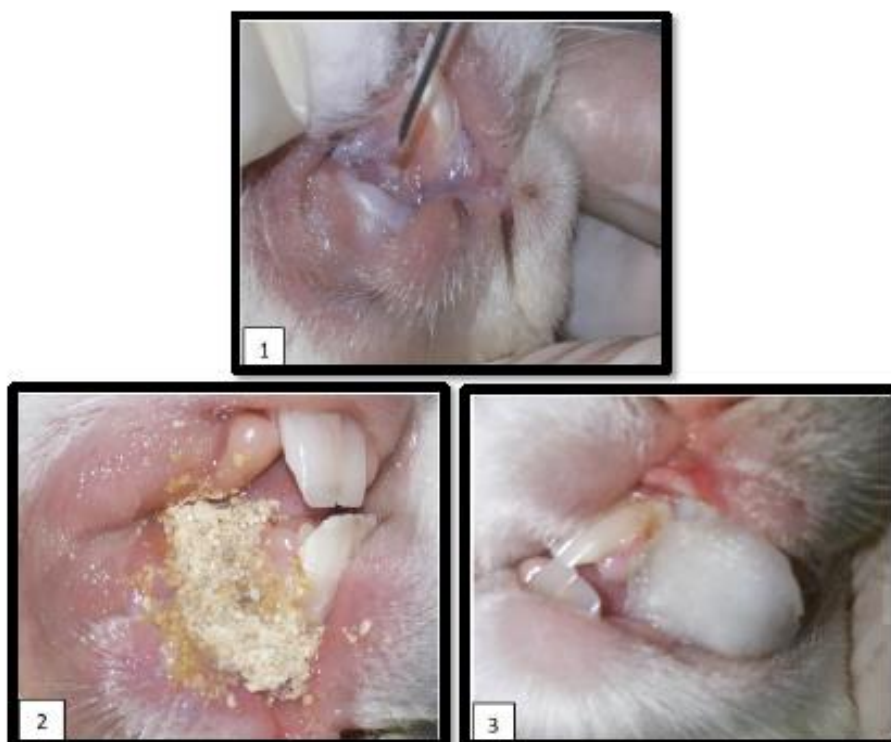


Figure 1: (1) Shows induce ulcer in mucus membrane of left cheek. (2) Shows chitosan powder application on induced ulcer. (3) Shows saturated gauze with 5drops of hyaluronic acid application on induced ulcer.

clinical fallow up was performed for 7 days to determine the changes in ulcerative site grossly and biopsies of ulcers were taken from buccal mucosa on days 3 and 7 after ulceration , biopsies were fixed with Hematoxylin-Eosin stains for histopathological examination for each group.

Results and Discussion:

Grossly finding: at day 3, presence of necrotic area surrounded by elevated congestion mucous membrane was observed in the control group, while in the second group at the same period showed presence of limited area of necrotic mucous membrane with area of sluphing, whereas in the third group showed area of necrotized mucous membrane surrounded by congested area (Fig. 2).



Figure 2: (4) Shows presence of necrotic area of mucus membrane also ulcer site surrounded by elevated congestion mucus membrane in control group at 3rd day. (5) Shows presence of limited area of necrotic mucus membrane with presence area of sluphing in second group at 3rd day. (6) shows presence of necrotized mucus membrane surrounded by congested area in third group at 3rd day.

At day 7 in control group recorded decrease in necrotized area surrounded by marked congested area and also small ulcer area was found, while at the same time in the second group showed regression of necrotic area with definite re-epithelization area, at the same period in the third group showed advance in regression of necrotic area and beginning of re-epithelization, as well as congested area was found (Fig. 3).



Figure 3: (7) Shows decrease in necrotized area surrounded by marked congested area and also small ulcer area is found in control group at 7 day . (8): Shows regression of necrotic area with definite re-epithelization area in second group at 7 day. (9): Shows advance in regression of necrotic area and beginning of reepithelization surrounded by congested area in third group at 7 day.

Histopathological results : At day 3 in the control group showed vacuolar degeneration, neutrophils infiltration and congested blood vessels, as well as showed severe inflammatory cells particularly neutrophils infiltration between necrotic muscle fibers, edema and inflammatory cells infiltration with hemorrhage in subepithelial layer (Fig. 4). In the second group at the same time histopathological section showed granulation tissue consisting from congested blood vessels and marked fibroblast proliferation with collagen fibers deposition and mononuclear cells infiltration between muscle layers with collagen fibers deposition with congested blood vessels in the injured area (Fig. 5). Despite the fact that in the third group at the same period showed presence of inflammatory cells particularly neutrophils and macrophages between necrotic muscle fibers, with mononuclear cells infiltration in collagen fibers adjacent to muscular layers (Fig. 6).

At day 7 in control group showed inflammatory cells particularly neutrophils infiltration between marked necrotic muscle fibers also showed severe congested blood vessels with hemorrhage in subepithelial layer as well showed large abscess consisting from aggregation dead and neutrophils, necrotic tissue encapsulated by fibrous connective tissue with congestive blood vessels (Fig. 7). In the second group at the same period showed mononuclear cells infiltration in the subepithelial layer, while showed thickness collagen fibers with macrophages and lymphocytes in congested blood vessels (Fig. 8). At the same time in the third group showed inflammatory cells in congested blood vessels with edema, necrotic debris and vacuolar degeneration, as well as severe neutrophils infiltration between marked necrotic muscle fibers (Fig. 9).

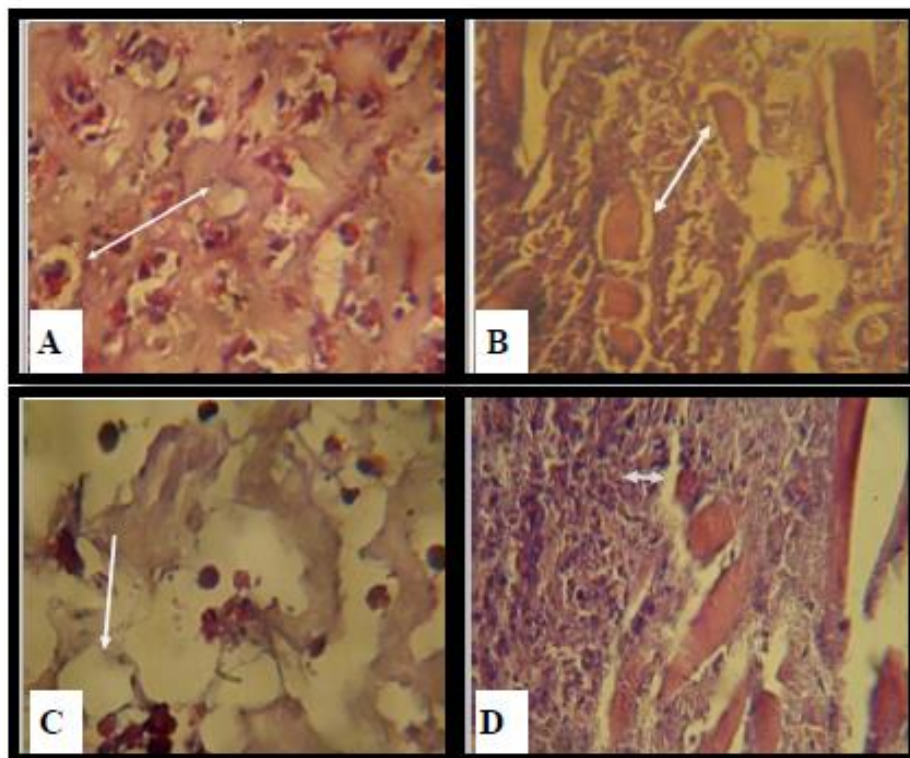


Figure 4: Histopathological section in the control group at day 3 post- injury (A) shows vacuolar degeneration, neutrophils infiltration and congested blood vessels (arrow). (B) shows severe inflammatory cells particularly neutrophils infiltration between necrotic muscle fibers (arrow) . (C) shows edema and inflammatory cells infiltration (arrow). (D) Shows inflammatory cells particularly neutrophils and macrophages between necrotic muscle fibers (arrow) (H&E stain 40).

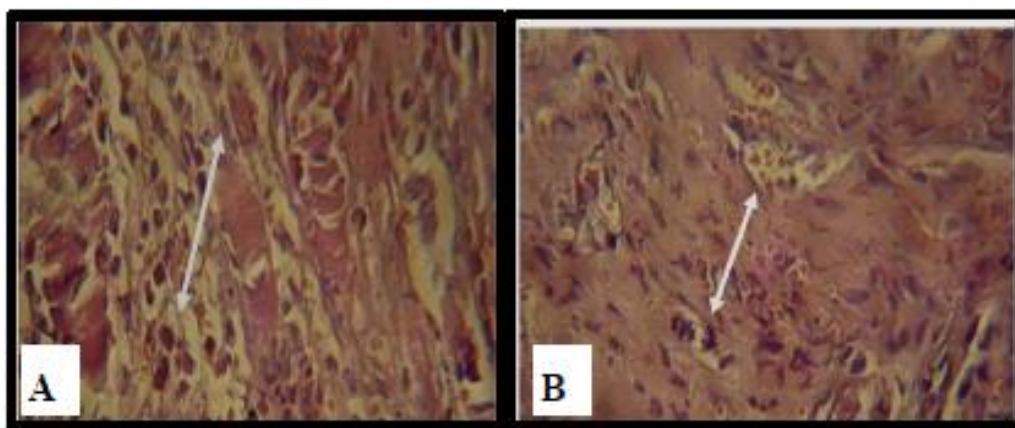


Figure 5: Histopathological section in the second group at day 3 post- injury. (A) Shows mononuclear cells infiltration between muscle layers (arrow). (B) Shows granulation tissue consisting from congested blood vessels and marked fibroblast proliferation with collagen fibers deposition (arrow) (H&E stain 40X).

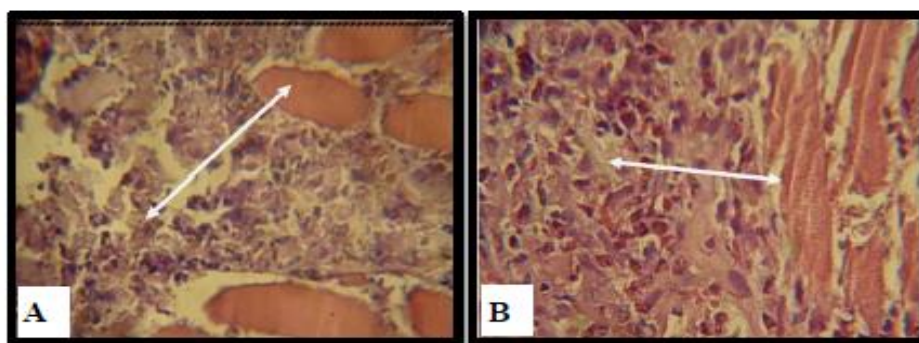


Figure 6: Histopathological section in the third group at day 3 post- injury. (A) Shows inflammatory cells particularly neutrophils and macrophages between necrotic muscle fibers (arrow). (B) Shows mononuclear cells infiltration in collagen fibers adjacent to muscular layers (arrow). (H&E stain 40X)

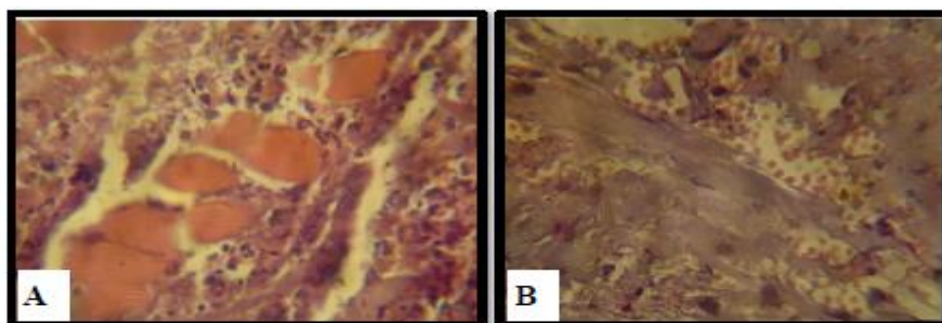


Figure 7: Histopathological section in the control group at day 7 post-injury. (A) shows severe inflammatory cells particularly neutrophils infiltration between marked necrotic muscle fibers. (B) Shows severe congested blood vessels with hemorrhage in subepithelial layer (H&E stain 40X)

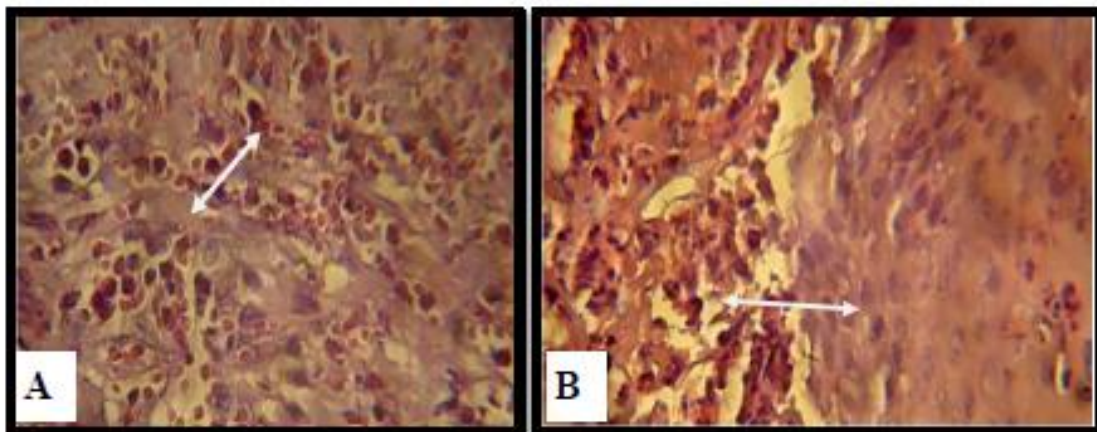


Figure 8: Histopathological section in the second group at day7 post-injury. (A) Shows mononuclear cells infiltration in the subepithelial layer (arrow). (B) Shows thickened collagen fibers with macrophages and lymphocytes in congested blood vessels (arrow) (H&E stain 40X).

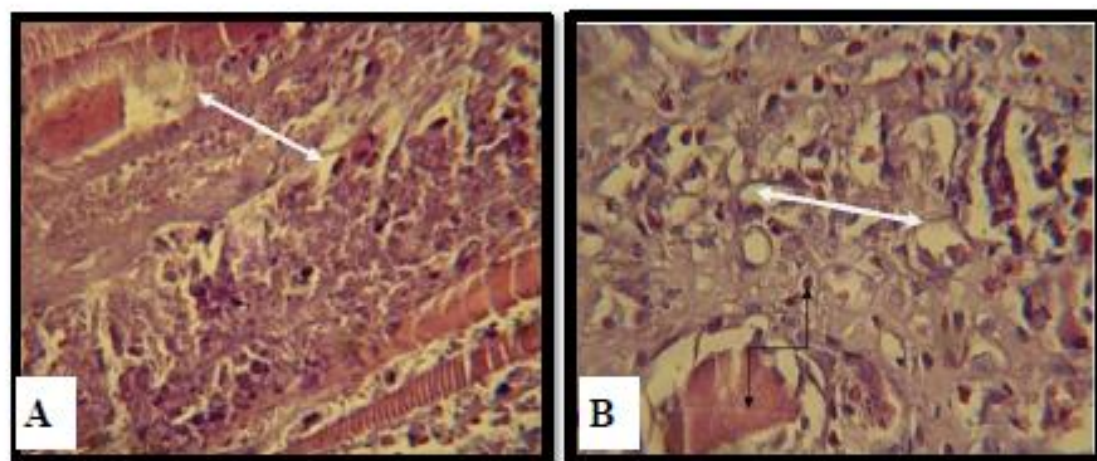


Figure 9: Histopathological section in the third group at day 7 post-injury. (A) Shows fibrous connective tissue replaced the necrotic area which extend to muscular layer fiber (white arrow). (B) Shows inflammatory cells in congested blood vessels(white arrow) with necrotic debris and vacuolar degeneration (black arrow) (H&E stains 40X).

The idea of choice chitosan powder 0.03 gm or hyaluronic acid 2.0 mg/ml topically in this study in treatment of oral ulcer are in relation to the knowledge that the chitosan has a recognized antimicrobial activity, being this, one of the main properties of the polymer. Several researchers observed that this polysaccharide has antimicrobial action in a great variety of microorganisms, including algae, fungi and bacteria and some researchers were used hyaluronic acid as a 0.2% solution for the treatment of recurrent aphthous ulcers in clinical trials and give a good results (6,13). Occasionally in this study didn't use any antibiotic to prevent any interfere with the action of chitosan or hyaluronic acid in treatment of buccal ulcer and the clinical follow up which recorded occurrence of ulcer infection actually one case in both control and third group, the occurrence of this infection may be due to the atrophy and ulceration of in case oral mucositis usually associated with an increased risk of infection, particularly when there is immunosuppressant and the most common pathogenic agent is *Candida* (14), this disagree with (15) who indicate that hyaluronic acid exhibits beneficial anti-inflammatory and antibacterial activity in the treatment of gingivitis and periodontitis, this condition not record in the second group this may be refer to the chitosan properties which is considered as an excellent candidate and antimicrobial properties for the treatment of oral mucositis(10), this accepted with the microscopic results that included less in inflammatory cell infiltration at this time when compared with the control or third group.

According to the grossly and histopathological results of this study showed vary in healing steps in all

groups that seen grossly progress in healing process in the second group

afterward third group when compared to control group at days 3 and 7, this differ in healing stages supported by histopathological results that found proliferation of fibroblasts and extensive fibrous connective tissue formation at day 3 and progress to replaced the ulcer site particularly by fibrous connective tissue at day 7 more earlier than the control and third group this agree with (7,8) the ideal treatment of aphthous ulcers would improve ulcer healing by stimulating mucosal cell growth and removing bacterial cells that otherwise retard the healing process and this agree with (16) offering that chitosan has not only delivery of therapeutic compounds but also exerting antimicrobial activity as well as the ability to stimulate cell proliferation and tissue organization agree with (17) who added accelerated ulcer healing also requires removal of bacterial contamination from the wound to provide favorable grounds for mucosal cell growth and repair. Microscopically at day 7 showed progress in replaced necrotic area by fibrous connective tissue in the second group while at this time showed start proliferation of fibrous connective tissue in the third group but the mucous membrane of ulcerative site not replaced in all group, this agree with (18) who refer course of oral ulcers healing varies from a few days to a little over 2 weeks, minor aphthae heal without scar formation, this agree with the grossly finding that showed complete healing record at day 9 in the second group and at day 13 in the third group while recorded at day 15 in the control group.

In conclusion oral ulcer healing accelerated by using of chitosan powder 0.03gm faster than hyaluronic acid 0.2 mg/ml when

used topically, while control group need more time to complete healing.

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