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THE EFFECT OF MEBO TOPICAL APPLICATION ON HEALING TONGUE WOUND OF ALBINO RATS

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ABSTRACT

Objectives: The tongue is an essential organ in the human body. Because of the mechanical forces that surround it, it is more prone to injuries than other organs. In this study we investigated and contrasted self-healing and treated healing tongue wounds, tracking histological changes over a period of 3 to 7 days following wound induction.

Design: 40 adult male Albino rats were utilized, 20 rats for group I and group II were sacrificed after 3 days from wound induction, group I was without taking any treatment, group II treated by MEBO topical application.20 rats were sacrificed after 7 days from wound induction for group III and IV. Group III was without treatment, group IV treated by topical application of MEBO. Tongue was dissected and examined by H&E stain and MT stains.

Results: The Histological results proved the potential effect of MEBO on wound healing of induced tongue wound. Theses results confirmed by statistical analysis by measuring the wound width and epithelium thickness. Wound width decreases during wound healing due to epithelial contraction. It decreases from 15.1 ± 2.30 in group I to 0.648 ± 0.840 in group IV with a high significant difference. Epithelium thickness increases from 2 ± 0.678 in group III to 4.28 ± 0.946 in group IV with a high significant difference. The Masson trichrome examination revealed a significant difference between in group III and group IV.

Conclusion: results confirmed the high impact of topical application of MEBO on accelerating tongue wound healing.

KEYWORDS β-sitosterol, Mebo, tongue wound, healing

INTRODUCTION

Tongue is a very vital organ in our body. The tongue's apex, body, and root make up its anatomy. Three layers make up its histology: the mucosa, submucosa and muscularis. The mucosa is a stratified squamous epithelium, while the submucosa is a thin layer of connective tissue that contains blood vessels. Bundles of skeletal muscle fibers organized in various directions make up the muscularis. The lingual mucosa of the dorsal, lateral

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border and to a certain extent the ventral surface of the apex had lingual papillae filiform and fungiform. Circumvallate papillae observed at the caudal part of body and root. All papillae are covered by stratified squamous epithelium that differs by the thickness and keratinization only. The distribution and random orientation of individual skeletal muscle fibers in the tongue allows for increased movement during chewing and swallowing (Abdel Moghith et al. 2023; Ahmed Hussien and Khalil Ibrahim 2023).

Seizures, self-harm, blunt force facial trauma, oral trauma while intubated and child abuse are some of the causes of tongue lacerations. The anterior dorsum of the tongue sustains the most cuts, followed by the mid-dorsum and anterior ventrum. Less frequently, posterior tongue lacerations occur. It is unlikely to result in a permanent deficiency if the tongue's tip or lateral edge is completely destroyed because there is nothing left to heal. The tongue will eventually enlarge after these kinds of injuries, masking the deficiency. Damage at the base of the tongue may be more troublesome than other. Because the hypoglossal nerve is located there, damage to the base of the tongue may be more serious than to other areas (Cavalcanti, Da Motta, and Marhino 2023; Farrag et al. 2022)**.**

Usually unintentional, tongue biting can result in discomfort, bleeding, and inflammation. Particularly in children, tongue-biting injuries are frequent and frequently small. In adults, they are typically more severe (Hennessy, 2024).

The body's natural response to tissue damage is wound healing. However, the vascular system, cytokines, mediators, and a variety of cell types interact intricately during wound healing, making it a complex process. The initial cascade of platelet aggregation and blood vessel vasoconstriction is intended to halt bleeding. A flood of different inflammatory cells, beginning with the neutrophil, follows. In turn, these inflammatory cells release a range of cytokines and mediators to encourage thrombosis, angiogenesis and re-epithelialization(Kangal and Regan 2023)**.**

Hemostasis, chemotaxis, and enhanced vascular permeability are characteristics of the inflammatory phase that prevent more damage, seal the wound, eliminate bacteria and cell debris, and promote cellular migration. The inflammatory stage typically lasts for a few days. Granulation tissue development, reepithelialization, and neovascularization are characteristics of the proliferative phase. This stage may continue for a few weeks (Coger et al. 2019)**.**

Although some scarring occurs during oral mucosal wound healing, it is less than that of skin, making the oral mucosa unique in its capacity for quick recovery. One important distinction between oral mucosa and skin is that oral mucosa heals under the continuous presence of saliva, which may be connected to the decreased inflammatory response observed in oral mucosal wound repair with decreased macrophage infiltration of the injured tissues. Crucially, saliva contains several biological components, including as mucins, antimicrobial peptidesand histatins, that can aid and encourage wound healing (Bartold & Ivanovski, 2024).

Since 1995, the Chinese burn ointment Moist Exposed Burn Ointment (MEBO) has held a validated US invention for pure herbal extract. Beta-sitosterol is the active ingredient of MEBO ointment (Moustafa 2016). It contains also, Sesame oil extract as a secondary component which plays a positive effect in wound healing that may be attributed to its ability to scavenge free radicals and antioxidant properties(AlAraby, Hassabou, and Shehab Eldin 2025).

MEBO is an analgesic and antibacterial natural agent. The major component of MEBO is β-Sitosterol with reportedly anti-inflammatory and antipyretic properties(Mabvuure et al. 2020). (Vaghardoost et al. 2018) also found on his study on the effect of herbal extracts on wound healing that sesame oil experienced the fastest rate of recovery.

β-Sitosterol is one of numerous phytosterols, or plant sterols, that share chemical similarities with cholesterol. One of the ingredients of the food

additive, it is a white, waxy powder with a distinct smell. Alcohols can dissolve phytosterols, which are hydrophobic(Michellod et al. 2023; Oja et al. 2009).

β-sitosterol has anticancer capabilities against leukemia, breast, prostate, colon, lung, and stomach cancers. Pharmacological screening has revealed that β-sitosterol disrupts several cells signaling pathways, such as cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and has anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardioprotective, and antidiabetic effects without causing appreciable toxicity (Khan et al. 2022).

In diabetic wounds, β-sitosterol can stimulate collagen synthesis, angiogenesis, and the proliferation of alternatively activated macrophages. Proteomic and transcriptomic studies showed that wounds treated with β-sitosterol had enriched VEGF, mTOR, and MAPK signaling pathways. Ndufb5 may be the target of β-sitosterol-treated wounds, according to molecular docking (Liu et al., 2024).

In this literature we evaluated the effect of topical MEBO application on tongue wound healing, through wound contraction, re-epithelialization and collagen formation assessment.

MATERIALS AND METHODS

40 adult male Albino rats weighing between 200 and 250 grams were utilized in this investigation. According to the Ethical committee of Nahda University animal house (NUB-025-035), the rats were kept in separate cages with suitable experimental settings. During the course of the trial, the animals were given tap water and a typical pellet meal. The rats were kept at room temperature (22–24°C) and subjected to light and dark cycles for 12:12 hours. The animals were classified into 4 groups as follow: Group I: 10 rats were subjected to tongue wound and scarified at day 3, they did not receive any treatment. Group II: 10 rats subjected to tongue wound and scarified at day 3, the wound treated by MEBO topical application three times / day from the first day of wound induction till scarification. Group III: 10 rats were scarified at day 7, they did not receive any treatment. Group IV: 10 rats were scarified at day 7from wound induction and treated with MEBO topical application three times /day from the first day of wound induction till scarification.

Wound induction:

0.1 ml of ketamine hydrochloride and 0.05 ml of xylazine hydrochloride, 100/g body weight of the animal, were typically injected intramuscularly to anesthetize rats. Following anesthesia, 2% chlorhexidine was used to antiseptically clean the tongue. To guarantee that every wound would be the same size, a biopsy punch (Acu-Punch, Acuderm Inc., Ft. Lauderdale, FL, USA) was then used to create a surgical mucosal incision. The round wound was roughly 2.5 mm in diameter and 2 mm in depth **(Fig.1).**

Fig. (1)

Tissue samples:

Light Microscopic Preparation: After being cleaned under running tap water, the formalin-fixed tongue samples were dehydrated using increasing ethyl alcohol grades, clarified in xylol, and embedded in paraffin wax. Sections of 5µ in thickness were cut, placed on sterile glass slides, and stained with Masson's trichrome (MT) to determine

the extent of collagen deposition and standard H&E stain to confirm histological features. All of these techniques were implemented in accordance with Drury (1980). Microscopic study of the histology slides: A Leica light microscope with a digital camera and image analysis software was used to evaluate the prepared slides (H&E and MT) in order to assess morphological alterations histologically.

RESULTS

Histological analysis Hematoxylin and Eosin stain: At day 3 from wound induction (group I) showed rupturing of epithelium coverage, inflammatory cells infiltration and granulation tissue in lamina propria (A. Fig.2). At day 3 in treated group (group II) showed starting of the healing process via proliferation of the epithelium and reduction in wound size. The new epithelium formed without corrugations (B. Fig.2). Granulation tissue appeared less than group I.

At day 7 in non-treated group (Group III) showed complete wound closure. Thin epithelial layer, without corrugations formed, tongue papilla and epithelial layers didn't restore its normal appearance **(C. Fig.2)**. At day 7 in treated group (Group IV) showed complete re-epithelialization, covered the underlying connective tissue stroma which appeared with number of fibroblasts, collagen bundles, and newly formed blood vessels **(D. Fig .2).**

Statistical analysis regarding the epithelium thickness revealed a significant difference between group I and group II, while the statistical difference between group II and III was non-significant. The difference between group III and group IV and the difference between group I and group IV were highly significant. Regarding width of the wound measured by Mm, a highly significant difference has been recorded between group I and group II, also between group III and group IV. A non-significant difference found between group II and group III. **(fig.3)**

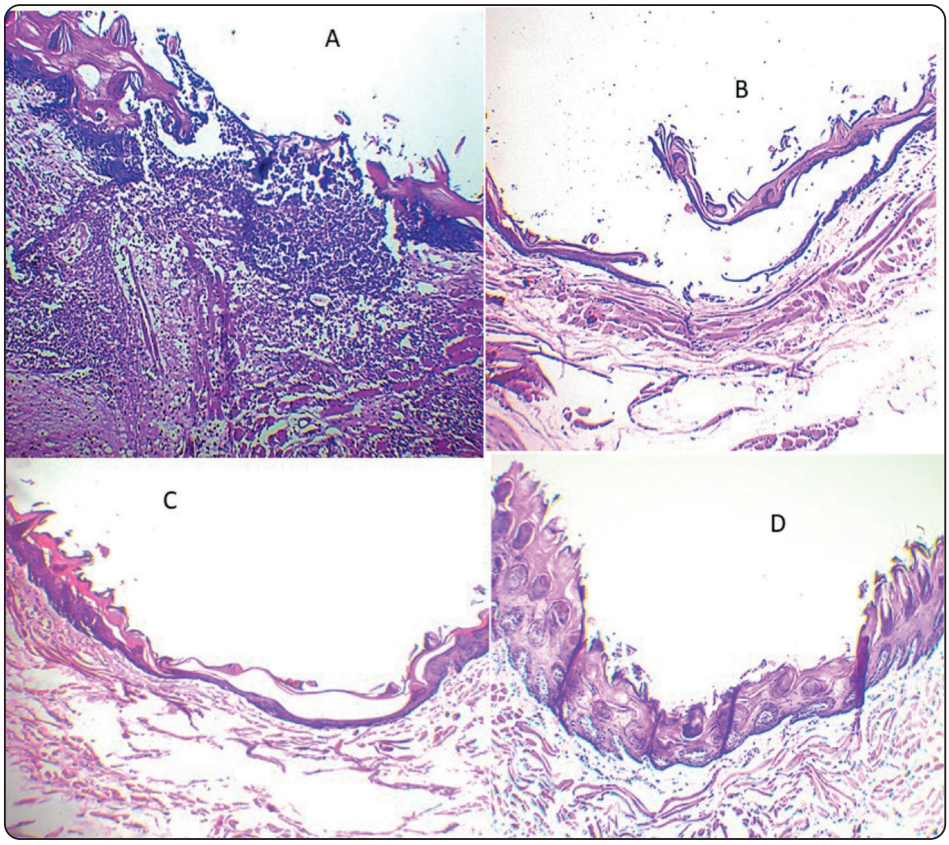

Fig. (2) Photomicrograph showing the wound area of the tongue: A showing group I cutting of the epithelium and formation of granulation tissue. B showing group II with proliferation of the epithelium and reduction on wound size. C showing group III, beginning of epithelial healing of the wound area. D showing group IV epithelium and connective tissue reorganization and restore its normal appearance {H&E x 100}.

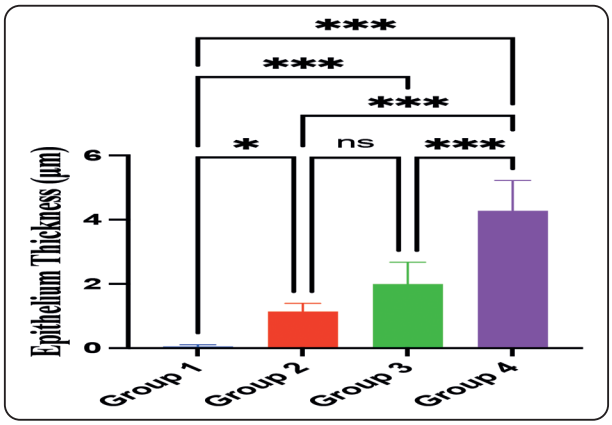

Fig. (3)

Masson's Trichrome Stain Results

At day 3 from wound induction Group I photomicrograph showed area of defect surrounded by a thin and mild stained collagen fibers (A. fig4). group II photomicrograph revealed thin collagen fibers, randomly organized and moderately stained with blue stain (B. fig4). At group III the collagen fibers appeared thick and intensely stained (blue color). Superficial fibers were parallel to the epithelial surface while deep fibers were perpendicular extending in between the muscle bundles (C. fig4). group IV revealed thick mildly stained collagen fibers were displayed in the lamina propria. Thin delicate collagen fibers between muscle fibers bundles were shown perpendicular to the epithelium (D. fig4).

Statistical analysis

Regarding area percentage of collagen fibers revealed a significant difference between group I and group II, also between group III and group IV. a highly significant difference was recorded between group I and group III. On the other hand, the difference between group II and group III was a non-significant difference (fig.5).

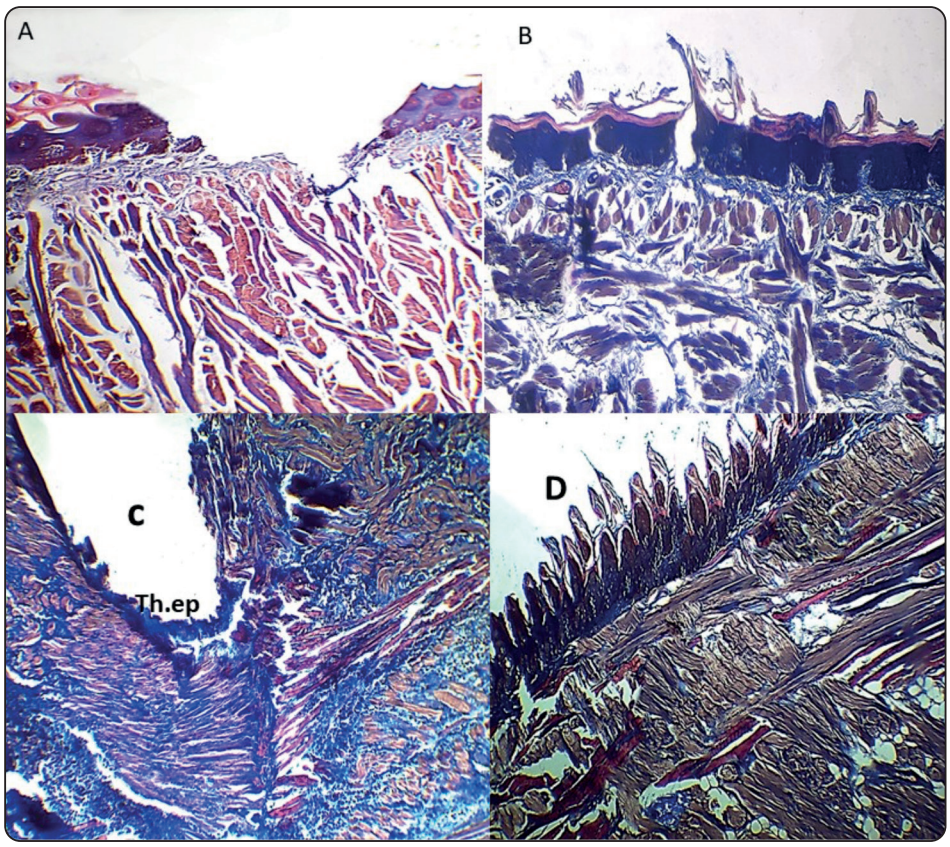

Fig. (4) Photomicrographs of wound area of the tongue A showing group I with epithelial and connective tissue obvious defect. Collagen fibers were shown to be thin, lightly stained . B showing group II thin collagen fibers, randomly organizes and moderately stained with blue. C showing group III the collagen fibers appeared thick and intensely stained (blue color) Superficial fibers were parallel to the epithelial surface while deep fibers were perpendicular extending in between the muscle bundles. D showing group IV thick mildly stained collagen fibers were displayed in the lamina propria. Thin delicate collagen fibers between muscle fibers bundles were shown perpendicular to the epithelium. (MT XIOO)

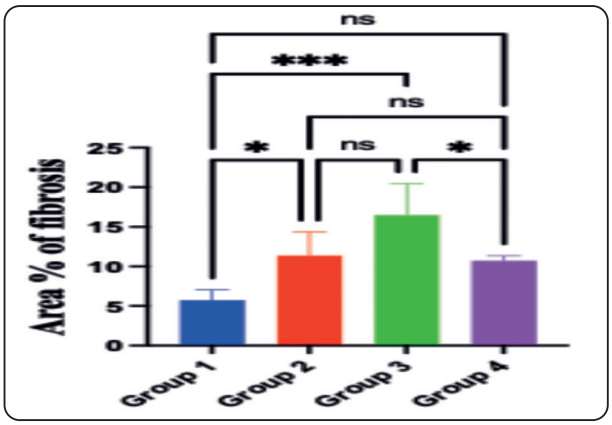

Fig. (5)

DISCUSSION

Many studies were done to evaluate the effect of MEBO in wound healing, but there aren't any studies on its effect on tongue wounds. Therefore, in the current study, the tongue was chosen as it would be subjected to friction by many factors as mentioned in introduction.

Using standard H&E histology stain and Masson's trichrome (MT) stain, we assessed the impact of (MEBO) on both epithelium and C.T. during wound healing. Collagen was clearly detected by the concentration of the blue stain in Masson's trichrome (MT) stain, which was used to assess the degree of collagen fibers formation during wound healing.

Inflammation, angiogenesis, fibroplasia, wound contraction and epithelization of the wound defect are all steps in the wound healing process, which is crucial for repairing injured tissue and preventing pathogen invasion. Depending on the extent of wounding, these processes begin as soon as an injury occurs and last for varying lengths of time(Panchatcharam et al., 2006).

In our study design we chose 2 intervals for scarification at day 3 and at day 7, as inflammatory phase peak at day 3 while healing begin at day 7 in oral mucosa. So, we can compare the treated groups with non-treated groups at the mentioned phases (Larjava 2012; Menezes et al. 2022; Toma et al. 2021).

In our H&E histological study in group I, we found that at day 3 from wound induction there is rupturing of epithelium coverage. Connective tissue was showing inflammatory cells infiltration and granulation tissue this agreed with (Larjava 2012) who reported that about two to four days after injury, new C.T. known as granulation tissue starts to form. The main constituents of granulation tissue are growing capillaries, inflammatory cells and fibroblasts.

Also (Menezes et al. 2022) found that the lamina propria of the tongue wound at day three was filled with acute inflammatory cells and granulation tissue, so day 3 is the best time to study the inflammatory stage.

In group II in the current study we observed the beginning of the healing process as wound contraction and resolving of granulation tissue. In MT results the blue stain of collagen fibers was moderate. These finding coincidence with (Larjava 2012) who found that tissue remodeling is a gradual process that transforms the granulation tissue into a mature C.T. The tissue is extremely hypercellular with condensed fibroblasts. Remodeling starts when wound contraction has assembled the collagen fibrils into thicker bundles and aligned them perpendicularly to the wound edges.

The group III results showing complete wound closure by thin layer of epithelium and intense blue stain in MT stain, these results confirmed by (Guo and DiPietro 2010) who explained the proliferative stage of wound healing, which is distinguished by the migration and proliferation of epithelial cells over the wound's temporary matrix (re-epithelialization). Fibroblasts and endothelial cells, which are the most common cell types in the reparative dermis, promote the development of capillaries, collagen, and granulation tissue at the site of damage.

The fibroblasts have begun forming new collagen and glycosaminoglycans by days five through seven. The center of the wound is formed by these proteoglycans, which also aid in wound stabilization. Cell migration from the wound's periphery and surrounding edges then initiates reepithelialization. Over time, a thicker and more resilient layer of cells will cover the wound, but initially only a thin superficial layer of epithelial cells is laid down(Wallace, Basehore, and Zito 2023).

At the last group (treated group) in this current study the remodeling stage typically found in H&E results. Epithelium and connective tissue restored its normal structures. In Masson's Trichrome, the collagen fibers in lamina propria appeared more arranged and the density of the blue color less than the third group with a significant difference**.** This finding agrees with (Campos, Groth, and Branco 2008) who explained the healing phase of remodeling, during which the wound returns to its typical state. Extra cellular matrix remodeling to resemble the architecture of normal tissue is a crucial aspect of the remodeling phase.

Statistical analysis indicated a highly significant difference between group III and group IV. A non-significant difference between group II and group III was revealed. these results confirmed the effect of MEBO in accelerating tongue wound healing.

As the major player of MEBO is β-sitosterol, (Babu and Jayaraman, 2020) proved that β-sitosterol has a variety of biological actions, including analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, lipid-lowering, hepatoprotective, protective against respiratory diseases, wound-healing, antioxidant, and anti-diabetic properties. The results of this investigation demonstrated that β-sitosterol gel (MEBO) has a perfect effect on tongue wound healing.

(Baskar et al., 2012) demonstrated that 5mg/kg of β-Sitosetrol can hasten wound healing by reducing inflammation, reviving oxidative stress in cells, and aiding in tissue repair.

β-Sitosterol has the potential to be a useful substitute for the conventional oral drugs that are currently used to treat and hasten the healing of traumatic mouth ulcers (Taha et al., 2024).

Sesame oil is the secondary component of MEBO and has a prominent effect on healing (Valacchi et al. 2011) founded that, the rate of wound closure is higher in the first seven days, when moderately ozonated sesame oil is used. Under the same therapy, a greater angiogenesis, an earlier and greater response of wound-repair cells.

(Mohammed et al. 2023) also proved that sesame oil showed higher statistically significant effect in the healing of recurrent aphthous ulcer and prophylactic effect up to three months from the start of the therapy.

(Jewo et al. 2009) thought that MEBO, can prevent infections and accelerate the healing process of wounds without having any of the negative side effects of refined chemicals. Also (Hassan et al. 2021) proved that MEBO is simple to use and delivers physiological moisture for enhanced wound healing and re-epithelialization. Regardless of the location, size, or local wound status. MEBO is an all-natural, pure herbal extract, making it neither poisonous nor irritating to the oral mucosa

In (Al-Numairy 2004) clinical trial study on the effect of MEBO in wound management, he proved that MEBO produces extremely good overall cosmetic outcomes and is safe, effective, and shortens the duration of treatment while lowering the risk of wound infection.

Limited studies done using MEBO intra-oral, more studies needed to investigate saliva and MEBO reaction as it still ointment with oily base.

CONCLUSION

H&E and MT results which confirmed by statistical analysis proved that group IV treated group with MEBO topical application has a highly significant effect in accelerating tongue wound healing, more clinical investigation is needed. A new form with acceptable taste and flavor is needed.

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