



EFFECT OF EUGENIA JAMBOLANA ON NUMBER OF INFLAMMATORY CELLS IN GINGIVITIS IN ALBINO RATS.

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INTRODUCTION

Oral cavity is the part of mouth lies posterior to the gums and teeth, bounded above by soft and hard palate, below by inner side of mandible and tongue. The oral mucosa is divided into three main types according to their primary function; 1st is masticatory mucosa, 2nd is lining mucosa and 3rd is specialized mucosa. Masticatory mucosa is present on hard palate, on the gums and dorsum of the tongue. Specialized mucosa is present on the dorsal surface of tongue which contains taste buds on the lingual papilla. The lining mucosa is found approximately 60% of the area in the oral cavity including the labial mucosa lining inside of

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ABSTRACT... Objectives: This study was conducted to study the effect of *Eugenia jambolana* extract on the thickness of epithelium in albino rats. **Study Design:** This experimental study. **Setting:** Conducted in the animal house, Anatomy Department, Post Graduate Medical Institute, Lahore, Pakistan. **Period:** July to August 2015. **Material and Methods:** Total 48 albino rats were taken in this study and were divided into three groups. Histological changes of the buccal mucosa were studied on 3rd, 4th, 10th and 20th day after the induction of gingivitis in both control and experimental group. **Results:** On day 3, the cellular infiltrate was three times in both experimental groups. The total count of neutrophils was 22.50 ± 2.082 , eosinophils was 50.00 ± 1.414 in group 1B. In group 1C, the neutrophils count was 27.50 ± 1.915 , eosinophil count was 5.00 ± 0.816 . On day 4, in group-2B, no eosinophils were seen. Lymphocytes was 19.75 ± 1.708 , neutrophils was 6.50 ± 1.219 and macrophages was 3.25 ± 1.258 . In group 2C, lymphocytes and macrophages present, very few neutrophils were also seen. Some fibroblast nuclei were seen. In group 2C, no eosinophils were seen. The lymphocytes count was 9.00 ± 1.155 , neutrophil count was 4.00 ± 1.414 and macrophage count was 1.25 ± 0.050 . On day 10, lymphocytes, plasma cells, macrophages and few neutrophils were seen. In group 3B, macrophages with large nucleus 3.25 ± 0.95 lymphocytes 1.25 ± 0.500 and few neutrophils 1.00 ± 0.000 were also present. No eosinophils were present. In group 3C, less inflammatory cells (table 4) beneath the epithelium. The subepithelial tissue consisted of more lymphocytes 1.75 ± 0.500 and neutrophils 1.25 ± 0.500 . On day 20, very few lymphocytes 1.75 ± 0.500 and neutrophils 1.50 ± 0.577 in experimental group 4B. In group 4C, lymphocytes 1.75 ± 0.500 and neutrophils 1.50 ± 0.577 were present, no evidence of macrophages and eosinophils in group 4B and 4C. **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.

Key words: Buccal Mucosa, *Eugenia Jambolana*, Gingivitis, Healing.

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the cheeks.¹

Masticatory mucosa has orthokeratized epithelium. The part of the oral mucosa that immediately surrounds the erupted teeth is called gingiva. Gingiva consists of the mucosal tissue surrounding the roots of the teeth and covering the tooth bearing part of the mandible and maxilla. Gingival tissue is described as stippled, the colour is pink.² It is compactly tailored to the tissues surrounding the tooth, has a prominent margin where it tightly surrounds the tooth. 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. Inflamed condition of gingiva is called gingivitis.³ When plaque accumulates around gingival margin, inflammation begins within gingival connective tissue and epithelium. Within 3 to 4 days connective tissue destruction starts, 70% collagen lost. Plaque is a yellowish sticky material made up of bacteria, mucus and food debris that deposits on the exposed parts of the teeth. Plaque deposition is one of the main causes of tooth decay.

Gingivitis initially affects the gums. Sign and symptoms include: painful or tender gums, swelling, softness, dusky red color, bleeding after brushing or flossing teeth. General illness, age, medication, poor dental hygiene, hormonal changes occurs in pregnancy increase the sensitivity of the gums, uncontrolled diabetes, misaligned teeth, rough edges of fillings and ill-fitting or dental mouth appliances such as braces, dentures, bridges and crowns when become unclean also raise the risk of gingivitis.⁴ Systemic diseases linked to periodontal disease include: renal, hepatic, pulmonary and cardiac diseases; osteoporosis, adverse pregnancy effects, and diabetes mellitus.

The inflamed gums have a “puffy” or “rolled” margin, with swollen and mushy appearance. Epithelial thickness increases with elongated papillae in gingival overgrowth and fibrosis occurs in the lamina propria with increased number of fibroblasts. The thickness is 5 to 10 times greater than normal gingival.⁵

The clinical picture of chronic gingivitis is indistinct and patients have no complaints of pain. So most patients remain unaware and are not diagnosed

by the dental surgeons. Spontaneous bleeding in chronic gingivitis is rare. Actually when the gingival margins touch by a sharp instrument, gingival connective tissue starts bleeding shows that the changes in the epithelium and the vascular transfigurements are quite obvious.

There are four stages of gingivitis. In the initial lesion, the initial changes occur after 2-4 days of plaque accumulation.⁶ Vascular changes include arteriolar dilation, capillaries and venules of dentogingival plexus also dilate. Blood flow and gingival crevicular fluid increases, leukocytes (PMN) are predominant in connective tissue, junctional epithelium and gingival sulcus. In early lesion, changes occur within 4-7 days.⁷ Erythema occurs and increased formation of capillary loops between rete pegs and bleeding on probing is seen. Lymphocytes and PMN are predominant. 70% of collagen is destroyed. In established lesion, gingiva gives bluish hues, blood flow becomes sluggish. Extravasation of RBC into connective tissue, causes continuous collagen loss as inflammatory cells infiltrates increases within 2-4 weeks after accumulation of plaque accumulation, gingivitis established.⁸ In advanced lesion, more fiber damage occurs, sulcus depth increases due to the apical migration of junctional epithelium from cementoenamel junction. Plasma cells are prominent.

For treatment of gingivitis, the goal is to reduce inflammation. Recommended treatment includes brushing, flossing and antibacterial mouth rinses or other aids. Any other related illnesses or conditions such as oral ulcers, diabetes, renal, cardiac problems should be treated. Early stages of gingivitis may regress with proper oral hygiene, whereas the advanced forms of gingival inflammation may not heal without specific dental

Categories	FNAC, n (%)	Histopathology, n (%)	
		Benign	Malignant
Unsatisfactory	7(9%)		
Benign	57(71%)	56(70%)	1(1%)
Atypia/follicular lesion of undetermined significance	3(4%)	2(3%)	1(1%)
Suspicious for follicular neoplasm	8(10%)	6(7%)	2(3%)
Suspicious for malignancy	3(4%)	1(1%)	2(3%)
Malignant	2(2%)	0	2(2%)
Total	80(100%)	65(81%)	8(10%)

Table-I. Cytologic and histologic correlation according to Bethesda system.

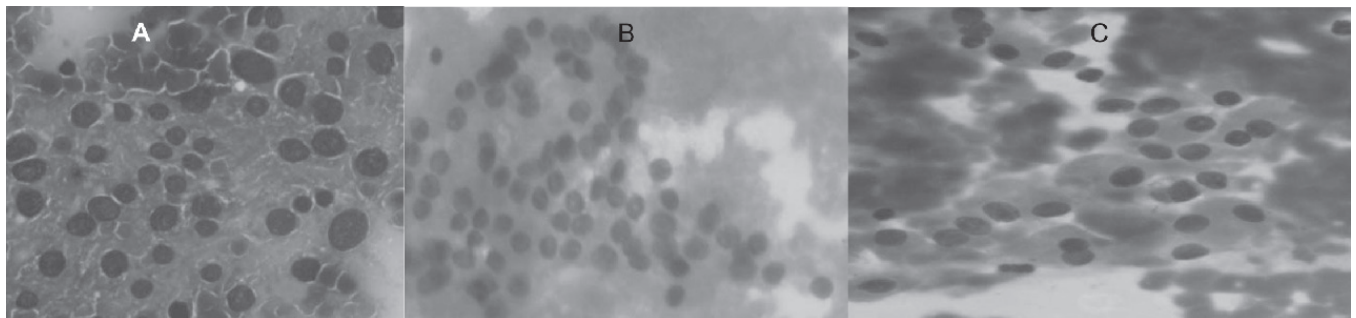


Image-A. Showing nuclear enlargement and architectural atypia, atypia of undetermined significance (AUS) was given (H&Ex40). B. suspicious nuclear grooves and occasional pseudo inclusions, AUS is the proper diagnosis (H&Ex40). C. Hurthle cell change Follicular neoplasm / Hurthle cell neoplasm (FN/Hurthle cell neoplasm) is the correct diagnosis (H&Ex40).

Cytological	Cases no	Histological Diagnosis	Frequency	PPV	
Bethesda 3 (AUS)	3	Benign	Multinodular hyperplasia Hashimoto thyroiditis	1 1	
		Malignant	Follicular variant of papillary carcinoma	1	33.3%
Bethesda 4 (FN)	8	Benign	Adenomatous hyperplasia	1	25%
			Follicular adenoma	3	
			Hurthle cell adenoma	1	
			Hashimoto thyroiditis	1	
			Follicular carcinoma	1	
		Malignant	Follicular variant of papillary carcinoma	1	
Bethesda 5 (Suspicious for malignancy)	3	Benign	Hashimoto thyroiditis	1	66.6%
		Malignant	Papillary carcinoma	2	
Total	14				35.71%

Table-II. Cytologic and histologic correlation of atypical cases with PPV according to the Bethesda system (n=14)

treatment.⁹ Honey has a therapeutic effect in the treatment of gingivitis and periodontitis. There are many different types of tooth pastes available such as tooth pastes for sensitive teeth, tooth paste with flouride, bleaching tooth pastes, whitening tooth pastes. Toothpastes with triclosan and copolymer combination reduce dental plaque and symptoms of gingivitis. Dentifrices such as stannous fluoride significantly reduce gingival bleeding during gingivitis.

Eugenia jambolana is a common, large evergreen tree of the Subcontinent. Syzygium cumini linn is another scientific name of Eugenia Jambolana, belongs to the myrtaecea family. It is commonly known as ‘Jamun’ in Urdu language. Other names used frequently are java plum, black plum, Jambu,

Jamun, Jambul, Indian blackberry, doowet, faux pistachier, etc. It grows naturally in tropical and subtropical zones. Eugenia Jambolana tree reach upto30 meters height and its life is about 100 years. The bark is rough and colour is grey. The juice is carminative, diuretic and gives a soothing effect on digestive system. Sauces and beverages are prepared from the juice of ripe fruit. Churan is a form of digestive powder made from the dried powder of fruit with salt. The bark, flowers and seeds of Eugenia Jamboalana have hypoglycemic property used for the treatment of diabetes. Fruits and especially leaves juices were recommended for treating dysentery and gingivitis.¹⁰

There are two main types of Eugenia jambolana

in subcontinent: (1) Ra Jaman and (2) Kaatha. The main cultivars between named cultivators are 'Early Wild', 'Late Wild' and 'Pharenda'; and the secondary ones are 'Small Jaman' and 'Dabka. In Java, Djoowet kreekil is the small form of the fruit; although the Djoowet booten is the seedless form of the fruit. In south areas of Malaya, leaves and flower clusters are small. In North, the type called 'Krian Duat' has different features. Leaves are large, thicker and inner side of bark is red.¹¹

Fruits which have flesh of purple colour show more astringency as compared to the fruits with white-flesh. The leaves possesses antibacterial property are used for strengthening the teeth and gums. The fruit and seeds of *Eugenia jambolana* are sour, acrid, sweet, have tonic and cooling effects, and are used in treating diabetes mellitus, diarrhoeal problems and ringworm infections. The bark is sour, astringent, sweet, diuretic, digestive and antihelmintic. Research has shown that it has significant hypoglycemic action in both urine and blood.

MATERIALS AND METHODS

An experimental animal study was conducted at Experimental Research Laboratory of Post Graduate Medical Institute Lahore to study histological changes in the buccal side of the gingival mucosa of 1st right maxillary molar of adult albino rats on 3rd, 4th, 10th and 20th day after inducing gingivitis in the adult albino rats. Adult animals of weight 200-250 gm were chosen. Adult rats of both sexes were used in this study. 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 University of Health Sciences, Lahore and Ethical Committee of Postgraduate Medical Institute, Lahore.

PROCEDURE

Forty eight adult albino rats 55-60 days old, weighing (200-250g) were procured from National Institute of Health Islamabad. They were individually housed in a climate controlled environment and maintained on normal food and water. 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). They were housed in cages with wire bar lids used to hold the water bottle and feed to prevent contamination with urine or feces. Animals were placed in their respective cages which were labeled by tags. Bedding was placed directly into the cage to allow the absorption of the urine. They were kept in well ventilated room at ambient temperature of $28.0 \pm 2.0^{\circ}\text{C}$ and humidity ($60 \pm 10\%$) under 12 hr light/dark cycles and well provided with food and water ad libitum.

All experimental protocols were performed in accordance with the guidelines for the humane use of laboratory animals established at our institution. Following acclimatization for a period of one week, procedure was started. Each animal was weighed before and at the end of the study. Weight was recorded on an electronic scale (Sartorius Precision Balance, Germany). Rats were divided into three equal groups by using random number generator. (Table-I)

1. Control group A
2. Experimental group B: These animals were not giving any dose after inducing gingivitis.
3. Experimental group C: These animals were given *Eugenia jambolana* extract after inducing gingivitis.

Histological changes of the buccal mucosa were studied on 3rd, 4th, 10th and 20th day after induction of gingivitis in both control and experimental group animals.

Induction of Gingivitis

For accurate examination of curing of gingivitis in inflamed gingiva, moderate gingivitis was induced in rats. For this purpose, a cotton thread placed between right maxillary molars. The present study was confined to the histological and anatomical examination of 48 adult albino rats.

The animals were allotted to three groups of 16 animals each: Control group A (1A,2A,3A,4A), Experimental group B (1B,2B,3B,4B) was not given *Eugenia Jambolana* extract, while the

Experimental group C (1C,2C,3C,4C) was given Eugenia Jambolana extract.

The rats were anesthetized with ketamine (100 mg/kg body weight) and xylazine (10mg/kg body weight) by an intraperitoneal injection.¹² The procedure was carried out under sterile conditions. The anesthetized animal was placed on its right side on wooden table. The area selected for inducing gingivitis was cleaned with piodine to remove saliva or any food particles that may be present. The cotton thread was placed between first and second maxillary molars of right quadrant of the group B and C rats for inducing gingivitis.^{13,14}

Collection of Eugenia Jambolana

Eugenia jambolana seeds were obtained by getting fruit from University of the Punjab.

Preparation of Eugenia Jambolana

Ethanollic extract of Eugenia jambolana seeds was prepared by getting fruits of Eugenia jambolana (jamun) from University of the Punjab. Pulp was removed from the seeds and washed several times with distilled water to remove the traces of pulp; the seeds were then dried at room temperature, kernel of the seeds was separated from the seed coat, powdered in an electrical grinder and stored at 5 °C. Hundred gm of kernel powder was suspended in 250 ml of distilled water and allowed it to stand overnight in refrigerator; it was then sieved through several layers of muslin cloth. 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 °C.

The yield of kernel was 3.2 g/100 g of seed powder.

Administration of Eugenia Jambolana

The extract of Eugenia jambolana was given orally with the help of insulin syringe for 10 days.

Tissue Sampling and Processing

At 3rd day after inducing gingivitis, four animals from each group were placed in a chloroform chamber and decapitated under deep

anesthesia. (Figure-1) Rest of the animals were decapitated by the same procedure and number at 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 Hematoxylin before microscopic study.

RESULTS

The general histological study of a normal gingival mucosa revealed the following layers;

1. Epithelium
2. Lamina propria

The epithelium was stratified squamous keratinized, with four basic layers. It consisted of a darkly stained layer of tall columnar cells known as stratum basale (basal layer), over which there were 7-8 strata of polyhedral cells forming the stratum spinosum (prickle layer). On top of these were 3-5 layers of flat cells forming stratum granulosum (granular layer). These layers were covered by a fine layer of anucleated corneocytes forming the stratum corneum (cornified layer). The colour of normal gingiva was pink. The normal gingival had scalloped margin. Rete ridges extending down towards the connective tissue had a usual conical shape. The lamina propria displayed fine network of collagen bundles.¹⁵ Fibroblasts and blood vessels were also present (Figure-2).

On day 3, on gross examination of the selected area in both experimental group 1B and 1C, marked redness and hypertrophy was also observed. Some ulceration also seen and there was also a tendency to bleed spontaneously. Junctional epithelium migrated apically from the cemento-enamel junction and the gingival sulcus depth increased in experimental group 1B and 1C. The color of gingiva also changed from pink to red. The texture of gingiva also affected because of inflammation.¹⁷ The inflamed gingiva was swollen and had puffy and rolled margins. The selected area of all the experimental animals was viewed under light microscope.¹⁸ On day 3, sign of severe gingivitis has been appeared in

all the animals of both experimental groups 1B and 1C. It was observed that there was severe inflammation in the buccal surface of the gingival mucosa of the maxillary right quadrant. There were bundles of collagen fibers showing the evidence of fibrosis.¹⁹ There were short rete pegs. The cellular infiltrate was three times in both experimental groups as compared to normal group.²⁰ In both experimental groups, vascular congestion was seen (Figure-3). The total count of neutrophils was 22.50 ± 2.082 and eosinophils was 50.00 ± 1.414 (Table-II) in group 1B. In group 1C, the neutrophils count was 27.50 ± 1.915 and eosinophil count was 5.00 ± 0.816 .

On day 4, the connective tissue of the two groups presented differences as well; in group-2B, abundance of inflammatory cells was visible in the lamina propria. Neutrophils with multilobulated nuclei were also recognized, so were a few lymphocytes accompanied by plasma cells (Figure-4). No eosinophils were seen in the connective tissue. The total count of lymphocytes was 19.75 ± 1.708 , neutrophils was 6.50 ± 1.219 and macrophages was 3.25 ± 1.258 (Table-III). The epithelium breaks, short rete pegs also seen. Fibrosis was three times more in group 2A (given no dose) as compared to group 2C (given Eugenia /jambolana dose).

In group 2C, long rete pegs were seen as compared to group 2B. In group 2C, fibrosis was near to normal. In group 2C, lymphocytes and macrophages were present and very few neutrophils were also seen. Some fibroblast nuclei were seen. In group 2C, no eosinophils were seen. The lymphocytes count was 9.00 ± 1.155 , neutrophil count was 4.00 ± 1.414 and macrophage count was 1.25 ± 0.050 . The difference between inflammatory cell count between the two groups was statistically significant ($p < 0.005$ Table-III).

On day 10, There was evidence of very less cellular infiltrate in group 3B. There was no vascular congestion. Numerous small capillaries can be seen beneath the regenerating epithelium. Lymphocytes, plasma cells, macrophages and few neutrophils were seen. The connective tissue

of group3 B showed evidence of macrophages with large nucleus 3.25 ± 0.95 lymphocytes 1.25 ± 0.500 and few neutrophils 1.00 ± 0.000 were also present. The granulation tissue also contained fibroblasts forming collagen fibers. No eosinophils were present.

In contrast to which group 3C, Inflammatory cells clear out with very minimal cells still visible in the connective tissue. The group 3C showed less inflammatory cells (Table-IV) beneath the epithelium. The subepithelial tissue consisted of more lymphocytes 1.75 ± 0.500 and neutrophils 1.25 ± 0.500 . The difference in values of a number of inflammatory cells in the two groups was statistically significant.

On day 20, Normal stratification of cells was visible. Collagen bundles were seen in the connective tissue. Healthy mucosa was seen in both experimental groups. Rete ridges goes up towards the cornifium layer. Connective tissue showed fibroblasts, loosely arranged collagen fibres were present. There was slight evidence of collagen bundle formation too. There was evidence of very few lymphocytes 1.75 ± 0.500 and neutrophils 1.50 ± 0.577 in experimental group 4B. In group 4C, lymphocytes 1.75 ± 0.500 and neutrophils 1.50 ± 0.577 were present (Table-V). There were no evidence of macrophages and eosinophils in group 4B and 4C.

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

Table-I. Details of study groups

Parameter	Control Group (mean ± S.D)	Experimental group 1 (mean ± S.D)	Experimental group 2 (mean ± S.D)	Number of Animals (N)	P-Value
Number of lymphocytes	1.75 ± 0.500	0.00 ± 0.000	0.00 ± 0.000	4	<0.001
Number of neutrophils	1.25 ± 0.500	22.50 ± 2.082	27.50 ± 1.915	4	<0.001
Number of macrophages	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.00	4	
Number of eosinophils	0.00 ± 0.000	5.00 ± 1.414	5.00 ± 0.816	4	<0.001

Table-II. Comparison of number and types of inflammatory cells between three groups on Day-3

Parameter	Control Group (mean ± S.D)	Experimental group 1 (mean ± S.D)	Experimental group 2 (mean ± S.D)	Number of Animals (N)	P-Value
Number of lymphocytes	1.75 ± 0.500	19.75 ± 1.708	9.00 ± 1.155	4	<0.001
Number of neutrophils	1.25 ± 0.500	6.50 ± 1.291	4.00 ± 1.414	4	<0.001
Number of macrophages	0.00 ± 0.000	3.25 ± 1.258	1.25 ± 05.00	4	<0.001
Number of eosinophils	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	4	

Table-III. Comparison of number and types of inflammatory cells between three groups on Day-4

Parameter	Control Group (mean ± S.D)	Experimental group 1 (mean ± S.D)	Experimental group 2 (mean ± S.D)	Number of Animals (N)	P-Value
Number of lymphocytes	1.75 ± 0.500	1.25 ± 0.500	1.75 ± 0.500	4	<0.001
Number of neutrophils	1.25 ± 0.500	1.00 ± 0.000	1.25 ± 0.500	4	<0.001
Number of macrophages	0.00 ± 0.000	3.25 ± 0.957	0.00 ± 0.000	4	<0.001
Number of eosinophils	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	4	

Table-IV. Comparison of number and types of inflammatory cells between three groups on Day-10

Parameter	Control Group (mean ± S.D)	Experimental group 1 (mean ± S.D)	Experimental group 2 (mean ± S.D)	Number of Animals (N)	P-Value
Number of lymphocytes	1.75 ± 0.500	1.75 ± 0.500	1.75 ± 0.500	4	<0.001
Number of neutrophils	1.25 ± 0.500	1.50 ± 0.577	1.50 ± 0.577	4	<0.001
Number of macrophages	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	4	
Number of eosinophils	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	4	

Table-V. Comparison of number and types of inflammatory cells between three groups on Day-20



Figure-1. Photograph showing anesthetization of experimental rat by an intraperitoneal injection

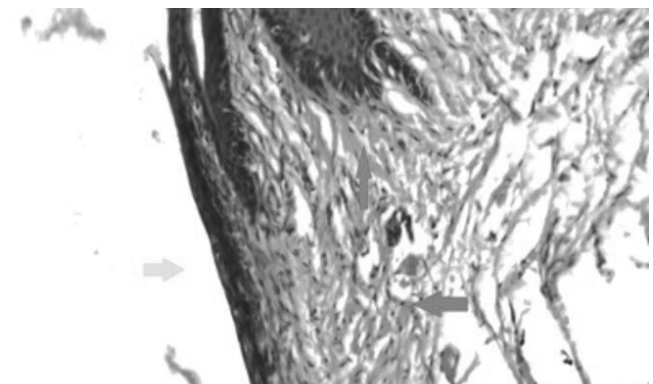


Figure-2. Section of right gingival mucosa of group-1 A on post gingival day-3 showing keratinized epithelium (black arrow), nonkeratinized epithelium (yellow arrow) rete pegs (green arrow). The lamina propria contains fibroblasts (F), collagen fibers (C), blood vessels (BV) and inflammatory cells (L) (blue arrow). H & E stain. Photomicrograph approx. 200X.

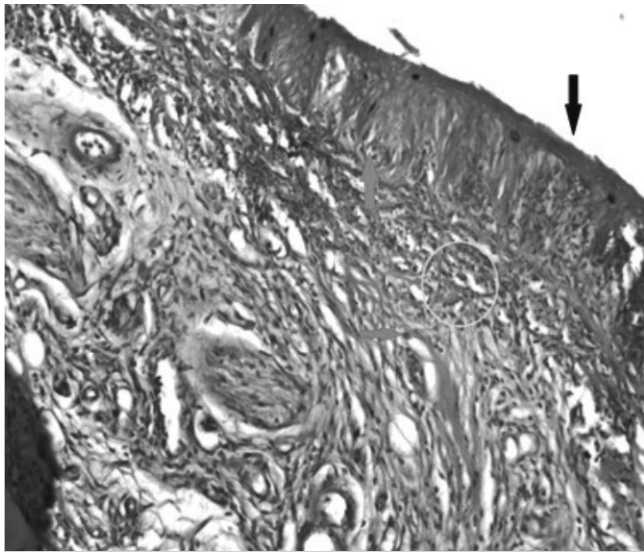


Figure-3. Section of right gingival mucosa of group-1 B on post gingival day-3 showing keratinized epithelium (black arrow), rete pegs (green arrow). The lamina propria contains fibroblasts (F), collagen fibers (C), blood vessels (BV) and inflammatory cells (L) (blue arrow). The area in yellow circle shown in the next picture. H & E stain. Photomicrograph approx. 200X.

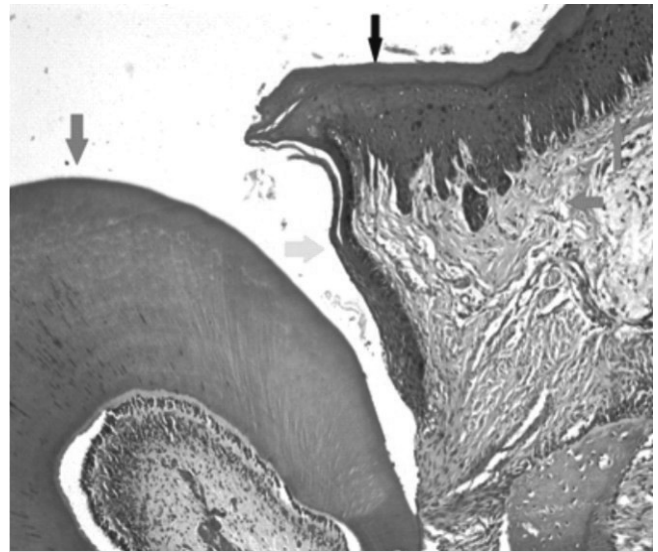


Figure-4. Section of right gingival mucosa of group-2 A on post gingival day-4 showing keratinized epithelium (black arrow), nonkeratinized epithelium (yellow arrow) rete pegs (green arrow), tooth (purple arrow). The lamina propria contains fibroblasts (F), collagen fibers (C), blood vessels (BV) and inflammatory cells (L) (blue arrow). H & E stain. Photomicrograph approx. 200X.

DISCUSSION

Current study presents an account of the effects of *Eugenia jambolana* extract on experimentally-induced gingivitis in albino rats. Histological evaluation was performed on the three groups A, B and C of albino rats on post gingivitis days 3, 4, 10 and 20. The group A was control group which showed normal histology and other two groups were experimental groups i.e., Group B (giving the food and water) and group C (giving *Eugenia jambolana* extract). The results showed remarkable differences between the healing pattern of the two experimental groups B and C. In the present study, cotton thread was placed between the first and second molars of the right maxillary quadrant of the albino rats to induce the lesion of interest – gingivitis, in both experimental groups B and C. The cotton thread acted as an irritant and caused gingival inflammatory reaction composed of leukocyte infiltration to the gingival mucosa through chemotaxis and increased vascular permeability. According to a recent study¹⁴, gingivitis was induced by placing cotton thread at the level of gingival crevice between 1st and 2nd molars of albino rats. In one of study, gingivitis was induced in maxillary molars of

albino rats by using cotton thread between 1st and 2nd molars. Inflammation occurred because the thread acted as a foreign object which caused irritation.

In current study, on day-0, the gingivitis was observed on the buccal surface of the gingival mucosa of the first right maxillary molar. On day-3, the gingiva became inflamed. According to the study conducted by Steenberg, (2006), gingivitis was induced after 4 days of giving injection of irritant to the mice. In the present study, the sign of gingivitis appeared after 3 days of placing cotton thread. The inflamed gingiva was red in colour. After inducing gingivitis, the gingiva became swollen. There were rolled margin of the inflamed gingiva instead of scalloped margin which was present in healthy gingiva. There was bleeding when probing was done with the periodontal probe. A study¹⁵ stressed on the fact that during gingivitis, the colour of the gingiva was changed from pink to red¹⁶ In one of the recent study¹⁷, the same gingival findings were found. The texture of the gingiva was also affected. The gingiva became swollen because of inflammation. Alterations occurred in the vascular

network and many capillaries bed opened. There was increased exudation of GCF and proteins in the gingival plexus. Because of this increased exudation, tissue made edematous and swollen.¹⁸ Bleeding occurred during probing and brushing because of moderate gingivitis.¹⁹ Researchers consider that bleeding on probing is the result of an inflammatory reaction in the tissues surrounding the epithelial junction, and it was an objective sign of incipient periodontal changes.²⁰

Microscopic examination reveals that the inflammatory cells mainly consisted of neutrophils and eosinophils were present. The inflammatory cells played important role in inflammation. The inflammatory cells neutrophils, lymphocytes and monocytes were found in the gingiva. There was increased number of leukocytes in inflamed gingiva.

Many changes occurred in the periodontal tissue during the pathogenesis of inflammation. Gingival overgrowth was accelerated by plaque accumulation. Many events occurred, due to the attachment of pathogens on the gingival tissue disturbing connective tissue homeostasis and alveolar bone starts destroying. The most distinct feature of gingival overgrowth was inflamed and swollen gums (free gingival margin and interdental papilla became enlarged and thickened gradually covering crown of the tooth). Gingival overgrowth can be idiopathic, inherited, also associated with other systemic diseases (such as renal or hepatic diseases). It was believed that all gingival lesions connective tissues became more fibrotic, with varying degree of inflammation and increase in size occurred gingival epithelium. The dose, duration and identification of the drug were main factors affecting degree of inflammation, cell sample and fibrosis.

On day-4, group 2B rats showed the signs of acute gingivitis. Lymphocytes and macrophages dominated the inflammatory infiltrate developed in the gingival connective tissues. Neutrophils were also present in this infiltrate, although most of the neutrophils move from gingival epithelium and connective tissues, established a relationship

with plaque. Lymphocytes and macrophages accelerated fibrosis by the releasing chemotactic factors. These factors recruited fibroblast to the site of inflammation, produced FGF which activates the fibroblasts, results in fibrosis to replaced lost collagen.

In contrast to this, in group 2C, the inflammatory cells count was also less than group 2B because of the anti-inflammatory property of *Eugenia jambolana*. The inflammatory cell infiltrate mainly consisted of neutrophils, lymphocytes and macrophages. The leaf extract of *Eugenia jambolana* was rich in flavonoids. The anti-inflammatory activity of *Eugenia jambolana* had been correlated with the methanolic leaf extract of *Eugenia jambos*, a closely related species containing same flavonoids present in *Eugenia jambolana*. Researchers showed that some isolated flavonoids and catechins were possessing anti-inflammatory, anti-allergic and analgesic activities. On the other hand, crude extracts of *Eugenia jambolana* administered orally in rats, showed gastro protective and antiulcerogenic property due to the presence of tannins. However, *Eugenia jambolana* leaves or flowers contained few known flavonoids.

On day-10, the inflammatory cells count was also less as compared to the inflammatory cell count of day 4. Histology of lamina propria and epithelium of the gingiva disturbed. On day 10, lymphocytes, neutrophils and macrophages were also present. According to one of the recent study²¹, 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 initiate its 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 were the severities of inflammation, tissue destruction and healing.

Inflammatory cells such as lymphocytes and macrophages played an important role in

inflamed gingiva. Cytokines and growth factors were mainly released by macrophages and lymphocytes.²¹

In contrast to group 3B, the group 3C the inflammatory cell count was low. The group 3C showed almost complete healing at day 10.

On day-20, the group 4B showed complete healing. Lymphocytes and neutrophils were present. Gingival tissues had a capability of repair, regeneration, renewal and healing after inflammation and wounding. 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 regeneration ability. Researchers showed interest in the natural processes which control periodontal tissues response to wounding, and how cellular interaction occurred between different periodontal tissues.

In contrast to this, the group 4C also showed complete healing. Microscopic examination revealed normal histology. The cell count was normal consisting mainly of lymphocytes and neutrophils. In the present study, gingivitis was treated with the ethanolic extract of *Eugenia jambolana*. The extract of *Eugenia jambolana* was given orally to albino rats for 10 days with 1 week follow up. The extract of *Eugenia jambolana* reduced inflammation more quickly in experimental group C as compared to experimental group B. In one of study conducted in Bangladesh.²² people lived in villages were unaware about taking care for their eye and teeth. So that majority of the rural people who were suffering from dental problems mainly swollen gums, toothache, dental caries, hallitoses, gingivitis etc and eye problems like conjunctivitis. For treating oral diseases, one kaviraj (people living in village) used the paste formed by crushing the roots of *Eugenia jambolana*. The powder formed by crushing roots of *Mangifera indica* in combination with the roots of *Areca catechu* and *Aegle marmelos*. This mixture was added in the *Eugenia jambolana* paste. This paste was applied for treating tooth problem like gingivitis and helitosis.

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 known 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 jambolana* daily to treat gingivitis. The leaves of *Eugenia jambolana* had anti-inflammatory effect so that gingivitis heals rapidly.

CONCLUSION

The *Eugenia jambolana* is a cheap fruit and is part of everyday life in lower socioeconomic class. Their effects on diabetes, odema, inflammation, constipation, bloody stools and liver inflammation were known, but not enough evidence was available to determine its effects on gingivitis. This study proved its beneficial effects on the healing of gingivitis. There was a marked difference in the healing pattern between the two groups. In the *Eugenia jambolana* extract group, there was marked acceleration in the healing pattern, epithelium regenerated more rapidly, inflammatory cell count were low and sulcus depth were within normal range at the end of the experiment with a subsequent rapid healing. 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*. *Eugenia jambolana* extract must be used for oral problems, especially in case of gingivitis.

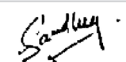
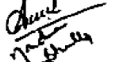


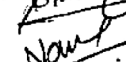
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AUTHORSHIP AND CONTRIBUTION DECLARATION

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3	Nadia Bhutta	Discussion Review.	
4	M. Behzad Salahuddin	Data analysis.	
5	Muhammad Bilal Arshad	Critical review of article.	
6	Nasrullah Khan	Article composition.	