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Anti-ulcer effect of megosin in a model of acute experimental colitis in RATS

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***Abstract*---**Megosin is the original derivative of gossypol. Previously, the drug was obtained by condensation of gossypol with β-aminoethylsulfate sodium and used as an immunosuppressive agent, which was a low-toxic substance and contributed to a longer engraftment of skin flaps in mice, kidneys and lungs in dogs. Further, it was used as an antiherpetic agent, in the form of a 3% ointment. At present, the Institute of Bioorganic Chemistry, on the basis of existing dosage forms, has developed megosin enteric capsules for the treatment of ulcerative colitis and conducted histological studies, which proved the ulcer-healing effect of the new dosage form of megosin.

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Keywords---ulcerative colitis, antiulcer activity, necrosis, tissue, micropreparation, enteric capsules.

Introduction

All over the world, various biologically active substances of plant origin are widely used in traditional medicine, pharmaceuticals and in various sectors of the national economy. One such substance is gossypol, which is secreted from the bark, stems, and seeds of the cotton plant. Its derivatives have antiproliferative, anti-inflammatory and antiulcer activity. Megosyn is one such gossypol derivative that has been developed in several dosage forms for the treatment of a number of pathologies. In this regard, the purpose of this study was to study the specific pharmacological (antiulcer) activity of megosin in the form of enteric capsules at a dose of 40 mg/kg in the therapeutic and prophylactic route of administration in a model of experimental ulcerative colitis in rats.

Methodology

Tested drug: Megosin enteric capsules at a dose of 40 mg/kg, developed in the laboratory of original substances and dosage forms of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. Reference drug: Sulfasalazine enteric tablets 500 mg, manufacturer KRKA d.d. Slovenia. (Clinical and pharmacological group: NSAIDs - Salicylic acid derivatives. Antiulcer drug).

The studies were carried out on white outbred rats weighing 180–210 g in accordance with the bioethics requirements of the National Pharmaceutical University, which are consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. After the completion of the experiment, rats were withdrawn from the experiment in accordance with the ethical principles of animal experiments [6].

Experimental colitis with ulcerative lesions of the colon mucosa was induced with acetic acid according to the modified Osikov M.V. with co-authors of the method [4]. Experimental animals (rats) were kept hungry with free access to water for 48 hours. Then the rats were anesthetized by intraperitoneal administration of sodium etaminal at a dose of 30 mg/kg. The pathology was reproduced by introducing 1.0 ml of 4% acetic acid into the large intestine of anesthetized rats to a depth of 8 cm. Experimental animals after modeling colitis for 9 days were orally administered the studied drugs in appropriate doses. Animals were divided into 4 groups: group 1 - intact animals, group 2 - animals with model pathology (control group), group 3 - animals treated orally with enteric capsules of Megosin at a dose of 40 mg/kg, the 4th group, who received the reference drug Sulfasalazine orally. On the 3rd, 6th and 9th days of treatment, the animals were taken out of the experiment under anesthesia. To ascertain microscopic changes, pathological material was selected for histological examination (colon), which was fixed in 10% aqueous neutral formalin, dehydrated in alcohols of increasing strength, embedded in paraffin blocks, histological sections were made on an MS-

2 sledge microtome, 4- 6 μm and stained with Ehrlich's hematoxylin and eosin [2].

Micropreparations were visualized using a ZEISS Primo Star trinocular microscope, SONY 1606 camera, 1/1.8, 64MP at 4x10, 10x10, 40x10 magnifications.

The results of histological examination. Ulcerative colitis in the large intestine in rats in an experiment to compare specific pathomorphological changes resulting from the use of the dosage form of the drug Megosin (intestinal capsules at a dose of 40 mg/kg) and the reference drug Sulfasalazine was caused using a special probe. Based on the results obtained in the course of dynamic observations after 3- and 9-day studies of the effect of the dosage form of the drug Megosin on the regenerative and antiulcer effect on the colon mucosa, morphological changes were revealed.

Discussion

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon mucosa of unknown etiology with the development of ulcerative necrotic changes in it, localized mainly in its distal parts, initially affecting the rectum with subsequent spread in the proximal direction and in 10% of cases involving the entire colon intestine (rarely - with reflux leotiflitis); proceeds with various local and systemic complications and extraintestinal manifestations [5]. The most optimal approach for modeling UC is the rectal administration of 4% acetic acid. This model implies the induction of UC by rectal administration to a depth of 8 cm with 1 ml of 4% acetic acid. The use of acetic acid in UC modeling is one of the most widely used modeling methods. As a result of the introduction of acetic acid into the intestinal cavity, clinical signs are observed: bloody diarrhea, weight loss; morphological features: an intense inflammatory response characterized by massive bleeding, focal ulceration, thinning of the intestinal wall, a decrease in the number of crypts, and neutrophilic infiltration [7]. Rectal administration of acetic acid for modeling UC is also possible in a 10% concentration, but in a smaller volume - 0.5 ml. As a result, there are clinical signs of intoxication, diarrhea with an admixture of mucus and blood, a decrease in body weight of animals; morphological features: pronounced hyperemia and edema of the mucous membrane, intense hemorrhages, numerous areas of necrosis [3].

Morphological structure of rats colon of the 1st group, intact animals. Rats are warm-blooded animals belonging to the class of mammals, the family of rodents, their gastrointestinal tract belongs to the labile group by the property of splitting the cells of the mucous surface. Labile cells are cells that are characterized by the rapid restoration of lost cellular components, and also rapidly multiply by mitosis. These cells quickly respond to the impact of exogenous or endogenous factors. In rats, damaged or apathetic cellular components and damaged mucosal lamellae are capable of rapid recovery, on average, from 1 hour 40 minutes to 2 hours 20 minutes [1].

The digestive tract in rats consists mainly of 4 layers of mucous membrane: mucosubcutaneous, muscular and serous covering. Depending on the functional

nature of the gastrointestinal tract is characterized by different levels of development of the mucous membrane and muscle layers. Let us briefly dwell on the normal histological structure of the large intestine.

As can be seen in Figure 1, the texture of the mucosa in intact rats has the same appearance, the mucosa in the form of a fold also has a smooth surface. The surface of the mucous membrane consists mainly of prismatic epithelium, mainly involved in the absorption of trace elements, water and vitamins. 80% of the structure of the glands are goblet cells. These cells are involved in the production of mucin-2, as well as in the evacuation of used waste products. Rare endocrine glands located near the basal layer produce biologically active substances such as serotonin, melatonin, etc. One of the properties in the specific structure of the large intestine is the absence of villi in the lymphatic vessels, and is also characterized by the fact that numerous lymphocytes are widely distributed precisely in the private plates of the mucous membrane.

Obtaining acute ulcers on the mucous membrane of the large intestine by experiment in rats of the control, 2- and 3-groups. When modeling UC with acetic acid, clinical signs are observed in the intestinal cavity: bloody diarrhea, weight loss; morphological features: an intense inflammatory response, characterized by massive bleeding, focal ulceration, thinning of the intestinal wall, a decrease in the number of crypts, and neutrophilic infiltration. In animals of the control pathology, multiple hemorrhages, edema, severe hyperemia, and impaired folding of the colon mucosa were observed. In animals of the control pathology, the processes of regeneration occurred slowly, as evidenced by the remaining signs of inflammation: hemorrhages, swelling, hyperemia (Fig. 4).

Morphological changes revealed on the 3rd day after the use of the pharmaceutical preparation megosin. The study was aimed at enhancing the regenerative action of the pharmaceutical drugs Megosin and Sulfasalazine on the mucous membrane of labile cells during pathological processes that occur in the gastrointestinal tract due to erosive (superficial destruction of mucous membrane cells) and necrotic-wound defects (sharp damage to the mucous membrane and subcutaneous tissue fiber and deep defects in muscle tissue).

In the 3rd group of the experiment, the effect of megosin on the epithelial cells of the damaged single-layer prismatic epithelium and the structure of the glands on the mucous membrane of the large intestine of rats was studied. Experimentally, changes were found in the mucous membranes of the large intestine of rats, which were studied during dynamic observations after the introduction of megosin experimentally on induced ulcers.

In particular, the effect of megosin, which was applied in the case of the so-called anti-ulcer disease, showed the same changes as after 3 days. The regenerative activity of the mucosal prismatic epithelium is determined initially by the fact that up to 25% of the group of damaged cells is restored in a 40x10 field of view (Fig. 5).

It was found that the repair regeneration of cellular structures that had undergone progressive dystrophic and necrotic changes in the goblet cells of the

mucosal villi increased by 25-45% on a 40x10 surface when visually examined (Fig. 5).

It was determined that the infiltration of lymphocytes is formed in the apical part of the villi of the mucous membrane and the infiltration of lymphocytes is relatively preserved on surfaces close to the basal layer. At the same time, secondary changes were found in ulcerative processes, that is, masses of detritus appeared between the villi in the form of a cavity.

In conclusion, it should be noted that on the third day after the experimentally induced ulcer, the damaged components of the mucous membrane (prismatic epithelium, connective cells, etc.) on the general background indicate an increase in their regenerative activity under the influence of megosin by 25%. In particular, it was found that the regenerative activity of the prismatic epithelium and the resulting necrobiosis of the goblet cells of the glandular structures are restored by 25-35%. These indicators indicate a rapid reparative regeneration of the wound process.

Morphological changes revealed on the 9th day after the use of the pharmaceutical drug megosin. With the help of the experiment, an acute ulcer was induced on the mucous surface of the colon, with the dynamics of observation on the 9th day, morphological changes were revealed after the use of the pharmaceutical drug megosin. Dark basophilic staining of the single-layer prismatic epithelium on the mucosal surface indicates high reparative regeneration. In the 40x10 field of view, it is visually observed that the coverage of the prismatic epithelium has increased by 65-85%, and at the same time, the increase in goblet cells, one of the main structures of the villus glands, shows that they are relatively closer to the norm in number and structure (Fig. eight).

Restoration of repair regeneration of 5/4 of the prismatic epithelium on the surface of the mucous membrane, a sharp decrease in the edema processes occurring between the mucosal cells and suckers, is shown in Figure 8. Hyperplasia of the structure located under the mucous membrane has sharply decreased. There was a decrease in edema processes in the extracellular matrix.

The proliferation of fibroblasts in the suction components is determined, which undergo necrosis and are lost as a result of reparative regeneration of the prismatic epithelial coating on the surfaces of the defect.

It was found that the infiltration of lymphocytes formed between the glands decreased in the same way as in the tissue of capillary cells, there was a sharp decrease in dystrophic changes, and homogenization of the cytoplasm occurred. The fact that in some germinal goblet cells the mechanism of focal apoptosis is activated, the appearance of granules in the cytoplasm and nucleus is restored, is shown in Figure 8.

A relative decrease in intraepithelial lymphocytes in the villi and a decrease in the number of lymphocytes near the basal layer indicates the cessation of the inflammatory process and a relative decrease in the amount of edema in the

tissue when Sulfasalazine is used. It has been ascertained that the completeness of the inflammatory process in the serous and muscle layers decreases (Fig. 9).

In the group of animals that were injected with the reference drug Sulfasalazine, positive changes were also observed in the condition of the colon mucosa in comparison with pathology, but the regeneration processes were less pronounced than in animals that were treated with megosin at a dose of 40 mg/kg. Thus, megosin in the form of enteric capsules at a dose of 40 mg/kg has anti-inflammatory and antiulcer effects on the model of experimental ulcerative colitis, which is confirmed by indicators of the condition of the colon mucosa.

A comparative histological analysis of the pharmacological preparations of megosin at a dose of 40 mg/kg and Sulfasalazine revealed the following changes: under the influence of the drug megosin at a dose of 40 mg/kg, a sharp decrease in interstitial edema was revealed, in which the activity of reparative regeneration, necrotic and inflammatory infiltration was stopped in such labile mucosal cells as in the prismatic epithelium, connective cells, enterocytes and components of mesenchymal cells. The drug megosin at a dose of 40 mg/kg showed the appropriate therapeutic effect, i.e., it has a high activity of anti-inflammatory and antiulcer action.

Conclusion

1. Megosin in the form of enteric capsules at a dose of 40 mg/kg has a pronounced anti-inflammatory and antiulcer effect on the model of acute experimental colitis in rats, which manifests itself in a decrease in the area of ulcers and the degree of damage to the colon mucosa.

2. The pronounced therapeutic and prophylactic effect of megosin in the form of enteric capsules at a dose of 40 mg/kg on the model of ulcerative lesions of the large intestine allows us to recommend the studied drug for use in the complex therapy of inflammatory diseases of the large intestine.

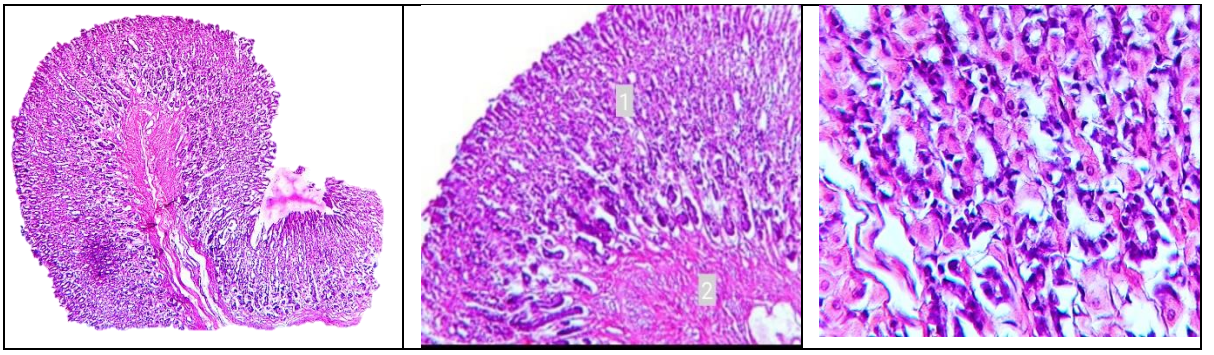

Fig. 1. Histoarchitectonics of the colon mucosa. Hematoxylin -Eosin. 4x10.	**Fig. 2.** Colon mucosa: goblet cells (1). basal surface and around stem cells (2) Hemotoxylin-eosin. 10x10.	**Fig.3.** Mucosal goblet cells, Lymphocytes located intraepithelial. Hematoxylin-eosin. 40x10.
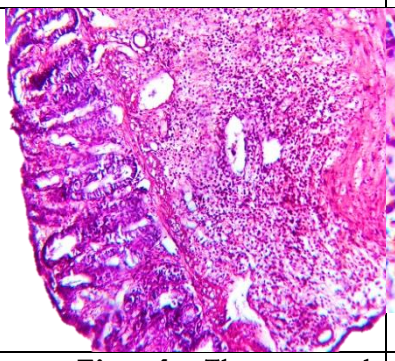	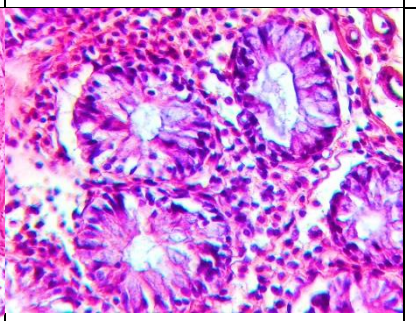	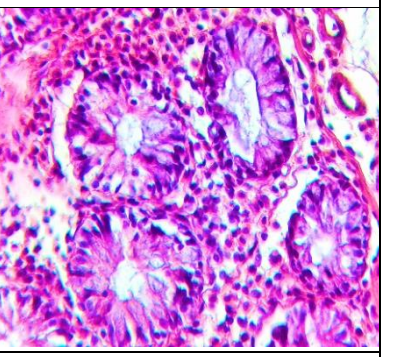
Fig. 4. The general background of the mucous surface of the large intestine on the 3rd day of the control group. Pink foci of necrosis. Hemotoxylin-eosin. 10x10.	**Fig. 5.** Influence of megosin on the ulcer in the mucous surface of the large intestine on the 3rd day. The foci of lymphocytic infiltration are visible. Hemotoxylin-eosin. 40x10.	**Fig. 6.** Application of Sulfasalazine for 3 days. Lesions in the cells of the gland on the 3rd day are relatively rare. Hemotoxylin-eosin. 40x10.
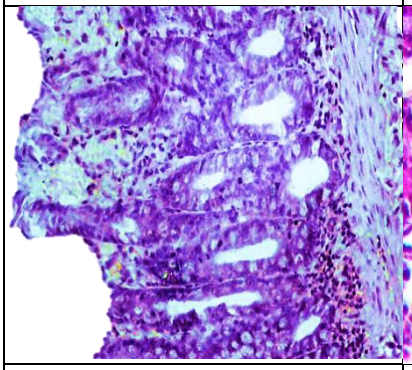	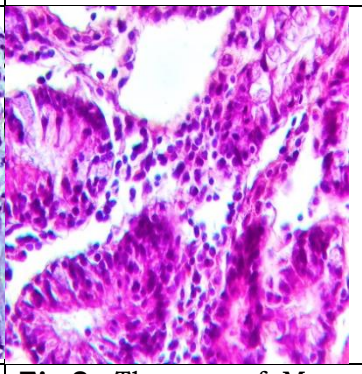	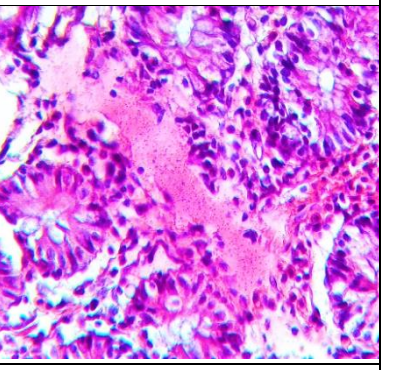
Fig.7. The general background of the mucous surface of the large intestine on the 9th day of the control group. Hematoxylin-eosin. 40x10.	**Fig.8.** The use of Megosin for 9 days goblet cells acquired a uniform appearance. The infiltration of lymphocytes is reduced. Hematoxlin-eosin. 40x10.	**Fig.9.** Application of Sulfasalazine for 9 days. The appearance of rare fibrous structures, proliferation of fibroblasts, the amount of lymphocytic infiltration around the necrosis is reduced. Hematoxylin-eosin. 40x10.

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