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Histological Evaluation of the Effect of Pure Nicotine and Pentoxifylline Gel on Oral Ulcers: Experimental study

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Abstract:
Background*: Ulcerative conditions are common and are observed in almost 25% of the younger generations including children and young adults, which requires profound attention. Among the available topical therapeutics in treatment of oral ulceration is pure nicotine. It is an alkaloid derived and found in the Solanaceae plants and exerts its effects through suppression of inflammatory pathway. The therapeutic role of pure nicotine on regression of oral ulcers has been studied and showed effectiveness in reducing pain and ulcer erythema*.
The most appropriate channel for delivering local drug therapy to the oral cavity seems to be gel based topical formulations. Pentoxifylline is an anti-inflammatory xanthine derivative that has been approved nowadays for peripheral arterial disease therapy, moreover it exhibits an anti-tumor necrosis factor (TNF)-α effect, reverses fibroblast proliferation and may act to decrease the oral ulcers pain and duration.
Materials and Methods*: Thirty six male Albino rats weighing 200–250 gm were used in this study. Oral ulcers were induced in the right buccal mucosa all rat population. The rat population was divided into three main groups, twelve rats each. Topical pure nicotine, pentoxiphylline gel and placebo were applied for each group four times daily on the ulcers.Four rats from each group were sacrificed at day two, four, and eight right cheek mucosa was removed and prepared for histological examination.*
Results*: The results of our study showed complete healing of the ulcer with absence of any sign of inflammation and increase in keratinization by day 8 in pure nicotine group, while in pentoxiphylline group the ulcers did not heal completely or produce any keratinization when compared to the nicotine group. In regards to control group, profound ulceration and inflammation were seen from day 2 until day 8.*
Key Word*: Ulcer;Pure nicotine; Pentoxiphylline; Keratinization; Anti-Inflammatory.*

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I. Introduction

Oral ulcers have a great impact on optimal wellness and good health of patients, it affects their daily activities and interferes directly with the quality of their lives. An oral ulcer is usually painful and could result from multiple factors including infections, malignancy, squamous cell carcinoma and trauma. Trauma is considered one of the most common causes of oral ulceration preferably occurring on various locations of the mucous membrane of the oral cavity. Ulcers are generally reviewed as chronic if they persist more than two weeks without response to treatment, a situation that mandates biopsy. Ulcerative conditions are common and are observed in almost 25% of the younger generations including children and young adults that requires profound attention. [(1, 2)]

Nicotine is an alkaloid that derived its name from its origin Nicotiana tabacum. It is found in the Solanaceae plants and exerts its effects through suppression of inflammatory pathways. Nicotine induces T-cells energy, and inhibits the production of many cytokines including tumor necrosis factor and hence is able to modulate local immune responses. Different forms of nicotine replacement materials are available these include, lozenges, transdermal patches, tablets and chewing gums. [(3)]

It is important to mention that pure nicotine has been reported by some studies as a safe component, although this still depends on the dose used. The therapeutic role of pure nicotine on regression of oral ulcers has been studied and showed effectiveness in reducing pain and ulcer erythema, never the less additional researches are needed to examine larger samples and try variable nicotine doses giving more consistent and reliable results.[(4)]

Pentoxifylline is an anti-inflammatory xanthine derivative that has been approved nowadays for peripheral arterial disease therapy. Most importantly, this drug is suggested to act on red blood cells, platelet adhesion and aggregation resulting in improving blood properties by making it less viscous. [(5)] Pentoxifylline exhibits an anti-tumor necrosis factor (TNF)-α effect, reverses fibroblast proliferation and enhances tissue oxygenation by

increasing blood flow to the affected microcirculation. [6] Some studies reported milder pain and less ulcer numbers in patients receiving pentoxifylline. However, though it could have an effective role in ulcer healing, immunodeficiency, and RAS treatment, still it has minor side effects including vomiting, GIT disturbances, headaches and dizziness. Patients on certain medications should be cautious when taking pentoxifylline to avoid possible complications. In this regards and owing to its high cost, pentoxifylline was reported by different studies to be used in combination with other drugs or as an adjunct to other treatments but not as a first line choice therapy. If patients are unresponsive to other modes of treatments then it could have a second line role. [7,8]

II. Material And Methods

An experimental study was performed to evaluate the advantageous effect of topical application of pure nicotine liquid and pentoxiphylline gel on oral ulceration. The study was approved Ethics Review Board of Faculty of Dentistry, Pharos University in 2018.

Animals:

Thirty six male Albino rats weighing 200–250 gm were used in this study. The animals were kept in polypropylene cages at the animal house of Faculty of Dentistry, Pharos University. All rats were fed with standard laboratory pellets and tap water ad libitum throughout the study period. The room temperature was about 22-24°C and the animals were exposed to 12:12 hours light dark cycles.

Ulcer induction:

Oral ulcers were induced in all rat population. Rats were fixed on their backs and all animals were anesthetized with an intra-peritoneal injection of ketamine* (90 mg/kg) and xylazine† (15 mg/kg). Round filter papers, 5.5 mm in diameter were soaked in 15 ml of 50% acetic acid and were applied over the right buccal mucosa for 60 seconds to create round uniform ulcer.

Study groups:

The rat population was divided into three main groups, 12 rats each.

- Nicotine group: rats treated by topical pure nicotine liquid.‡
- Pentoxifylline group: rats treated by topical pentoxifylline gel.
- Control group: rats treated by placebo.

A cotton-tipped applicator was used to apply the drug for each group 4 times daily on the ulcers.

Topical pentoxifylline preparation:

Carbopol (Cbp 934P), a bioadhesive polyacrylic acid polymers powder; was used in the present study to prepare pentoxiphylline gel. Cbp powder § was slowly added into water under constant stirring using a magnetic stirrer. The mixture had been kept at room temperature for 24 hours to eliminate the entrapped air bubbles and about 2–3 drops of triethanolamine** was added, and mixed until the gel was formed.[9]Consequently, an adequate weighed quantity of PTX (1%)§ was sprinkled gently onto the prepared gel using a magnetic stirrer until a homogenous clear gel was obtained. The gels were kept at room temperature for 24 hours. The pH of the gels was within neutrality: 7.1, which is considered acceptable to avoid the risk of any possible irritation in the oral cavity. It exhibited satisfactory bioadhesive properties and a pseudo-plastic rheological behavior.

Topical placebo preparation

A soft base was formed by melting 30 gm of white soft paraffin with continuous stirring.

Histological Evaluation:

Four rats from each group were sacrificed at day 2, 4, and 8 by an overdose intra-peritoneal injection of 100 mg/kg Phenobarbital sodium. Subsequently, right cheek mucosa was removed from each animal with surgical scissors and scalpel blade number 15 and then placed in 10% neutral buffered formalin solution for 48 hours. Specimens were dehydrated, diaphonized, fixed in paraffin, and cut transversely into 5 µm-thick slices on a microtome (Leica Biosystems, Wentzler, Germany). Mucosal samples were stained with hematoxylin-eosin (HE) and Masson'strichrome. All sections were mounted on glass slides with cover slips using Entellan (Merck, Darmstadt, Germany) for microscopy.[10]

*Eipico, Egypt.
†ADWIA, Egypt.
‡Chansflavor, RM509, Shenical, Mansion, Shenzhen, Guangdong, China.
§Pharaonia Pharmaceuticals, Alexandria, Egypt
**Sigma-Aldrich, Germany

III. Result

To compare the effect of pure nicotine and pentoxifylline gel on ulcer healing, several histopathological findings are essential to detect microscopically. These findings depend mainly on four overlapping dynamic phases. Hemostasis considered as the preliminary phase of healing since it occurs within the first minutes after the initial injury. This hemorrhage activates platelets to the site of ulcer which will play major role at this phase and the entire healing pathway. Homeostasis detected microscopically through Fibrin mesh formation. This mature scaffold holds the infiltrating cells including leukocytes, keratinocytes, and fibroblasts. [(11)] The inflammatory phase started by the attraction of PMNS, macrophages and chronic lymphocytes to the site of ulcer. Within first 48 hours of injury, dense acute and chronic inflammation intermingled to the fibrin mesh forming mature granulation tissue base located immediately under the injured site. [(11)] Next phase usually can be monitored after 4 to 5 days of initial injury. [(11,12)]

The reparative phase initiates as form of re-epithelialization. At this phase, increased vascularization and extracellular matrix production can be noted histologically. As a result of this phase, the ulcer granulation tissue base comprises fibroblasts, new budding vessels, and immature collagen type III.[(11)] Some fibroblasts will also begin to differentiate into myofibroblasts that have contractile function aiding reduction of ulcer size and facilitate adjacent keratinocytes at the ulcer edges to begin the process of Epithelialization.[(11)] Interestingly, at the maturation and remodeling phase, the pure nicotine treated ulcers showed full thickness healing and epithelial maturation at the eighth day. The epithelium showed abundant ortho-keratinization, which is known effect of pure nicotine on keratinized epithelium.

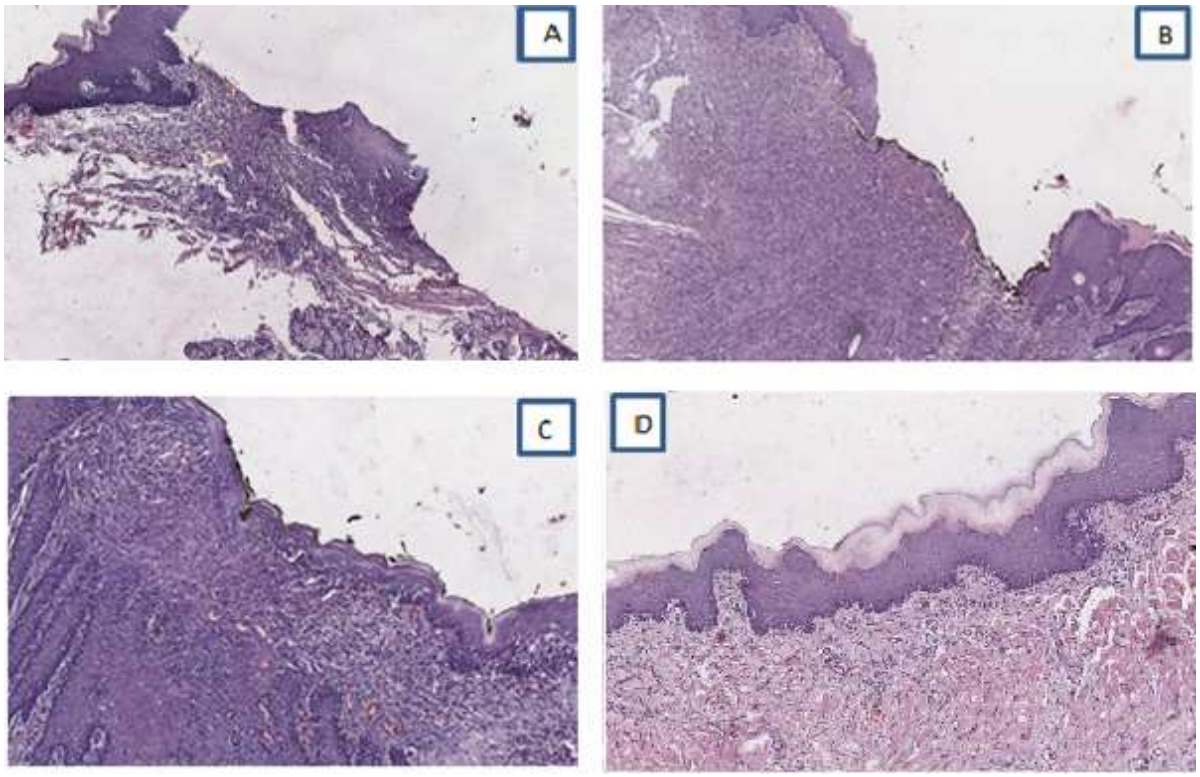

FIGURE 1. Albino rat ulcerated buccal mucosa treated by direct application of pure nicotine. A, (day 1) of the induced ulcer. Note the complete loss of epithelial surface. B, (day 2) ulcer started to form fibrin bed infiltrated by acute and chronic inflammatory cells. The inflammation involving deep connective tissue. C, (day 4) note the reepithelization and chronic inflammation limited to superficial lamina propria. D, (day8) complete healing and absence of inflammation. Note epithelial layers and thick corrugated ortho-keratin formation. (hematoxylin–eosin [HE], original magnification x100). [(13)]

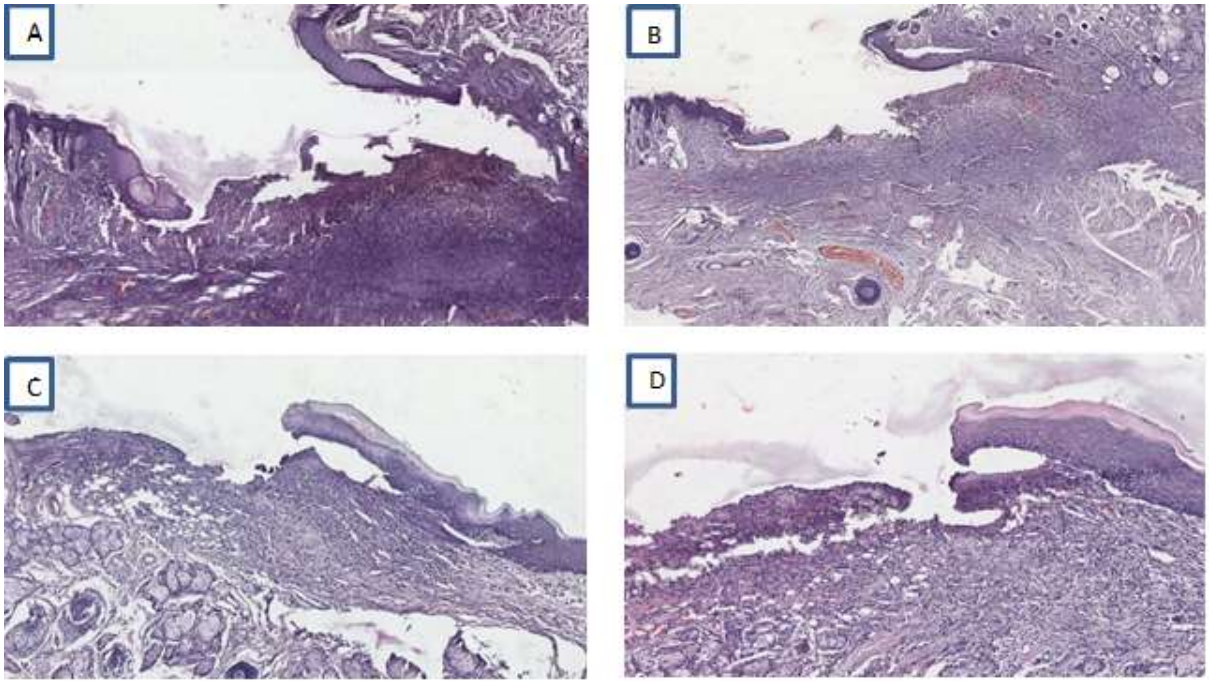

FIGURE 2. Albino rat ulcerated buccal mucosa treated by direct application of pentoxifylline gel. A, (day 1) large ulcerated mucosal surface exhibiting dense acute and chronic inflammatory cells through underlying connective tissue. B, (day 2). Marked reduction of the dense inflammation demonstrated at the ulcer site. The ulcer size still remarkable .C, (day 4) note the slight reduction of ulcer size. D, (day8) no changes identified. (hematoxylin–eosin [HE], original magnification x100)

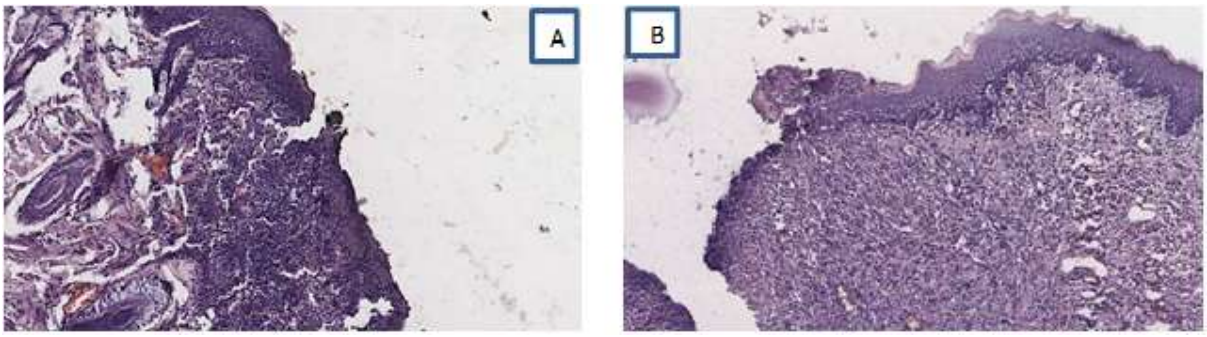

FIGURE 3. Albino rat ulcerated buccal mucosa treated by placebo (control). A, (day 2) deep ulceration with dense neutrophilic infiltration. B, (day 8) the ulcer ped retained exhibiting chronic inflammatory infiltration and fibroblast proliferation. (hematoxylin–eosin [HE], original magnification x200).

IV. Discussion

An ulcer is a tissue defect that involves damage to both epithelium and lamina propria. A breach of the oral mucosa probably affects most people at various times during life. Multiple factors could cause chronic oral ulcerations. These defects are painful and are common reasons for people to seek medical or dental advice. [1,14]

Nicotine is considered an efficient para sympathomimetic alkaloid and a vastly used stimulant that is produced naturally in a certain family of plants known as nightshade. (Most notably in tobacco). Some investigators embrace the belief that nicotine may be of great help in the patients suffering oral ulcerations hence serves for oral mucous membrane protection through certain immune-regulatory mechanisms.[15,16]The most appropriate channel for delivering local drug therapy to the oral cavity seems to be gel based topical formulations. Several advantages relates to these systems these include drug release and dispersion, being non-greasy, the ease of administration, and patient acceptance. Pentoxifylline is an anti-inflammatory gel xanthine derivative that may

act to decrease the oral ulcers duration. In diabetic patients, this gel was found to fasten the wound healing process in oral ulcerations and also in animal models and patients with venous ulcers. [5,17]

In this study, thirty six male Albino rats were divided into 3 groups. Ulcers were induced orally on their buccal mucosa and the effect of pure nicotine and pentoxifylline gel on healing were evaluated at 2, 4 and 8 days.The results of our study in regards to control group, showed profound ulceration and inflammation that prevailed and increased from day 2 till day 8. Pure nicotine application resulted gradually in complete healing of the ulcer with absence of any sign of inflammation by day 8, this was observed through subtle histological changes including, reduction in number of inflammatory cells, increased in blood vessels hence tissue vascularity, re-epithelialization and very importantly the increase in keratinization seen as a corrugated thick layer. These findings were in agreement with Mai Zakariaa, and Aliaa El-Meshadb who recorded in their studies a lower mean of ulcer in the nicotine group, with a significant variation in ulcer healing after 4 and 6 days. Also, our results were in accordance with Mahmood Reza Kalantar, and Zahra Golestannejad, who reported in their studies, not only that ulcers regressed, and healed but also new ulcers did not appear as long as nicotine application continued.[3, 4]

These results could be explained by the therapeutic effect of nicotine that results in tissue healing through various possible mechanisms; including inhibition of pro-inflammatory cytokines production involving a variety of interleukins; (IL)-2, IL-6, IL-8, IL-10, tumor necrosis factor through its direct effect on macrophages and induction of T-cells energy. [3,18,19] Cortisol and adrenocorticotrophic hormones delivery could also be activated by nicotine resulting in further suppression of inflammatory pathways. In addition, as shown in a vet study, the remedial effect of pure nicotine can be related to its role in the angiogenesis promotion and wound healing through activation of nicotinic acetylcholine receptors. Three case reports, support the hypothesis that nicotine confirming its protective part, as they observed prevention and healing of aphthous when patients used nicotine. [3,20] Some researchers thought that this protective effect is related to the increased keratinization of the oral mucosa in smokers and that this keratin layer acts as a mechanical and chemical barrier against trauma or microbes. In contrast, some have hypothesized that nicotine may be the responsible agent for the reduction in ulcer size. [16]

Our results regarding pentoxifylline group showed that ulcers started alleviating gradually but did not heal completely or produce any keratinization when compared to the nicotine group. The histological changes included severe inflammation followed by marked reduction in inflammatory cells and decrease in ulcer size in following days without full attenuation. These results were in line with Lee et al who reported that the wound size started to diminish within the first 24 hours reflecting the progress of healing, and by the end of a 10 days period, the percentage of pentoxifylline gel treated ulcers were much higher than the control group. It should be mentioned that in their study there was complete wound healing and probably this goes back to the longer duration of the gel application (8 days versus 10 days). Also in accordance to Hatemi et al, the gel decreased the duration of oral ulcers in their analytical study on 21 patients which might make it a positive favorable agent for patients who agonize oral ulcers.[21,22]

The ulcerative healing effects of pentoxifylline is not precisely defined but could be verified through its ability to increase red blood cells flexibility, having an anti-tumor necrosis factor (TNF)-α effect, acting as a vasodilator enhancing tissue oxygenation, and the strong role in cessation of inflammatory reactions in vivo. Pentoxifylline has been shown to increase leukocyte deformability and to stop neutrophil adherence and activation in animal and human in vitro studies and in addition, it participates in inhibition of fibroblast proliferation and extracellular matrix production. [6]

V. Conclusion

Treatment of ulcers is a challenging situation. Over the years, diverse kinds of therapies have been tired, but till date still no specific treatment has been proposed as being the most appropriate.

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