THE CLINICAL AND HISTOPATHOLOGICAL EFFECT OF CHITOSAN APPLIED LOCALLY ON THE HEALING OF EXPERIMENTALLY INDUCED TEAT FISTULA IN GOATS.

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Keywords; histopathological, Animals, Ruminal contraction

ABSTRACT

The clinical and histopathological the effect of chitosan applied locally on the healing of experimentally induced teat fistula in goats. Sixteen local breed goats have been used, aged range between (1.2-2) years with body weight (30+-5) kg. Animals were randomly allotted into two equal groups.

An artificial teat fistula of one cm.length was done for all animals under the effect of local analgesia and deep sedation. The first group, fistula was treated with chitosan 0.5gm and then closed by suture; while the second group (control one), fistula was closed routinely "via suture without any addition. After daily clinical follow-up, mild local reaction include local redness and swelling of the operative site and systemic mild hyperthermia, decrease ruminal contraction, increase respiratory rate.

Histopathological examinations were performed on all animals. Eight animals used for each group :(2 for each period).

Sections were stained with hematoxylin –eosin stain. Results of Histopathological examination in treated group reflected the presence of large numbers of neutrophils

In the first three days postoperatively , granulation tissue were seen in the sixty days which converted to cellular connective tissue in the ninth day with congested blood vessels .in addition there are lymphocytic aggregations ,arrangement of collagen fibers and formation of new epidermis during the 12th .days.

In conclusion we can say that chitosan play an important role in enhancing repair of teat fistula.

INTRODUCTION

Animal protein is essential for foodstuff and protects to biochemical and metabolic activation .economic evaluation of farm animals, depend on level of fertility and its production activity. Milk production depends on physical condition of udder and teats; the location of goat's udder exposes their teats to many adverse conditions Such as, moisture, cold, infection and various types of trauma and wounds. In many cases serious injury occur to the teat and udder from striking the udder and teats against solid objects frequently traumatize the tissue. The most important surgical affection of teats are wound and laceration which lead to incontrollable loss of milk when wounds penetrating the milk cistern which cause economic loss of production.

Strict antiseptic precaution and immediate surgical correction of wounds produced complete heal of the wound may occur by first intention. At the same time systemic treatment and local application of different chemical or materials will improve wound healing. [1]

Chitosan is a natural polymer obtained by deacetylation of chitin. Chitin is the second most abundant polysaccharides in nature after cellulose. The main commercial source of chitin is the shell waster of shrimp, crab, lobster, krill, and squid. It's a biologically safe, non toxic, biocompatible and biodegradable polysaccharide. Begin a bio adhesive polymer and having antibacterial activity, chitosan is a good candidate for site-specific drug delivery. [2].Chitin and chitosan have the same chemical structure, chitin is made up of a linear chain of acetyl glucosamine groups .Chitosan is obtained by removing enough acetyl groups (CH3-CO)for the molecule to be soluble in most diluted acids . this process, called deacetylation,releases amine groups (NH)and gives the chitosan a cationic characteristic. This is especially Interesting in an acids environment where the majority of polysaccharides are usually neutral or negative charged. [3][4] Chitosan exhibits myriad of biological actions, namely hypercholesterolemia, antimicrobial and wound healing properties low toxicity coupled with wide applicability makes it a promising candidate not only for the purpose of drug delivery for a host of drug moieties,((anti inflammatory, peptides, etc)) but also as a biologically active agent. Chitosan nano and micro particles are also suitable for controlling drug release. Association of vaccines to some of these particulates system has shown to enhance the antigen up take by mucosal lymphoid, these by inducing strong systemic and mucosal, immune response against the antigens. [5]

In this study we evaluate the effect of local application of chitosan Powder on the penetrating wound of teat which induced surgically (teat fistula) in goats in lactation period.

MATERIALS AND METHODS

The experiment was performed on 16 clinically healthy goats in lactation period. The animals were maintained under similar condition of feeding and management. Goats were randomly divided into two equal groups. the animals was fastened for 24 hours prior to operation ,the operative site prepared by clipping , shaving ,complete evacuation of udder from milk ,thorough cleaning and disinfecting of the teats surgery was performed under combination of sedation (xylazine hydrochloride in a dose of 0.2 mg /kg bw intramuscularly) .local analgesia by ring block technique infiltration of 2% lidocaine solution all around the base of the teat involved all the layer of teat wall .(mucosa, muscular layer ,skin) .Vasoconstrictors should not be added to solution used to produce ring block in teats for prolonged vasoconstriction may result in ischemic necrosis of the end of teat. tourniquet applied at the base of the teat for hemostasis .The site of operation covered with thin film of cotton saturated with 70%alcohol.Draping the operative site introduce teat siphon through the teat orifice as guide for surgical incision. 1cm surgical incision induced by scalpel on the medial aspect of the teat include All the thickness of the teat wall .

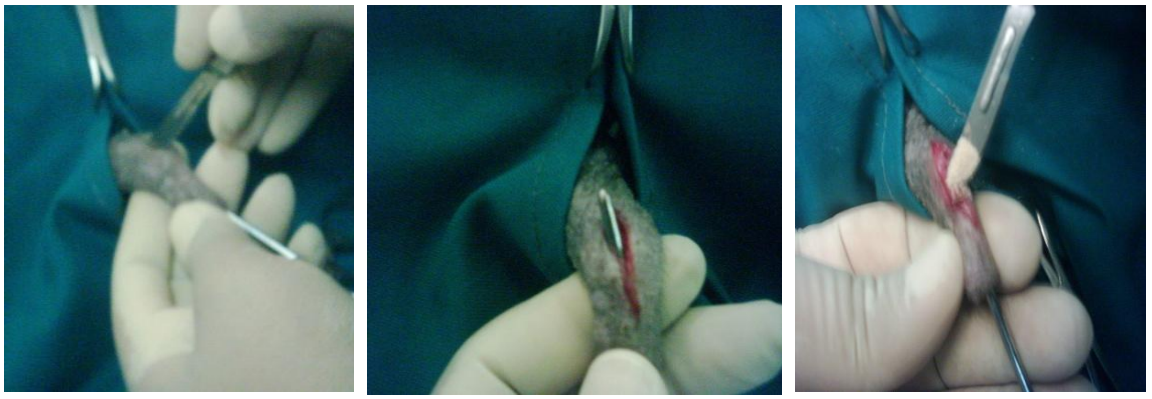
Treated Group:-

Consist of 8 goats; one cm length teat fistula was induced surgically in the wall of teat. Local application of 0.5 gm of sterile chitosan powder to evaluated the effect of chitosan on wound healing (The sterilization time should be as short as possible to attain both sterility and minimal degradation of the polymer. 10 min. autoclaving time is sufficient to sterilize the chitosan. [6] Was added after closure of mucosal layer, Suturing the mucosa alone by 3.0 chromic cat gut by simple continuous pattern. And then closure of the remaining layer of the teat with 2.0 non absorbable silk by simple Interrupted suture pattern dressing the site of operation with plane sterile gauze and support the gauze dressing with adhesive tape.

Control Group:-

Same number of animals and same technique was performed with out any addition to the wound .The animals in both groups put under observation follow up. Biopsy samples were taken at interval of 3, 6, 9 and12 days post operatively for histopathological examination.

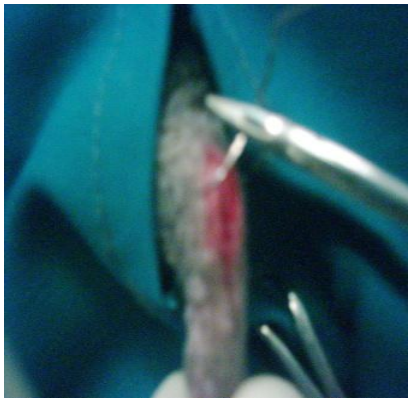
Intramuscular injection of penicillin –streptomycin (pen-strep) at a dose of 10.000 i.u,20 mg /kg b.w daily for four days. The main important management step in the post operative care was evacuation of the udder from milk by sterile teat siphon to prevent pressure on the site of operation, prevent leakage and to control mastitis .The kids should be kept far away from his mother. Suture material was removed after 6 days. For histopathological examination, biopsy was taking at interval of 3, 6, 9 and 12 days post operatively .1cm of full thickness teat layers from the incision site was taken surgically and put in 10%neutral buffered formalin for fixation, processed routinely in histo-kainite and cut at 5Mm thickness by microtome. The histopathological slides were prepared and stained with hematoxeline and eosin stain.



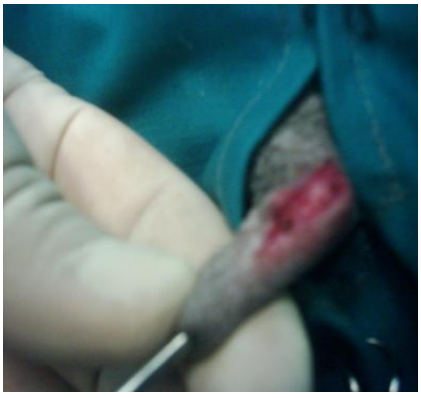
A) 1 cm surgical incision induced Include all the thickness of the Teat. Include all the thickness of the Teat

B) Surgical incision involves all Layer of the teat.

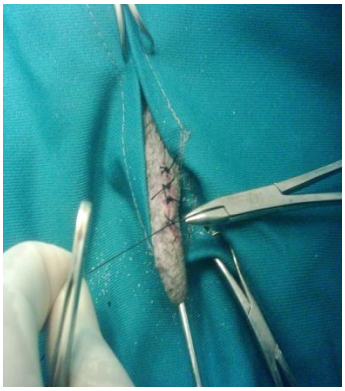
C) Local application of 0.5 sterile chitosan powder



D) Suturing the mucosa by (Simple continuous pattern).



E) Complete closure of the mucosa checked by teat siphon.



F) Suturing the remaining layer of the Teat with 2.0 non absorbable silk (simple Interrupted suture pattern).

RESULTS

The assessment of clinical results indicates that there is systemic reaction in both groups represented by slightly increasing body temperature and decreasing in ruminal contraction and increasing the average of respiration and locally represented by redness and swelling at the site of operation.

After removing the stitches, it is noticed that there is good apposition of the wound edges and no milk leakage from the site of operation, and there is no necrosis or infection and no change in milk consistency, as for as the color. At the examining of the teats after one month post operative it is noticed that there is few scar tissue.

In one cases of treated group there was a defect in the milk evacuation and consequently there was a failure in the surgical operation,

As for as the other cases of this group the healing was good and there is no complications.

While in control group there was delay in healing and this clear in the histopathological section despite of presence of systemic and local reactions.

It is noticed that there is change in milk consistency and the examination of operative sites after one month post operation indicates to the presence of clear scar tissue as compared with treated group.

The histological section3 days postoperatively of the treated group explains the treated material (chitosan) surrounded by large no. of neutrophils, fibrin network, and edema. The fibroblast embedded the fibrin network in the dermis area.(Fig-1A), Also the

section shows initiated hyperplasia of epithelial line of the teat canal with mononuclear cells infiltrated in sub epithelial layer (Fig-1B).

6 days postoperatively of the treated group the microscopic examination reveals that the chitosan (treated material) are surrounded by dens granulation tissue which consist from immature fibroblast and congested blood vessels in the dermis and in the sub epithelial layer of the teat (Fig-2). Also we can see repair of the endothelium of the teat canal by a layer of epithelial cells thichness1-3 cells. In addition to that there is a line of cellular connective tissue extending from epidermis to the dermis characterized by hyperplasia in which the dermis is thicker layer compared with surrounding, with formation of rete pige in the dermis with large rounded cells directed toward the epidermis (Fig-3).

9 days of the treated group the section shows large amount of granulation tissues have been change to cellular connective tissue which characterized by different size and shape of the fibroblast with different direction. Also there is complete repair of the dermis while the blood vessels Congested and proliferation of fibroblast which release a thick network Of connective tissue similar to normal dermis .Also there is mature fibrous connective tissue in the sub epithelial layer Of the teat canal infiltrated with lymphocytes, and there is increase Thickness of epithelial linen of the teat canal to about 3-5 cells. (Fig-4).

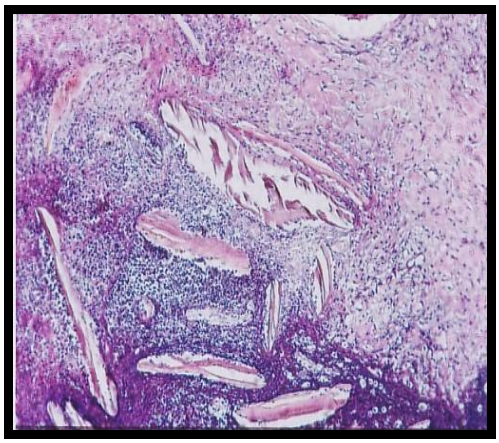
12 days of the treated group this section shows mature fibrous connective tissue which characterized by more collagen fibers regular in direction, less cellular contact the edge of the wounds with lymphocytic infiltration. And there is complete regeneration of the epidermis and epithelial linen of the teat canal (Fig-5).

In control group 3 days postoperatively longitudinal section shows the line of wound is filled with network of fibrin infiltrated with neutrophils, macrophages and lymphocytes which Spread in the dermis adjacent to the site of incision (Fig-6).

6 days of the control group the microscopic section reveals that the inflammatory exudates consist from fibrin network and neutrophils with the clotting blood in the line of incision also there is granulation tissue formation in the dermis and in the sub epithelial line of the teat canal (Fig-7).

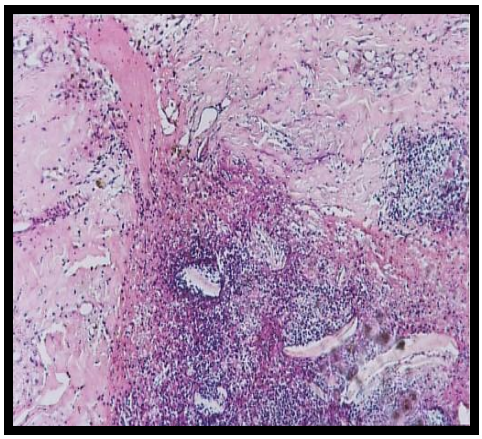
9days of the control group the microscopic section reveals presents of dens cellular fibrous connective tissue which contain mayo fibroblast and collagen fibers and polymorphic fibroblast and inflammatory cells (Fig-8).

12 days of the control group the section shows dens cellular fibrous connective tissue conduct the edges of the wound with lymphocytic infiltration and regeneration of the epidermis which characterized by wide rounded rete pige.(Fig-9).



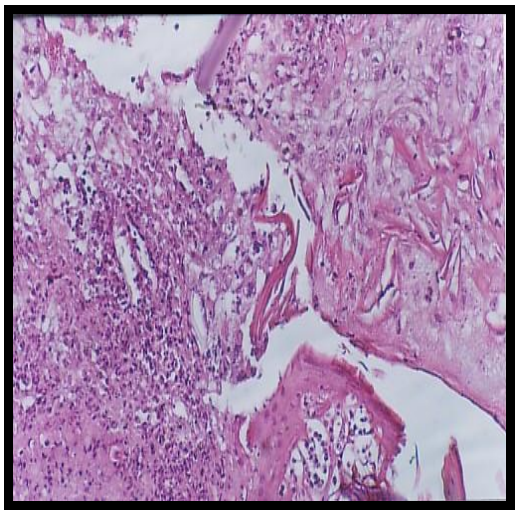
(Fig.1A)

3 days postoperatively the histological of the treated group explains the treated material (chitosan) surrounded by large no. of neutrophils, fibrin network, and edema. The fibroblast embedded the fibrin network in the dermis area.



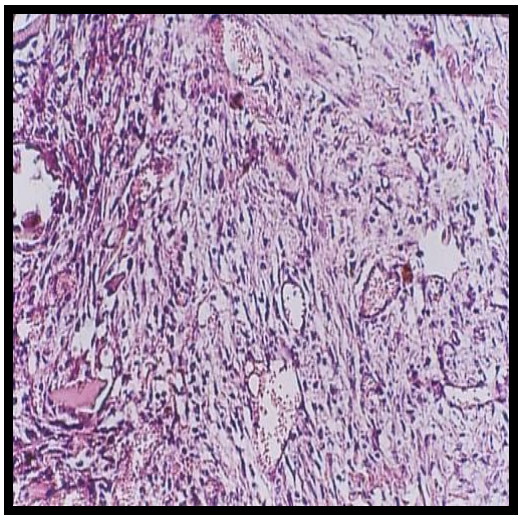
(Fig.1B)

3 days treatment: Section shows initiated hyperplasia of epithelial line of the teat canal with mononuclear cells infiltrated in sub epithelial layer. (H&E stain X10)



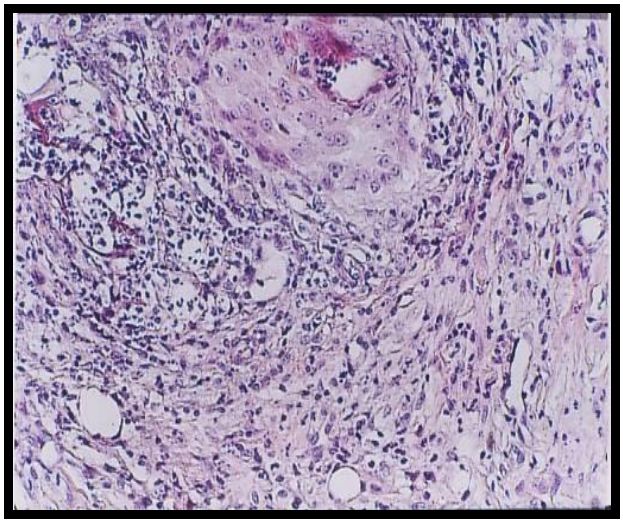
(Fig.2)

6 days treatment: microscopic section reveals that A, treated materials (chitosan) are surrounded by dense fibrous tissue which consist from B, fibroblast and C, congested blood vessels in the dermis (. H&Estain.X20)

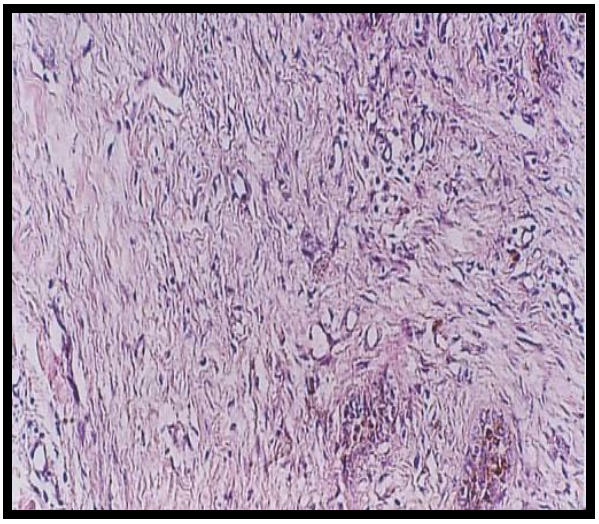


(Fig.3)

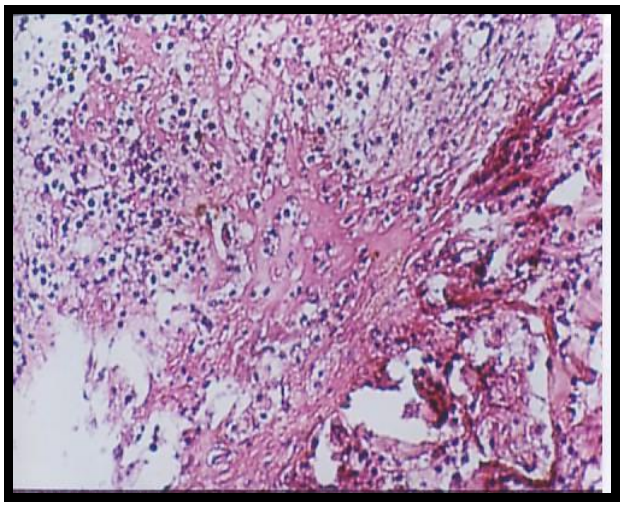
6 days treatment: the section shows repair of the epithelial line of the teat canal by a layer of A, epithelial cells contain 1-3 cells . in addition there is B, a line of cellular connective tissue. (H&E stain.X20)



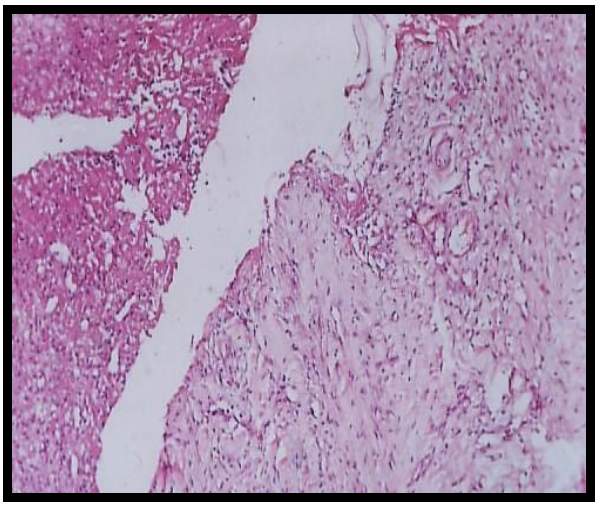
(Fig. 4) 9 days treatment: the section shows cellular connective tissue characterized by different size and shape of the fibroblast with different direction,(H&E stain.X20)



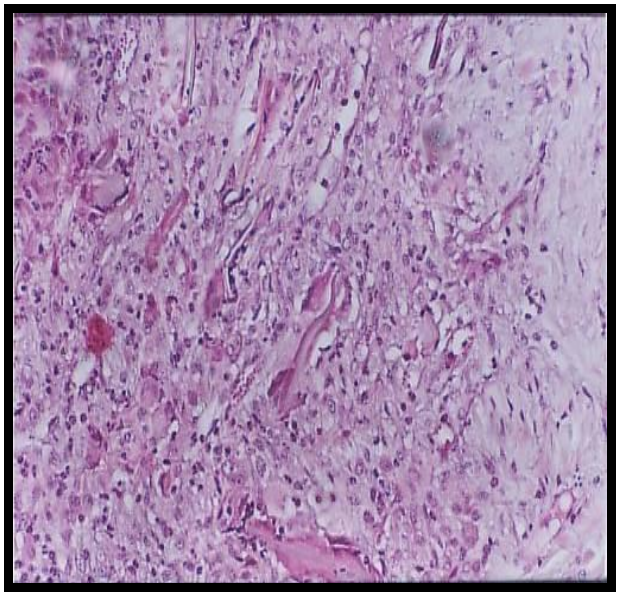
(Fig.5)12 days treatment: the section shows mature fibrous connective tissue by more collagen fibers regular in direction, (.H&E stain.X20)



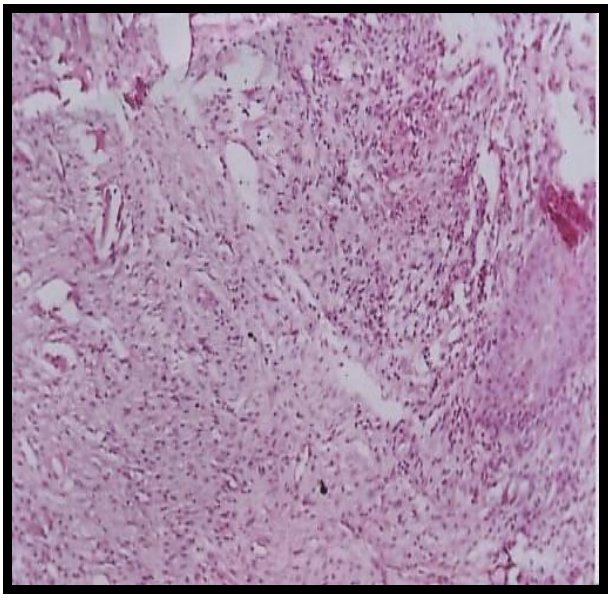
(Fig. 6) 3 days control :longitudinal section shows the line of wound is filled with A, network of fibrin infiltrated with inflammatory cells which spread in the dermis adjacent to the site of incision(.H&E stain.X20) B,



(Fig. 7) 6 days control: the microscopic section reveals the exudates consist from A, fibrin network and neutrophil and there is B, granulation tissue formation in the dermis (.H&E stain X10).



(Fig.8) 9 days control: the section show the dense fibrous tissue contain A, my fibroblast proliferation with, granulation tissue in the sub epithelium of the teat (.H&E stain.X20)



(Fig.9)12 days control: the section shows A, dense cellular fibrous tissue conduct the edges of the wound with lymphocytic infiltration and C. regeneration of the epidermis(.H&E stain X10). B,

DISCUSSION

This is referred by histopathological finding which observe a large numbers of inflammatory cells in the site of the wound and surrounding the treated material (chitosan) this is may be due to the effect of chitosan in the infiltration of the inflammatory cells these results come in agreement with studies done by [7][8][9].

The clinical follow-up of control group indicates that there is systemic and local reaction and delay in the healing of the wound , this is referred by histopatholgical finding were there is few of inflammatory cells as compared with treated group .

There is also change in the milk consistency as for as the color, density It is also noticed that there is a lot of scar tissue at the site of operation.

The histopathological follow-up revealed that there is a clear difference in time of healing between the control and treated group.

Wound healing clearly appear clinically in three days after operation in treated group and this referees to the role of chitosan as accelerated to the healing of wounds and there is identical to [10][11][1].

The inflammatory reaction in the treated group started directly and severely after induced the wound and this is due to the role of chitosan in attracting the inflammatory cells and this is clearly showing through histopathological finding which revealed that the inflammatory cells reaches to the peak in the third day and these cells play a vital role in the healing and this agreed with [12][13][14][7].

The histopathological finding also revealed that there were remnant of chitosan material surrounding by macrophages in the wound and presence of layer of collagen fiber parallel with blood vessels and surrounding by a large number of mononuclear cells, giant cells and surrounding them by connective tissue which indicate that the role of chitosan in organization of tissue effect and stimulation of fibroblast this agreed with [15]. After 6 days of removing of stitches it appeared that the edge of wound have good apposition indicate that the chitosan act as hemostasis through joining with R.B.Cs by the presence of polycations which are able to form gel heparinized leading to reducing firm coagulum due to negative changes of the cell membrane this agreed with [16].

Fast healing of wound in treated group indicates the role of chitosan in the enhance infiltration of the inflammatory cells in the early stages of the healing and then increase collagen production by fibroblast this return to effect of chitosan in enhance the job of mononuclear cells through production of leukotrien –B4 which derived from arachnoid acid and production of interleukin -8 which promote the fibroblast these results come in agreement with study done by [17][18][19].

Few scar tissues in the clinical and histopathological results indicate that chitosan has been proposed to enhance wound healing by inhibiting fibroplasias and promoting organized tissue, reconstruction wound healing with chitosan processed a manner that reconstruct tissue in a normal architecture without fibroplasias or scaring ,these results come in agreement with study done by [20].

The histopathological finding show presence of granulation tissue in the wound site in treated group and this indicates the role of chitosan as antibacterial factor which prevent growth of bacteria by production of lactic acid and this result come in accordance with other studies done by[21][22].

دور الكايتوسان في معالجة ناسور الحلمة المستحدث تجريبيا في الماعز

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الخلاصة

تضمن البحث دراسة التأثيرات السريرية والنسجية المرضية لأضافة مادة الكايتوسان موضعيا على التأم ناسور الحلمة المحدث تجريبيا في المعز. استخدمت ستة عشر معز من السلالة المحلية تتراوح اعمارها (1.2-2) سنة بمعدل وزن (30 ± 5) كيلو غرام. قسمت الحيوانات عشوائيا الى مجموعتين متساوية. تم استحداث ناسور الحلمة جراحيا بطول 1 سم لجميع الحيوانات تحت تأثير التخدير الموضعي والتسدير العميق. في المجموعة الاولى (مجموعة المعالجة) تم اضافة مادة الكايتوسان(0.5 غرام) بعد اغلاق الغشاء المخاطي في حين اغلقت مجموعة السيطرة بدون اضافة. تم متابعة الحالات يوميا من الناحية السريرية حيث لوحظ وجود تفاعل بسيط فبي موقع العملية متضمنا احمرار،انتفاخ لموقع العملية مع ارتفاع بسيط لدرجة حرارةالجسم وزيادة في سرعة التنفس مع انخفاض لتقلصات الكرش. تم اخذ خزعة نسيجية لثمان حيوانات بواقع حيوانين لفترات زمنية مختلفة. تم التقطيع النسيجي بالطرق الروتينية وصبغ الشرائح بصبغة هيموتوكسيلين ايوسين. كانت نتائج الفحوصات المرضية النسجية لمجموعة المعالجة وجود عدد كبير من الخلايا الالتهابية في الايام الثلاثة الاولى بعد العملية. في اليوم السادس بعد العملية لوحظ وجود نسيج حبيبي اخذ بالتحول الى خلايا نسيجية رابطة في اليوم التاسع مع وجود اوعية دموية محتقنة بالاضافة الى وجود تجمع من الخلايا اللمفاوية مع الياف الكولاجين مع تكون ادمة في اليوم الثاني عشر. استنتجت الدراسة بأن اضافة الكايتوسان موضعيا ساعدت وحسنت التأم واصلاح ناسور الحلمة.

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