

HISTOLOGICAL STUDY OF THE EFFECT OF PROPOLIS ON GINGIVAL TISSUE OF ALBINO RAT

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ABSTRACT

Aim The aim of the present work was to study the proliferative and maturative influence of propolis on the gingival tissues in rats using routine H&E stain and immunohistochemical detection of any possible changes in proliferating cell nuclear antigen (PCNA) and E-cadherin in the surface epithelium of the gingiva so that we get an answer for the question; Is it beneficial if the dentist use propolis as a constituent of surgical dressing as well as mouth wash?

Materials and methods Thirty adult male albino rats with 120-150 gram body weight were used in this investigation. The animals were divided into 2 groups 15 animals each; **Group I** animals served as controls, and supplied with a daily oral dose of 1ml distilled water. **Group II** animals were treated with propolis in a daily oral dose of 50 mg/kg body weight using curved metallic oro-pharyngeal tube. Propolis tablets were dissolved in distilled water to a concentration of 5mg/1ml. The animals were fed natural diet and given drinking water ad libitum throughout the whole experimental period, which lasted for 3 months. At the end of the experiment, the animals of both groups were sacrificed by cervical dislocation. gingival Specimens of both sides were taken from the region of the first molars, The specimens were fixed in 10% neutral buffered formalin, processed to be embedded in paraffin. Six microns thick sections were cut and stained Hematoxylin and eosin stain for histological detection any structural changes in the epithelium, lamina propria. Immunohistochemical staining procedure using a- The universal dakocytomationlabeled streptavidin- biotin system horseradish peroxidase enzyme for detection of any possible changes in (PCNA) of the surface epithelium. b- The ultravasion mouse tissue detection system using antimouse monoclonal antibody for demonstration of E-cadherin to detect any possible changes.

Results The present work revealed that supplementation of propolis to the rats may activate the proliferation, regeneration and maturation of the epithelial cells the gingival tissue and improve vascularity and organization of collagen fibers of gingival subepithelial lamina propria

Conclusion Propolis solution enhances the integrity and barrier function of the gingival epithelial cells and collagen fiber, thus it increases the viability and physiological health of gingival cells.

Recommendation Propolis is recommended to be used as a beneficial constituent of mouth wash and surgical dressing after fulfilling all the required investigations on human.

KEY WORDS: Propolis, gingiva dressing, wound healing, proliferation, gingiva,

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INTRODUCTION

Propolis, a natural antibiotic, is a resinous yellow to dark brown substance that honey bees collect from tree buds, sap flows or other botanical sources to seal unwanted open spaces in the hives protecting them from outside contaminants. Propolis is a complex mixture of different naturally-occurring constituents with more than 300 constituents identified to date, which includes phenolic acid, terpenes, cinnamic acid, caffeic acid, aromatic aldehydes, alcohols, amino acids, fatty acids, vitamins (A, B1, B2, B3, and B7), several esters, minerals, essential oils, and flavonoids (flavones, flavonols, and flavanones)¹. Propolis possess a broad spectrum of biological activities and advise to use it extensively in the diet to improve health and prevent diseases². It exhibits several pharmacological properties such as antimicrobial, anti-inflammatory, healing, anaesthetic and cariostatic properties³.

Orsolic⁴ studied propolis antitumour action in vivo and in vitro and demonstrated antitumor effects in different cancers. Propolis used in treatment human papilloma virus infection which can lead to cervical cancer of women⁵.

In dentistry propolis has been used for treatment of moniliasis, acute necrotizing ulcerative gingivitis, gingivitis and periodontitis, dentinal hypersensitivity and caries. It is very widely used in dentistry, since it inhibits different oral pathogenic microbes such as bacteria, fungi and viruses⁷. Thus Propolis can be applied in the form of spray, mouthwashes and toothpastes⁸. Most of the previous researches have been done to study the effectiveness of propolis on wound healing of skin so our investigation interested to study the histological effect of propolis on the oral mucosa as trial for its application as beneficial constituent of intra oral surgical dressing. A dressing is a sterile pad or compress applied to a wound to promote healing and protect the wound from further harm. A dressing is designed to be in direct contact with the wound. Many modern

dressings are self-adhesive. A dressing can have a number of purposes, depending on the type, severity and position of the wound, although all purposes are focused towards promoting recovery and protecting from further harm⁹.

Proliferating cell nuclear antigen (PCNA), a cell cycle marker protein, is well known as a DNA sliding clamp for DNA polymerase delta and as an essential component for eukaryotic chromosomal DNA replication and repair. Due to its mobility inside nuclei PCNA is dynamically presented in a soluble or chromatin-associated form¹⁰. It was originally described as a 36 KD protein synthesizes during the S phase of the cell cycle and named as cyclin. Subsequently this protein was renamed as PCNA to distinguish it from the cyclin family of proteins. PCNA is a ring-shaped protein that encircle duplex DNA and play an essential role in many DNA metabolic processes in archaea and eukarya. The eukaryotic and euryarchaea genomes contain a single gene encoding for PCNA¹¹. In addition to their function as processivity factors replicative polymerases, PCNA protein also associate and modulate the activities of a large number of other proteins involved in nucleic acid metabolic transactions¹².

PCNA is useful marker of proliferating cells because its expression and distribution correlate with cellular proliferating rate. Immunohistochemical expression of PCNA increase during G-1 phase, peaks at the S- phase and declines during G-2 phase of the cell cycle. Anti- PCNA antibodies provide an appropriate method for clarifying all phases of the cell cycle of proliferating cells. PCNA being involved in DNA repair, suggesting that it may be expressed by cells that are not cycling¹³. PCNA exist in two forms during S- phase, the nucleoplasmic form found soluble in the nucleoplasm and the form associated with sites of DNA replication. Both forms are expressed in formaldehyde fixed tissues.

PCNA labeling index escalates significantly

in the order of normal oral mucosa, oral epithelial hyperkeratosis and oral squamous carcinoma, and therefore it has been used as indicator of the malignant potential of oral lesion.

Cadherins (CDS) are family of cell surface glycoproteins of 723-747 aminoacids that act as intercellular adhesion molecules by calcium dependant hemophilic binding¹⁴. It is one of a large family of genes which code for calcium dependant cell adhesion molecules. The E cadherin gene is located on the long arm of chromosome 16 (16q). It is designed as 16q22.1 and consists of 16 axons¹⁵. Cadherin are Ca^{2+} dependant cell adhesion molecules that bind homotypically to identical cadherins molecules on opposing cells. The homotypic, adhesion and Ca^{2+} binding activity reside in the extracellular region of cadherin, whereas the intracellular domain, together with adapter proteins such as α , β and γ catenines, is responsible for linking cadherin to cytoskeleton actin filaments. The coordinated intercellular and intracellular protein-protein interaction are crucial for the formation of tissue structure and proper physiological functioning of tissue¹⁶.

Cadherin are currently divided into four typed: Epithelial(E), neural (N), Placental (P) and Vascular (V)¹⁷. These groups of Cell Adhesive Molecules were first discovered by Dr. Takeichi in 1982. In 1984 he gave the name "Cadherin" meaning, "Adhesion molecules that work in the presence of calcium ions". He successfully cloned the molecules in 1987¹⁸. There are more than 120 types of cadherin which form the "Cadherin super family". This super family with at least 10 sub-families which can be distinguished on the basis of protein domain composition, Genomic structure and phylogenic analysis of the protein sequences¹⁹. These cadherins are similar to each other in amino acid sequence and consists of three domains. They are; 1- cytoplasmic domain (COOH-terminal/ C-terminal). 2- Transmembrane domain

(Hydrophobic region). 3- Extracellular domain (NH₂- terminal / N- terminal)²⁰. The epitope for antibody of blocking cadherin action is located in the 113 amino acids of the NH₂ terminal (extracellular domain) region and this region is essential for binding specificity of cadherin²¹. Cadherins are found in membrane structures including adherens junctions, desmosomes, tight junctions and gap junctions²².

Cadherin cell to cell adhesion proteins play an important role in modulating the behavior of tumor cells. Cadherin serves as a suppressor of tumor cell invasion, and when tumor cells turn on the expression of cadherin, they often express less cadherin, enhancing the tumorigenic phenotype of the cells²³.

Epithelial cadherin is a classical cadherin and forms the key functional component of adherent junctions between the epithelial cells. It is expressed in all the living cell layers and associated with general differentiation features of the epithelia¹⁴. In epithelial cells, tight adhesion mediated by E – cadherin receptors is essential for the differentiation and functionality of epithelial sheets, and cell to cell contact. Altered expression of E cadherin may result in the loss of contact inhibition and abnormal cell proliferation, hence tumorigenesis takes place²⁴.

Loss of the cell adhesion and E-cadherin plays an important role in progression of oral squamous cell carcinoma or its down regulation is associated with de- differentiation and metastasis²⁵.

The objectives of the present work were to study the proliferative, regenerative and maturative influence of propolis on the gingival tissues in rats using routine H&E stain and immunohistochemical detection of any possible changes in proliferating cell nuclear antigen (PCNA) and E- cadherin in the surface epithelium of the gingiva. To get an answer for the question; Is it beneficial if the dentist use propolis as a constituent of surgical dressing as well as mouth wash?

MATERIALS AND METHODS

Thirty adult male albino rats with 120-150 gram body weight were used in this investigation. The animals were divided into 2 groups 15 animals each.

Group I animals served as controls, and supplied with a daily oral dose of 1ml distilled water.

Group II animals were treated with propolis in a daily oral dose of 50 mg/kg body weight²⁶ using curved metallic oro-pharyngeal tube. Propolis tablets were dissolved in distilled water to a concentration of 5mg/1ml. Pollen Bee propolis (500 mg tablets) were purchased from CC Pollen Co, U.S.A

The animals were kept in specially designed cages, 5 animals per cage. They were fed natural diet and given drinking water adlibitum. They were kept under proper condition of temperature and ventilation throughout the whole experimental period, which lasted for 3 months

At the end of the experiment, the animals of both groups were sacrificed by cervical dislocation. Gingival Specimens of both sides were taken from the region of the first molars, fixed in 10% neutral buffered formalin, for 24 hours, washed, dehydrated in ascending grades of ethyl alcohol, cleared in zylene and embedded in paraffin. 5-6 micron thick sections were cut and stained with:

I- Hematoxylin and eosin stain for histological detection any structural changes in the epithelium, lamina propria.

II- Immunohistochemical staining procedure;

- a) The universal dakocytomationlabeled streptavidin- biotin system horseradish peroxidase enzyme for detection of any possible changes in (PCNA) of the surface epithelium.
- b) The ultravesion mouse tissue detection system using antimouse monoclonal antibody for demonstration of E- cadherin to detect any possible changes.

Immunohistochemical staining procedure used for PCNA detection:

- Sections were mounted on positively charged slides.
- Section were preincubated in absolute methanol containing 0.5 percent hydrogen peroxide for 10 minutes at room temperature to block any endogenous peroxidase activity without affecting the immune-reactivity of the antigen.
- Sections were rinsed gently with phosphate buffer solution (PBS PH 7.4) 3 times 2 minutes each.
- Sections were incubated for 10 minutes with non- immune serum (reagent 1A) by adding two drops of serum blocking solution to each section. After that the solution was blotted off.
- Two drops (equivalent to 100 µl) of primary antibody were applied to each section and left for 30-60 minutes.
- Sections were rinsed well with PBS three times two minutes each.
- Excess buffer was removed and slides were wiped.
- Two drops of biotinylated second antibody (reagent 1B) containing antimouse antibody were applied to each section and incubated for 10 minutes.
- Sections were rinsed in 3 changes of PBS two minutes each. Slides were blotted and wiped.
- Two drops of enzyme conjugate (reagent 2)- which is streptavidin conjugated to horseradish peroxidase enzyme in PBS- were added to each section and incubated for 10 minutes.
- Sections were rinsed in PBS.
- 3,3'- diaminobenzidine (DAB) substrate chromogen solution was applied to cover tissues section and incubated for 5-10 minutes.
- Sections were then rinsed gently with distilled water.

- Sections were then counter stained with hematoxylin.
- Slides were examined under the light microscope and any nuclear staining regardless the intensity was considered immunopositive.

Immunohistochemical method used for detection of E cadherin

- The immunohistochemical detection system was ultravision mouse tissue detection system: Antimouse HRP/DAB which was brought together with primary antibody (mouse monoclonal antibody of E- cadherin clone 36B5) was used.
- The reagents in the kit constitute a labeled streptavidin- biotin immunoenzymatic antigen detection system.
- This technique involves the sequential incubation of the section with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody that react with the primary antibody enzyme labeled streptavidin and DAB chromogen.

Steps of staining

- Sections were deparafinized by incubating them in xylene time, 5 minutes each.
- Sections were rehydrated in descending grades of ethyl alcohol, 3 minutes in each concentration then immersed in water 5 minutes.
- Endogenous peroxides actively was blocked by immersing the slides in 3% hydrogen peroxide solution for 10 minutes at room temperature, then washed two times with PBS 3 minutes each.
- Slides were dipped in antigen retrieval solution, heated to 95-100°C for 10-20 minutes, then cooled in the buffer at room temperature for 20 minutes.
- Slides were rinsed in buffer three times, one minute each.

- One to two drops of the prediluted primary antibody (mouse monoclonal antibody of E-cadherin clone 36B5) was added to each section, slides were incubated for 30 minutes in humid chamber, then washed in PBS 3 times minutes each.
- One to two drops of the biotinylated secondary antibody (ultravisionbiotinylated goat antimouse secondary antibody) were applied to each section and incubated for 10-15 minutes then rinsed with PBS 3 times, 3 minutes each.
- Two drops of streptavidin peroxidase were added to each section, incubated for 10-15 minutes then rinsed in PBS at room temperature 3 times, 3 minutes each.
- One to two drops of DAB chromogen were applied to each section, slides were then incubated for 10 minutes at room temperature, followed by washing in PBS for 3 minutes.
- Slides were then counterstained with Mayers hemotoxyline for 1-5 minutes, washed in water for 7-8 minutes, PBS for 1 minute, then tap water for 3 minutes.
- Slides were left to dry in air, then mounted in Canada balsam to be examined with light microscope.

Positive staining section appeared brown in color. The intensity of the immunohistochemical staining results for both PCNA and E- cadherin was assessed semiquantitatively and scored as follows: negative, weakly positive, moderately positive and strong positive staining reactions.

RESULTS

I-Histological results:

Hematoxylin and eosin stain

1- Group I animals (Control Group):

The gingiva of control animals showed the normal histological features of the surface epithelium and lamina propria. The gingival epithelium was of the

keratinized stratified squamous type characterized by numerous folding towards the underlying connective tissue of lamina propria forming numerous, slender, long and irregular epithelial ridges. The epithelium was formed of four categories of cells, the basal cell layer formed of a single row of low columnar cells resting on the basement membrane, prickle cell layer formed of several rows of polyhydral cells with intercellular spaces and intercellular bridges could be seen giving them the spinous or prickly appearance. Then the granular cell layer formed of 2-3 rows of large flattened granular cells and then the most superficial hornified layer with its eosinophilic amorphous appearance. Clear cells have been encountered among the basal cells. The underlying lamina propria was formed of the two indistinct layers, the papillary layer characterized by having thin collagen fibers and small blood capillaries and reticular layer having coarser collagen fibers, large blood vessels, and nerves. Various connective tissue cells were found between the collagen fibers, the prominent cells were the fibroblasts. Few chronic inflammatory cells were sometimes encountered (Fig. 1).

2- Group II (*Propolis treated group*):

The gingiva of the animals of group II that received propolis as a daily oral dose 50 mg/kg body

weight, showed nearly the same histological features of surface epithelium of the control group with the four categories of cells with apparent increase in number of cells leading to slight acanthosis, mild increase in granular cell layer and its keratohyaline granules in addition to slight hyperkeratosis. Numerous clear cells were observed not only among the basal cells but also among the upper spinous cells having clear cytoplasm around their nuclei. The lamina propria showed slight increase in condensation of collagen fibers in addition to an apparent increase in vascularity (Fig. 2).

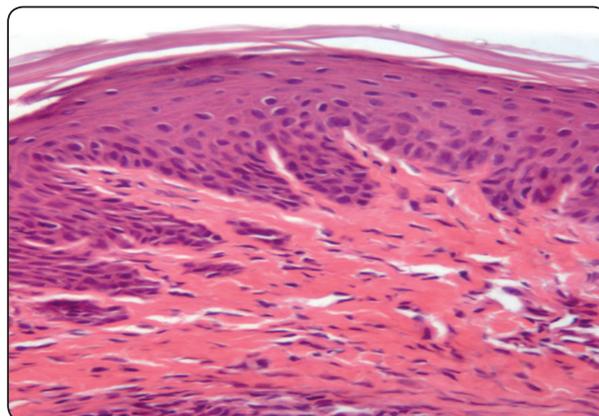


Fig. (1) A photomicrograph of the gingiva of a control animal showing normal histological features of the surface epithelium of the keratinized stratified squamous type and lamina propria. (HX&E. orig. mag. 400)

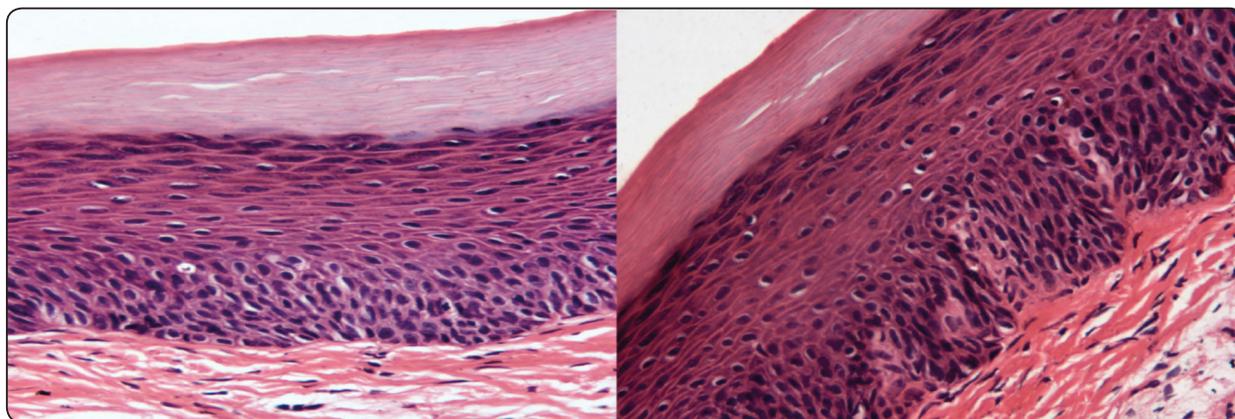


Fig. 2: Photomicrographs of the gingiva of group II animals received propolis as a daily oral dose 50 mg/kg body weight showing slight acanthosis, mild increase in granular cell layer and its keratohyaline granules in addition to slight hyperkeratosis. The lamina propria showed slight increase in condensation of collagen fibers (HX&E. orig. mag. 400).

II- Immunohistochemical results:

A) Immunohistochemical localization of PCNA:-

1- Group I animals (Control Group):

The basal and suprabasal cells of the surface epithelium of the gingiva of the control animals were presented with moderately positive staining reactivity for PCNA (Fig.3).

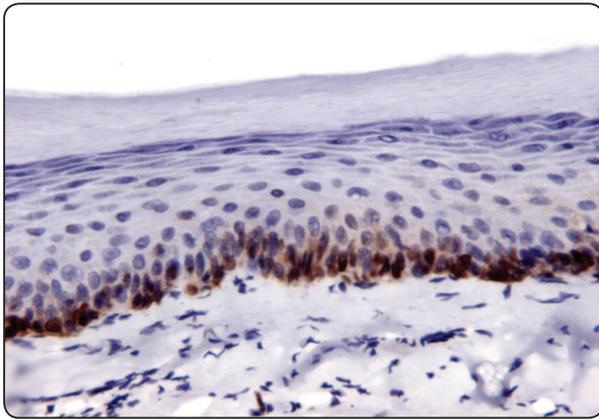


Fig. (3) A photomicrograph of the gingiva of a control animal incubated with PCNA monoclonal antibody showing moderately positive staining reactivity of the basal and suprabasal cells to PCNA (orig. mag. 400)

2-Group II (Propolis treated group):

The surface epithelium with acanthosis of the gingiva of rats received propolis as a daily oral dose 50 mg/kg body weight, showed strong staining reactivity of their basal and suprabasal cells to PCNA (Fig.4).

B) Immunohistochemical localization of E-Cadherin:

1-Group I animals (Control Group):

Examination of sections taken from the gingiva of rats of control groups and incubated with mouse monoclonal antibody of E- Cadherin clone 36B5 revealed a strong positive reaction in the epithelial

cells of the different strata of the surface epithelium of the gingiva (Fig.5)

2-Group II (Propolis treated group):

Gingiva of rats of group II incubated with mouse monoclonal antibody of E- Cadherin clone 36B5 demonstrated a strong positive reaction in the epithelial cells of the different strata of the increased thickness surface epithelium of the gingiva (Fig.6).

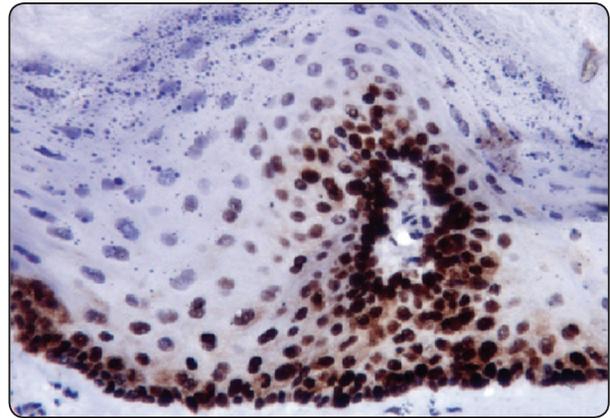


Fig. (4) A photomicrograph of the gingiva of rats from group II incubated with PCNA monoclonal antibody showing strongly positive staining reaction in the basal and suprabasal cells (orig. mag.400).

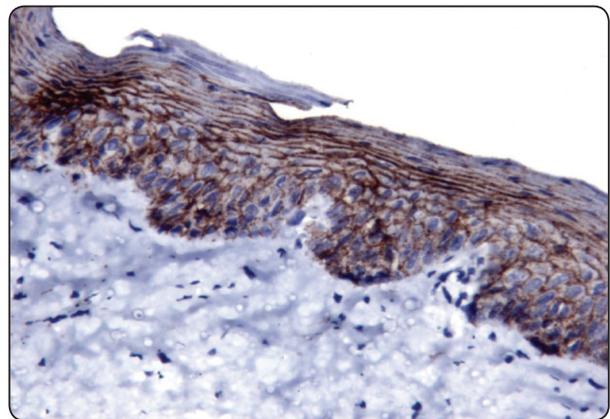


Fig. (5) A photomicrograph of the gingiva of control rats incubated with monoclonal antibody of E- Cadherin clone 36B5 showing strongly positive staining reactivity of the epithelial cells (orig. mag.400).

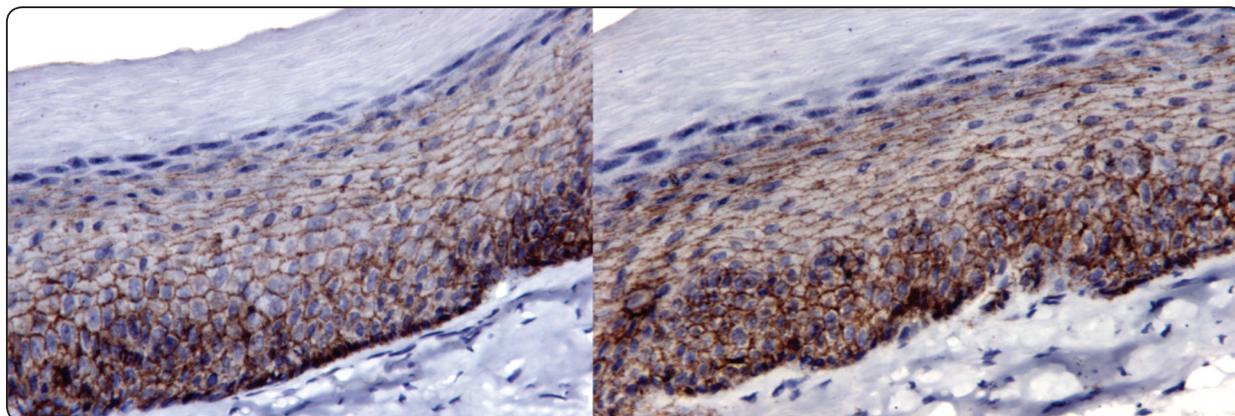


Fig. (6) Photomicrographs of the gingiva of rats from group II incubated with monoclonal antibody of E- Cadherin clone 36B5 showing strongly positive staining reactivity of the epithelial cells (orig. mag. 400).

DISCUSSION

Propolis is an ancient and important medical remedy that has been used in various clinical situations, with many biological properties, including immunomodulatory⁴, anti-inflammatory²⁷, antiulcer²⁸, antioxidant²⁹, antibacterial³⁰, antiviral³¹, antifungal³² and antiparasite³³ activities, hepatoprotective³⁴, cariostatic³⁵ and anticancer³⁶. However the use of propolis in dentistry is relatively new; but considering the effects of propolis on infection, inflammation and carcinogenesis, it may have more potential uses in the treatment as well as the prevention of oral disease. While the composition of the different type of propolis differs depending on its botanical and geographic origin, the biological effects of the different propolis types are similar. These effects are suggested to be due to the presence of flavonoids and polyphenols especially hydroxycinnamic acid derivatives which are present in most types of propolis. Flavonoids are well known plant compounds which have antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties³⁷.

Our histological examination of gingival specimens taken from group II rats that received a daily oral dose of propolis appeared normal with nearly the same histological features of normal

tissues. However apparent increase in the number of cells of the surface epithelium was seen leading to slight acanthosis, this mild epithelial hyperplasia might be due to decrease in the rate of the physiologic cell death (apoptosis) of keratinocytes under the effect of propolis application. In our opinion the decrease in the rate of cell turnover which might be associated with increased mitochondrial enzymatic activity of keratinocytes is similar to the results have been reported by³⁸ in the effect of propolis on periodontal ligament fibroblasts. They found increases in the metabolic activity and proliferation of PDL cells and decreases apoptosis and suggested that propolis has a beneficial role on the viability and physiological health of periodontal ligament cells.

Magro- Filho³⁹ stated that topical application of propolis hydro-alcoholic solution was found to accelerate epithelial repair after tooth extraction but had no effect on socket wound healing. After few years the same authors⁴⁰ studied the topical effect of propolis in the repair of sulcoplasties by the modified Kazanjian technique: cytological and clinical evaluation and found that propolis in aqueous alcohol solution exerted a small analgesic and anti-inflammatory effect and also aided repair of the intra-buccal surgical wounds.

Present study showed mild increase in granular cell layer and its keratohyaline granules in addition to orthokeratinized epithelia with slight hyperkeratosis. In our opinion keratinization of the superficial layers might be a kind of epithelial cell protection against mechanical injury and irritation. Keratinocytes undergo a program of terminal histophysiological and morphological differentiation, expressing a set of structural proteins, keratins, which assemble into filaments and function to maintain cell and tissue integrity. Normal terminal differentiation is associated with increased keratohyaline granules and a reduction in nuclei in superficial layer cells⁴¹. Li and coworkers⁴² stated that the amount of keratohyaline granules determines the extent of the keratinization process. Our investigations are in accordance with⁴³ who reported that propolis significantly improved the total protein content of the liver and kidney.

Numerous clear cells were observed in the present study in the basal and parabasal cell layers of the gingiva in the group II rats treated with propolis. They include melaocytes, Langerhans cells, Meckel cells and inflammatory cells⁴¹.

In our opinion the most predominant one was Langerhans cells (LCs), and this is accordance to Sforzin and his colleague⁴⁴ who stated that propolis enhanced natural killer cell activity and increased dendritic cell function. Many studies demonstrated that propolis increases the cellular immune response through the stimulation of human peripheral blood leukocyte proliferation⁴⁵, increase the activity of helper T-lymphocytes, causes increase in the levels of cytokines IL-2 and interferon IFN- γ , and helps to activate macrophages and is thus useful in the treatment of several diseases caused by immune dysfunction⁴⁶. We suggested that the immunostimulatory effect produced by the propolis may be due to cell mediated and humoral antibody mediated immune response. In our opinion the antioxidants present in propolis play a great role in its immunomodulatory properties.

Some anti-inflammatory substances found in propolis have been isolated. According to Mirzoeva and Calder⁴⁷, these substances are caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester (CAPE). These compounds contribute to the suppression of prostaglandins and leukotrienes synthesis by macrophages and have inhibitory effects on myeloperoxidase activity, NADPH-oxidase, ornithine decarboxylase and tyrosine-protein-kinase. Other study attributed propolis anti-inflammatory activity to other compounds, including salicylic acid, apigenin, ferulic acid and galangin⁴⁸.

The results of the present study revealed an increase in the fibroblasts, collagen fibers and vascularity in lamina propria of the gingiva which may be due to the effect of propolis in activation of immune system as mentioned before where cytokines, growth factors, and enzymes released by immune cells directly promote fibroblast activation. These results are in accordance with Kilicoglu⁴⁹ who observed that fibroblast proliferation, activation and synthesis capabilities were better in the presence of propolis than in its absence. Other study done shared a similar view that propolis speeds up the healing process not only through its anti-inflammatory effect, but also by direct action on fibroblast proliferation⁵⁰. On the other hand Günay et al.⁵¹ used propolis gel containing flavonoids and Caffeic acid phenylethyl ester (CAPE) to assess its effect on fibroplasia and epithelialization in post tooth extraction wounds and observed that although it had no significant action on fibroblast growth, it enhanced epithelialization. Borges and coworkers⁵² reported an opposing observation on the effect of Tubi-bee propolis on glioblastoma and normal fibroblast cell lines. They found that propolis exerted a strong inhibition on the proliferation of both cell lines. There was another study done by⁵³ revealed that propolis has antiproliferative action on human cultured fibroblasts and caused mild necrosis.

Our immunohistochemical investigation of the gingiva of group II rats received propolis resulted in surface epithelium with acanthosis showed observed increase in the staining reactivity of their basal and suprabasal cells to PCNA. These immunostaining characteristics allow the identification of proliferating cells in the different phases of the cell cycle. This result confirm our histological results which revealed acanthosis of the surface epithelium with hyperkeratosis. This correlate with cellular proliferation and differentiation. This is in accordance with ³⁸ who stated that propolis not only decreased apoptosis but also increased the metabolic activity and proliferation of cells.

PCNA is also involved in DNA repair and so our finding is in agreement with those of⁵⁴ who found that propolis significantly reduced the level of DNA fragmentation due to its antioxidant activities, DNA fragmentation is used for detection of apoptotic cells in tissues. Chen et al⁵⁵ reported that propolis can have a double benefit of protecting healthy cells whilst killing cancer cells.

The present immunohistochemical work revealed the gingival epithelium of group II rats with strong positive reaction in the epithelial cells of the different strata of the increased thickness surface epithelium to E- Cadherin clone 36B5. The appropriate regulation of cell-cell adhesion an important event in the homeostasis of different cell types. In epithelial cells, tight adhesion mediated by E cadherin receptors is essential for the differentiation and functionality of epithelial sheets, upon assembly of cadherin- mediated cell-cell contact.

Based on our histological and immunohistochemical results from group II that received daily oral dose of propolis we suggest that supplementation of propolis to the rats may activate the proliferation, regeneration and maturation of the keratinocytes and non- keratinocytes of the gingival tissue and improved vascularity and organization of col-

lagen fibers of gingival subepithelial lamina propria thus enhancing the integrity and barrier function of the gingival epithelial cells and collagen fibers, supported the great antioxidants and immunomodulatory activities of propolis. It can be concluded that propolis can be used as a beneficial constituent of surgical dressing as well as mouth wash.

Ultimately, the aim of a dressing is to promote healing of the wound by providing a sterile, breathable and moist environment that facilitates granulation and epithelialization. This will then reduce the risk of infection, help the wound heal more quickly, and reduce scarring. Our observation is in accordance with Sutta⁵⁶ who used propolis alcoholic solutions at animal wounds treatment in clinical and also experimental cases. Histologically, they observed that propolis treatment induced better healing by reducing the inflammatory response; consequently, epithelial healing was faster with propolis. The authors considered propolis suitable for wound treatment, following elimination of the infection. It is known that healing is directly related to the inflammatory process and if the latter is less pronounced, production of healing molecules and deposition of collagen fiber bundles increase. In our opinion propolis tissue regeneration properties, including healing, are possibly due to its antioxidant activity in addition to the prevention of wound infections and inhibition the spread of the surface contamination. Whenever free radicals are produced, they hamper or even block cells regeneration. Removal of free radicals by propolis flavonoids would allow regeneration of an ill organ or tissue in an ordinary way similar to previous studies reported by Marcucci⁵⁷ and Małgorzata et al⁵⁸.

Research done by Kilicoglu and his colleagues⁴⁹ studied the effect of propolis on the healing of colon anastomosis in rats and observed that in the propolis group, fibroblast proliferation began soon after the number of neutrophil cells decreased. They also found that lymphocytes appeared earlier

in that group compared to the control group. This suggests that propolis quickens the wound healing process by reducing acute inflammation and stimulating macrophage and T-lymphocyte activity. The hydro- alcoholic propolis extract, at very low concentrations can inhibit hyaluronidase activity, this enzyme is responsible for several inflammatory processes and so propolis has a greater potential as an anti-inflammatory. In addition, propolis evidenced considerable antimicrobial activity, as it inhibited the growth of yeasts, Gram- negative and Gram-positive bacteria⁵⁹.

Propolis also increases expression of a number of genes that promote wound healing such as fibroblast growth factor 18 (FGF18) and vascular endothelial growth factor A (VEGFA). FGF18 is a pleiotropic growth factor that induces proliferation in various tissues. Hu et al⁶⁰ observed that FGF18 caused a dose-dependent increase in the DNA synthesis of NIH3T3 fibroblast cell line. Through an MTS cell proliferation assay, they further established that FGF18 stimulates growth of fibroblast cells. Vascular endothelial growth factor A (VEGFA), also known as VEGF, is a member of the PDGF/VEGF growth factor family and is most commonly associated with induction of endothelial cell growth and migration. However, studies have also shown that VEGF stimulates fibroblast proliferation⁶¹.

Propolis is remarkably used in dermatology for wounds healing, burn and external ulcers treatment, healing time reduction, wound contraction increase, and tissue repair acceleration. During wound healing, perfect synchronized cellular and molecular interactions occur to repair damaged tissue⁶². And so we can say the role of propolis in dentistry to improve the oral health and used in the treatment of oral lesions and repair of surgical wounds. This is in accordance with⁶³ who showed that caffeic, ferulic, *p*-coumaric and cinnamic acids found in Brazilian Green propolis displayed antiulcer activity. Published evidence has indicated

that propolis, can improve tissue healing, especially following pathological insults such as burns and periodontal diseases⁶⁴.

CONCLUSION

Our histological and immunohistochemical results from group II that received daily oral dose of propolis revealed that supplementation of propolis to the rats activated the proliferation and maturation of the surface epithelium of the gingival tissue and improved vascularity and organization of collagen fibers of gingival subepithelial lamina propria thus enhancing the integrity and barrier function of the gingival epithelial cells and collagen fibers, increasing not only the viability but also the physiological health of gingival cells

We recommend using Propolis as a beneficial constituent of mouth wash and surgical dressing after fulfilling all the required investigations on human. In addition to our results suggest that propolis solution can be used as a beneficial constituent of mouth wash and surgical dressing for treating various types of intra oral wounds and ulcers.

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