

The Research Of The Application Possibility Of The Composition Based On Lipid-Transporting Protein (Ns-Ltp1) From The Seeds Of Nigella Sativa In The Treatment Of Ulcer Collites

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INTRODUCTION

The urgency of the problems associated with the current state of research and treatment of gastrointestinal diseases in Uzbekistan is due to a large number of patients with diseases of the digestive system, their severe course, leading to premature disability and death of patients, the need to improve treatment methods. The prevalence of certain diseases of the digestive system in our country has its characteristics, which are manifested in the prevalence of inflammatory bowel diseases. Accordingly, the prevalence of diseases of the digestive system in our republic ranks third after diseases of the respiratory system and the cardiovascular system [1].

Ulcerative colitis is a chronic inflammatory disease of an autoimmune etiology with ulcerative destructive changes in the mucous membrane of the rectum and colon, which proceeds with various complications, such as narrowing, perforation, bleeding, pseudopolyposis, dystrophy, cachexia, etc. [2]. According to the World Health Organization, the incidence of ulcerative colitis is 50–80 people per 100 thousand people [3, 4]. Currently, the anti-inflammatory and wound-healing properties of medicinal plants and preparations from them are not widely used, which, perhaps, have a slightly less pronounced effect, but better tolerance and less toxicity. In this regard, the search for new effective anti-inflammatory drugs of plant origin is urgent. The advantages of using herbal remedies are due to a wide range of pharmacological properties, mildness, and the absence of side effects with prolonged use since they are close in chemical nature to the human body and are easily included in biochemical processes [5].

Biologically active substances of peptide nature, belonging to the class of lipid-transferring proteins, are most actively involved in the process of healing and tissue regeneration, due to interaction with bilayer lipid membranes in the presence of such phospholipids as diacylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine inositol-phosphatidyl-serine.

For the lipid-transfer protein Ns-LTP1 from the seeds of *Nigella sativa*, it was shown in [6-8] that this peptide contains phosphatidylcholine and, therefore, can participate in the process of tissue healing and regeneration.

The work aimed to study the specific pharmacological (antiulcer) activity of a composition based on lipid-transferring protein (Ns-Ltp1) from seeds of *nigella sativa* in a therapeutic and prophylactic route of administration on a model of experimental ulcerative colitis in rats.

MATERIALS AND METHODS

The studies were carried out on white outbred rats weighing 180–210 g following the bioethics requirements of the National University of Pharmacy, which are consistent with the provisions of the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasbourg, 1985). After the end of the experiment, the rats were taken out from experience following the ethical principles of animal experiments [9]. For histological examination, after the end of the experiment, 1 cm³ of organs was taken from experimental animals. This area was fixed in 10% normal formalin. Then the tissue was embedded in paraffin and sections of histological preparations with a thickness of 3-5 µm was prepared, staining them with a mixture of hematoxylin + eosin. The resulting preparations were analyzed under a model: ZEISS microscope. Primo Star, camera SONY 1606, 1 / 1.8, 64MP "x = 4x10, 10x10, 10x20, 10x40.

Ulcerative colitis (UC) is a pathology in which inflammation of the colon is diffuse in nature, begins within the mucous membrane, often affecting the submucosa and muscle layers of the colon wall. Experimental colitis with ulcerative lesions of the mucous membrane of the sigmoid colon was induced with acetic acid according to the method of Fitzpatrick et al. This method, according to histomorphological and biochemical parameters, corresponds to ulcerative colitis in humans [10].

RESULTS AND DISCUSSION

Ulcerative colitis caused by acetic acid is accompanied by the development of pronounced destructive changes in the conditions of the colon mucosa with edema, hyperemia, and hemorrhages. During the reproduction of the model pathology in rats, signs of general intoxication of the body were observed, which was expressed by an increase in motor activity, a state of anxiety, and aggressiveness.

The comparison drug was Sulfasalosin, an anti-inflammatory drug used to treat Crohn's disease and ulcerative colitis (KRKA, Slovenia).

The animals were divided into 4 groups: group 1 - animals with model pathology, group 2 - animals receiving Ns-LTP1 at a dose of 10 mg/kg, group 3 - animals receiving Ns-LTP1 at a dose of 20 mg/kg, group 4 - animals receiving Sulfasalosin at a dose of 1 mg/kg. Experimental animals 4 days before and 9 days after modeling of colitis were administered orally drugs in appropriate doses.

In animals that were injected with Ns-LTP1 and sulfasalazine, these signs were manifested to a lesser extent and were absent by the 3rd day of treatment, while in animals of the control pathology group, these signs persisted throughout the experiment. The first symptoms of colitis were diarrhea mixed with mucus and blood, frequent urge to defecate, changes in the dynamics of body weight of animals.

To assess the healing processes of the large intestine lining, the rats were taken out of the experiments on the 3rd and 9th days, 3 animals from each group, conducting their postmortem examination with a visual assessment of the state of the mucous membrane. The samples were preserved in a 12% formalin solution, then stained with hemotoxylin-eosin and examined under a microscope.

When opening on the 3rd day of treatment, animals in the control group with an acute erosive ulcer of the superficial epithelium, part of the mucous layer of the colon cavity, phagocytic cells around the defect, fibrinous exudation is observed at the edges of the wound. Young connective tissue cells tend to form granulation tissue, consisting of fibroblasts, which have many small capillaries and microcirculatory elements. The process of increased formation of granulation tissue moderately developed infiltration and numerous microcirculatory vessels in the stroma, perivascular and interstitial edema were revealed (Figure 3.5).

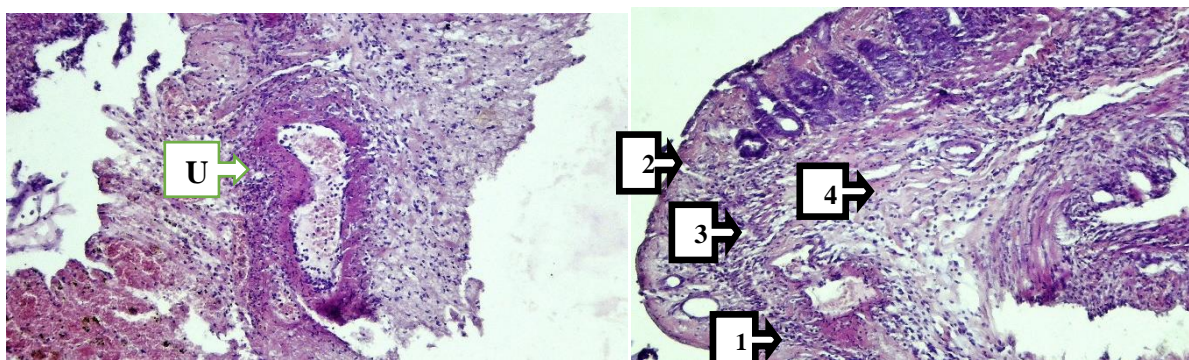


Fig. 3.5: 3rd day of treatment in the control group. In acute erosive ulcers (U) of the superficial epithelium, the process of increased formation of granulation tissue (1), moderately developed lymphoma-macrophage infiltration (3) in especially eroded foci (2), numerous microcirculatory vessels in the stroma, perivascular and interstitial edema (4) were revealed.

The autopsy on the 3rd day of treatment of the animals treated with Sulfasalazine around the wound revealed relatively uneven boundaries of serous-hemorrhagic exudate and necrotic changes, accumulation of brownish-red pigments (hematin) around the wound, thin membranes swelling on the surface of the mucous membrane. Microscopic examination revealed moderate neutrophilic infiltration, poorly developed histiocytic and macrophage foci of infiltration around the damaged lesion (Fig. 3.6.).

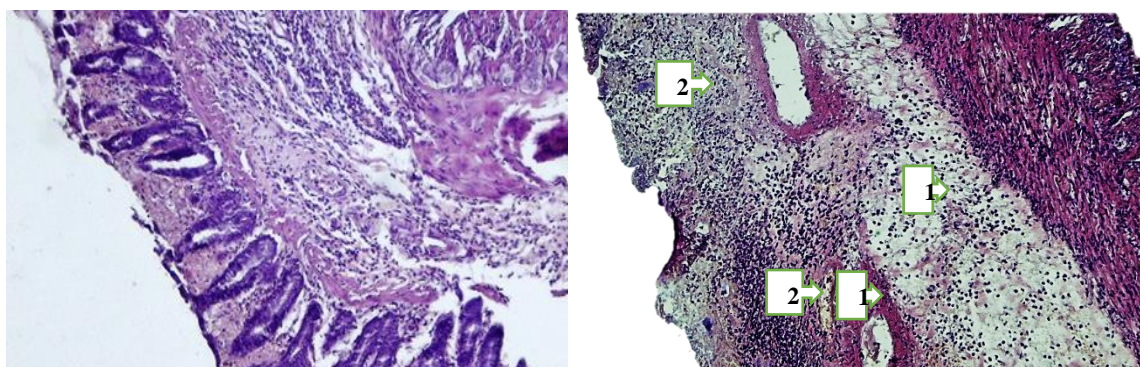


Fig. 3.6: 3rd day of treatment in the group treated with Sulfasalazine. A microscopic image of local changes on the 3rd day of the experiment, the formation of sparse fibrous connective tissue (granulation tissue) (1), the formation of leukocyte infiltration (2), a microcirculatory reaction, the detection of fibrous elements in the affected areas is described. On the intestinal mucosa, areas of clear proliferation and differentiation of connective tissue cells are observed (1). Stain: H-E. 4x10.

It has been established that many epithelial cells in a state of necrobiosis around erosive-necrotic foci are surrounded by neutrophil infiltration. It was determined that the diffuse spread of the inflammatory infiltrate: neutrophilic infiltration is extended to the submucosa, muscular and serous membranes. Revealed uneven vascular filling, uneven interstitial edema, fibrinoid foci in the stroma, and foci of focal mucoid edema (the phenomenon of metachromasia). These changes are caused by the inhibition of pro-inflammatory mediators (leukotrienes, cytokines, lymphokines, interleukins) under the action of the drug Sulfasalazine. Therefore, the traditional drugs used in the experiment have an antiulcerogenic effect (non-steroidal anti-inflammatory drugs).

The autopsy on the 3rd day of treatment in animals treated with Ns-LTP1 revealed several changes in the damaged surfaces of the intestinal mucosa. It was determined that neutrophilic infiltration around necrotic erosive foci on the surface of the mucous layer is reduced, the border of the demarcation plane

is located in a certain sequence. Revealed a sharp development of foci of reparative regeneration in the epithelium of the mucous membrane around the foci (Fig. 3.7.).

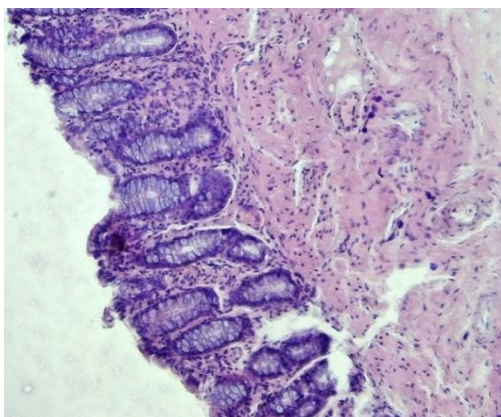


Fig. 3.7: 3rd day of treatment in the group treated with the Ns-LTP1 composition. With erosive-necrotic changes arising from direct damage to the mucous membrane after 72 hours, a demarcation line was formed in the intestinal cavity after the application of Ns-LTP1 around the damaged focus at the edges of the injury as a result of infiltration of the mucous membrane epithelium (absorption of organic components), indicating that the border of the inflammatory line has ceased. Hematoxylin and eosin stain. Size: 4x20.

At autopsy on the 9th day of treatment of animals in the control group, the changes are local in nature. In the damaged areas of the mucous membrane, a proliferative reaction of epithelial cells is revealed, in the process of regeneration, the exposed areas of tissue are covered, the average metaplasia of the covering epithelium is revealed. Glandular narrowing occurs due to scarring of the interstitial tissue. There is a strong proliferation and differentiation of young connective tissue cells (Fig. 3.8.).

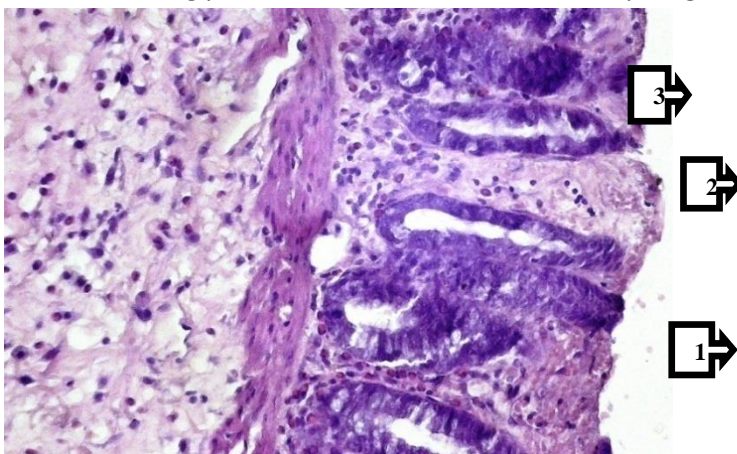


Fig. 3.8.: Control group. Changes after 9 days after injury are local in nature. middle metaplasia of the covering epithelium (1). Glandular narrowing (3) occurs due to scarring of the interstitial tissue (2).

The autopsy on the 9th day of animals treated with Sulfasalazine during the macroscopic examination after necrotic, erosive ulcerative changes on the surface of the affected mucosa, it was found that brownish-red foci on the wound surface turned into pinkish-red granulation tissue. In the healthy layer of the mucous membrane of the edge of the wound, hyperplastic changes are revealed (Fig. 3.9.).

Microscopic analysis revealed the relative formation of the epithelial lining, consisting of several mucocytes on the surface of the mucous membrane, the proliferation of numerous rarefied fibrous granulations in the crypts of the mucous membrane. In the preserved glandular cells in the area of injury, foci of hyperplasia and hyperproduction are revealed. Discovered goblet cell hypertrophy. Around the granulation tissue, a large amount of proliferation of endothelial cells and many forming vascular foci are found. In the stroma of the mucous membrane, infiltration of poorly formed lymphocytes, macrophages, histiocytes, and some neutrophils is revealed.

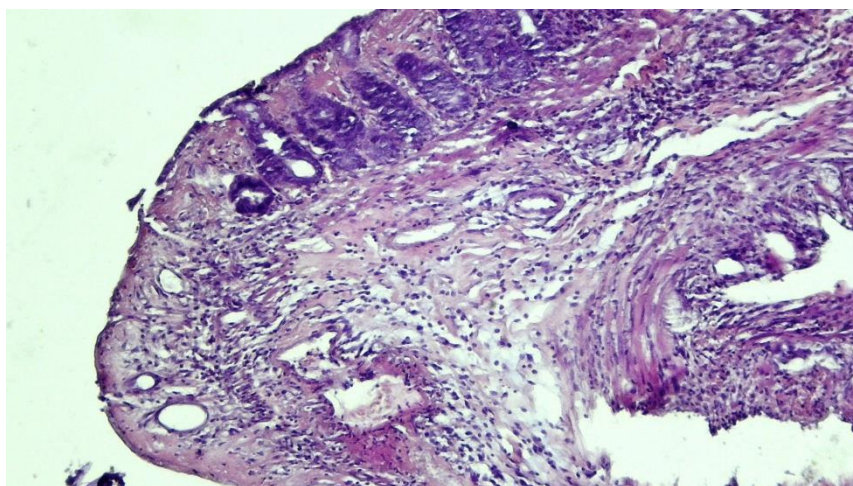


Fig. 3.9: Day 9 of treatment in the Sulfasalazine group. After 9 days after special injury to the mucous membrane of the colon, at the site of acute erosive changes, sparse connective tissue scars, moderate traces of serous exudate are formed. The stroma is made up of connective tissue, fibroblasts, and fibrocytes. There are areas where fibrous (collagen) structures are formed, and capillary hyperemia is not detected. Hematoxylin and eosin stain. Size: 4x20.

Focal foci are distinguished, consisting of sparse fibrous connective tissue, in which the subcutaneous layer and the border of the basement membrane are not defined. In the myocytes of the muscle layer, atrophic, dystrophic, and degenerative changes are revealed. In the interstitial tissue, uneven edema and fibrinoid foci are found.

Thus, the suppression of the inflammatory process in the mucous membrane under the action of the drug Sulfasalazine leads to the development of dystrophic and dysregenerative (atrophy, hypertrophy, hyperplasia, and sclerosis) processes in the parenchymal elements.

The autopsy on the 9th day of animals treated with the composition with Ns-LTP1 revealed hyperplasia of border cells, hyperplasia of the mucous membrane around the wound, an increase in squamous cells, a decrease in tumors in the interstitial tissue, the formation of fibroblasts from transformed macrophages around the damaged basal lamina. This is evidenced by a decrease in the foci of neutrophilic infiltration in the wall of the damaged vessel and the appearance of mitotic foci in the preserved glandular epithelium. The formation of a protective layer on the surface of the mucous membrane, resembling a lipid membrane, affects the development of secondary changes and creates conditions for the proliferation of mucocytes. Reduction of foci of fibrinoid necrosis, which usually should occur in the submucosa and muscle layers in the area of damage, is shown in Fig. 3.10. At the same time, staining for H-E revealed a slight development of interstitial edema in muscle tissue, a decrease in the reaction of basophilic cells (mast cells) in a special lamina of the mucous membrane.

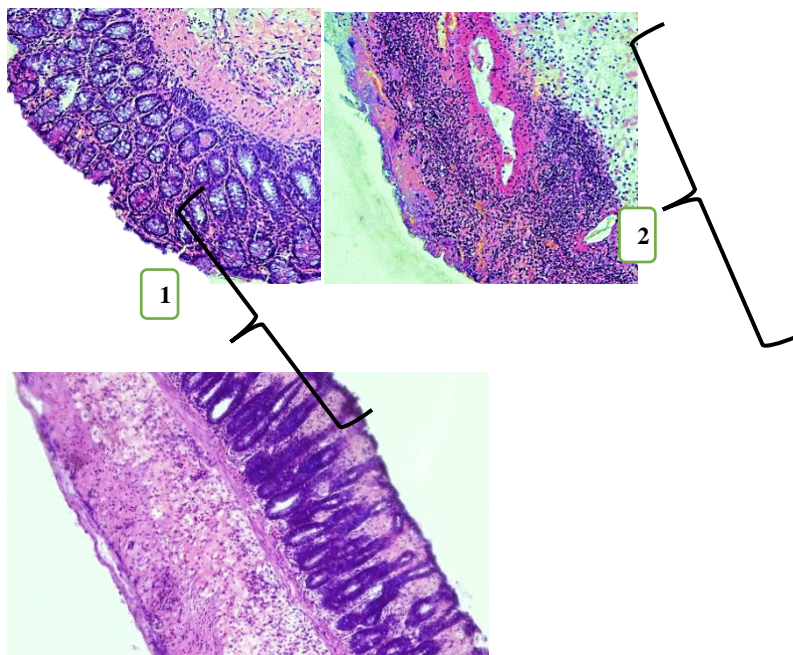


Fig. 3.10: 9th day of treatment in the group treated with the composition with Ns-LTP1. The final duration of the experiment is determined by the intestinal mucosa, replacement, and restoration (incomplete and complete regeneration) of the covering epithelium. Ns-LTP1 has a stabilizing effect on the cell membrane (glycolipid and glycoprotein layer - glycocalyx). Regeneration, the transformation of cells, and differentiation of erosive (1) necrotic (2) lesions are achieved through the regeneration of epithelial cells of the mucous membrane. Paint H-E. Size: 4x10. 4x20.

Phospholipids included in Ns-LTP1 stabilize the cell membrane by suppressing destructive changes in damaged cell membranes. The appearance of heterochromia granules in the nuclei of damaged cells in preparations indicates cell division and regeneration. Based on this, partial (substitution) and complete (restitution) foci of reparative regeneration around the affected area are identified.

Thus, microscopic examination of eroded samples of the large intestine determined that the effect of Ns-LTP1 on the gastrointestinal mucosa is faster and more complete than that of traditional drugs, without a sharp limitation of the area of inflammatory infiltration, thereby accelerating wound healing.

CONCLUSION

1. Composition based on lipid-transfer protein (Ns-Ltp1) from seeds of nigella sativa has a pronounced anti-inflammatory and antiulcer effect in a model of acute experimental colitis in rats, which is manifested in a decrease in the area of ulcers and the degree of damage to the MMC.
2. The pronounced wound-healing effect of the composition based on lipid-transferring protein (Ns-Ltp1) from the seeds of Nigella Sativa in the model of ulcerative lesions of the large intestine makes it possible to recommend the investigated drug for use in the complex therapy of inflammatory diseases of the large intestine.

LIST OF REFERENCES

1. Karimov M.M., Saatov Z.Z., Sobirova G.N. Diseases of the digestive system. A guide for doctors. "BAKTRIA PRESS". Tashkent, 2015, 402 p.

2. Langan R.C., Gotsch P.B., Krafczyk M.A. et al. Ulcerative colitis: diagnosis and treatment. *Am. Fam. Physician*, 2007, 76 (9), pp. 1323–1330.
3. Ivashkin V.T. Rational pharmacotherapy of diseases of the digestive system. a guide for the practice of doctors. M: Litterra, 2007, 1056 p. (In Russ.)
4. Yu.S. Egamov, A.E. Ruziev, S.A. Khaidarov. Evaluation of the results of the analysis of conservative and surgical methodstreatment of ulcerative colitis. *Moscow surgical journal*. 1 (75) 2021.S. 5-9.
5. E.A. Belousov, D.I. Abdulganieva, O. P. Alekseeva et al. Socio-demographic characteristics, course features and treatment options for inflammatory bowel diseases in Russia. Results of two multicenter studies // *Almanac of Clinical Medicine*. - 2018. - T. 46, No. 5. - P. 445–463.
6. Klimova, S.V. Clinical significance of the activity of mitochondrial enzymes of lymphocytes in inflammatory bowel diseases in children: author. dis. ... Cand. honey. Sciences: 14.01.08 // Moscow, 2010.-- 108 s
7. V.I. Sovalkin, G.R. Bikhavova, Yu.A. Emelyanova Modern view of the pathogenesis and laboratory diagnosis of ulcerative colitis (literature review) // *Archive of Internal Medicine*. - 2017. - T. 7, No. 4. - P. 252–259.
8. *Handbook of Coloproctology* / ed. prof. Yu.A. Shelygin, prof. L.A. Thankful. - Litterra, 2012.-- 608 p.
9. European convention for the protection of vertebral animals used for experimental and another scientific purpose: Council of Europe 18.03.1986. - Strasbourg, 1986. - 52 p.
10. Fitzpatrick L. R., Bostuick G. S., Renzetti M. [et al.] // *Agents and Actions*. - 1990.- Vol. 30, N 3–4. - P. 231.