

Histological Determination of Cinnamon and Olive Oil Extract on Traumatic Oral Ulcer in Laboratory Rabbit

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Abstract

Background: The mucosa of oral cavity is the mucous membrane which covers the tissues of the mouth cavity. In order to repair damage from a local aggressor, many cell strains and their byproducts work together throughout the vital physiological process of wound healing. This process culminates in tissue repair and starts relatively early in the inflammatory phase. The supplements of cinnamon and olive oil can dramatically raise blood levels of antioxidants while lowering those of inflammatory indicators like C-reactive protein.

Objective: The aim of the study was to determine the local histological effect of topical application of cinnamon and olive oil extract on the rabbit oral mucosa

Patients and Methods: 20 adult male rabbits that weight about 700-900 Kg and age about (6-8) months where used in this experimental study. Ulcer induction: Prior to the creation of the ulcers, rats were fixed on their backs and all animals were anaesthetized and induction the ulcer with round filter papers 5.5 mm in diameter were soaked in 15 ml of 50% acetic acid. In order to create round ulcer, an acid-soaked filter paper was pressed onto the right buccal mucosa for 60 seconds. Then divided the groups according to the healing time with 10 rabbits as a control group left healed normally and 10 rabbits as an experimental group that daily used mixture of cinnamon extract and olive oil ready extract topically applied on the traumatic ulcer. The animals were sacrificed along three- and seven-days healing periods and then prepared H&E stain for analyzed the results.

Results: In comparison to the control group, the histological results of oral ulcers that were created and treated with a daily application of a herbal mixture consisting of cinnamon extract and olive oil extract showed greater epithelization, reduced inflammation, and increased angiogenesis, all of which sped up the healing process. Furthermore, there was a noteworthy distinction in the formation of extracellular matrix and collagen fiber synthesis between the experimental and control groups.

Conclusion: Topical treatment using ready herbal extracts of olive oil and cinnamon was more successful in facilitating the recovery of traumatic ulcers.

Keywords: herbal extract, traumatic ulcers, cinnamon extract olive oil extract.

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Introduction

Mucosa of oral cavity is the term for the mucous membrane that covers the structures inside the mouth cavity. Three layers make up the oral mucosa histologically. The first layer is the epithelium of oral cavity, which is a surface squamous stratified epithelium (1). The submucosa, which is located at the lowest level, is a dense, irregular connective tissue that lies beneath the lamina propria, all is connective tissue (2). The production of necessary nutrients, defense of the underlying tissues against mechanical, chemical, and biological stimuli, and the development of a sensory function that permits the experience of temperature, touch, pain, and taste are just a few of the many functions of the oral mucosa plays (3-4). The keratinized or para-keratinized stratified squamous epithelium that lines these surfaces awards the masticatory mucosa its capability to withstand the stress that it experiences at mastication time. Lastly, a particular mucosa on the back of the tongue has a squamous stratified epithelium which may or may not be keratinized (2). Furthermore, the non-keratinized epithelium, found on the lining mucosa, losses the granular layer, and the spinous layer is recognized to be in general thinner (5). Desmosomes hold the epithelium's cells together as they progressively flatten from the stratum basale to the stratum corneum, where they take on a scaly or squamous look. Beneath the epithelium lies a layer of connective tissue called the lamina propria, which is composed from blood vessels, neurons, mast cells, fibroblasts, macrophages, and inflammatory cells (6). Oral ulcers are a common presenting indication of a variety of disorders affecting the mouth that have multiple causative causes. Because various types of ulcerated lesions share similar clinical and histologic characteristics, these lesions may present a special diagnostic difficulty for medical professionals. (7,8). A biopsy may be necessary to rule out neoplasia or other disorders in chronic ulcerations, which may not always show a clear and obvious trauma source. Acute trauma ulcers typically heal on their own without complications after 14 days (9).

Cinnamomum spp., as the plant is known scientifically, is a versatile herb used in herbal therapy. Mucilage, tannin, sugar, resin, limonene, safrole, and essential oil with antimicrobial, antiseptic, antiviral, and antifungal qualities are among its constituents (10). With strong antioxidants on par with synthetic antioxidants, cinnamon bark has the potential to enhance the oxidative stability of food (11). Studies have indicated that olive oil consists a major part in the health benefits of the Mediterranean diet (MED) (12). The chemicals found in olive oil have demonstrated potential as antibacterial, anti-inflammatory, and antioxidant agents (13). The aim of the study was to determine the local histological effect of topical application of cinnamon and olive oil extract on the rabbit oral mucosa.

Patients and Methods

Study protocol

The experiment done at Diyala province-Baqubah and from 1st November 2023.

Study population and Study design

The 20 male rabbits were randomly assigned and used in the work into two groups consisting of 10 animals each, the experimental group and the control group. Each group was divided into two group according to healing periods to 3 days and 7-days healing intervals (5 rabbits to each interval). The combination of xylazine 2% (0.08 ml/kg B.W.) and ketamine 10% (3 mg/kg B.W.) was administered intramuscularly (IM). Prior to the procedure, all surgical supplies and towels were autoclaved for 30 minutes at 121°C and 15 bar/cm² of pressure to sterilize them.

Ethical considerations

This study was approved by the Ethics Committee of College of Medicine,

University of Diyala and according to the ethical approval (2024MAH823).

Ulcer Induction

Circular filter sheets soaking in 15 milliliters of 50% acetic acid, with a diameter of 5.5 mm. An acid-soaked filter paper was applied to the right buccal mucosa for 60 seconds in order to induce a circular ulcer [figure 1]. Ten milliliters of sterile distilled water were used topically once a day to treat the ulcer (Control Group). A single daily micropipette dose of 10 mL of ready extract mixture (buying from herbal company)

that diluted to 1 g/ml was used to treat the ulcer (14). Animals were euthanized with an excess of general anesthesia at the conclusion of the three and seven-day healing periods after ulceration in order to obtain ulcer samples for histological and histochemical analysis. In order to create slides, the specimens were embedded in paraffin, fixed in 10% formalin solution, and sectioned into thin 5 m slices. Hematoxylin and eosin (H&E) staining was carried out for histological assessment under a light microscope (15, 16).



Figure 1: induce ulcer

Statistical analysis

The detailed explanation of all variable was analyzed by used the Statistical Package for the Social Sciences (SPSS) version 25. The data were expressed using the mean, standard deviation, standard error, 95% confidence interval, minimum, and maximum values. Mann-Whitney The test was employed to examine the relationship between the variables that were being studied. If the confidence level was 95% and the P-value was 0.05 or below, it was deemed to have statistical significance.

Results

Ulcer size analysis

The average ulcer size for each groups on days 3, and 7 is showed decreasing induced ulcer sizes with time, with the mixture of herbal material group showing a greater mean value on day seven of the healing periods. The percentage of rabbits who reported a recovery in ulcer size at day 3 and day 7 for the groups that were examined showed an increase over the duration of the study. The mixture of two extract group had the highest mean percentage value at day seven, as indicated in (Table 1).

Inflammatory cells

According to the quantity of inflammatory cells that were scored the mean number of inflammatory cells to be at its maximum level after just 7 day in the extract mixture group, while it was at its lowest level after seven days in the oil group Table (2). Mann-Whitney U test was used in order to test the correlation between variables of two groups that were researched and presented in Table 3. Highly significant difference was between the mixture of two herbal material group and the control group (P = 0.013) at 3 days healing periods, and there was also a highly significant difference between the mixture of two herbal material group and the control group (P = 0.013).

Epithelial cells

The mean epithelial thickness of each of the groups that there was not a significant difference between any of the groups that were evaluated during any of the healing periods. Whereas this is evident on day 3 (P=0.631) and

day 7 (P=0.109), respectively Table 4.

Mann-Whitney U test was used in order to test the correlation between variables of two groups showed Non-significant difference was between the mixture of two herbal material group and the control group (P = 0.631) at 3 days healing periods, and there was also a non-significant difference between the mixture of two herbal material group and the control group (P = 0.109) at 7 days (Table 5).

Blood vessels and cells

The average number of blood vessels in the examined groups during all healing phases there was high average number of blood at 3 & 7 days in compare to control group and there was high important distinction between the researched groups through any of the recovery intervals. Where on day 3 the (P=0.004) and on day 7 the (P=0.002) between the studied groups (Tables 6,7).

Table (1): Descriptive statistics of mean of ultimate ulcer size in researched groups in all recovery intervals

Day	Group	N	Mean	SD	SE	95% Confidence interval for mean	Min.	Max.
Day 3	Extract mixture	6	6.1	0.43	0.11	5.7-6.5	5.72	6.3
	Control	6	7.3	0.31	0.12	6.7-7.3	6.74	7.4
Day 7	Extract mixture	6	1.7	0.92	0.45	0.7-2.5	2.1	2.9
	Control	6	2.9	0.71	0.32	2.2-3.7	2.4	3

Table 2: Descriptive statistics of the mean of inflammatory cells at day 3 and 7

Day	Group	N	Mean	SD	SE	95% Confidence interval for mean	Min.	Max.
Day 3	Extract mixture	5	25.9	25.9	3.21	1.31	19.5-26.3	19.2
	Control	5	20.8	27.8	1.42	0.62	23.3-26.2	23.42
Day 7	Extract mixture	5	20.4	20.4	3.41	1.45	15.8-23.0	15.21
	Control	5	22.6	22.6	2.14	0.91	24.4-28.8	24.43

Table 3: Comparison of the studied groups by Mann-Whitney test, according to the mean of inflammatory cells

Day	Group	Mean rank	P value
Day 3	Extract mixture	5.58	0.010*
	Control	7.42	
Day 7	Oil	3.92	0.013*
	Control	9.08	

Table 4: Descriptive statistics of the mean of epithelial cells at day 3 and 7

Day	Group	N	Mean	SD	SE	95% Confidence interval for mean	Min.	Max.
Day 3	Extract mixture	5	224.2	19.4	7.9	193.9-234.6	187.36	233.27
	Control	5	202.7	22.5	9.2	195.0-242.3	187.23	247.08
Day 7	Extract mixture	5	341.6	46.3	18.9	253.0-350.2	235.27	360.05
	Control	5	259.6	46.7	19.1	203.5-301.6	197.06	312.23

Table 5: Comparison of the studied groups by Mann-Whitney U test, according to the mean rank of epithelial cells

Day	Group	Mean rank	P value
Day 3	Extract mixture	6	0.631
	Control	4	
Day 7	Extract mixture	8.17	0.109
	Control	6.83	

Table 6: Descriptive statistics of mean of blood vessels in studied groups in all healing periods

Day	Group	N	Mean	SD	SE	95% Confidence interval for mean	Min.	Max.
Day 3	Extract mixture	5	13.7	2.4	1	9.2-14.2	9	15.3
	Control	5	10.5	3.9	1.6	5.4-13.6	6	17
Day 7	Extract mixture	5	15.7	2.1	0.9	10.5-14.8	10	15.6
	Control	5	12.3	3.1	1.2	8.1-14.5	7	16

Table 7: Comparison of the studied groups according to blood vessels

Day	Group	Mean rank	P value
Day 3	Extract mixture	8.08	0.004
	Control	4.92	
Day 7	Extract mixture	7.33	0.002
	Control	5.67	

Histological results

The histological finding at 3 days, the oral ulcer that created in control group revealed presence of granulation tissue and inflammatory cell less than that at experimental group figure 2-3, while at 7 days with complete healing the ulcer in experimental group and presence rate ridges and

papillary portion with normal lamina propria while the ulcer in control group that revealed newly formed thin epithelium in ulcer area The lamina propria showed granulation tissue formation with moderate to severe number of inflammatory cells, with scanty collagen fibers and few blood vessels figure 4-5. Histological finding (Hematoxylin and Eosin staining).

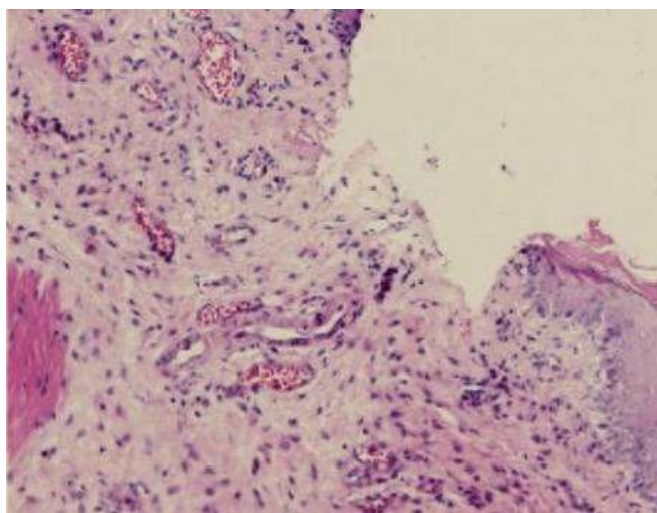


Figure 2: At the 3rd day in control group shows limited epithelium migration from the margin toward the center area with less number of the inflammatory cells H& E stained slide (x10).

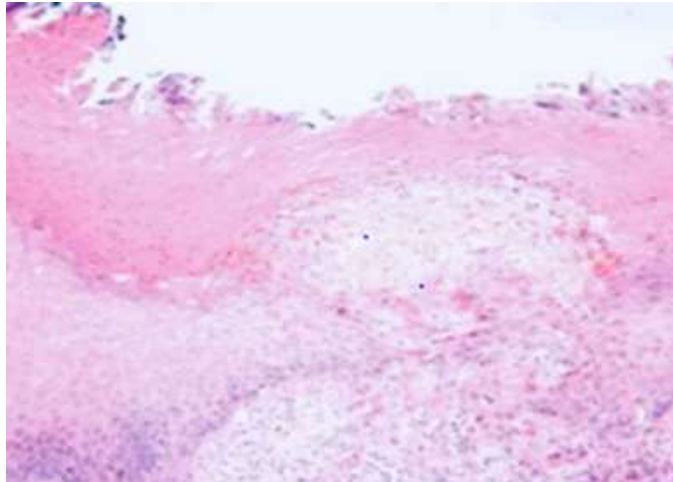


Figure 3: View of the study group at 3 day reveals an ulcer with epithelial and connective tissue migration and large number of the inflammatory cells H& E stained slid (x10).

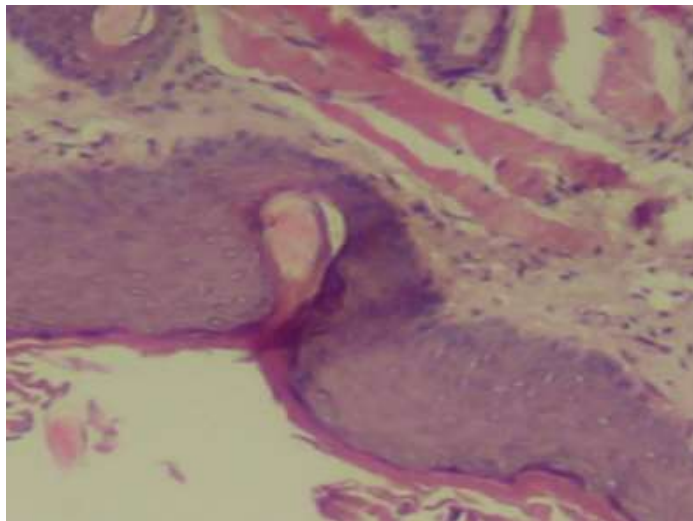


Figure 4: View of the control group at 7 day reveals control group, showed newly formed thin epithelium in ulcer area cells with early mature rete ridges H& E stained slid (x10).

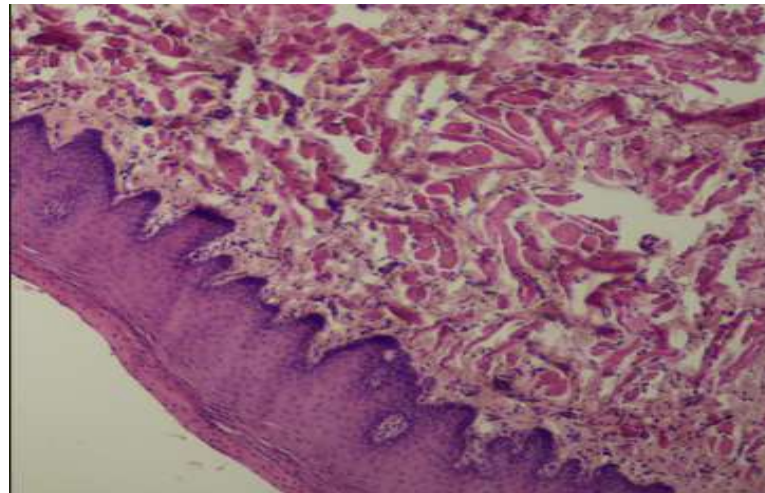


Figure 5: View of the study group shows at 7 day reveals mature keratinized stratified squamous epithelium with mature rete ridges H& E stained slid (x10)

Discussion

Trauma induced ulcers are damages to the mucosa of oral cavity performed by physical or mechanical trauma, or chemical burning for example acetic acid, unexpected chewing through mastication, chewing while talking, piercing by sharp objects, clefted, distorted, or Caries teeth or sharp edge (17). Olive oil and cinnamon extract has the ability to accelerate incision recovery because its influence on incision contraction, re-epithelization, early neovascularization, and enhanced collagen intensity (18). In every group, the average percentage of healing ulcer size increased over time, with the monitoring group owning the lowest mean value compared to the mixture extract group. This result of olive oil's & cinnamon effect due to antibacterial characteristics, which reduce the period wanted for incision contraction and cancel bleeding after surgery this finding agree with (19). In the present study, histological finding of inflammatory cells revealed varying degrees of variability across all healing intervals. The experimental groups' inflammatory response decreased over time,

whereas the monitoring group's elevated. This perhaps because the mixed oil contains anti-bacterial and anti-inflammatory actions (18) that are absent in the control group. This is agreed with (20), that employed myrrh powder in their work and diluted with sodium chloride to treat intraoral mucosal ulcers and showed that this enhanced the anti-inflammatory impact of myrrh in comparison to the control group this similar to the action of cinnamon and olive oil extract. It's also agree with (21) who utilized myrrh oil in the therapy of skin wound healing and improved the anti-inflammatory effect of myrrh in comparison to the control group. In the current research, the new blood vessels in both the monitoring and experimental groups was examined with a clear difference between the groups. The mixture extract group had the highest mean value of blood vessels on the first and seventh day compared to the control group. In agreement with (21) the present study revealed that experimental groups had more angiogenesis than control groups. Re-epithelization is the

process by which basal and suprabasal cells proliferate and migrate through the recovery stage in an attempt to mend a wound. The current study showed that re-epithelialization increased over time for both the experimental and control groups. The mixture extract oil group recorded a high mean value on days 3 and 7, which was marginally higher than the monitoring group. This was attributed to exceed neovascularization, fibroblast cells, and collagen fiber, as well as increased epithelial cell proliferation and progression. This is consistent with earlier researches (20,21). The keratin layer, subepithelial infiltration of mononuclear cells, and an obvious grade of re-epithelialization with the recuperation of rete ridges were all visible with the use of H&E stain. A significant rise in the combination extract groups in the basement membrane, especially on days 3 and 7 of the recovery periods; the histochemical results corroborate this (22).

Conclusion

According to the findings of this study, the topical application of essential olive oil extract when mixed with cinnamon extract is significantly more beneficial than the control group in promoting the healing of oral traumatic ulcers.

Source of funding

No source of funding

Conflict of interest

The author acknowledges no conflict of interest in this study

Recommendations

Herbal elements are among the most important lines of treatment that are recommended due to their many uses in healing oral and skin wounds. It is necessary to take into consideration

additional matters such as the type, size, and location of the wound, in addition to the blood supply, infection, and other matters that may hinder the healing process and hinder the action of the materials used in treatment.

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التقييم النسيجي والنسيجي لشفاء جروح تجويف الفم لدى الأرانب عن طريق تغذية الأحماض الأمينية القابلة للامتصاص

منار عبد الرزاق حسن^١

الملخص

الخلفية: قرحة الفم المؤلمة هي آفة منخفضة ومحدودة بشكل جيد مع خلل ظهاري مغطى بجلطة الفيبرين، مما يؤدي إلى مظهر أصفر-أبيض، يحدث بسبب إصابة كيميائية أو ميكانيكية أو حرارية للغشاء المخاطي للفم مع تآكل مؤلم. الأحماض الأمينية ضرورية لشفاء الجروح لأنها تعزز نمو النسيج الضام وكذلك تنشيط وتكاثر الخلايا الليفية.

الهدف من الدراسة: تقييم تأثير التطبيق المنهجي لجمع الأحماض الأمينية (تناول الفم) في علاج تقرح الفم المؤلم خلال فترة زمنية محددة عن طريق التقييم النسيجي والنسيجي لشفاء الأنسجة الرخوة.

المواد والطرق: تم استخدام ٢٠ ذكر أرنب بالغ بوزن حوالي ٧٠٠-٩٠٠ كغم وأعمار حوالي (٦-٨) أشهر حيث تم استخدامها في هذه الدراسة التجريبية. تم إنشاء القرحة المؤلمة بقطر (٨ مم)، و(١ مم) بواسطة مثقاب ماسي دائري جراحي في الغشاء المخاطي للخد الأيمن، ثم تم تقسيم المجموعات إلى مجموعتين، ١٠ أرانب للمجموعة الضابطة التي تلتئم بشكل طبيعي، و ١٠ أرانب للمجموعة التجريبية التي يستخدم يومياً خليط من الأحماض الأمينية نظامياً من خلال خلطه مع الماء لمدة شهر. تمت التضحية بالحيوانات خلال فترات شفاء مدتها ٣ و ٧ أيام وتم فحص الأنواع تشريحياً بعد التحضير النسيجي للقرحة المؤلمة.

النتائج: أظهرت النتائج النسيجية والنسيجية انخفاض الالتهاب، وتسريع إعادة تنسج سطح القرحة، وتولد الأوعية الدموية بشكل أفضل، وتعزيز إعادة تشكيل المصفوفة خارج الخلية مما يؤدي إلى تعزيز نضج الأنسجة والشفاء الكامل في جميع الحالات لمجموعات الدراسة منها في المجموعة الضابطة.

الاستنتاج: الدواء الكيميائي الذي يمثل التطبيق الجهازى للأحماض الأمينية فعال في تسريع شفاء القرحة المصابة في المجموعة التجريبية مقارنة بالمجموعة الضابطة عن طريق تكاثر الخلايا المتسارع وإعادة تنسج الغشاء المخاطي.

الكلمات المفتاحية: القرحة المؤلمة، الأحماض الأمينية، إعادة التظاهر

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