

# Efficacy of Coconut Milk on Healing of Oral Ulcers in Experimental Model; A Comparative Study



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**OBJECTIVE:** This study was carried out to observe the morphological changes in healing of oral mucosal ulcers in experimental animals by applying coconut milk.

**METHODOLOGY:** This experimental study was spanned over one month. It was carried out at animal experimental lab of University of Health Sciences Lahore, Pakistan. A total of 30 rabbits were divided into 2 groups (Group A & B, control & experimental respectively). Oral ulcers were induced in all the rabbits by using 4mm diameter punch biopsy apparatus through standard protocol. Normal healing in Control group was compared with the healing effects of medicament (coconut milk) which was applied topically thrice daily for fourteen days in Experimental group. Biopsies from both the groups were taken on 1, 4th, 7th and 14th day to see gross morphological as well as histological changes.

**RESULTS:** Effects of coconut milk on healing of oral ulcers in experimental group, showed a sharp contrast with that of control group ( $p=0.001$ ) especially on day 7th where maximum healing was induced by coconut milk.

**CONCLUSIONS:** Coconut milk enhanced the healing of oral ulcers. Coconut milk has proved far superior healing effects on oral mucosa and therefore recommended as a natural healing medicament to be used as an alternate ulcer healing agent which is easily available, safe and inexpensive.

**KEYWORDS:** Coconut milk, healing medicament, histopathology, oral ulcer

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## INTRODUCTION

An ulcer is a breach in epithelium or mucous membrane resulting in loss of superficial epithelium and exposing connective tissue, muscle or bone, pus discharge or necrosis may also occur.<sup>1</sup> Recurrent aphthous ulceration (R.A.U) or recurrent aphthous stomatitis is the most frequent oral mucosal disease known to human beings

affecting 10 -20 % of the population.<sup>2</sup> Recurrent Aphthous Stomatitis (RAS) is a condition in which ulcers repeatedly occur in the oral cavity.<sup>3</sup> Despite of much clinical and research attention, the cause still remains inadequately understood.<sup>4</sup> Management is directed at minimizing the swelling & soreness, speed up healing by topical or systemic steroids, NSAIDS or antiseptics to protect the ulcerated areas.<sup>5</sup>

Antibiotics are fundamentally essential in minimizing the infectious diseases globally. Unfortunately, the resistance is increasing in the pathogens, enhancing their spread and minimizing the helpfulness of the antimicrobial agents.<sup>6,7</sup> Use of plants is dated back since prehistoric eras as nutritional sources and for curing a huge number of diseases known to human beings. As an alternative substitute, the application of plants has been promoted by the World Health Organization (WHO) through various schemes.<sup>7</sup>

Coconut milk and coconut water provides anti-

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ulcerogenic properties as good as to those of sucralfate, which coats the gastric lining with sticky complex proteins, thereby protecting it.<sup>8</sup> Antioxidants and high levels of potassium make coconut water unique.<sup>9</sup> Cytokinins are also present in coconut which promotes plant cell division and growth with astonishing anti-cancer, anti-thrombotic and anti-ageing properties.<sup>10-12</sup> Coconut milk is obtained from fresh white meat of coconut and oil is obtained after drying the coconut and then squeezing it, fatty acid contents of both the extracts are equal.<sup>13</sup> In a study conducted on peptic ulcers induced by Indomethacin, it showed a 54% reduction by coconut milk in the mean gross ulcer index as compared to 56% by sucralfate and only 39% by coconut water. Another study reported that the administration of coconut milk improved ethanol-induced gastric ulcer and worked as a possible choice in the treatment of ulcers.<sup>14</sup>

Similarly, topical application of dexamethasone also decreases the size of oral ulcers in addition to pain relief.<sup>15</sup>

Many specific therapies and the new biologic agents have reformed the treatment of cancers, autoimmune and inflammatory diseases are also associated with adverse events in the oral cavity including oral ulcers and other reactive symptoms.<sup>16</sup>

Current experiment was conducted on minor aphthous ulcers to observe the morphological sequence and changes in healing of oral mucosal ulcers in experimental animals by applying coconut milk used as healing agent.

## METHODOLOGY

This Experimental Study was spanned over a period of one month from September to October 2014. After the procurement of animals and other required material the study was conducted in the Department of Oral Pathology/Morbid Anatomy and Histopathology, University of Health Sciences (UHS), Lahore. Permission to conduct the study was obtained from the Institutional Review and Ethical Committee of UHS (UHS/EDUCATION/126-13/3357) where animal house facilities were also available. The sample size of 15 animals per group was calculated by the statistical formula and through OpenEpi software version 2.20 keeping the power of study equal to 80% and level of significance equal to 5%. All the processes of this research including the examination were conducted by a single, trained and calibrated examiner (principal investigator) who was trained in the Department of Oral Pathology (UHS).

Rabbits of New Zealand breed were segregated. Those who were of 8-10 weeks old, male with approximate weight between 1.5-2.5 kg and were Fed on normal diet for at least two weeks at our experimental laboratory were included in this study. However, Animals that were sick or dead during

the study period and had improperly fixed / processed tissue were excluded.

A total of 30 rabbits were randomly divided into two groups (Group A & B, control & experimental respectively) by drawing lottery/ balloting method. Each group equally had 15 rabbits. The study animals were maintained in the animal house of University of Health Sciences, Lahore, under controlled temperature (24 -260C) and humidity of 45 - 55 %. The light and dark cycles were maintained for 12/12 hours each. The animals were fed on standard rabbit diet and tap water. They were adapted for two weeks before starting the experiments. All procedures were performed under hygienic environment.

1) Experimental **Group A** (Control group): Induction of ulcers & healing without any medicament to observe the natural mechanism of healing and to compare it with experimental group

2) Experimental **Group B** (Case group): Induction of ulcers & applying coconut milk thrice daily at 8hours interval with cotton pellets right after inducing the ulcers for 14 consecutive days.

*Coconut milk extraction:* After the water dispersion was removed, the fleshy endocarp (white meat) was obtained by using a knife to isolate it and then blended in a blender processor adding one and a half cup (200 ml) of warm water. The milk was obtained by filtration with filter cheesecloth which was squeezed to get all the milk out. The water dispersion and milk extracts were refrigerated and used within 16 hours of cracking and extraction, respectively, to avoid microbial activities.<sup>15</sup>

*Ulcer induction:* Healing by secondary intention was the goal of this procedure. The animals were locally anaesthetized with xylocaine 2% solution containing lignocaine HCL + adrenaline 1:200,000. The mucosa was sterilized by using a swab covered in 0.12% chlorhexidine digluconate. About 2mm thick buccal mucosa was punched out with a punch biopsy apparatus (4 mm in diameter) and separated with the help of a scalpel in all the rabbits of both groups.

*Biopsy Procedure:* Specimens were taken from the buccal mucosa on Day 1, Day 4, Day 7 and Day 14 with the help of punch biopsy apparatus (8mm in diameter). Tissue specimens were fixed in 10% formalin and brought to the department of Oral Pathology/Histopathology.

Detailed gross examination findings were recorded. Paraffin embedded blocks was made after taking the tissue through ascending grades of ethanol. The sections 3-4 micro meters were cut using rotary microtome and stained using

Haematoxylin and Eosin stain and submitted for microscopic examination.

The scores were self-generated however; they were first piloted on the 10% study subjects and were therefore the scores were reliable and valid to be applied in main research. The overall score of each tissue was assessed on following criteria.

0. Focal or Complete re epithelialization/ Remodeled connective tissue

1. Focal or Complete re epithelialization with Fibrosis + Mild chronic inflammation (lymphocytes)

2. Ulcer present with Fibrosis or chronic inflammation, Focal re epithelialization may be seen

3. Ulcer present with chronic inflammation or granulation tissue. No re epithelialization

4. Ulcer present with acute process (dilated vessels, mixed inflammatory infiltrate with neutrophils) No re epithelialization.

### STATISTICAL ANALYSIS

The data was entered and analysed using SPSS 20.0. A scoring criterion was devised for qualitative histopathological variables and Fisher's exact test was applied. A p value of < 0.05 was considered as statistically significant.

### RESULTS

All the rabbits were weighed before the start of experiment and at the end of experiment. Average weight of each group at the beginning of experiment, mean age in months and the site of biopsy taken are given in Table 1.

**Table 1:** The table shows the mean weight & age of rabbits of each group at induction of ulcer

Groups and number of rabbits	Mean Average Weight in kg	Site of biopsy taken	Mean age in Months ± 2
Group A rabbits (n=15)	1.89	Buccal mucosa	11
Group B rabbits (n=15)	2.08	Buccal mucosa	12

The mean Kappa value of intra-examiner reliability was identified as 0.95. The follow-up attrition was 0% as no subject lost to follow-up during the total study period.

*Macroscopic features:* Gross morphology of the ulcers at 1st, 4th, 7th and 14th day were observed in terms of their size initially, at the time of biopsy, the consistency and texture of ulcer, and hemorrhage/necrosis at the site of ulcer. These observed basic features in Groups A & B are shown

in Table 2. Clinically the buccal mucosa of rabbits in Group A & B on the day of induction of ulcer (day 1) is shown in Figure 1(A&B), whereas, much healed buccal mucosa at day 7th is represented in Figure 2(A&B).

**Table 2:** Gross/macroscopic observations at 1st, 4th, 7th and 14th day of biopsies

Groups	Size of the initial ulcer	Size at the time of biopsy	Color of tissue	Consistency/ Texture	Hemorrhage
Day 1					
Group A	4mm	4mm	Red	Soft	Present
Group B	4mm	4mm	Red	Soft	Present
Day 4					
Group A	4mm	4mm	White	Soft/Friable	No
Group B	4mm	2.8mm	White	Soft/Friable	No
Day 7					
Group A	4mm	2.25mm	White	Soft/Friable	No
Group B	4mm	0 mm	White	Soft	No
Day 14					
Group A	4mm	0mm	Normal	Soft	No
Group B	4mm	0mm	Normal	Soft	No

**Figure 1: (A & B)**



**Figure 2: (A & B)**



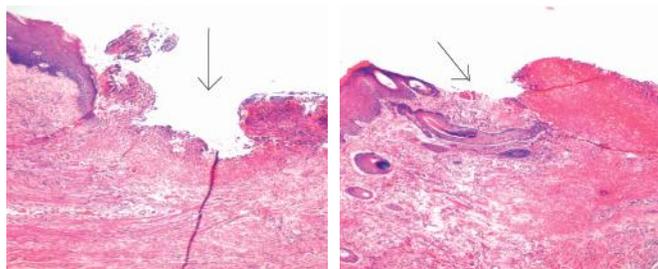
**Table 3:** Microscopic observations at 1st, 4th, 7th and 14th day of biopsies and the corresponding scores.

Groups	Microscopic Score according to scoring criteria	P-Value (Fisher's exact test)
Day 1		
Group A	4	P=0.226
Group B	4	P=0.315
Day 4		
Group A	4	<0.024
Group B	2	
Day 7		
Group A	3	<0.012
Group B	1	
Day 14		
Group A	0	<0.032
Group B	1	

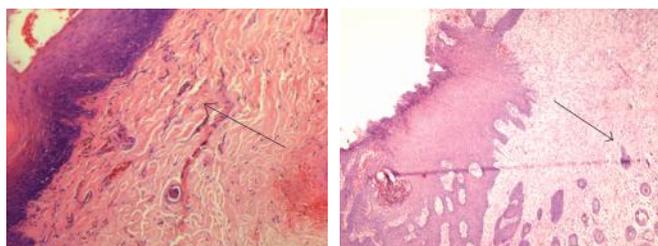
**Microscopic features:** Microscopic observations at 1st, 4th, 7th and 14th day of biopsy along with the corresponding score accordingly to the mentioned criteria and the associated P-value is mentioned in Table 3.

Photo micrographic representation of the buccal mucosa of rabbits in Group A & B on the day of induction of ulcer (day 1) is shown in Figure 3(A & B). Initiation of fibrous band formation, remodeling and more cellular connective tissue on day 14th in Group A & B is shown in Figure 4 (A & B).

**Figure 3: (A & B)**



**Figure 4: (A & B)**



## DISCUSSION

Current research was conducted to observe the morphological and histological variation in oral mucous membrane of rabbits after ulcer induction and to compare those morphological events with the oral mucosal ulcers where the natural medicaments were used in rabbits. There is no unique eliciting agent responsible for oral ulceration. Mucosal damage appears to characterize a T cell mediated immunological reaction. Peripheral blood investigation of patients with apthae indicates a reduction in ratio of CD4+ to CD8+ T lymphocytes.<sup>17,18</sup> Over the years extensive research work has been carried out to find the etiological factors and treatment modalities of oral ulcerations. Current study was focused to find an alternative treatment option for the patients experiencing recurrent oral ulcers or any form of mucosal ulcers due to any reason.<sup>15</sup> There are lots of medicaments and brand products in the market for this problem but we tried to discover and advocate natural remedies which are readily available and inexpensive for

the patients. Every ingredient of the coconut has therapeutic value. Nneli et al in their study suggested that a 54% reduction in the mean ulcer index was caused by coconut milk against 56% by sucralfate while only 39% reduction in mean ulcer index by coconut water. Hence, the outcome of both the sucralfate and coconut milk were identical in induced peptic ulcers in rats' stomach.<sup>15</sup>

As far as microscopic variables are concerned, day 1 biopsies showed breach in epithelial lining and hemorrhage in all the biopsies. Biopsies taken on day 4 showed variable microscopic appearances among the groups. Ulcer with hemorrhage (extravasated RBC's) was found to be present in all the biopsies of group A while group B showed reepithelialization microscopically and healing ulcer clinically ( $p < 0.001$ ). Ulcer was recorded as healing ulcer microscopically when there was evidence of beginning of re epithelialization. Group A showed predominantly moderate acute inflammatory infiltrate. Group B showed more or less mixed type of inflammation that is neutrophils and lymphocytes both present with rest of the constituents of granulation tissue. Using the scoring criteria when comparison was made by using fisher exact test it was found significant among the groups ( $p < 0.001$ ). Neovascularization (component of granulation tissue) was observed and compared among the groups. It was mild in group A (control) but its presence was marked in all experimental group as compared to group A ( $p < 0.001$ ).

As no study was found in literature on the healing of oral ulcers to compare the results of the present study, a study by Folkman et al on experimental duodenal ulcers in rats was taken into account which reported the stimulation of angiogenesis in granulation tissue by administration of basic fibroblast growth factor that dramatically speeded up healing of ulcers in their studies.<sup>19</sup> Collagen lay down and fibrosis is the ultimate part of healing.<sup>20</sup> At day 4, there was no significant change in fibroblast density in Group A (control) but half of the biopsies of group B showed plump fibroblasts and increase in density of fibroblasts per unit area ( $p < 0.001$ ). Typical mitosis was counted per high power field in adjacent epithelium (40x) as total number of cells undergoing mitosis with visible mitotic figures. The diagnostic value of mitosis count is highly significant whether a lesion is precancerous or neoplastic (atypical mitosis) or normal (typical mitosis). No atypia or basal cell layer hyperplasia was found in any biopsy of experimental group as well as control.

Biopsies taken on Day 7 showed no evidence of ulcer in the entire experimental group biopsies while in group A (control), breach in epithelium was evident along with extravasated red blood cells (hemorrhage). When compared among the groups using fisher exact test it was found to be statistically significant ( $p = 0.002$ ). At this stage, group A

(control) showed mild chronic inflammatory cells in the connective tissue. Newly formed blood vessels (Neovascularization) were microscopically analyzed and their presence was found to be marked in Group A and 50% of Group B. It was not found statistically significant either ( $p > 0.005$ ). Density, shape and size of fibroblast (fibrosis) were noted in all the biopsies. There was not any significant change in fibroblastic proliferation of Group A biopsies while all the experimental groups showed marked evidence of plump fibroblasts in the connective tissue. This correlation was found to be statistically significant ( $p < 0.001$ ). No signs of necrosis, basal layer atypia or loss of polarity were seen.

Final biopsies were taken on day 14, no evidence of ulcer presence was found in any biopsy as well as no signs of inflammation. As far as connective tissue remodeling and fibrosis is concerned, Biopsies taken from Group A (control) showed complete fibrosis while Group B showed 50% biopsies with fibrosis and rest 50% were cellular and showed plump fibroblasts (fibroplasia). These findings can be correlated with a study done on gastric mucosal healing which is similar to its stage of proliferative healing stage in which spindle shaped cells are seen to form bundles and extend in radiating form to center of ulcer in palisaded form with hyperplasia of numerous capillaries in regenerated tissue. Acanthosis was seen in all healed epithelium of all the groups with some spongiosis as well.<sup>19,21</sup>

As no similar studies were found in the literature to compare the results of the present study, therefore; the current findings are establishing the initial preliminary facts which in future recommend analyzing the molecular basis and the contents of coconut water and coconut milk that can be isolated and used as healing medicaments. Having emphasis more on using natural remedies will lead to minimum side effects and less expensive to the patients. Further similar studies should be conducted to establish the evidence based healing properties of coconut especially in immune-compromised models.

## CONCLUSION

Findings of this study lead to the conclusion that the Coconut milk enhanced the healing of oral ulcers. Coconut milk has proved far superior healing effects on oral mucosa and therefore recommended as a natural healing medicament to be used as an alternate ulcer healing agent which is easily available, safe and inexpensive.

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## CONFLICT OF INTEREST

There is no conflict of interest that may profit or loss through the publication of this paper.

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