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Experimental study of treatment effectiveness of the natural drug – Naran S in inflammatory conditions of the oral cavity in animals

Mouth washes, applied at home after tooth brushing are considered to be the best carriers of the means counteracting building up of the bacterial deposit and aiding the treatment of inflammatory conditions of the mucous membrane of the oral cavity and periodontal tissues (4, 5, 6, 9, 12, 14). Most commonly, mouthwashes are the preparations of natural origin displaying anti-inflammatory, astringent and antiseptic action and they come as infusions, decoctions, ready-to-use tinctures or extracts. It was shown that the specially chosen herbal compositions, applied after ridding of the irritating agents may make the subsidence of inflammation faster and can ease the healing of the ill tissues (4, 5. 6,12, 14).

The new natural drug elaborated in the Chair and Department of Inorganic Chemistry of the Medical University of Lublin (patent license P. 332066) was the subject of the study. The constituents of this drug are the pharmacopoeic healing plants (chamomile flower, plantain leaf, euphrasy herb, oak bark, the flower of dead-nettle) containing mainly phenol acids, iridoids, flavonoids and tannins.

MATERIAL AND METHODS

The experiment that lasted 4 weeks was conducted on Wistar male rats from the inbred breed of the average body mass amounting to 200 g. The animals were divided into two control groups – I and III and two experimental groups – II and IV with 10 animals in each group. The animals were fed with standard fodder for rodents. All animals, both experimental and the control ones were subjected to injection performed intragingivally into the interdental papilla of the lower incisors. After that the oral cavities of the animals were rinsed two times during 24-hour period with the natural drug (two sachets of the mixture were inundated with 250 ml of water in the temperature of 80° C and left for cooling) or with physiological salt according to the following scheme:

Group I – injection of 0.025 ml of 0.9% NaCl, rinsing – 0.9% NaCl
Group II – intragingival injection of 0.025 ml of complete Freund (AF)(Calbiochem) – adjuvant for inducing the topical benign gingivitis, rinsing with 0.9% NaCl Opinion number 347/2002
Group III – injection of 0.025 ml of 0.9% NaCl, rinsing with Naran S preparation (20 ml)
Group IV – intragingival injection of 0.025 ml of complete Freund adjuvant, rinsing with 20 ml of Naran S

The study material was taken after 24 hours' time after injection and after 1, 2, 4 weeks following the experiment. Staining with hematoxyline and eosin as well as the one according to the Masson's method was performed, where the cytoplasm of the cells was stained pink with eosin and the nuclei were stained violet with hematoxylin.

RESULTS

Morphology of the mucous membrane of the oral cavity of the experimental animals with induced inflammation of the mucous membrane with Freund adjuvant and later treated with water infusion of Naran S was studied.

The observed epithelium and the mucous membrane of the oral cavity, control group I, displayed normal image of the histological structure. The high epithelium was situated on extremely folded basilar membrane and the collagen fibers of the mucous membrane were regularly placed among fibroblast cells.

In group II preparations, typical features of inflammation were observed. The epithelium of the mucous membrane was low and was characterized by blurred areas of cellular layers, the mucous membrane contained great quantities of the amorphous fluid which led to a disorderly arrangement of collagen fibers and tissue cells. Blood vessels were dilated and filled with blood and many cells of inflammatory infiltration were observed (Fig.1).

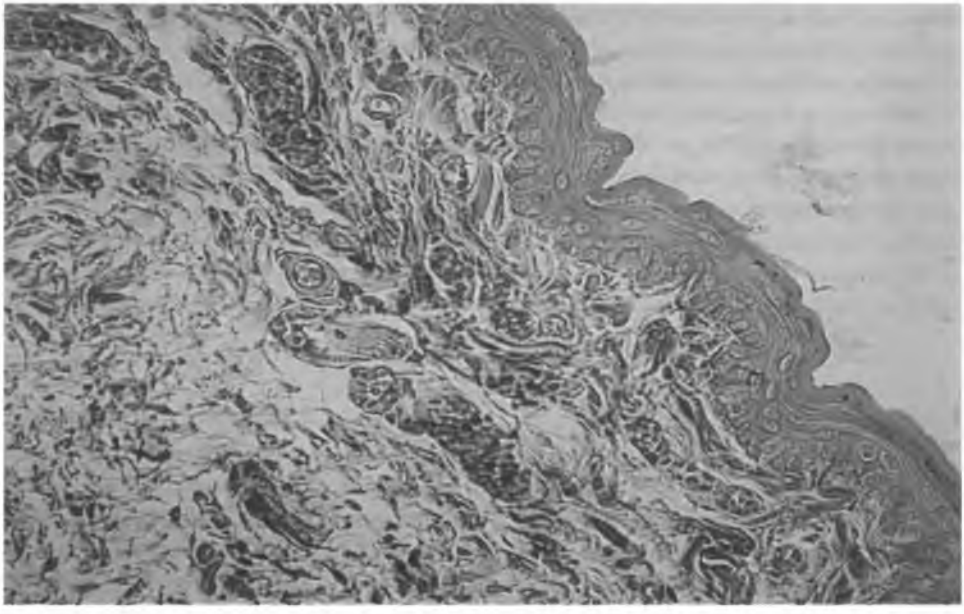

Fig. 1. Inflammatory infiltration of the mucous membrane after application of Freund adjuvant. Staining according to the Masson's method. Magn. 100x

In group III, the mucous membrane of the oral cavity displayed correct histological structure; there was only a small amount of dilated blood vessels filled with blood. Surface layers of the epithelium displayed the presence of intensely decolorized grains of the stain from the drug.

The results in the studied group IV were similar to those in group II in the initial stage of the experiment and were characterized by the inflammation, but in the final stage, the preparations displayed normal histological structure. The changes affected the high epithelium in which one could observe numerous inflow, one-nucleus cells in the parabasilar region of the epithelium and many granular bodies intensely discoloured in the surface epithelial layers (Fig. 2).

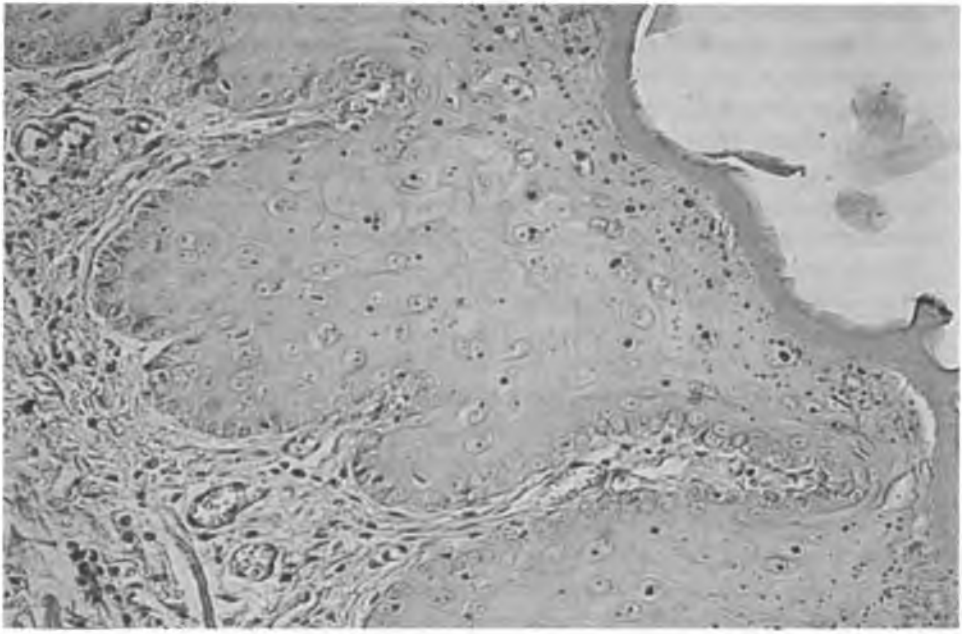

Fig. 2. The epithelium of the mucous membrane after the treatment. Visible granular character of the dye from the drug in the granular layer of the epithelium. Hematoxylin and eosin staining. Magn. 200x

DISCUSSION

Iridoids are characterized by a wide spectrum of biological activity. Antibacterial and antifungal activity (1, 18) has for long been ascribed to aukubine and its derivatives, but the proper acting substance is aglicon- aukubigenine – the product of aukubine decomposition under the influence of â-glycosidase.

Citing M i ł k o w s k a - L e y c k et al., E l i c h et al. in 1962, while conducting their studies with the use of *Staphylococcus aureus* strain, confirmed that 1 ml of 2% water solution of aukubine in the presence of β-glycosidase gives the same effect as 600 I.U. of penicillin. In 1986, D a v i n i et al. obtained aglicon- aukubigenine – aukubigenine in the crystalloid form and they proved that it acts strongly against *Staphylococcus aureus*, a little weaker against *Bacillus subtilis* and *Bacillus cereus*. The most probable mechanism of its action is blocking of the enzymatic systems of the bacterial cell by originating product of aukubine decomposition of a structure similar to iridodial one (13). Anti--inflammatory action is exhibited by aukubine (1, 16), harpagozyde (10, 11) and gencyanine – the alkaloid from *Gentianaceae* family (10).

Flavonoids exhibit, among others, the sealing and tightening effect on capillary walls, which is connected with the inhibition of hialuronidase enzyme activity; by stopping histamine excretion, they act anti-inflammatorily and antiallergically, for example apigenine, luteoline, quercetine.

According to the latest research, anti-inflammatory and anti-aggregation properties are connected with the ability of inhibiting the biosynthesis of the factors causing inflammation and blood plates aggregation. Some of these factors are prostaglandines, super-oxide anions, leukotriens, nitrogen oxide, and isoprostanoids. Apigenine influences the metabolism of steroids by the inhibition of estrogen synthesis.

Phenol acids have not been very well known pharmacologically. Regarding the fact that they appear in the majority of the raw materials as the compounds accompanying main active components, their synergic effect must be taken into account. Coffeic acid displays antiseptic, anti-inflammatory, hepatoprotective and immunotropic properties; it is oxidized in the organism creating ferulic acid, isoferulic, cycolignan, eskuletine, which are all biologically active substances. Chlorogenic acid, however, the depside of coffeic acid with quinine acid displays immuno-stimulating properties, the ability of changing lipid concentration, free oxygen radicals, it also inhibits histamine release; rosemary acid presents anti-inflammatory, anti-viral and anti-oxidative properties (2).

Plantain leaves, regarding high content of flavonoids, saponins, steroids, and alkaloids possess anti-inflammatory activity in relation to the mucous membrane of the oral and pharyngeal cavity. They diminish congestion of mucous membranes and the excessive permeability of the capillaries. This action has been tested on rats with different kinds of inflammations (15). Plantain juice or the decoction from its dried leaves eases wound healing, skin regeneration, heals irritations and acts astringently (3, 10). *Plantago Lanceolata* (*Plantaginaceae*) displays anti-microbial action, which is ascribed to aukubine present in the leaves, but the proper acting substance is anglicon-aukubigenine.

Camonile capitulum (*Anthodium Chamomillae*) is predominantly an oily raw material, however, it has to be stressed that in its action other compounds also play an important role as e.g. flavonoids. Volatile oil occurs in it in the quantity of 0.5–1.5% (FP IV requires 0.3%), and is a dark blue, oily liquid. But the most important group in chamomile are sesquiterpenes – (-)-α bisabolol- 0.3–77.2% and its oxides A and B, farnezane occurring in the amount of 0.1–42% and chamazulene in the concentration of 0.6–49.9% (7, 8, 10, 17). Regarding high content of proazulens, this raw material belongs to the group of azulene materials.

Agreeing with Schilcher (18) one should stress that the most important role in anti-inflammatory action of chamomile of inhibiting histamine secretion from tissue cells, among others the so-called fattening cells is played by the lipophilic components such as chamazulen and (-)-α bisabolol and hydrophilic flavonoids and most importantly – apigenine. Strong antimicrobial action is displayed by chamazulen and (-)-α bisabolol which act against Gram-positive and Gram-negative bacteria as well as against human pathogenic fungi. For example, α bisabolol inhibits the development of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Corynebacterium diphteriae* and *Bacillus subtilis* in the range of 32-64 $\mu g/cm^3$, and the growth of *Enterobacter aerogenes* was inhibited in the concentration of 64 $\mu g/cm^3$.

Water extracts from euphrasy also act anti-inflammatorily because the aukubine inhibits the release of histamine and has anti-allergic effect. Tannins present in euphrasy, oak bark and polyphenol acids act anti-inflammatorily and astringently. Tannins also bind with the proteins of the microbial cells, effecting in this way bactericidal action or they inhibit pathogen microorganisms in the alimentary canal and on the skin (antisepticum). Apart from that, tannins also deactivate bacterial toxins created by different disease causing microorganisms. Worth stressing is the influence of piro-catechine tannins and catechine itself on the capillary walls of the alimentary canal and oral cavity by decreasing their permeability, weakening of plasma permeability out of vascular bed and by inhibiting micro bleedings from damaged capillaries and minute vessels (antihaemorrhagicum) (19).

CONCLUSIONS

Basing on the results obtained so far we could say that the thick epithelium, correct arrangement of collagen fibers and the lack of tissue fluid in the mucous membrane proper, the correct shape of the cells of different epithelial layers from

the last stage of treatment in group IV and the absence of these features in group II may testify to a wanted medical action of water infusion of Naran S.

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SUMMARY

The aim of the study was the evaluation of the action of a new natural drug – Naran S, elaborated in the Department of Inorganic Chemistry of the Medical University of Lublin (patent registration P.332066) in the treatment of inflammatory conditions of the oral cavity mucous membrane. The experiment was conducted on male Wistar rats. Topical inflammation of the gum was induced by injecting a complete Freund adjuvant (AF)(Calbiochem) into the inter-dental papilla of the lower incisors. The material was taken, fixed and stained with hematoxylin and eosin and by the use of the Masson's method after 24 hours, 1, 2, and 4 weeks following the injection time. Morphology of the mucous membrane was examined and it was noticed that in the group treated with Naran S infusion the complete rebuilding of the epithelial strata, collagen fibres took place. The shape of the cells was regular and there occurred the elimination of the tissue fluid in the proper mucous membrane. The results obtained in the study allow to claim that the topically given plant drug thanks to its contents of biologically active substance such as: iridoids, flavonoids, phenol acids, tannins, displays curative activity in the therapy of the inflammatory conditions of the oral cavity mucous membrane.

Doświadczalne badanie skuteczności leczenia stanów zapalnych jamy ustnej zwierząt lekiem naturalnym – Naranem S

Celem pracy była ocena działania nowego leku naturalnego Naranu S, opracowanego w Katedrze i Zakładzie Chemii Nieorganicznej AM w Lublinie (zgłoszenie patentowe nr P.332066), w leczeniu stanów zapalnych błony śluzowej jamy ustnej. Doświadczenie przeprowadzono na szczurach–samcach rasy Wistar. Miejscowy stan zapalny dziąseł wywołano poprzez iniekcję kompletnego adjuwantu Freunda (AF) (Calbiochem) w brodawkę międzyzębową siekaczy dolnych. Materiał pobrano, utrwalono i barwiono hematoksyliną i eozyną oraz metodą Massona po 24 godzinach od wykonania iniekcji oraz po 1, 2 i 4 tygodniach. Badano morfologię błony śluzowej jamy ustnej zwierząt i zauważono, że w grupie leczonej naparem Naranu S doszło do szybkiej odbudowy poszczególnych warstw nabłonka, układ włókien kolagenowych i kształt komórek stał się prawidłowy oraz nastąpiła eliminacja płynu tkankowego w błonie śluzowej właściwej. Osiągnięte wyniki pozwalają stwierdzić, że zastosowany miejscowo lek roślinny, dzięki zawartości związków biologicznie czynnych, m.in. irydoidów, flawonoidów, kwasów fenolowych, garbników, wykazuje działanie lecznicze w terapii zapaleń błony śluzowej jamy ustnej.