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held on April 29th, 2022 by

NGO European Scientific Platform (Vinnytsia, Ukraine)
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USE OF HUMIC SUBSTANCES IN CASES OF TRAMATIC DAMAGE OF PERIODONTS IN LABORATORY ANIMALS

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The object of the study were 30 sexually mature eight-month-old female rats, whose average body weight was 0.4 kg. Further calculation of the administration of feed and drugs was performed based on the body weight of the animals.

Feeding was performed 2 times a day with an interval of 12 hours. The main diet was a mixture of wheat and oatmeal; cabbage and apples were added 3 times a week. The water consumption was not limited.

The rats were divided into 3 groups of 10 animals in each group.

The inflammatory process in the periodontium was mechanically caused in the first and second groups of animals. Under the sedative effect of Meditin and local anesthesia with Septanest 1: 100000, trauma to the mucosa of oral floor and gum margin of the central incisors of the lower jaw was caused with polyamide thread, which sewed the mucosa and was wound around the central incisors with following fixation of the thread, Figure 1.

The rats developed an inflammatory process after 5 days. The polyamide thread was removed on the sixth day.

The animals of the first group were kept on a standard diet of feeding and drinking. The second group of animals was transferred to the standard feeding regime, but at the same time they received water with the addition of humic substances. The third group of animals - the control group did not undergo surgical interventions, was on the usual diet of feeding and drinking.

After 14 days, the rats of the studied groups were withdrawn from the experiment. The animals were removed from the experiment under anesthesia using sodium thiopental. After the administration of anesthesia, blood was taken for a blood count. Blood sampling was performed from the heart according to the standard method.



Fig. 1. **Traumatization of periodontal tissues of rats of the studied groups**

Subsequently, the blood was sent to a veterinary laboratory for a blood count.

After decapitation and isolation of the lower jaw with underlying tissues, the test material was fixed in 10% formalin and transferred to the histological laboratory for morphological study.

Histological study was performed on biopsy samples of wound edges, the nature of which largely determines the activity of healing. For morphological studies 10% neutral formalin was used that provides a relatively good fixation of pieces of tissue and individual cells [Меркулов Г.А. Курс патогистологической техники. - Ленинград: Медицина, 1969. - 422 с.]. The main stages of the study: fixation of biopsy samples (tissue fixation time — 1-5 days); dehydration (dehydration of objects) and pouring in paraffin; preparation of sections with thickness of 6 - 8 microns; staining of samples with hematoxylin and eosin, as well as picrofuxin by Van Gizon and Mallory-Slinchenko to study the fibrous structures of connective tissue, as these techniques give a sufficient picture of dystrophic and regenerative processes, allowing to assess the effectiveness of treatment.

The sections were studied and photographed under a Zeiss "PrimoStar" microscope with a DCM 500 camera.

The following indicators were studied on the biopsy samples: the relative volumes of granulation tissue and necrotic masses; the number of vessels of granulation tissue in the field of view of the microscope at a magnification of x 400 was counted; the characteristics of the shape, size and degree of heterochromia of endothelial cells. The quality of blood supply to granulation tissue was assessed by the level of morphofunctional activity of fibroblasts through determination of the average area of fibroblast nuclei. The morphometry was performed on microphotographs using Image-ProPlus computer program calibrated on the morphometric line ОМОУ 4.2 ГОСТ 7513-75, the division price of 0.01 mm, ЛОМО.

In addition, to clarify the mechanism of action of the analyzed treatments on the reparative processes, the method of immunohistochemical determination of CD34 + in wound biopsies using monoclonal antibodies to CD34 (MCA CD34) was

used, to study the expression of this antigen in proliferating vessels of granulation tissue (Патент на корисну модель №39445 – Спосіб діагностики загоєння трофічної виразки нижньої кінцівки, опубл. 25.02.2009р. - Г. Н. Герасимов; Г. И. Губина-Вакулик).

The results of histological study revealed that rats of the first group, who did not receive humic substances, showed swelling of submucosal layer with areas of growth of granulation tissue and the presence of thin-walled capillaries. The inflammatory infiltrate was represented by lymphocytes, histiocytes, segmental leukocytes, an additive of eosinophils. Figure 2

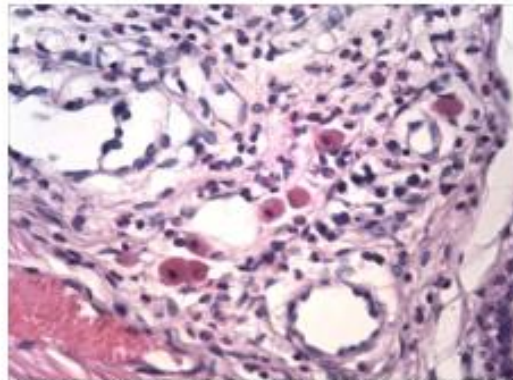


Fig. 2. **Histological slide of the mucous membrane of animals of the first group**

The histological slides of animals of the second group, which received water with humic substances, showed uneven thickening of the submucosal layer due to fibrous tissue with moderate lymphohistiocytic infiltration. Figure 3.

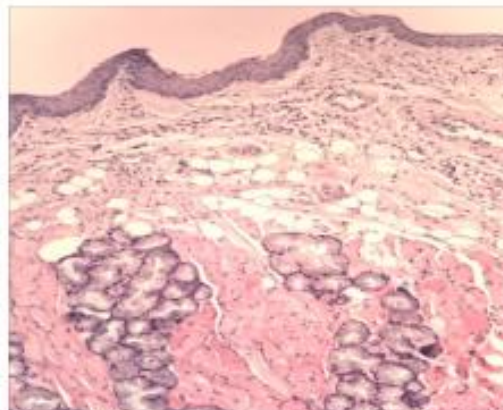


Fig. 3. **Histological slide of the mucous membrane of animals of the second group**

Histological slides of the third group - the control group showed focal hyperplasia of the non keratinized stratified squamous epithelium, and a slight thickening of the submucosal layer due to fibrinous tissue. Figure 4.

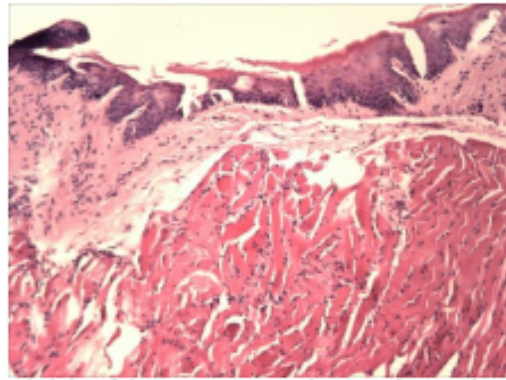


Fig. 4. Histological slide of the mucous membrane of animals of the third group

The results of a blood count revealed that rats, who did not receive humic substances, had leukocytosis with an increase of the index by 2 units, an increase in banded neutrophils was 2 times higher than normal, as well as an increase of ESR by 2.

The animals of the second group (who received humic substances) showed a decrease in the number of leukocytes per 1 unit relative to the group, who did not receive humic substances, also increased ESR and segmental neutrophils increased by 1.5 times higher than normal.

The rats of control group showed no special abnormalities of the blood count. Figure 5

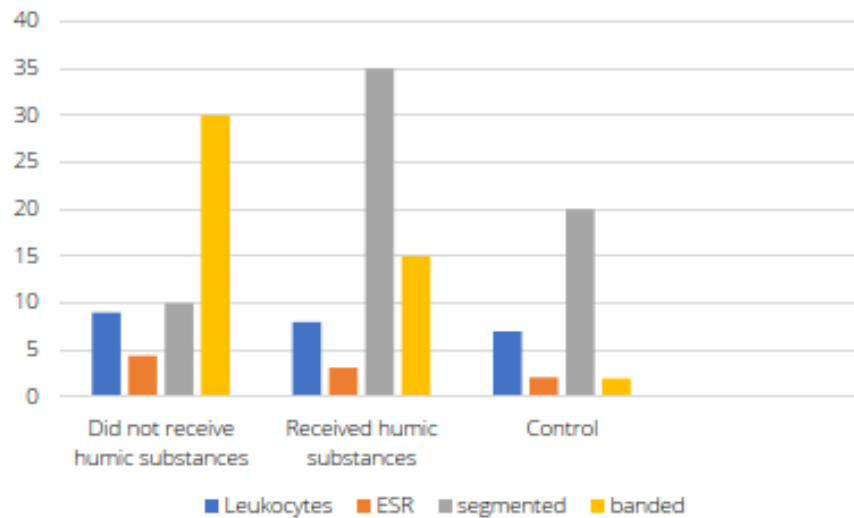


Fig. 5 The result of the indicators of the blood count in the studied groups

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