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Anti-Inflammatory Effect of Eugenia Jambolana on Epithelial Thickness in Induced Gingivitis

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ABSTRACT

Objective: The objective of this study was to correlate the histological changes in gingival epithelium, after tropical application of Eugenia jambolana and to observe the effect of Eugenia jambolana extract on the thickness of epithelium on induced gingivitis in albino rats.

Materials and Methods: This experimental study was conducted in the animal house, Anatomy Department, Post Graduate Medical Institute, Lahore, Pakistan. A total of 48 albino rats were selected and were further divided into three main groups i.e. Group A (Control; healthy mucosa, no intervention), Group B (Experimental B; inflamed mucosa with an application of extract), and Group C (Experimental C; inflamed mucosa with no intervention) having 4 subgroups each based on the number of days i.e. 3rd, 4th, 10th and 20th day. Histological changes in the buccal mucosa were observed on the respective days, after inducing the gingivitis in both the control and experimental group.

Results: Results were recorded on the 3rd, 4th, 10th and 20th days. On the 3rd day, signs of severe gingivitis appeared in both experimental groups 1B and 1C. An epithelial thickness of 12.00±2.160µm was observed in group 1B and, 10.2/5±1.708 µm in group 1C. On day 4th, in group-2B, the epithelial thickness was 12µm±1.633µm. The epithelial thickness was 10.25µm±1.708µm in group C. On day 10th, group 3B exhibited, a thickness of 20.00 µm±1.633µm. In group 3C, the thickness of epithelium was 23.75µm±1.258µm. On day 20th, the thickness was 23µm ±1.155µm in group 4B. In group 4C, the thickness was 24µm ±1.414µm.

Conclusion: This study proved the beneficial effects of Eugenia jambolana on the healing of gingivitis. The contents in Eugenia jambolana have an anti-inflammatory action on soft tissues which could be beneficial to treat gingivitis.

Keywords: Anti-inflammatory, Buccal mucosa, Eugenia Jambolana, Gingivitis, Healing

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INTRODUCTION

The oral cavity is lined by the mucous membrane called oral mucosa which is histologically a stratified squamous epithelium and has a protective function as a barrier against pathogens.[1] The mucosa of lips or cheeks transcends towards the mucosa of the alveolar process by forming a vestibular fold. Oral mucosa is folded in the cheek area, where it is known as the buccal frenulum and on the median part of the upper and lower lip area is the labial frenulum.[2]

The part of the oral mucosa that immediately surrounds the erupted teeth is called the gingiva. Gingiva consists of the mucosal tissue surrounding the roots of the teeth and covering the tooth-bearing part of the mandible and maxilla. The gingival tissue is stippled, pink in colour. Periodontal diseases such as periodontitis and gingivitis are inflammatory processes that destroy the periodontal tissues supporting the gums, the periodontal ligaments and the alveolar bone, affect about 50% of the world population.[3] When plaque accumulates around the gingival margin, inflammation begins within gingival connective tissue and epithelium. Within 3 to 4 days connective tissue destruction starts, 70% of collagen lost. Masticatory mucosa has orthokeratinized epithelium, in which stratum granulosum is not so prominent and covers those regions of the mouth which are exposed to strong forces such as hard palate, attached gingiva and tongue.[4]

The histological evaluation shows that in the absence of the concerned etiological factor, gingival mucosal changes involve lamina propria as well as the epithelium of the gingiva. The epithelium becomes enlarged and connective tissue shows more fibrosis which extends with various levels of inflammation in all gingival overgrowth lesions.[5] Epithelial thickness increases with elongated papillae in gingival overgrowth and fibrosis occur in the lamina propria with an increased number of fibroblasts. The thickness is 5 to 10 times greater than the normal gingiva.

Eugenia jambolana is an evergreen plant is originally from India, Pakistan, and Indonesia. Leaves, bark, stems and seeds of the plants are used herbally. Eugenia jambolana contains anthocyanins which suppress inflammation. Effective application of the extract may assist in the healing of inflamed gingiva. Extract of seeds of Eugenia jambolana was found to have anti-diabetic, anti-inflammatory, hepatoprotective, antihyperlipidemic and antibacterial properties.[6] The antioxidant capacity of anthocyanins prevents oxidants from destroying connective tissue in capillaries.[7] Based on its medicinal properties the objective of this study was to correlate the histological changes in gingival epithelium, after tropical application of Eugenia jambolana and to observe the effect of Eugenia jambolana extract on the thickness of epithelium on induced gingivitis in albino rats.

MATERIALS AND METHODS

This experimental animal study was conducted at the Experimental Research Laboratory of Post Graduate Medical Institute Lahore. The purpose was, to study histological changes in the buccal side of the gingival mucosa of 1st right maxillary molar of adult albino rats on the 3rd, 4th, 10th and 20th day after inducing gingivitis. Adult animals weighing 200-250 gm of both genders, were chosen. The reagent used in this study was Eugenia jambolana known as Jamun. The study protocol was approved by the Advanced Studies and Research Board of the University of Health Sciences, Lahore and the Ethical Committee of Postgraduate Medical Institute, Lahore.

Forty-eight adult albino rats were procured from the National Institute of Health Islamabad. All animals used in this study were handled with the international, natural, and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care - 1984.[8] Following acclimatization for one week, the procedure was started. Each animal was weighed before and at the end of the study. Rats were divided into three equal groups by using a random number generator as shown in Table 1. Histological changes of the buccal mucosa were studied on the 3rd, 4th, 10th and 20th day after induction of gingivitis in both control and experimental group animals.

The rats were anaesthetized with ketamine (100 mg/kg body weight) and xylazine (10mg/kg body weight) by intraperitoneal injection.[9] The area selected for inducing gingivitis was cleaned with pyodine to remove saliva or any food particles that may be present. The cotton thread was placed between the first and second maxillary molars of the right quadrant of the group B and C rats for inducing gingivitis.

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Table 1: Details of study groups

Groups	Subgroups	Number Of Animals	Remarks
Control group A	1A (day3)	4	Without given any dose
	2A (day4)		
	3A (day10)		
	4A (day 20)		
Experimental group B	1B (day3)	4	Without Eugenia jambolana extract
	2B (day4)		
	3B (day10)		
	4B (day 20)		
Experimental group C	1C (day3)	4	With Eugenia jambolana extract
	2C (day4)		
	3C (day10)		
	4D (day20)		

Eugenia jambolana seeds were obtained by getting fruit from the University of Punjab. Ethanolic extract of *Eugenia jambolana* seeds was prepared by 100 gm of seed-kernel powder which was suspended in 250 ml of distilled water and allowed to stand overnight in the refrigerator. After sieving, the filtrate (water extract) was discarded. The residue was extracted with 95% ethanol using sox halation/ wherein ethanol was evaporated in a rotatory evaporator at 40–50 ºC. The yield of the kernel was 3.2 g/100 g of seed powder. The extract of Eugenia jambolana was given orally with the help of an insulin syringe for 10 days.

On 3rd day after inducing gingivitis, four animals from each group were placed in a chloroform chamber and decapitated under deep anaesthesia. The rest of the animals were decapitated by the same procedure and number on the 4th, 10th and 20th day after inducing gingivitis. The whole right maxillary quadrant was dissected and after washing with saline it was fixed in neutral 10% buffered formalin for 48 hours at room temperature. Later, the specimens were processed for histological slides stained with Eosin and Haematoxylin before the microscopic study.

RESULTS

The general histological study of a normal gingival mucosa revealed the following layers: the epithelium and lamina propria. The normal gingiva in the control group had a scalloped margin, pink in colour with an epithelial thickness of 23.25±1.500µm. The lamina propria had fine bundles of collagen.

On day-3, gross examination of the selected area in both experimental groups (1B and 1C) showed marked redness, hypertrophy, slight ulceration and a tendency to bleed spontaneously. Junctional epithelium migrated apically from the cementoenamel junction, and the gingival sulcus depth increased in experimental groups 1B and 1C. The inflamed gingiva was swollen and puffy with rolled margins. The selected area was viewed under a light microscope. Signs of severe gingivitis including inflammation on the buccal surface of the maxillary right quadrant was also observed. An epithelium thickness of 12.00±2.160µm and 10.25±1.708 (Table 2) was noted which is less than the normal thickness. There was evidence of fibrosis with short rete pegs.

On day-4, group 2B exhibited epithelial breaks and short rete pegs with an epithelial thickness of 12µm±1.633µm. In group 2C, long rete pegs were seen, fibrosis was near to normal. It was observed that attaining the thickness of epithelium in group 2C was faster than that in experimental group 2B. The epithelium thickness was measured to be 10.25µm±1.708µm in group 2C. The difference between the epithelial thickness of the two groups was

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TABLE 2: Comparative analysis of epithelial thickness (µm) between groups on Day-3, -4, -10 and -20.

Parameter	Days	Control Group (mean ± S.D)	Experimental group 1 (mean ± S.D)	Experimental group 2 (mean ± S.D)	Number of animals (N)	*p*-value
Thickness of epithelium (µm)	3	24.00±1.414	12.00 ± 2.160	10.25 ± 1.708	4	<0.001
	4	23.50±1.291	12.00 ± 1.633	10.25 ± 1.708		
	10	23.25±1.500	20.00 ± 1.633	23.75 ± 1.258		
	20	23.25 ±1.500	23.00 ± 1.155	24.00 ± 1.414		

found to be statistically significant as shown in Table 2.

On day 10, there was a prominent increase in the thickness of epithelium in both groups. The gingival epithelium was closer to attain its normal thickness. In group 3B, the keratinized stratified squamous epithelium of a mean thickness of 20.00 µm±1.633µm (Table 2) was present. The epithelium consisted of a single layer of large columnar cells constituting stratum basale, 4-5 layers of polyhedral cells forming stratum spinosum and 2-3 layers of flat cells with keratohyalin granules forming stratum granulosum (on top). In group-3C however, the keratinized stratified squamous epithelium was about the mean thickness of 23.75µm±1.258µm (Table 2). The epithelium thickness was normal as compared to group 3B. The difference in values of thickness of epithelium in the two groups was statistically significant as shown in Table 2.

On day 20, there was complete regaining of gingival epithelium in experimental groups 4B and 4C. The gingival epithelial thickness was 23µm ±1.155µm in group 4B. In group 4C, the gingival epithelial thickness was 24µm ±1.414µm. Normal stratification of cells was visible. Collagen bundles were seen in the connective tissue. Healthy mucosa was seen in both experimental groups. Rete ridges moved up towards the cornified layer. Connective tissue showed fibroblasts and loosely arranged collagen fibres. There was also slight evidence of collagen bundle formation. The gingival epithelium and connective tissue of both experimental groups were almost similar to the gingival epithelium and connective tissue of the control group.

DISCUSSION

In the current study, on day-0, gingivitis was induced on the buccal surface of the gingival mucosa of the first right maxillary molar. On day-3, the gingiva became inflamed. Margins of this swollen and reddish gingiva were rolled instead of scalloped. According to Amoian and colleagues[10], bleeding occurs during probing and brushing because of moderate gingivitis. Researchers consider that bleeding on probing is the result of an inflammatory reaction in the tissues surrounding the epithelial junction and it is an objective sign of incipient periodontal changes. According to Dongari-Bagtzoglou and colleagues, gingival overgrowth was accelerated by plaque accumulation.[11] Many events occurred due to the attachment of pathogens on the gingival tissue disturbing connective tissue homeostasis and alveolar bone starts destroying. Gingival overgrowth can be idiopathic, inherited or associated with other systemic diseases (such as renal or hepatic diseases). It was believed that in all gingival lesions, connective tissues become more fibrotic with varying degrees of inflammation and an increase in size occurs in the gingival epithelium. The dose, duration and identification of the drug were the main factors affecting the degree of inflammation, cell sample and fibrosis.[12]

On day-4, group 2B rats showed the signs of acute gingivitis. The thickness of the epithelium was reduced. Subjects in which bleeding on probing occurred showed more connective tissue than epithelium. A significant rise in the inflamed component caused an overall increase in the connective tissue and because of this increase, epithelial thickness decreased. A study done

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by Polson and co-workers[13] stated that the structural and functional integrity of epithelium was dependent on the status of connective tissue. This was based on the concept that very thin epithelium is present in the inflamed connective tissue.

In contrast to this, in group 2C, the thickness of the gingival epithelium was reduced as compared to the control group. The extract of Eugenia jambolana was given so that the thickness of epithelium was more than the group 2B rats. Rapid healing in group 2C was observed. The leaf extract of Eugenia jambolana was found to be rich in flavonoids. The anti-inflammatory activity of Eugenia jambolana has been correlated with the methanolic leaf extract of Eugenia jambolana, as closely related species containing the same flavonoids present in Eugenia jambolana.[14] Researchers showed that some isolated flavonoids and catechins were possessing anti-inflammatory, anti-allergic and analgesic activities.[15] On the other hand, crude extracts of Eugenia jambolana administered orally in rats showed gastroprotective[16] and antiulcer properties due to the presence of tannins. However, Eugenia jambolana leaves or flowers contained few known flavonoids.

On day-10, the thickness of the gingival epithelium of group 3B rats was near to attain the normal thickness. Histology of lamina propria and epithelium of the gingiva was found to be disturbed. According to a study by Bartold and Narayanan[17], in all gingival lesions, extensive fibrosis occurred in connective tissue which became enlarged with varying degrees of inflammation and inflamed gingival epithelium. Immediately after plaque deposition started to the gingival margin, subjacent connective tissue became infiltrated with inflammatory cells and initiated destruction. At the same time, tissue repair occurred, showing fibrosis at the site of inflammation. The sequence of events in the development of periodontal diseases was the severity of inflammation, tissue destruction and healing. The main function of CTGF was to activate fibroblasts to produce extracellular matrix constituents, that produced more collagen fibres.[18,19]

In contrast to group 3B, group 3C attained normal thickness. The healing was more rapid in group 3C as compared to group 3B because the Eugenia jambolana extract was given. The inflammatory cell count was low. Group 3C showed almost complete healing at day 10. The gingival index showed a grade of 0, which means no inflammation. According to the previous studies on periodontal diseases, in epithelial hypertrophy, the stratified squamous epithelium was thicker due to the increase of the spinous layer (acanthosis) associated with acantholysis.[5, 20] The ratio between keratinized and non-keratinized areas was also affected. Rete pegs were formed by the deep epithelial bending into the lamina propria.

On day-20, group 4B showed complete healing. The thickness of the epithelium was normal. Lymphocytes and neutrophils were present. The gingival index showed grade 0, which means no inflammation. Gingival tissues could repair, regenerate, renew and healing after inflammation. This regeneration ability of the gingival epithelium was necessary for maintaining homeostasis in the gingival mucosa. Lamina propria also healed very rapidly after inflammation due to this regenerative ability. Similarly, group 4C also showed complete healing. Microscopic examination revealed normal histology. Researchers showed interest in the natural processes which control periodontal tissue's response to wounding and how cellular interaction occurred between different periodontal tissues.[21]

In one of the studies conducted in Bangladesh, by Zakaria and colleagues[22] it was investigated that majority of the rural people, living in villages, were suffering from dental problems mainly swollen gums, toothache, dental caries, halitosis, gingivitis. They were also suffering from eye problems like conjunctivitis. For treating oral diseases, one kaviraj (people living in the village) used the paste formed by crushing the roots of Eugenia jambolana. The powder was formed by crushing roots of Mangifera indica in combination with the roots of Areca catechu and Aegle marmelos. This mixture was added to the Eugenia jambolana paste. This paste was applied for treating tooth problems like gingivitis and halitosis.

According to one of the recent studies in India by Sangeeta and Mall[23], rural people of the Bahraich district are socioeconomically very poor so these uneducated poor people were completely dependent on the previous knowledge of herbal medicines for the treatment of different ailments. These people were aware of the medicinal importance of these herbal plants because of the knowledge transferred from their forefathers. The rural people of Bahraich chewed two leaves of Eugenia

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jambolana daily to treat gingivitis. The leaves of Eugenia jambolana had an anti-inflammatory effect so that gingivitis healed rapidly.

CONCLUSION

The Eugenia jambolana is a cheap fruit and part of everyday life in the lower socioeconomic class. This study proved its beneficial effects on the healing of gingivitis. There was a marked difference in the healing pattern between the two experimental groups. In the Eugenia jambolana extract group, there was marked acceleration in the healing pattern, epithelium regenerated more rapidly. Oral intake of Eugenia jambolana can heal gingivitis rapidly. The contents in Eugenia jambolana have an anti-inflammatory action on the soft tissue. Results suggest that epithelial changes seen in the experimental group-C could be a result of constant healing caused by the anti-inflammatory nature of Eugenia jambolana.

DISCLAIMER

None.

CONFLICT OF INTEREST

None to declare.

ETHICAL STATEMENT

The study protocol was approved by the Advanced Studies and Research Board of the University of Health Sciences, Lahore and the Ethical Committee of Postgraduate Medical Institute, Lahore.

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AUTHORS CONTRIBUTION

Conception and design of the study: S. Chaudhry

Acquisition of data: S. Chaudhry

Analysis and interpretation of data: S. Chaudhry

drafting of the manuscript: N. Munir

Critical review of the manuscript: M. Wajahat, S. Zia

Approval of the final version of the manuscript to be published: S. Chaudhry, N. Munir, S. Hameedi, S. Zia, A. Ahmed, M. Wajahat

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