

Effects Of Topical Application Of Variety Of Betel Nuts, Betel Quid With And Without Tobacco On The Buccal Mucosa Of Wistar Albino Rats - A Histopathological Study



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Abstract

An animal study was conducted to assess the effects of white and red betel nut and betel quid in Wistar albino rats. Fifty-six rats were divided equally into 7 groups. Betel nut or quid paste was applied on the buccal mucosa for 6 months. Biopsies were done at 2, 4 and 6 months for histopathology. Epithelial, submucosal collagen and vascular changes, and inflammatory infiltrate were significantly altered in the study groups. The white variety of betel nut and betel quid revealed more epithelial and connective tissue changes than the red variety. The degree of change increased on adding slaked lime and tobacco.



Background

The use of white and red betel nut is indigenous to Southeast Asia with strongly interwoven religious practices and cultural rituals¹. White betel nut is a matured fruit containing less polyphenols, more alkaloids whereas red betel nut is a tender fruit with more polyphenols and less alkaloids. Oral submucous fibrosis is a high risk precancerous condition². The hypothetical etiology include chronic irritant, collagen, genetic or immunological disorder³. The rate of malignant transformation is 3% to 19%⁴.

Objective

To study the effects of red and white betel nut and betel quid on the buccal mucosa of Wistar albino rats.

Methods

On approval from Institutional Animal Ethics Committee, the study was done on 56 Wistar albino rats divided equally into 7 groups:

Group	Details
1	Painted with white variety of betel nut on the buccal mucosa
2	Painted with red variety of betel nut on the buccal mucosa
3	Painted with white betel nut + betel leaf + slaked lime
4	Painted with red betel nut + betel leaf + slaked lime
5	Painted with white betel nut + betel leaf + slaked lime + tobacco
6	Painted with red betel nut + betel leaf + slaked lime + tobacco
7	Control

1 gm of betel nut or quid paste (freshly prepared) was applied bilaterally on the buccal mucosa on alternate days for 6 months.



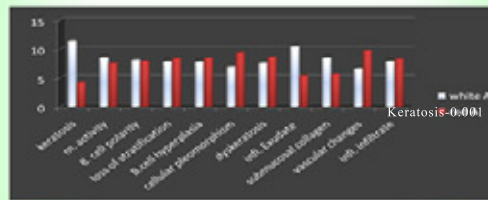
White & red betel nut

Betel quid preparation

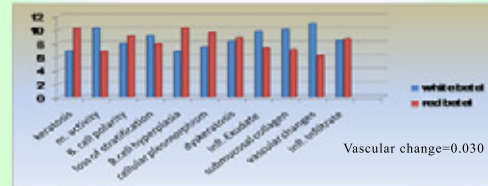
Applications and biopsies at 2, 4 & 6 months were done using Ketamine (intraperitoneal). Biopsy specimens were stained with haematoxylin, eosin and Van Geison's. Histopathological criteria was based on that followed by **Khrime et al.** and **Wahi et al.** for epithelial change, **Khrime et al.** for connective tissue change and **Sirsat and Pindborg et al.** for vascular change.

Results

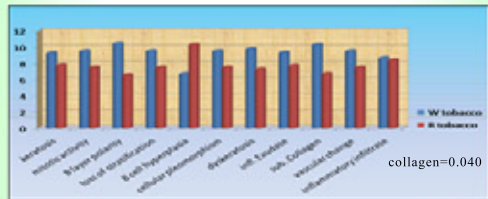
Graph 1: Group 1 and 2



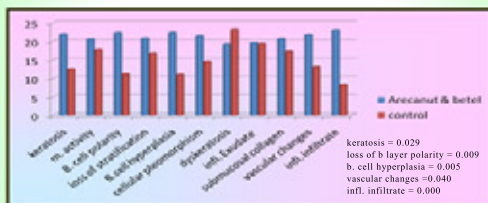
Graph 2: Group 3 and 4



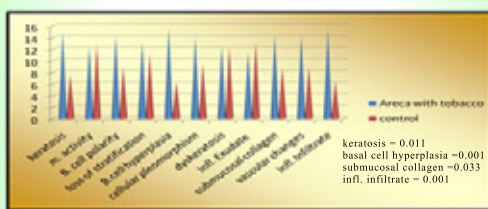
Graph 3: Group 5 and 6



Graph 4: Group 1, 2,3,4 and control

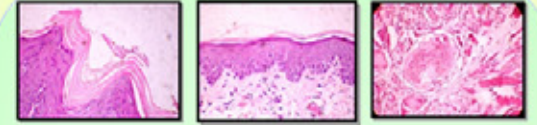


Graph 4: Group 1, 2,3,4 and control

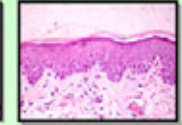


Discussion

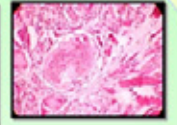
Group 1 & 2: Keratinosis in group 1 is attributed to coarseness of the white betel nut paste leading to chronic irritation or the loss of certain chemical contents of red betel nut during processing. **Group 5 & 6:** Submucosal collagen in group 5 may be because white betel nut contains less polyphenols (17.8%), more alkaloids (arecoline 0.2%) whereas red betel nut has more polyphenols (47.9%) and less arecoline (0.06%).⁵ **Group 1 & 5:** Dyskeratosis is one of the late features of epithelial dysplasia^{6,7}. **Group 2 & 6 :** Keratinosis is the initial change of epithelium under chronic irritation; tobacco & slaked lime are known to induce such change.



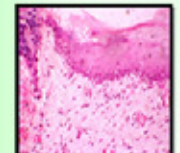
Microphotograph showing orthokeratinosis in group 1 (100X)



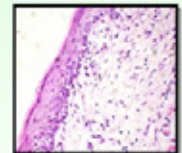
Microphotograph showing loss of basal layer polarity, loss of stratification, cellular pleomorphism, increased mitotic activity, lymphocytic infiltration in group 3 (100X)



Microphotograph showing inflammatory infiltrate & multinucleated giant cells in the connective tissue in group 4 (100X)



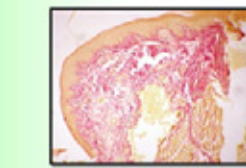
Microphotograph showing loss of basal layer polarity, loss of stratification, cellular pleomorphism in group 5 (100X)



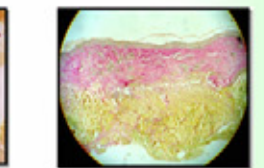
Microphotograph showing dyskeratosis, loss of basal layer polarity, loss of stratification, lymphocytic infiltration in group 6 (100X)



Microphotograph showing congestion of blood vessels along with eosinophilic infiltrate in group 1 on 6 months application. (40X)



Microphotograph showing increased submucosal collagen fibres in connective tissue in group 5 (40X)



Microphotograph showing increased submucosal collagen fibres in the connective tissue in group 6 (40X)

Comparison of 1,2,3,4 with 7: Significant epithelial dysplasia, vascular changes & inflammatory infiltrate support the hypothesis that slaked lime hydrolyses arecoline & arecadaine of betel nut to facilitate fibroblast stimulation.⁸

Comparison of 5, 6 with 7: Presence of submucosal collagen implies the synergistic action of tobacco with betel nut (white/red).

Pindborg & Zachariah reported 40% oral cancer patients had signs of OSF (all chewed betel quid with tobacco)⁹

Ranganathan et al reported 185 patients diagnosed as OSF had the habit of chewing betel quid with tobacco.¹⁰

Relative risk assessment: The risk factors for dysplastic changes - groups 1, 3, 6 - risk factors for leukoplakia. Risk factors for increase in submucosal collagen - group 5 - attributed to OSF.

Conclusion

White variety of betel nut & betel quid revealed more epithelial & connective tissue changes than the red variety. The degree of change increased with slaked lime & tobacco.

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