

## **CLINICAL AND HISTOLOGICAL EVALUATION OF THE EFFECTS OF BOVINE SALIVA ON EXPERIMENTALLY INDUCED OPEN WOUNDS IN RATS**

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### **ABSTRACT**

The use of bovine saliva as a wound dressing material. In the present study, bovine saliva was applied to experimental by second intention on the experimental wounds in rats , and it's effects on epithelization, wound contraction newly formed granulation tissue.

We evaluated the time interval required for wound healing using a standard wound with and without bovine saliva. The bovine saliva did interfere with a normal development of wound healing.

Histological evaluation was considered to parameters the effect of bovine saliva on wound healing. The effect appear to be due to an increase collagen activity , consequently improving the collagen matrix and enhancing the breaking strength by lysosomes with saliva.

### **INTRODUCTION**

Wound healing is the process of repair that fallows injury to skin and other soft tissue. It has got three phases inflammation, proliferation and remodeling. The aim of this study is to evaluate healing effect induced by topical use of bovine saliva on the process of healing of open wound in skin of sheep (1).

Saliva is the watery and usually some what frothy substance produced in mouth of animal by salivary glands the composition of saliva is 98% water electrolytes (2-21 m mol/l sodium 10-36m mol/L potassium, 1.2 – 2.8m mol/L calcium, 0.08 – 0.5m mol/L magnesium, 5-40m mol/chloride, 2-13m mol/L. bicarbonate and 1.4-39m mol/L phosphate) mucous (manly mucopolysaccharides and glycoprotein). Antibacterial compounds(IgM and IgA) and various enzymes (amylase, Lysome, lipase). However the researchers of the national institute of dental and craniofacial research demonstrated that the proteins called secretary leukocyte protease inhibitor (SLPI) plays critical role in normal wound healing (2). When (SLPI) was applied topically to non healing wound it actually reserved tissue distraction and hastened healing. Therefore common belief that saliva contained in the mouth has natural disinfectants which lead people to believe it is beneficial to lick their wound,

Researchers at the university of Florida at Gainesville have discovered a protein called nerve growth factors (NGF) in the saliva of mice when wounds doused with NGF healed twice as fast on untreated and un licked wound (Cited by wikipedia the free cyclopedia) However saliva his multifunctional like antibacteria (Amylase, cystatins, Histatine, mucins). Antiviral cystatine, mucins). Antifungal (Histatins) Tissue coating (Amylases, cystatins mucins, praline-rich proteins , statherine), Lubrication and viscoelasticity, (Mucins, statherins) Mineralization (Cystatins, Histateins protine – rich – proteins, statherins) Dgesthion , Amylases mucine and lipase ) and Buffering (Cabonicn hydrases, and Histatins).

Cohen isolated polypeptide from secretion of the sub maxillary salivary gland of adult male mice, which stimulate the proliferation of various epidermis and epithelial tissue both in vivo and vitro (3). Bovine saliva with normal saline and Vaseline jell as dressing material however used in dressing a open wound of dog. (4). The healing of mucosal wound resembles that of coetaneous in mong respects but there are characteristic features of mucosa , including relatively high tissue turnover in a densely vascularized , saliva containing a specific microfilaria (5,6). However oral mucosal wound heal more rapidly than skin wound of similar length and depth, stated that oral wound repair take place with minimal scarring and profile of cytokines growth factors expressed different from that of coetaneous.

## MATERIALS AND METHODS

In this study saliva was collected from eight healthy cows at 500 ml each, in clean container and kept at room temperature 37C .

**Animals.** Twenty-four male Wistar rats weighing 200 g to 250 g were used. The animals were acclimatized for 1 week to the laboratory conditions prior to experimental manipulation. The animals were exposed to a cycle of 12 h/light and 12 h/dark at a room temperature of 25oC. The rats had free access to standard laboratory diet and water .

**Surgical procedure.** Anesthesia was induced via intramuscular injection of ketamine hydrochloride 50 mg per kg of body weight. After anesthesia was given, the animals were placed face down on a heated mat. After shaving and sterilizing the caudal area of the back, identical wounds (5 mm diameter) were punched bilaterally in the epidermal and dermal layers of the skin. All animals were housed in individual cages specially designed to prevent bedding from entering the wound. The animals were randomly subdivided into 3 groups. In each group, 8 rats were randomly selected for laser irradiation of the right-side wound. The left-side wound was left untreated and served as control.

In studied groups the wounds were treated with topical application of Bovine saliva 3 time/daily while control group wound treated with normal saline in similar way. However all the animals were kept under similar type of management.

**Histological observations.** The animals were sacrificed by high-dose anesthetization 3, 7, 15 and 21 day days post injury. The bilateral wounds were immediately excised and placed in 10% neutral formaline. Routine tissue processing for light microscopy was performed on all specimens. The skin samples were embedded in paraffin. Serial sections (5 mm) were cut by microtome and stained with hematoxylin and eosin (H&E) to assess the wound healing process, leukocyte infiltration, and fibroblast count. Masson's trichrome was used to evaluate the position of connective tissue elements in this process. Slides were examined using an Olympus light microscope (Olympus Inc, Center Valley, Pa) and photographed with an Olympus photograph system.

## RESULTS

The study show histological changes between study groups treated with Bovine saliva and control group (treated with normal saline) in different period.

On third day Biopsy showed that epithelialization filled the wound with new blood vessels compared with that of control which showed sponginess and edema with inflammatory cell study group (Fig.1) control group (Fig.2).

The 7<sup>th</sup> day study group shows inflammatory cell and dense collagen fiber with decrease number of blood vessels (Fig .3), while in control group mainly increase of collagen fiber and disappear of blood vessels (Fig 4).

After two weeks the inflammatory cell, and monocyte cells were present and the wound space filed with collagen fibers (Fig 5, 6)

On day 21<sup>st</sup> there were decrease in monocytes and collagen fiber and epidermis, derm, shows normal structure of skin (Fig 7).



**Fig 1:** cross section in wound 3 days treated group shows fibroblast (A) and blood vessels (B) (H and E stain 400X)



**Fig 2:** cross - section in wound 3 days control group shows inflammatory cells (Monocyte) (A) (H and E stain 400X)



Fig 3: cross - section in 7 days treated group shows increase in inflammatory cells (D) and disappear of blood vessels (H and E stain 400X).

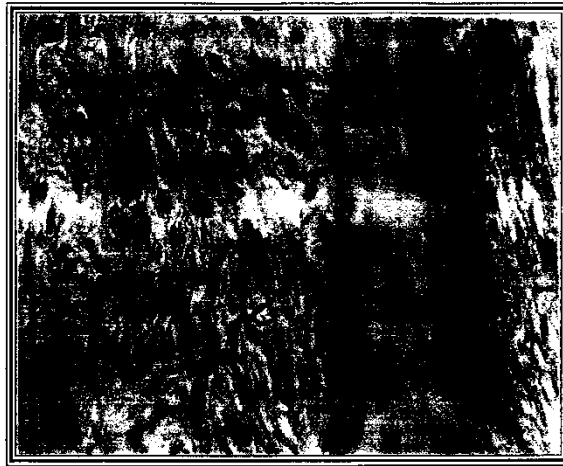
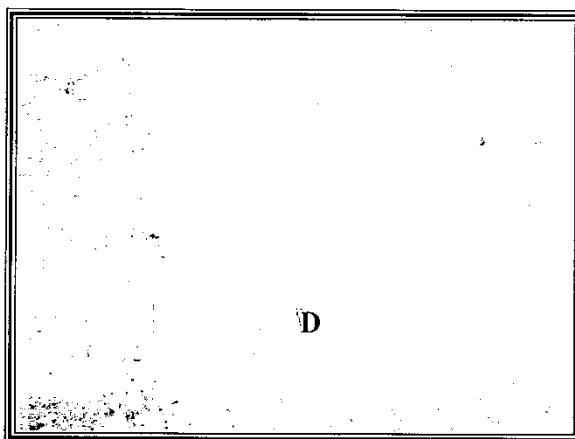


Fig 4: cross - section in 7 days control group shows increase in collagen fiber (C) and disappear of blood vessels (H and E stain 400X).



**Fig 5:** cross - section in wound 15 days shows monocytes (D) fill the line of wound and dense of collagen fiber (O) (H and E stain 100X).



**Fig 6:** cross - section in wound 15 days shows monocytes (D) increase and dense collagen fiber (O) (H and E stain 100X).



Fig 7: cross - section in wound 21 days shows epidermis (K), Dermis (P), subcutaneous tissue (R), and all structure of skin (H and E stain 100X).

### DISCUSSION

The result of this study suggested that the bovine saliva collected from cow possesses considerable accelerate activity on wound healing. The finding of early formation of epithelization and new blood vessels in rats biopsy of study groups on 3 day compares to that from control group. This finding agreed with other study the press release and topical applicator of saliva and Natonou institutes of health 2000 suggested that when a wound occurs, a wide variety of structural and functional proteins such as proteoglycans, collagen and fibro protein are marshaled to the wound site by (SLPI) elastase was degrade these proteins causing break down in tissue matrix for there more (SLPI) controls the activity of leukocytes. Inflammatory cells are attached a wound by multiple stimuli. Including protein (Transforming growth factor beta) which produced in wound, and controls cellular proliferation and differentiation. In the absence of SLPI, TGF.B lyses overdrive and exacerbates inflammation. The finding of the study shown on 3 day, and 7 suggest the same (10,11,12).

However the anti microbial effect and enzymatic activity of the Bovine saliva help in accelerating the healing process. Epithelization and contraction as a part of natural healing have been stimulated by Bovine saliva (13,14,15).

The study clarified the role of bovine saliva on wound healing have been show that the protein secretory leukocyte protease inhibitor (SLPI) know amylase enzyme has ability to reverse tissue destruction can hasten healing when applied topically (16, 17).

## CONCLUSION

The conclusion draws from this study that the bovine saliva has been shown to accelerate healing time of soft tissue wounds (12, 18, 19). According to the result the bovine saliva when used the wound healed faster than that of control group.

Therefore we conclude that bovine saliva can be used as a wound dressing material in surgery leading to healing by second intention without deleterious effects to the connective tissue.

The study recommends the use of bovine saliva as wound dressing to animalize the cost of treatment when compared with conventional treatment and by shorting the time required.

## التقييم السريري والنسجي للتأثير الموضعي لعاب الأبقار على الجروح الجلدية المفتوحة والمستحثة تجريبيا في الجرذان

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### الخلاصة

استخدم في هذه التجربة لعاب الأبقار كأحد مواد التضميد بوضعه تجريبيا على الجروح المستحثة في الجرذان من خلال تأثيره على عملية الترميم الظهاري، تقلص الجرح، وتكوين نسيج حبيبي جديد. تم تقييم الفترات الزمنية لالتئام الجروح من خلال استحداث جروح نموذجية باستعمال وبدون استعمال لعاب الأبقار الذي يتداخل مع التطور الطبيعي لعملية التئام الجروح. أظهر التقييم النسيجي تغيرات كبيرة لتأثير لعاب الأبقار على التئام الجروح نتيجة للزيادة في الفعالية الكولاجينية، إضافة إلى زيادة في الوسادة الكولاجينية وزيادة قوة التحطيم الأيسوسومي لوجود اللعاب.

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