

Active Hexose Correlated Compound improved the gingival integrity of Albino rats.**Zoba H. Ali¹ and Rabab Mubarak^{1&2}**¹Oral Biology Department, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt²Oral Biology Department, Faculty of Oral and Dental Medicine, Nahda University, Beni suef, **Egypt**rawya_h2a@yahoo.com rababmubarak2010@hotmail.com

Abstract: Background: Nutrition is an integral component of oral health. There is a continuous synergy between nutrition and the integrity of the oral cavity in health and disease. **Objective:** The aim of this study was to investigate the effect of active hexose correlated compound (AHCC) on the integrity of rat gingival tissue. **Material and methods:** Twenty adult male albino rats (250±10 g) were divided equally into two groups, control and AHCC groups. AHCC was administered orally by oesophageal tube at a concentration of 1g AHCC/ kg of body weight/ day in distilled water. The rats were sacrificed after three months; the gingival specimens were dissected out and prepared for light and electron microscopic examinations. **Results:** AHCC improved the gingival integrity. It produced normal histological features of the surface epithelium with mild acanthosis, mild hyperkeratosis. Lamina propria demonstrated more condensed collagen fibers, increased number of fibroblasts and clusters of lymphocytes and macrophage. Several ultrastructural changes were observed, some of which suggested that AHCC treatment resulted in the formation of more efficient permeability barrier in the gingival area. Keratin filaments were appeared in the stratum basal, as the keratinocytes migrate apically; tonofilaments in the cell continue to accumulate and increased in number. In the strata spinosum and granulosum numerous membrane-coating and keratohyalin granules were synthesized. Membrane-coating granules were fused with the cell membrane and increased epithelial barrier function. The keratohyaline granules released their content to the cytoplasm making the tonofilaments to aggregate into tonofibrils. In the stratum corneum the keratin fibrils (tonofibrils) completely replace the cytoplasmic contents of the cell. **Conclusion:** AHCC supplemented to the rats may be increase the integrity of the gingiva epithelial cells through regulating the keratinization, tonofilaments, keratohyalin and membrane-coating granules formation.

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1. Introduction

The primary role of the oral mucosa is protection of the underlying structures from foreign agents. The surface of the oral mucosa consists of a stratified squamous epithelium which is separated from the underlying connective tissue by an undulating basement membrane⁽¹⁾. The oral mucosa forms a major interface between the animal and the environment. Its integrity and appropriate response of the mucosa-associated immune system to antigens are crucial to maintain oral and general health^(2,3). Epithelia throughout the body function as a physical barrier against invading bacteria and also provide effective innate immune defenses by producing antimicrobial peptides⁽⁴⁾. Healthy attached gingiva is composed of keratinized stratified squamous epithelium and a lamina propria. Cellular infiltration, consisting mainly of plasma cells, lymphocytes and histocytes can be observed in healthy gingival connective tissue⁽⁵⁾. Epithelial cells (keratinocytes) in the normal gingival mucosa were eventually differentials into stratum corneum. The degree of keratinization is reflected in the degree of packing and orientation of tonofilaments. In addition to the

keratinocytes in the gingival tissue, dendritic cells of two types have been distinguished; melanocytes and Langerhans' cells. They resemble each other in structure but differ in cell content and in position^(6,7). Non-keratinocytes have been termed clear cells in haematoxyline and eosin section⁽⁸⁾.

A nutritious diet, including adequate amounts of protein, vitamins, essential fatty acids and micronutrients, play an important role in the resistance to infectious conditions including gingivitis and periodontitis⁽⁹⁾. There is an interdependent relationship between nutrition and health of oral tissues. The well-being of the oral tissues, depend on the intake of nutrients. Oral health determines the type of food consumed and ultimately the nutritional level⁽¹⁰⁾.

Active Hexose Correlated Compound (AHCC) was developed in 1987 at the University Of Tokyo Faculty Of Pharmaceutical Sciences along with other researchers as a natural product used for regulating high blood pressure. However, AHCC is now primarily known for its immune stimulant potential in protection against viruses, cancers, and infections⁽¹¹⁾. AHCC supplement is a super food

nutrient supports normal immune function and improves the number of immune system cells in health and disease conditions. Moreover AHCC increased the cell activity^(12,13).

The predominant components in AHCC are oligosaccharides, totaling approximately 74% of the total dry weight. Of these, nearly 20% are partially acetylated α -1, 4-glucans, which are believed to constitute the active compounds in AHCC⁽¹⁴⁾. Health care professionals advocate the use of AHCC for the treatment of cancer, AIDS, hepatitis C, diabetes, hypertension and autoimmune diseases. There is no reason to doubt that AHCC is one nutritional supplement that has universal application not only for treatment of disease but also for maintenance of optimum health⁽¹⁵⁾. AHCC is mixture of polysaccharides, amino acids, lipids and minerals extracted from the culture of the basidiomycete mushroom *Lentinula edodes*^(16, 17). AHCC also contains high levels of antioxidants such as L-ergothioneine, which helps to reduce the risk of oxidative cause of mutation in cell division and alternation⁽¹⁸⁾.

Therefore, the aim of the present study was to determine the light and electron microscopic changes in rat gingival tissue induced by oral administration of active hexose correlated compound.

2. Materials and methods:

Twenty adult male albino rats weighing approximately 250±10 g were used in the present study. They were divided equally into two groups of ten rats each. The animals were obtained from the Research Institute of Ophthalmology. They were kept in especially designed wire mesh bottom cages 5 animals per cage to maintain them under optimum condition of good ventilation. The animals were supplied by standardized laboratory diet & water and were classified into following groups:

Control group: consisted of 10 rats and represented the untreated control group. They had the normal diet.

Experimental group (AHCC group):

Consisted of 10 rats and they had the normal diet. AHCC was administered orally by oesophageal tube at a concentration of 1g AHCC/ kg of body weight/ day in distilled water.



Each rat received about 2.5 -3ml (100mg/ml) of active hexose correlated compound in distilled water for three months. This dose of AHCC has been used previously and did not produce toxic effects in young mice⁽¹⁹⁾. The type of AHCC used in this study was Kinoko AHCC 250mg capsules (AHCC, Amino Up Chemical Co, Ltd., Sapporo, Japan Quality of life LABS).

At the end of the experimental period the rats of the two groups were sacrificed by carbon dioxide inhalation. The gingival specimens were dissected out from the region of the mandibular molars of both sides.

Histological study: The gingival specimens of left side in each group were fixed in 10% calcium formol for 12 hours, washed by tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in paraffin wax. Section of 6-7 μ m thickness was prepared and stained with H & E for histological study by light microscope.

Transmission electron microscopic examination:

Specimens of the right side were cut into small fragments of about 1×1mm³ and were fixed in a solution prepared by mixing equal volumes of 3 % glutaraldehyde and 0.1 phosphate buffer (pH 7.4) at 4°C for 3-5 hours. Specimens were washed in three changes of phosphate buffer. Secondary fixation was achieved in 1% osmium tetroxide at 4°C, for 1.5 hours and then rinsed in PBS. Specimens were then dehydrated in ascending grades of ethyl alcohol, finally dehydrated and cleared in propylene oxide and embedded in epoxy resin capsules. Semi thin sections of 0.5-1 μ m were cut, fixed on glass slides and stained with 1% toluidine blue and examined by light microscope for detection of the site to be studied by TEM. Ultra thin sections 60-100A thick were cut using the ultra microtome, mounted on copper grids and stained with uranyl acetate and lead citrate⁽²⁰⁾. The grids were examined by Joel 100 S TEM at different magnifications and photographed using C.C.D camera in the electron microscope unit at the National Cancer Institute.

3. Results

Light microscopic results:

I-Control group:

The gingiva of the rat was composed of surface epithelium of the orthokeratinized stratified squamous type with slender, long, irregular and numerous epithelial ridges. The epithelium lies on a basement membrane which separates it from the connective tissue (Fig.1). Four layers can be observed in the gingival epithelium, basal layer of cuboidal or columnar cells, a spinous layer consisted of several rows of irregular polyhedral cells, a granular layer of flattened cells containing basophilic granules called keratohyaline granules and a surface acidophilic amorphous cornified layer that made up of keratinized squamae, devoid of organelles and nuclei (Fig.2). The lamina propria was formed of two indistinct layers, the papillary layer having thin collagen fibers and small blood capillaries and the reticular layer having coarse collagen fibers and large blood vessels the predominant cells were the fibroblasts and few chronic inflammatory cells were sometimes detected (Fig.3).

Experimental group:

The gingiva of rats received a daily AHCC showed nearly the same histological features of the surface epithelium of the control rats with the four categories of cells with apparent increase in number of cells leading to slight acanthosis (Fig.4). Mild increase in the granular cell layer with enlargement of the keratohyaline granules, which appeared as basophilic particles and mild hyperkeratosis were obvious. Some areas of squamoid proliferation were detected within the upper prickle and granular layers (Figs.5). Numerous clear cells were observed in the basal and parabasal cell layers, which differ in appearance from other epithelial cells in having a clear halo around their nuclei. The basal cell layer showed focal basilar hyperplasia (Fig.6). The lamina propria showed collagen fibers arranged in thick bundles with increased number of fibroblasts, blood vessels and clusters of lymphocytes and macrophage (Figs.4&6).

Transmission electron microscopic results:

Control group:

The electron microscopic examination of the gingival specimens of the control group showed different layers of keratinized stratified squamous epithelium. The basal cells were cuboidal or slightly columnar in shape and contained a single, large, irregularly-shaped nucleus with a single, eccentric nucleolus. Numerous mitochondria were concentrated in the bases of the cells deep to the nucleus (Fig.7). The melanocytes were situated on the basal layer of the gingival tissue and they lack desmosomes and tonofilaments. Melanin was appeared within the cytoplasm of the melanocytes as

small dark granular structures called melanosomes (Fig.8).

The cells of stratum spinosum were larger than the basal cells. The cells appeared polyhedral with spherical central nuclei and prominent nucleoli. The nuclear membrane had shallow indentations. Cell organelles were fewer, but tonofilaments were numerous. The intercellular spaces were small and adjacent membranes were connected by desmosomes of variable length and also microvillus-like configuration extended into the intercellular spaces was obvious. The granular layer was composed of flattened cells with elongated nuclei. The nuclear membrane showed deep indentation (Fig.9). Obvious changes were seen in the cells of stratum corneum. These varied from a loss of internal cell detail to a great increase in electron density. The wide and irregular intercellular spaces were detected and desmosomes were no longer recognizable (Fig.10).

Experimental group:

Electron microscopic examination of the gingiva of experimental group revealed normal appearance of the basal cells with oval nuclei, prominent nucleoli, shallow invaginated nuclear membrane and junction complex between cells. The cytoplasm contained the usual cell organelles such as mitochondria, Golgi apparatus, lysosomes, rough endoplasmic reticulum and free ribosomes. Also dendritic cell was detected between the basal cell with long dendritic processes (Fig.11).

The prickle cell layer was composed of several rows of polyhedral cells with irregular nuclei and sometimes with prominent nucleoli. These cell layers constituted the largest portion of the gingival epithelium. In the cytoplasm rough endoplasmic reticulum, mitochondria, well developed Golgi apparatus and various amounts of vacuoles were visible. Many tonofilaments (bundle of keratin intermediate filaments) were present in the form of bundles either lying free in the cytoplasm or run into the dense plaque of the desmosome. Intercellular spaces were narrow. The numbers of desmosomes are much greater in the stratum spinosum (Fig.12). The filaments condensed into dense bundles, forming networks around the nuclei and at the periphery in association with desmosomes. The cytoplasmic contents appeared with variable electron density. Filaments, ribosomes and occasional lipid droplets were seen in some specimens. Numerous granules were found in the cells of the upper layers of the stratum spinosum. These granules were called membrane-coating granules (Fig.13).

The granular cell layer was increased in thickness and the granular cells appeared with thickened cell membrane especially at their inner side also irregular nuclear membrane was detected.

Keratohyalin granules were seen in the cells of the stratum granulosum. They appeared homogeneously electron-dense and surrounded by ribosomes and also closely related to tonofilaments. The granules were variable in size. Two types of keratohyalin granules were detected in the cytoplasm of granular cell layer. First type appeared as highly electron dense granules, irregular in shape and associated with tonofilaments. The second type is more regular in outline and the granules were embedded in an electron-dense matrix (Fig.14).

The surface was covered by the keratinous layer and the cells appeared extremely flattened and the cytoplasm was markedly electron dense and homogeneous. The nucleus, keratohyalin granules and all cell organelles were disappeared and the cells appeared to be packed with tonofilaments. Intercellular junctions between the flattened cells are still distinguishable. This form of keratinization is referred to as orthokeratinization (Figs.15&16).

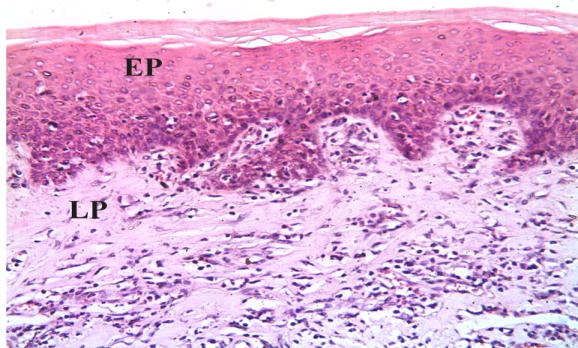


Fig.(1): A photomicrograph of the gingiva of a control animal showing the histological structure of the gingiva consisting of keratinized stratified squamous epithelium(EP) and lamina propria (LP) (H&E. X 200).

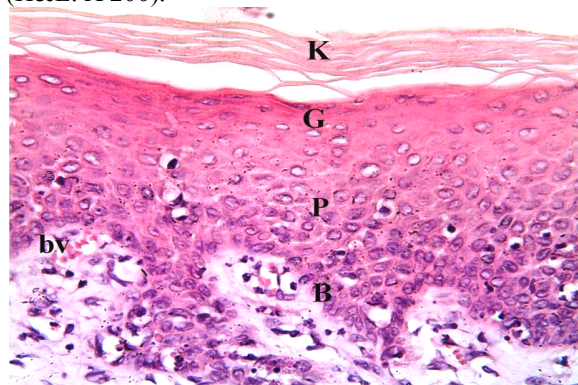


Fig.(2): A higher magnification of previous photomicrograph showing basal (B), prickle (P), granular (G) and keratinous (K) layers of the epithelium, clear cells (arrows) and the underlying lamina propria with small sized blood vessel (bv) (H&E. X 400).

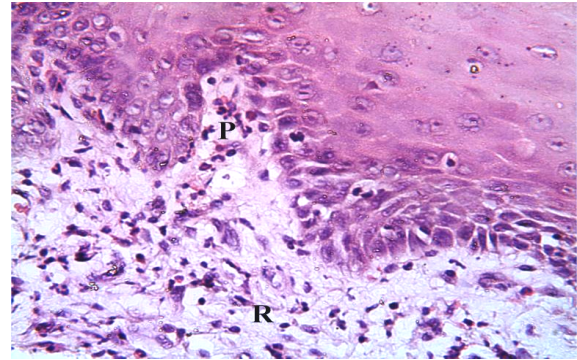


Fig.(3): A photomicrograph of the gingiva of a control animal showing lamina propria with the papillary (P) and reticular layers (R) in addition to connective tissue cells between the collagen fibers and mild chronic inflammatory cell infiltration (arrows). (H&E. X 400).

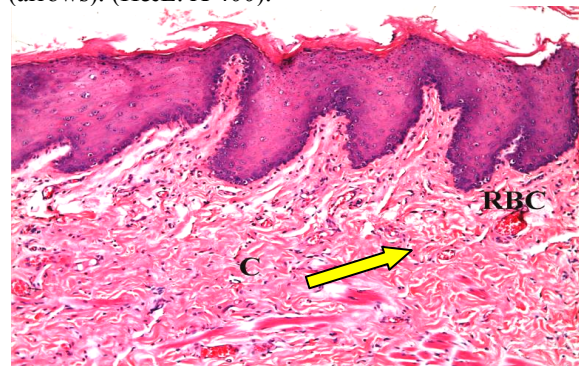


Fig.(4): A photomicrograph of the experimental group showing surface epithelium with slightly broad epithelial ridges and thick condensed collagen fibers (C) associated with dilated blood vessels engorged with RBC and clusters of subepithelial lymphocytes in lamina propria (arrows). (H&E. X 200).

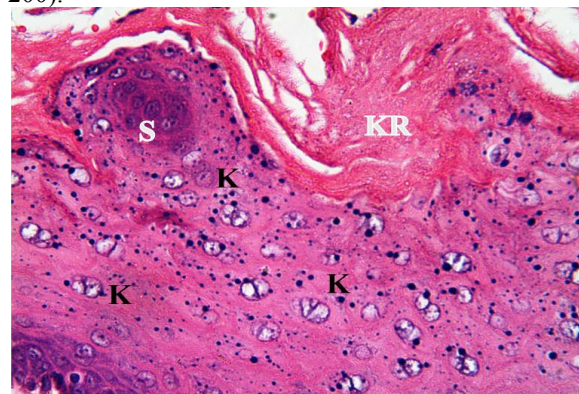


Fig. (5): A photomicrograph of the experimental group showing increased keratinization (KR), numerous enlarged keratohyaline granules in the prickle, granular and cornfield layers (K) and squamoid proliferation at the granular cell layer (S) (H&E. X400).

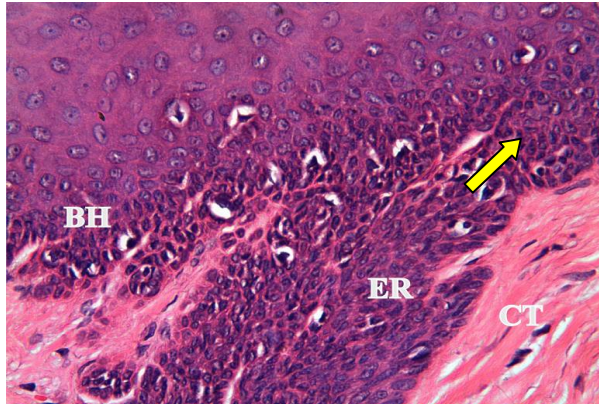


Fig. (6): A photomicrograph of the experimental group showing the irregular epithelial ridges (ER) extended towards the underlying C.T., clear cells with dark nuclei surrounded by a light halo (arrows) and focal basilar hyperplasia (BH) (H&E. X 400).

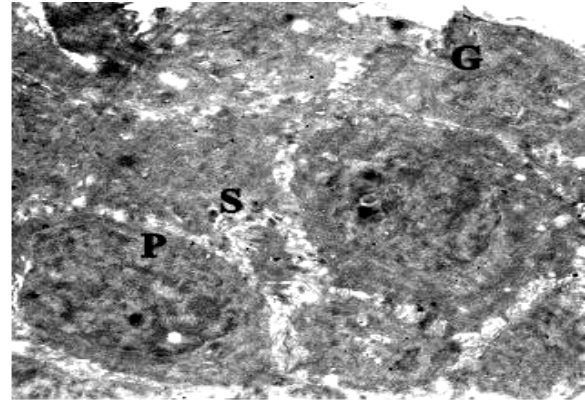


Fig. (9) An electron micrograph of gingiva of the control animal showing polyhedral cells of prickle (P) with small intercellular spaces (S) and interdigitation between cells (arrows) and flattened granular cells (G) (Urany acetate & lead citrate x 1000)

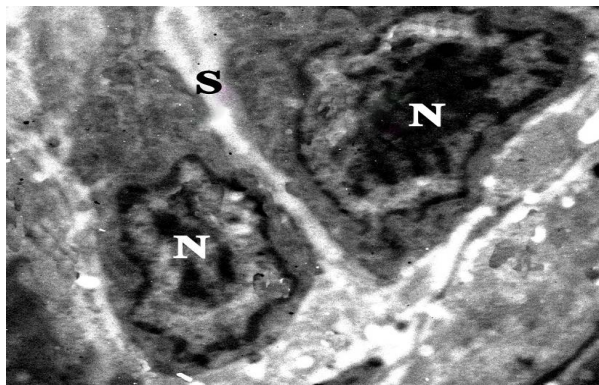


Fig. (7) An electron micrograph of gingiva of the control animal showing columnar basal cells with small intercellular spaces (S), the nuclei appeared oval with shallow invaginations of the nuclear envelope (arrows) (Urany acetate & lead citrate x3000)



Fig. (10) An electron micrograph of gingiva of the control animal showing keratin layer (K) with almost structureless cytoplasm (Urany acetate & lead citrate x2000)

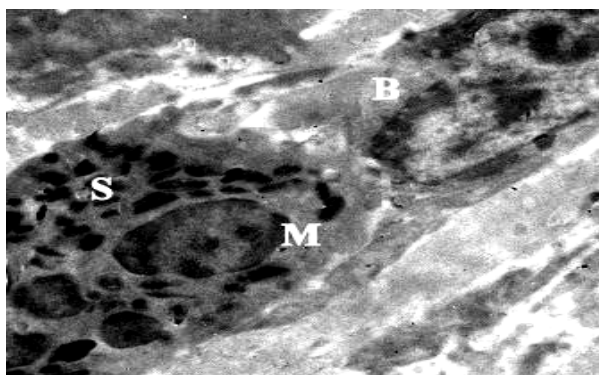


Fig. (8): An electron micrograph of gingiva of control group showing a melanocyte (M) with abundant dense melanosomes (S) in the basal layer (B) (Urany acetate & lead citrate x3000).

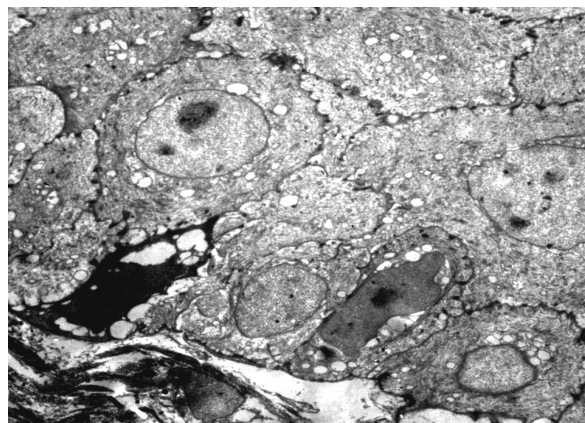
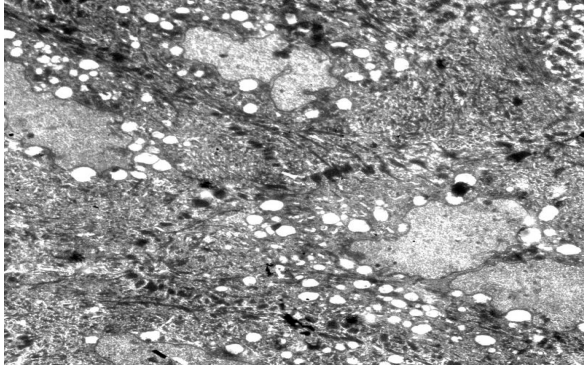


Fig. (11) An electron micrograph of the gingiva of experimental group showing cuboidal and columnar basal cells and dendritic cell (arrow) (Urany acetate & lead citrate x1000)



(Fig.12). An electron micrograph of gingiva of the experimental animal showing prickle cell layer, the nuclei having irregular nuclear membranes, numerous desmosomes along the cell circumference, numerous longitudinal and cross-sections of collagen bundles (Urany acetate & lead citrate x1000)

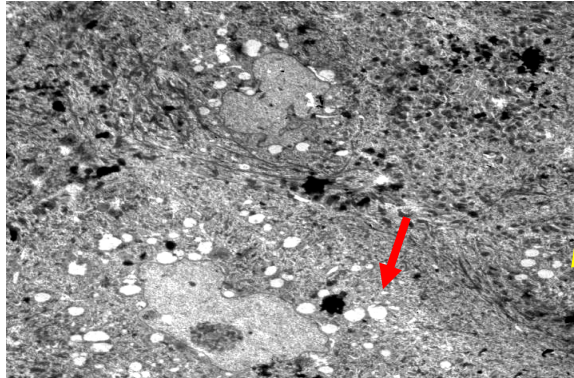


Fig. (13) An electron micrograph of the gingiva of experimental group showing prickle cell layer with nucleus and prominent nucleolus, dense filament bundles formed networks around the nuclei (yellow arrow) and lipid droplets (red arrow) (Urany acetate & lead citrate x1000)

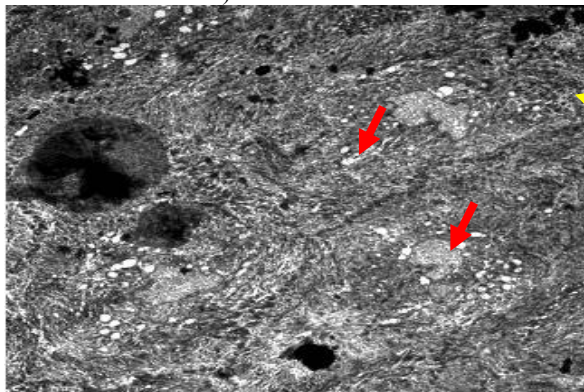


Fig.(14). Electron micrograph of cell from stratum granulosum showing numerous enlarged keratohyaline granules with variable sizes (yellow arrows), the second type of keratohyaline granule, granules are embedded in an electron-dense matrix (red arrows) (Urany acetate & lead citrate x500)

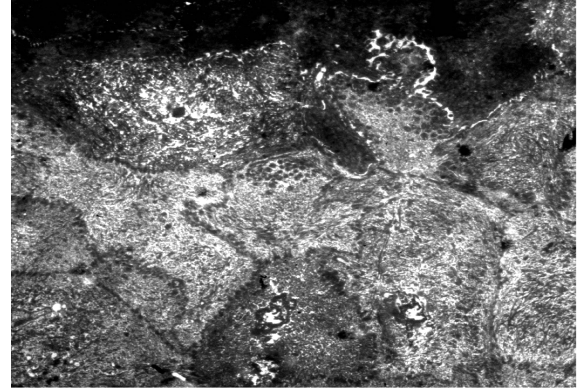


Fig. (15) An electron-micrograph of gingiva of experimental group showing granular and stratum corneum layers with well developed cell membranes and rich in dense tonofilaments. (Urany acetate & lead citrate x500).

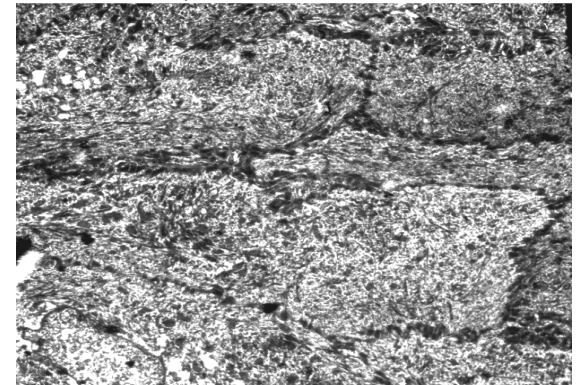


Fig. (16) An electron-micrograph of gingiva of experimental group showing stratum corneum cells packed with tonofilaments (Urany acetate & lead citrate x1000).

4. Discussion

The avascular nature of a complex and metabolically active tissue such as stratified squamous epithelium makes the problems of nutrient distribution to various cell strata and subsequent nutrient utilization particularly important. Amino acids incorporated by stratified squamous epithelial cells, in addition to participating in metabolic functions common to all cells, are utilized in the synthesis of specialized structures such as tonofilaments, keratohyalin granules (KHG's), desmosomes and membrane coating granules (MCGs)⁽²¹⁾.

The oral cavity has sometimes been described as a mirror that reflects the health of the individual. Nutrition affects oral health and there is an interdependent relationship between nutrition and health of oral tissues⁽²²⁾. Studies on various natural and nutritional therapies showed that many aspects of the deterioration of immune response that occurs during life can be prevented.

AHCC is a nutritional food and supplement ingredient that has been widely sold in Japan and Asia for the past 15 years, and increasingly in the US and Europe. AHCC has proven extremely effective for activating vital parts of the immune system leading to both prevention and treatment of serious diseases⁽¹⁴⁾. AHCC had antioxidant, anti-inflammatory effects and increased nitric oxide release by peritoneal cells⁽²³⁾. The safety and efficacy of AHCC supplementation have been assessed in healthy adult populations, noting an increase in dendritic cell number and function⁽²⁴⁾. Clinical effects and safety of the AHCC have been demonstrated in humans with malignancy, as well as in healthy subjects⁽²⁵⁾. AHCC induced the production of the cytokines that stimulate cellular immunity. It increased the number of T lymphocytes by up to 200 percent. There was also evidence that AHCC increased the population of macrophages, even doubling them⁽²⁶⁾.

The structural and ultrastructural results of the gingival epithelia of the control group in this study showed normal keratinocytes and keratinization layers. Moreover normal and healthy gingival tissues were observed in rats of the experimental group. The histological results of the present study revealed that, the application of AHCC in drinking water for three months resulted in the formation of mild epithelial hyperplasia which may be explained by either increasing mitosis or decreasing apoptosis of normal keratinocytes under the effect of AHCC application. These findings are in agreement with those of **Burikhanov et al.**,⁽²⁷⁾ they found that AHCC partially suppressed DNA fragmentation on thymus apoptosis induced by dexamethasone in rats. AHCC acts as an antioxidant that suppresses thymic apoptosis and enhances cell renewal by increasing the rate of mitotic cell division as well as it affects the metabolic cell activity. Also **Wakame**,⁽²⁸⁾ reported that AHCC prevented cellular damage induced by streptozotocin in rats and protected beta cells from degeneration.

In the herein study, keratinocytes of the experimental group as compared with the keratinocytes of the control group appeared more active with a considerable increase in the number of mitochondria, ribosomes and tonofibrils even in the basal cells. It is possible that AHCC exerts its effect on the keratinocytes by acting as an antioxidant.

Antioxidants play a vital role in maintaining the structural components of cells and aiding in immune defense⁽²⁹⁾. The anti-oxidant activity of AHCC has been reviewed by **Ye et al.**,⁽³⁰⁾ they suggested that AHCC acts as a potent antioxidant and protects against disorders induced by oxidative stresses.

Electron microscopic results in the present study represented overall increased in protein synthesis organelles in experimental group. Prominent nucleoli and well developed rough endoplasmic reticulum were detected in most cells. The presence of vacuoles of varying sizes and glycogen particles in the cells might be related to high metabolic activity of epithelial cells of experimental group. **Ye et al.**,⁽³¹⁾ and **Aviles et al.**,⁽¹⁹⁾ suggested that AHCC could act as a potential modifier of biological response. AHCC enhanced the activity of natural killer cell in cancer patients, enhanced spleen cell proliferation and cytokine production, as well as nitric oxide and cytokine production in peritoneal cells.

In the present study the amount of the membrane coating granules were increased and thickening of cytoplasmic membranes in the upper spinous and granular layers was detected in the experimental group. Most of these granules lie close to the plasma membrane, but some were scattered throughout the cytoplasm. Narrow intercellular spaces containing an amorphous granular material were seen in the experimental group. These findings were in accordance with **Squier**⁽³²⁾ who found small cytoplasmic granules, approximately 2 um in diameter, appear in the Golgi region of the prickle cell layer, migrate to the superficial region of cells at the midlevel of the epithelium, and apparently fuse with the cell membrane in the upper regions of the epithelium. Also **Meyer**⁽³³⁾ reported that when the membrane coating granules fused with the cell membrane, the contents of the membrane-coating granules were extruded into the intercellular spaces of the epithelium. Moreover **Swartzendruber**⁽³⁴⁾ suggested that the membrane-coating granules in keratinized epithelia contained electron-dense lipid lamellae and therefore the intercellular spaces of the stratum corneum were filled with short stacks of lipid lamellae which fuse at the edges to produce multiple broad lipid bi-layer sheets. **Christopher et al.**,⁽³⁵⁾ reported that the intercellular spaces contain a variety of proteins, proteoglycans, glycolipids, and lipids derived from the membrane-coating granules and secreted by the keratinocyte, which serve to modulate the passage of molecules across the epithelial barrier. The level at which permeability ceases coincides with the level of secretion of membrane-coating granules. In keratinized mucosa, this level is formed by the boundary between the stratum granulosum and the stratum corneum. **Ritz et al.**,⁽¹³⁾ found that supplementation with AHCC improved lung epithelial integrity following influenza infection. So we can suggested that AHCC might be increased the number of the membrane- coating granules which

increased the permeability barrier function of the gingiva.

In the present study, the keratohyaline granules were observed even by using the light microscopic examination of the stratum granulosum cells. Electron microscopic examination revealed that these granules were variable in size and the cytoplasm contained two types of keratohyaline granules. Keratohyaline granules were probably synthesized by ribosomes that can be seen surrounding them. Keratohyaline granules were also seen in association with tonofibrils. Similar results were obtained by **Adams**⁽⁶⁾ who found that the ultrastructural characteristics of the keratohyaline granules have complicated rather than simplified the picture in relation to their function. At least two types of granules exist. Keratohyaline granules were associated with ribosomes and tonofilaments. **Ten Cate**⁽⁸⁾ stated that the keratohyaline granules were thought to form the matrix in which filaments of the keratinized layer are embedded or aggregated.

In the present study, slight thickening of the surface corneum layer of the experimental group was detected. The stratum corneum cells were appeared flat with markedly electron-dense cytoplasm, and packed with tonofilaments. Increased in the degree of keratinization might be important in creating a more effective permeability barrier. Orthokeratinization constitutes a highly impermeable membrane. These findings were in agreement with those of **Elias & Friend**⁽³⁶⁾ they reported that the keratinization included modification of the cell membrane and the intercellular material. The cornified layer could be regarded as an efficient, well-integrated system of protection to the underlying tissues. Authors added that the orthokeratinized epithelia provide the best protection against mechanical injury. **Squier & Rooney**⁽³⁷⁾ suggested that a significant permeability barrier was present in the stratum granulosum of keratinized epithelia. **Larker**⁽³⁸⁾ found that the epithelial barrier coincided with the appearance of MCGs, which released their contents into the intercellular spaces. **Presland & Dale**⁽³⁹⁾ stated that each cornified cell had a cornified envelope composed of cross linked proteins and lipids that replaced the plasma membrane and formed a critical part of the epithelial barrier. **Christopher et al.**,⁽³⁵⁾ demonstrated that the major pathway of large molecules across the stratified epithelium was via the intercellular spaces and that there was a barrier to penetration as a result of modifications to the intercellular substance in the superficial layers. Moreover **Muller et al.**,⁽⁴⁰⁾ reported that the epithelia throughout the body function as a physical barrier against invading bacteria and also provide effective

innate immune defenses by producing antimicrobial peptides.

In the experimental animals of the present study the desmosomes and tonofilament bundles were markedly increased throughout the gingival epithelial layers. These findings are in accordance with other investigators **Einar et al.**,⁽⁴¹⁾ found that the number of desmosomes was being few in the basal layers and increased in the more superficial layers. The amount of filaments and the tendency for them to be arranged in bundles were a particularly characteristic common feature.

The observed increase in tonofilaments and keratohyalin granules implied that there was an increasing protein synthetic activity in stratified squamous epithelial cells. Keratinization of the superficial layers might be a kind of epithelial cell protection against irritation⁽⁴²⁾. Keratinocytes undergo a program of terminal differentiation, expressing a set of structural proteins, keratins, which assemble into filaments and function to maintain cell and tissue integrity. Authors added that there was two types of cell adhesion structures, desmosomes and hemidesmosomes, function to glue keratinocytes to one another and to the basement membrane, and connect the keratin cytoskeleton to the cell surface⁽⁴³⁾. AHCC administration reduced colonic inflammation, improved body weight, food intake and mucosal barrier defense⁽²³⁾.

In the present study numerous clear cells were observed in the basal and parabasal cell layers of the gingiva in the experimental group, this finding was in accordance with those of **Hutchens et al.**,⁽⁴⁴⁾ they found that the oral epithelial dendritic cells were present with the epithelium of gingiva, hard palate, tongue, cheek mucosa, and skin of the external lip; the presence of dendrites easily distinguished such cells from keratinocytes.

Murine studies suggest that buccal mucosa dendritic cells capture antigen and migrate to cervico-mandibular lymph nodes, where antigen is presented⁽⁴⁵⁾. Interleukin 1 β , secreted by dendritic cells, is the main cytokine responsible for mediating increased Human β -defensin 2 expression in gingival epithelial cells and stimulate keratinocyte proliferation⁽⁴⁶⁾. Cytokines play a prominent role in growth, differentiation, and host defenses⁽⁴⁷⁾. Cellular immune components of the oral mucosa are lymphoid follicles, aggregates and individual lymphocytes and dendritic cells. The most important being the oral mucosal Langerhans cells, macrophages and mast cells⁽⁴⁸⁾. Dendritic cell was making up only 0.4% of human skin and 0.1–2% of human gingiva⁽⁴⁹⁾. The ability of dendritic cells to stimulate immune responses was related to their activation and maturation status. Activated dendritic cells were a

significant sources of chemokines that recruit other immune cells, including T cells, natural killer cells and monocytes⁽⁵⁰⁾.

Yin et al.,⁽²⁶⁾ suggested that cytokines, chemokines and β -defensins were involved in interaction of gingival epithelial cells and dendritic cells. Differential and coordinated regulation between gingival epithelial cells and dendritic cells might be important in regulation of innate immune homeostasis and response to pathogens in the oral cavity.

AHCC increased the circulating levels of interferon gamma and interleukin β ⁽²⁷⁾. Moreover **Kenner**⁽¹¹⁾ reported that one important group of lymphocytes is the specialized group of cells called natural killer cells. Natural killer cells as well as macrophages can destroy tumor cells and microbes. Author added that AHCC had increased the activity of natural killer cells by 300 percent and even gradually increasing to as high as 800 percent over time. **Daddaoua et al.**,⁽²³⁾ found that AHCC increased T-cell proliferation, altered cytokine production and induced anti-inflammatory effects. Also **Terakawa et al.**,⁽²⁴⁾ suggested that AHCC enhanced natural killer cell activity and increased dendritic cell function.

In the present study the lamina propria showed an increase in collagen fibers bundles, fibroblasts, blood vessels and clusters of subepithelial lymphocytes, which may be due to the effect of AHCC in activation of immune system as reported by **Ishibashi et al.**,⁽⁵¹⁾ they stated that supplementation studies with AHCC have demonstrated positive effects on immune function and increased resistance to viral and bacterial infection in humans as well as animal models.

In summary: we suggest that supplementation with AHCC, a natural bioactive dietary supplement (mixture of polysaccharides, amino acids, lipids and minerals) may activate the proliferation and maturation of the keratinocytes and non-keratinocytes of the gingival tissue and enhanced the integrity and barrier function of the gingiva epithelial cells.

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