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**THE EFFECT OF A PLANT-DERIVED ANTIOXIDANT
ON THE CORRECTION OF GINGIVAL DYSTROPHIC-INFLAMMATORY CHANGES
IN PUBERTAL RATS**

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Impaired barrier properties of the gingival epithelium play a principal role in the pathogenesis of periodontal diseases in adolescents, especially secondary to diseases of the upper gastrointestinal tract. Microcirculatory disorders activate processes of free radical oxidation with subsequent structural disorganization of gingival tissues. The purpose of the study was to examine the effect of a plant-derived antioxidant on the correction of dystrophic-inflammatory changes in the gingival tissues in pubertal rats with simulated reflux gastritis by analyzing morphological alterations. The model of hyposecretory reflux gastritis was induced by intragastric administration of medical bile to 23 pubertal Wistar rats of both sexes once a day for 15 days. The animals received an herbal preparation with an antioxidant effect and placebo therapy for the next 15 days. Gingival specimens were processed for histological analysis and visualized using a microscopic method after animal sacrifice. Morphological alterations in the gingival tissues of rats with induced reflux gastritis represented phenomena characteristic of vascular and stromal protein dystrophies with an inflammatory component as a sign of dystrophic-inflammatory processes. The use of the plant-derived antioxidant stimulated processes of epithelial proliferation and promoted cell differentiation amid the recovery of microcirculation.

Key words: gingiva, hypoxia, free radical oxidation, dystrophy, reflux, gastritis, antioxidant therapy.

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**ВПЛИВ АНТИОКСИДАНТУ РОСЛИННОГО ПОХОДЖЕННЯ НА КОРЕКЦІЮ
ДИСТРОФІЧНО-ЗАПАЛЬНИХ ЗМІН ЯСЕН У ЩУРІВ ПУБЕРТАТНОГО ВІКУ**

У патогенезі захворювань пародонту у підлітків основну роль грає порушення бар'єрних властивостей епітелію ясни, особливо на тлі захворювань верхніх відділів шлунково-кишкового тракту. Порушення мікроциркуляції активують процеси вільнорадикального окиснення з подальшою дезорганізацією структури тканин ясни. Метою дослідження стало вивчення впливу антиоксиданту рослинного походження на корекцію дистрофічно-запальних змін тканин ясни у щурів пубертатного віку з відтвореним рефлюкс-гастритом шляхом дослідження морфологічних змін. Модель гіпосекреторного рефлюкс-гастриту відтворювалася за допомогою інтрагастрального введення медичної жовчі 23 щурам пубертатного віку обох статей лінії Вістар. Наступні 15 днів тварини отримували рослинний препарат з антиоксидантним ефектом та плацебо-терапію. Дослідження гістологічних препаратів ясни проводили мікроскопічним методом після виведення щурів з експерименту. Морфологічні зміни в тканинах ясни щурів із відтвореним рефлюкс-гастритом представляли явища, характерні для судинних і стромальних білкових дистрофій з запалювальним компонентом, що є ознакою дистрофічно-запальних процесів. Застосування антиоксиданту рослинного походження стимулювало процеси проліферації епітелію та сприяло диференціюванню клітин на фоні відновлення мікроциркуляторного русла.

Ключові слова: ясна, гіпоксія, вільнорадикальне окиснення, дистрофія, рефлюкс, гастрит, антиоксидантна терапія.

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Based on the epidemiological data analysis, the incidence of periodontal tissue diseases in adolescents varies from 40 to 100 % [7]. Periodontal diseases develop mostly in adolescents with concomitant pathology, most commonly with gastrointestinal disturbances [9]. Impaired barrier properties of the gingival epithelium are of primary importance to the pathogenesis of periodontal diseases [9]. The epithelial ability to protect against bacterial invasion depends on the intensity of cell proliferation and differentiation as well as adequate microcirculation [4, 5]. Violations of gingival microcirculation with subsequent hypoxia activate free radical oxidation processes [2, 6].

The process of free radical oxidation [13] is defined as a mechanism that compensates for a decreased level of oxygen in tissues, however, with a deficiency of antioxidant protective factors, the gingival tissue resistance decreases. Medications of natural origin (plant or animal), which do not accumulate in the body, without toxic or side effects, should be used to correct the antioxidant protection in adolescents. It is crucial to prescribe medicines with a complex effect in the case of concomitant somatic diseases in order to reduce a medication load on the adolescent body.

Antioxidants are divided into direct-acting and indirect-acting agents based on biological effects. Direct-acting medications (polyphenolic and thiol compounds, carboxylic acids, glycosides, and combined

drugs) neutralize free radicals in the process of autooxidation [1]. *Alnus incana*/*glutinosa* is a complex preparation made up of natural polyphenolic substances obtained from the catkins of grey alder and black alder. Flavonoids in the drug composition exhibit an antioxidant activity, prevent lipid peroxidation and activation of oxygen species. Quercetin, catechin, rutin, leucoanthocyan provide reparative properties of the drug through an activation of protein biosynthesis and increase in the microcirculatory area. This drug is used in the therapy of gastritis, however, given the common ontogenetic development of all parts of the digestive tract, including the oral cavity, its complex therapeutic effect on the gingival tissues should be studied.

The purpose of the study was to examine the effect of a plant-derived antioxidant on the correction of dystrophic-inflammatory changes in the gingival tissues in pubertal rats with simulated reflux gastritis by analyzing morphological alterations.

Materials and methods. The study was conducted in 2023. Experiments were done in the vivarium of Dnipro State Medical University (DSMU). Morphological examinations were carried out at the Histological Department of Dnipropetrovsk Regional Bureau of Forensic Medical Examination “DRC”.

The model of hyposecretory reflux gastritis [10] was induced to 23 Wistar rats of both sexes weighing 70–80 g by intragastric administration of medical bile at a dose of 10 ml/kg body weight once a day for 15 consecutive days. The control group consisted of 10 animals. The drug “Altan” (manufacturer PJSC SIC “Borshchahivskiy CPP”, Ukraine) and placebo therapy were given to the animals immediately following the gastritis model induction over the next 15 days. The dosage of the drugs was calculated based on the animal weight. The drugs were administered intragastrically at a single daily dose.

To analyze structural abnormalities in the gingival tissues in rats after the model induction and subsequent treatment, the following groups were formed:

Group 1 consisted of 8 animals injected with medical bile intragastrically. The rats were sacrificed immediately upon the disease model induction;

Group 2 included 7 animals subjected to an intragastric injection of sterile physiological solution after the disease model induction (placebo);

Group 3 was composed of 8 animals treated with an antioxidant (*alnus incana*/*glutinosa*) by intragastric injections.

The animals were housed at standard vivarium conditions and fed a normal diet. All experimental studies were carried out in accordance with the international recommendations for conducting biomedical research using animals, the requirements of the European Convention on the Protection of Laboratory Animals (Strasbourg), the standard regulation on ethics of the Ministry of Health of Ukraine No. 690 dated 23.09.2009.

Experimental study protocols and results were approved by the decision of the Bioethics Commission of the State Institution “DSMU of the Ministry of Health of Ukraine”. The animals were sacrificed at the end of experimental medical procedures under thiopental anesthesia (20 mg/kg of body weight intraperitoneally) via complete exsanguination by heart puncture.

For morphological evaluations, the gingiva was harvested from the jaws of animals and fixed in a 10 % neutral formalin solution. The gingival specimens were subjected to routine dehydration using an alcohol series with an increasing gradient and embedded in paraffin blocks of standard size. Tissue sections were stained with hematoxylin and eosin on glass slides. Histological specimens were evaluated microscopically (Axioskop 40 microscope (ZEISS)).

Results of the study and their discussion. Morphological examinations of the gingiva from the animals with reflux gastritis (Group 1) have revealed an uneven thickness of the gingival epithelium, the presence of dyskeratosis phenomena, incomplete keratinization. Dystrophic changes have been detected in the granular layer. In the epithelium, swelling of cells, pericellular edema, foci of hydropic degeneration and destruction of epithelial cells, as well as the presence of vesicles at the border of the horny layer have been visualized. Cells with phenomena of karyolysis, karyopyknosis and apoptosis have been observed in the spinous layer. Basal cell layer was characterized by uneven thickness, patchy mild dysplasia occurred. The basal layer cells were well-defined with cylindrical shape. Epithelial papillae were abnormally shaped due to irregular acanthosis and papillomatosis. Thickened and dense collagen fibers were clearly seen in the gingival lamina propria. Phenomena of fibrosis and microvascular congestion by collagen fibers have been found infrequently. An essential characteristic of the structural and functional disorganization of the gingival lamina propria was an overt fibrosis and sclerosis of the papillary and reticular layers. Collagen fibers have sometimes been assembled in extended massive fiber bundles oriented angularly related to each other. Foci of mild plasmorrhagia were visualized in the gingival lamina propria.

Focal basophilia indicated mucoid swelling of the connective tissue being a reversible stage of the tissue disorganization. This process is based on the accumulation of blood plasma products in the matrix resulted from increased tissue-vascular permeability (plasmorrhagia), destruction of connective tissue elements and accumulation of protein-polysaccharide complexes. Flat papillae were seen over a large area of the gingival lamina propria. Reduced height of the connective tissue papillae resulted in an impaired epithelial trophic and violated attachment to the gingival lamina propria. A few lymphocytes and single plasma cells were diffusely located in the gingival lamina propria. An increased number of mast cells indicated the beginning of an inflammatory reaction. Mast cells are known to secrete biologically active products – histamine and heparin, which affect the neutrophilic leukocyte chemotaxis. The identified changes have demonstrated vascular-stromal disorders with an inflammatory component, a testament to signs of chronic dystrophic-inflammatory periodontal diseases.

The placebo group (Group 2) has shown a decrease in the spinous cell layer thickness and manifestations of amitosis. But at the same time, active mitotic activity has been clearly detected in the basal and parabasal cell layers of the epithelium. This depiction was indicative of a hypoxia-induced incoordination of cell division in the gingival epithelium. Collagen fibers were thickened and dense in the lamina propria. Fibrotic alterations and foci of mild plasmorrhagia have been observed in the papillary and reticular layers (Fig. 1).

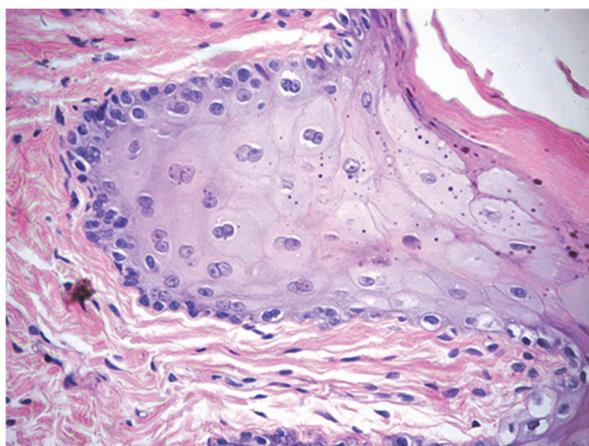


Fig. 1. Lamina propria of a Group 2 rat. Fibrosis. Staining with hematoxylin and eosin. 400 x magnification.

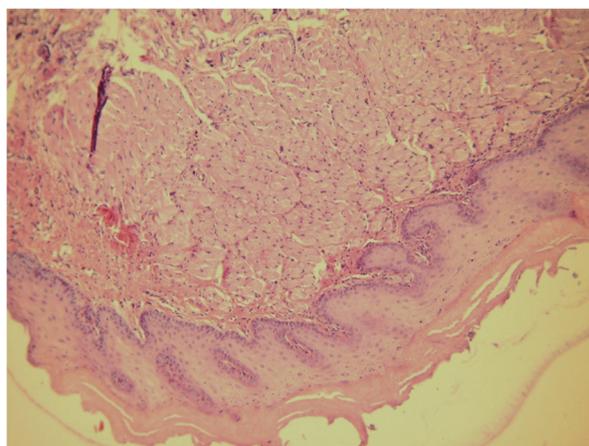


Fig. 2. Manifestations of hyper- and parakeratosis in the rat gingiva (Group 3). Staining with hematoxylin and eosin. 200 x magnification.

In the group of animals exposed to therapeutic effects of *alnus incana/glutinosa*, delayed epithelial cell exfoliation has been detected and, as a result, the horny layer was thickened. The granular cell layer was thin (Fig. 2).

The gingival epithelial basement membrane of the animals has been drawn irregularly with a moderate number of mitoses. Phenomena of acanthosis and papillomatosis have been seen. Mild diffuse lymphocytic infiltration has been found in the lamina propria. There were signs of minor edema in the gingival lamina propria, which was a sign of initial and reversible dystrophic changes (mucoid degeneration). Well-defined papillae have been identified in the gingival lamina propria. However, sclerosis of the papillary layer in the gingival lamina propria has testified to the insufficient nutritional support to the epithelium (Fig. 3).

But in combination with the phenomena of acanthosis and papillomatosis, this indicated the renewal of the vascular bed and, as a consequence, an increase in the epithelial trophism and regenerative processes. In this instance, acanthosis was uneven, with a significantly increased number of spinous cell rows, mainly between the papillae of the gingival lamina propria, being combined with papillomatosis.

After the antioxidant using, the epithelial thickness was increased mainly due to the spinous layer demonstrating proliferative processes in the epithelium due to active cell differentiation. Deformations of the epithelial papillae were caused by sclerosing within the papillary layer of the lamina propria close to the basement membrane (Fig. 4).

Oxidative stress plays a crucial role in the etiopathogenesis of all systemic diseases. The oral cavity is the only place in the human body that is exposed to many external and internal factors, such as food, bacteria [5], air, influences of stomach content components in digestive system diseases. Dystrophic-inflammatory gingival lesions are associated with oxidative-reductive imbalance [15]. Positive biological effects of reactive oxygen species in physiological concentrations are well known. However, reactive

oxygen species concentrations can increase significantly as a result of pathological changes, causing a state of oxidative stress. This phenomenon occurs when reactive oxygen species overproduction coexists with antioxidant system insufficiency [3]. Studies have shown that oxidative stress leads to the degradation of the extracellular matrix components in the periodontal tissue, including collagen, elastin, proteoglycans and glycosaminoglycans (for instance, hyaluronic acid). It leads to the destruction of periodontal attachment apparatus [11]. On the other hand, oxidative stress contributes to inflammatory reactions in the periodontium [13]. Saliva produced by the salivary glands has many functions and it is also a rich source of enzymatic and non-enzymatic antioxidants to support redox homeostasis in the oral cavity. Oxidative stress can be balanced or attenuated due to the action of endogenous antioxidant mechanisms (salivary protective components) as well as exogenous ones contained in food products and medicines. This offers an opportunity to develop preventive programs for oral cavity diseases. Pharmaceutical preparations containing flavonoids help to actively modulate the capillary bed, protect cells from damage by oxygen and prevent inflammation in various tissues and organs [12]. Our work has shown, that while there has certainly been signs of gingival tissue restoration under the influence of the plant-derived antioxidant, the regenerative process was not complete, since papilla deformities in the gingival lamina propria indicated partial restoration of the microvasculature [2]. The state of tissue hypoxia disrupts the histochematic barrier permeability giving rise to persistent plasmorrhagia and hemorrhagia, edema, dystrophy, and constantly occurring micronecrosis. On the other hand, chronic hypoxia stimulates plastic processes in the connective tissue [8].

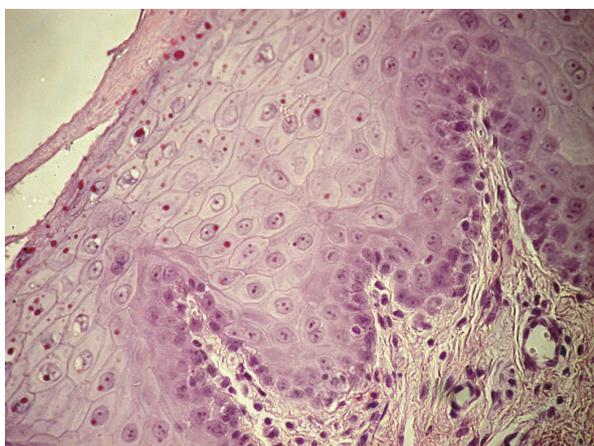


Fig. 3. Sclerotic alterations of the gingival lamina propria of a rat (Group 3). Staining with hematoxylin and eosin. 400 x magnification.

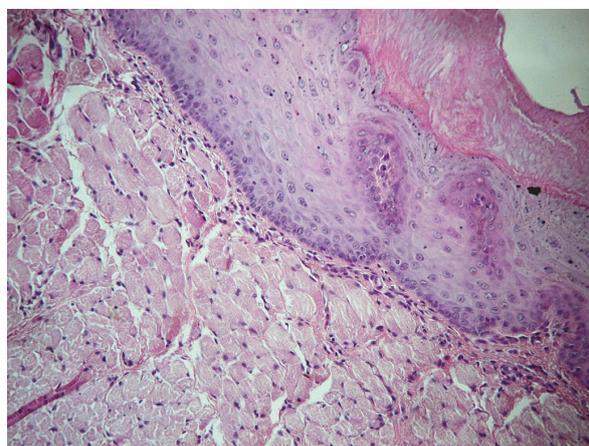


Fig. 4. Deformation of the gingival epithelial papillae. Staining with hematoxylin and eosin. 400 x magnification.

Regenerative processes were observed in the spinous layer located most closely to the vascular bed, but in the granular layer, farthest from the capillaries, dystrophic changes predominated due to a decrease in the biosynthetic activity of cells. The gingiva was affected by hyper- and parakeratosis suggesting metabolic process derangements and alterations in cellular structure due to impaired differentiation. It is therefore necessary to study combine effects of medicines aimed at increasing gingival blood flow as to provide the most complete recovery of its morphological structure after hypoxia.

The effect of arginine on regeneration processes in tissues is well-known. Endothelial cells can rapidly uptake extracellular L-arginine and oxidize it to nitric oxide. It has been demonstrated that taking arginine during tissue hypoxia stimulated reparative and regenerative processes by modulating angiogenesis [14].

The metabolism of L-arginine is catalyzed by arginase. High concentrations of this enzyme are found in regenerating tissues due to the expression in macrophages. Arginase stimulates the production of ornithine (a precursor for proline), which serves as a substrate for collagen synthesis. Therefore, arginine as a supplement may have multidirectional effects on tissue regeneration processes [14].

Thus, our following studies will focus on optimizing regenerative processes in experimental dystrophic-inflammatory gingival lesions.

Conclusions

1. Morphological alterations in the gingival tissues of rats with induced reflux gastritis have demonstrated vascular and stromal violations with the inflammatory component showing dystrophic-inflammatory processes in periodontal tissues.

2. In Group 2 (placebo), the incoordination in cell division and differentiation of the gingival epithelium was evidence of hypoxic-induced ineffectiveness of regenerative processes as there was no adequate treatment.

3. In Group 3, the use of antioxidant has enhanced the process of keratinization in the midst of destructive changes in the granular epithelial layer. At the same time, the drug has stimulated proliferative processes in the epithelium and promoted cell differentiation. The development of well-defined but deformed epithelial papillae has indicated the gradual recovery of transcapillary exchange and the improved trophic supply to the epithelium with treatment.

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