

## THE EFFECT OF DEALCOHOLIZED EXTRACT OF ACORUS CALAMUS ON MORPHOLOGICAL CHANGES OF THE GASTRIC MUCOSA UNDER THE CONDITIONS OF ALCOHOL-PREDNISOLONE ULCER IN RATS

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### Abstract

Modern pharmacotherapy of gastric diseases includes a significant number of drugs, among which the vast majority is synthetic drugs from the group of H<sup>+</sup>-K<sup>+</sup>-ATPase inhibitors, H<sub>2</sub>-receptors blockers, bismuth preparations, etc. At the same time many phytomedicines also have a gastroprotective effect, which allows them to be used for complex therapy of gastric diseases, or after classical therapy.

One of the phytomedicines that is traditionally used to treat diseases of the stomach is sweet flag (*Acorus calamus* L.).

The aim of the study was to experimentally investigate the effect of dealcoholized extract of *Acorus* leaves (DEAL) on morphological parameters under conditions of alcohol-prednisolone ulcer in rats.

The gastroprotective properties of DEAL were studied in a model of acute gastric ulcer in rats; it was stimulated by intragastric administration of a mixture of prednisolone at a dose of 20 mg/kg and 80% ethanol at a dose of 6 mL/kg. DEAL was administered intragastrically prophylactically once daily at a dose of 1 mL/kg for 3 days before the modeling of the pathology. The reference drug was chosen ranitidine ("Ranitidine-Zdorovya", tab. 0.15 № 20). The reference drug was administered intragastrically at a dose of 20 mg/kg in a similar regimen.

It has been found that the introduction of alcohol-prednisolone mixture in rats provoked in the latter the emergence of acute ulcerative-erosive process in the fundal and pyloric area of the gastric mucosa, which was associated with significant hemocapillary disorders and swelling of the stroma. In most cases, the ulcers spread to the entire depth or most of the glandular tubes. In areas outside the destruction zones, a decrease in mucoid secretion by mucosal cells of the superficial-foveolar epithelium and (according to the morphological state of the chief and parietal cells) some stimulation of pepsin production and excretion, increased acid production were observed.

Although DEAL did not prevent the development of a pathological process in the gastric mucosa of most rats, but under its influence limited the depth of lesions of the glandular tubes, decreased the severity of hemocapillary disorders, signs of stroma swelling, stabilized mucoid synthesis, improved functional state of the parietal and chief cells. All this confirms that in this experimental model of gastric pathology at this scheme of administration and dose, the extract of sweet flag leaf has a certain gastroprotective effect. The severity of the gastroprotective effect of DEAL is somewhat inferior to that of the comparison drug ranitidine.

**Keywords:** dealcoholized extract of *Acorus calamus* leaves, alcohol-prednisolone gastric ulcer, rats, morphological studies.

## Introduction

Modern pharmacotherapy of gastric diseases includes a significant number of medicinal products, among which the vast majority is synthetic drugs from the group of H<sup>+</sup>-K<sup>+</sup>-ATPase inhibitors, H<sub>2</sub>-receptors blockers, bismuth preparations, drugs used to treat *Helicobacter pylori* infection etc [1, 2]. At the same time many phytomedicines also have a gastroprotective effect, which allows them to be used for complex therapy of gastric diseases, or after classical therapy [3, 4, 5].

In the pathogenesis of gastric ulcers, gastritis and other gastrointestinal diseases, one of the links of damage is excessive activation of free radical oxidation, so the presence of antioxidant effect in a number of phytomedicines allows reducing the degree of free-radical damage, to improve the state of antioxidant enzymes, which in turn enhances cell proliferation and promotes regeneration processes [6, 7, 8, 9].

Quite a lot of phytomedicines have anti-*Helicobacter* and anti-*H. pylori*-induced gastric inflammatory effect, which makes their use in the combined treatment of gastric ulcer and gastritis etiologically and pathogenetically justified, especially given the data on the growth of *H. pylori* resistance to antibiotics [10, 11, 12].

Therefore, the significant role of phytotherapy in medicine is undoubted [13, 14, 15].

One of the phytomedicines that is traditionally used to treat diseases of the stomach is sweet flag (*Acorus calamus* L.). In official medicine, the rhizomes of sweet flag (*Acorus calamus* L.) are used in the form of an aqueous infusion and tincture, as well as in the composition of complex medicinal blends or medicinal teas. Galenic preparations from the rhizome of sweet flag are widely used for the treatment of chronic gastritis, peptic ulcer of the stomach and duodenum, especially in cases of low acidity of gastric juice, achylosis, diarrhea of various origins and other digestive disorders. Sweet flag rhizome powder is a part of tablets "Vikalin" and "Vikair", which are used in peptic ulcer of the stomach and duodenum and gastritis as antacids, antispasmodics, astringents and antiseptics in the acute phase [16].

In modern medical practice, the rhizomes of sweet flag (*Rhizomata Calami*) are used mainly as an aromatic bitterness. This increases appetite and improves digestion [16, 17]. Rhizomes of sweet flag are a component of complex drugs (Stovalid N, Hevert-Gall S, Gallexier (Germany), used to stimulate appetite and improve digestion in gastrointestinal disorders and in the treatment of PUD and gastritis [18]. There are data on the antibacterial properties of *Acorus calamus*, including in relation to *H. pylori* [19, 20, 21].

Antimicrobial properties are inherent not only in the rhizomes but also in the leaves of sweet flag. In the study [22] for hydrophilic and hydrophobic extracts of both *A. calamus* rhizome and leaves, a correlation was found between biological activity and total phenol content and flavonoids content.

However, despite the wide range of pharmacological properties of sweet flag extracts, its use in traditional medicine is quite limited.

Toxicity of sweet flag rhizomes is associated mainly with the chemical composition of its essential oil, in particular, with the content of  $\beta$ -azarone [21, 23, 24]. According to animal experiments,  $\beta$ -azarone, contained in the essential oil of sweet flag, has a carcinogenic effect. In the United States, the use of sweet flag oil in food is prohibited, and in Europe there are strict standards for its use [24]. Due to the likelihood of embryotoxic effects, the use of sweet flag drugs during pregnancy is contraindicated [24].

At the Department of Botany of the National University of Pharmacy under the supervision of Professor Gontova T.M. a dealcoholized extract of *Acorus* leaves (DEAL) was received.

Dealcoholized extract of *Acorus* leaves was obtained by evaporation, which was carried out on a rotary evaporator under the following conditions: temperature 50 °C, rotation speed - 100 rpm, and vacuum force - 900 mBar. 1 mL of the extract contains 235 mg of dry matter.

Using HPLC we have identified phenolic compounds and determined their quantitative content in the extract: hydroxycinnamic acids - caffeic acid (21.83  $\mu$ g/mL), *p*-coumaric acid (70.43  $\mu$ g/mL) and flavonoids: isoorientin (142.96  $\mu$ g/mL), robinin (2677.89  $\mu$ g/mL), apigenin-7-glucoside (65.56  $\mu$ g/mL), apigenin (9.98  $\mu$ g/mL) and acetatin (12.45  $\mu$ g/mL). Quantitative content of all identified

components is 3001.1 µg/mL. In the dealcoholized extract of *Acorus* leaves the content of the sum of hydroxycinnamic acids has been determined, which amounted to in terms of rosmarinic acid - 0.39 mg/100 mL. The total antiradical activity (697.72 ± 1.41 µg/g of trolox equivalent antiradical capacity) was determined. 3 compounds with antiradical activity were identified, their quantitative content in the extract was determined: isoorientin (62.27 µg/g), robinin (317.53 µg/g) and rutin (41.07 µg/g). Azarone was not detected [25].

The aim of the study was to experimentally investigate the effect of DEAL on morphological parameters under conditions of alcohol-prednisolone ulcer in rats.

## Methods

The gastroprotective properties of DEAL were studied in a model of acute gastric ulcer in rats; it was stimulated by intragastric administration of a mixture of prednisolone at a dose of 20 mg/kg and 80% ethanol at a dose of 6 mL/kg [26, 27]. Ethanol is an alimentary gastrototoxic substance that causes dehydration and coagulation of the gastric mucosa and its necrosis. Prednisolone is a glucocorticoid hormone that potentiates the ulcerogenic action of ethanol. As a result of the synergism of the ulcerogenic effect, the strength of the damaging effect of strong alcohol on the gastric mucosa increases tenfold [27, 28].

The drug combination is administered to rats after 24 h of fasting (free access to water). One day after the introduction of the combination of alcohol + prednisolone, the animals were euthanized with further examination of the gastric mucosa [28].

DEAL was administered intragastrically prophylactically once daily at a dose of 1 mL/kg for 3 days before the modeling of the pathology. The reference drug was chosen ranitidine ("Ranitidine-Zdorovya", tab. 0.15 № 20). The reference drug was administered intragastrically at a dose of 20 mg/kg in a similar regimen [26, 27, 28]. Experimental animals were divided into IV groups of 6 animals each: I - intact control (IC); II - control pathology (CP); III - ranitidine; IV - DEAL. The experiments were performed in accordance with the rules of the

"European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" [29].

24 h after reproduction of control pathology euthanasia of rats was performed; stomachs were removed for histological examinations.

Samples for histological examination were prepared according to general methods and stained with hematoxylin and eosin and used the Periodic Acid - Schiff (PAS) reaction according to McManus to detect neutral mucopolysaccharides and determine the activity of mucus secretion by the secretory elements of the stomach [30]. Micropreparations were studied under a Granum microscope, and images were documented with Granum DCM 310 digital video camera. Photographs were processed on a 2.4 GHz Pentium computer using ToupView program.

For the convenience of comparison and for greater objectification of the obtained results, we have conducted a semi-quantitative assessment of the state of gastric mucosa on the following indicators: a) the presence of ulcerative-erosive defects: 0 points - no destructive changes in gastric mucosa; 1 point - superficial erosions of the columnar epithelium of varying degrees of formation; 2 points - 1-3 small ulcers/1 medium ulcer in the micropreparation; 3 points - numerous small ulcers/ 2-3 medium ulcers in the micropreparation; 4 points - more than 4 medium ulcers in the micropreparation/extensive massive ulcer in the micropreparation. b) the depth of the lesion of the glands in the defect: 0 points - the glands are not damaged; 0.5 points - damage to the mucous-neck zone of glands; 1, 2, 3 points - lesions of 1/3 of the gland length, 1/2 of the gland length and 2/3 of the gland length, respectively; 4 points - total gland damage. c) hemocapillary disorders: 0 points - no disorders; 1 point - focal plethora of capillaries of the surface zones of the gastric mucosa; 2 points - widespread plethora of capillaries of the superficial zones of the gastric mucosa, erythrocyte stasis; 3 points - widespread plethora of capillaries of the superficial zones of the gastric mucosa, erythrocyte stasis and homogenization of capillary walls, plasmodiapedesis; 4 points - paralytic dilatation of blood vessels of various calibers, hemorrhages. d) edema of the gastric mucosa stroma: 0 points - no signs; 1, 2, 3 points - respectively weak, moderate

and pronounced signs of edema; e) the severity of mucous secretion by mucocytes of the columnar epithelium of the gastric mucosa outside the destruction in the severity of the PAS reaction: 1 point - weak staining; 2 points - moderate; 3 points - expressive staining [31]. To formulate statistical conclusions used analysis of variance (Kruskal-Wallis test), when comparing samples - Mann-Whitney test at a probability level  $p \leq 0.05$  [32, 33].

## Results and Discussion

Histological examinations of animals with alcohol-prednisolone gastric ulcer have revealed the following.

In animals of intact control (Fig. 1) the surface of the fundal area of the mucous membrane (MM) is covered with a single layer of predominantly cubic epithelium. Gastric fossae (microscopic recesses of the surface) are shallow. Own glands are densely located, long. In glandular tubes mucous (additional), parietal and chief cells which territorial arrangement is typical are clearly distinguished, visually secretory activity of all listed cells is within norm. Mucous cells are located mainly in the initial part of the glands, near the neck; their cytoplasm is quite clearly illuminated. Parietal cells predominate in number over other glandular cells, distributed on 2/3 of the body of glands, their cytoplasm is clearly oxyphilic. The chief cells predominate in the end sections of the glandular tubes. They have a well-defined secretory zone, the cytoplasm has a basophilic color. Stroma of the MM contains a moderate number of lymphocytes and eosinophils. A significant amount of neutral mucopolysaccharides was detected during the PAS reaction in the apical parts of the superficial epithelial cells, the cytoplasm of the foveolar epithelial cells and the mucous cells of the glandular tubes. In the pyloric part of the stomach, the surface is covered with a single layer of cylindrical epithelium, the gastric pits are deeper and wider. The pyloric glands are quite loose, the connective tissue layers between them are more pronounced. The glands are lined with a single layer of cells with a distinct light wide apical zone, in which the PAS reaction reveals a lot of PAS-positive mucoid

secretion (Fig. 1). The condition of the microcirculatory tract in all studied areas of the gastric mucosa (GM) is without features. The muscular membrane is not changed. The submucosa is relatively wide, consisting of loose connective tissue, which shows normal-looking blood vessels.

Morphometric assessment of the state of MM of the studied areas of rats is given in tables 1 and 2.

After administration of alcohol-prednisolone mixture on MM micropreparations in all studied areas of the stomach in 6 of 6 rats ulcerative defects have been revealed (table 1).

Ulcerative defects varied in both the number and depth of lesions of the glandular tubes. As a rule, the defect crater is filled with necrotic mass and cellular detritus, often, apparently due to damaged vessels and capillaries, among the necrotic masses can be seen basophilic homogeneous clusters - deposits of hydrochloric hematin.

In cases when the depth of the defect did not cover all the MM, in the basal parts of it the linearity of the glandular tubes was often disturbed, dissociation and necrobiosis of cells were noted. In addition to acute ulcers in different parts of the GM, the so - called superficial erosions were observed, which are characterized by desquamation and necrobiosis of superficial epithelial cells and cells of gastric pits, stroma exposure (Fig. 2).

In the areas of preserved MM of the fundus of the stomach, the cytoplasm of parietal cells is often vacuolated, clarified. In the chief cells, the secretory zone is clearly reduced, the cytoplasm is much less basophilic, the lumens of the glandular tubes are expanded, and sometimes they show visible remnants of secretions. This state of the chief and parietal cells morphologically reflects a certain increase in the functional activity of these cells, ie - acid formation and peptic activity. Mucosal cells of GM (superficial-foveolar epithelium, additional cells of own glands, pyloric cells), on the contrary, are visually reduced in size, the secretory zone of cytoplasm is also reduced, depleted of mucoid PAS-positive secretion, which is a sign of reduced mucoid synthesis process. That is, the digestive power of gastric juice increased with a decrease in the process of mucoid synthesis, and, as a consequence, - the protective effect of mucus. In the GM stroma often can be seen subepithelial edema of various severity, sharply stretched blood capillaries filled

with erythrocytes in a state of stasis. Diapedetic hemorrhages are met quite often (Fig. 2).

The vessels of the submucosal layer were often in a state of paralytic lesion and significant blood supply, the submucosal layer itself in the area of direct lesion of the mucous membrane is clearly swollen.

The results of semi-quantitative (score) assessment of the severity of the pathological process in the GM of rats of the control pathology group are presented in Table 2.

The introduction of the studied DEAL to rats, in general, improved the condition of the rats GM (Table 2).

So, in 1 of 6 rats ulcers in the studied areas are absent. Only a slight decrease in the height of the superficial epithelial cells in the area of the bottom was observed. Subepithelial edema and plethora of the capillary network are absent. The structure of the own and pyloric glands is preserved. The submucosa is either unaltered or moderately swollen. In the superficial-foveolar epithelium the content of glycoproteins is increased in comparison with the control pathology (Fig. 3, Fig. 4).

In 83.3% of rats in the area of both the bottom and the pylorus of the stomach, although ulcerative defects were detected, but 66.6% of them were single small defects, the depth of which did not exceed  $\frac{1}{3}$  the length of glandular tubes, and the glandular tubes themselves under defects are largely preserved. To a greater extent, rats had superficial erosions, which were mainly focal in nature and affected only the superficial-foveolar epithelium. In most of these animals, hemocapillary disorders were also pronounced (Fig. 5).

Outside the zones with erosive-ulcerative lesions, focal moderate stromal edema and plethora of the capillary network are visible; in the superficial-foveolar epithelium of most animals there is an increased PAS-positive reaction to the level of intact control (Fig. 6 a). Only in 1 rat GM defects in the area of the bottom of the pylorus were quite pronounced (Fig. 6 b).

It should be noted that in the vast majority of rats in this group, the morphological state of the parietal and chief cells of the own glands outside the zones of destruction was close to intact.

After administration to rats of the comparison drug ranitidine in 33.3% of cases observed the absence of ulcerative disorders in the area of the bottom and pylorus. In some places the flattening of superficial epithelial cells is visible, but in general, their morphological characteristics are preserved. The severity of the PAS reaction is comparable with intact control (Fig. 7).

In 4 rats in both studied areas of GM small innumerable ulcerative defects affecting no more than  $\frac{1}{3}$  of glandular tubes length, superficial erosions have been revealed. Focal edema and plethora of the capillary network of the subepithelial stroma persisted.

The severity of PAS-positive staining in the cells of the superficial-foveolar epithelium, cytoplasm of the mucous cells of the fundal glands and pyloric cells significantly exceeded that in the control pathology (Fig. 8, table 1), and the functional state of parietal and main cells of own glands visually acquired a typical level.

Therefore, summarizing all the data obtained, we can make the following generalizations.

The introduction of alcohol-prednisolone mixture in rats provoked in the latter the emergence of acute ulcerative-erosive process in the fundal and pyloric area of the gastric mucosa, which was associated with significant hemocapillary disorders and swelling of the stroma. In most cases, the ulcers spread to the entire depth or most of the glandular tubes. In areas outside the destruction zones, a decrease in mucoid secretion by mucosal cells of the superficial-foveolar epithelium and (according to the morphological state of the chief and parietal cells) some stimulation of pepsin production and excretion, increased acid production were observed.

Although DEAL did not prevent the development of a pathological process in the gastric mucosa of most rats, but under its influence limited the depth of lesions of the glandular tubes, decreased the severity of hemocapillary disorders, signs of stroma swelling, stabilized mucoid synthesis, improved functional state of the parietal and chief cells. All this confirms that in this experimental model of gastric pathology at this scheme of administration and dose, the extract of sweet flag leaf has a certain gastroprotective effect. The severity of the

gastroprotective effect of DEAL is somewhat inferior to that of the comparison drug ranitidine.

Probably, the antiulcer properties of DEAL are due to the synergistic action of the components of the extract (primarily, the presence in its composition of oxycinnamic acids and flavonoids), which stimulate the protective factors of the central nervous system, have antioxidant and anti-inflammatory effects [34, 35, 36].

Thus, in the model of alcohol-prednisolone ulcer in rats, it has been found that DEAL under the conditions of prophylactic intragastric administration at a dose of 1.0 mL/kg has an antiulcer effect.

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**Table 1.** Distribution of rats in the presence of acute ulcers in the gastric mucosa

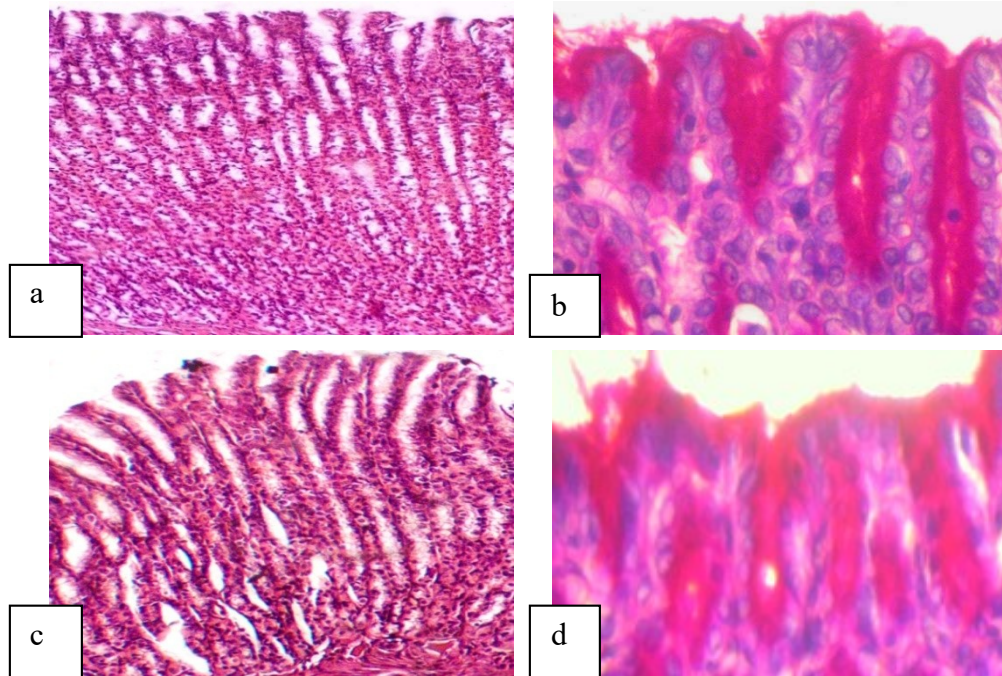
Groups of rats	Number of rats in the group	Gastric mucosa			
		no ulcers	acute ulcers		
			large	medium	small
Intact control (IC)	6	6	0	0	0
Alcohol-prednisolone mixture (CP)	6	0	4	2	0
DEAL, 1 mL/kg	6	1	0	1	4
Ranitidine 20 mg/kg	6	2	0	0	4

**Table 2.** The effect of DEAL on the semi-quantitative assessment of the severity of the pathological process induced in the gastric mucosa of rats with an alcohol-prednisolone mixture (n = 6), Me (LQ; UQ)

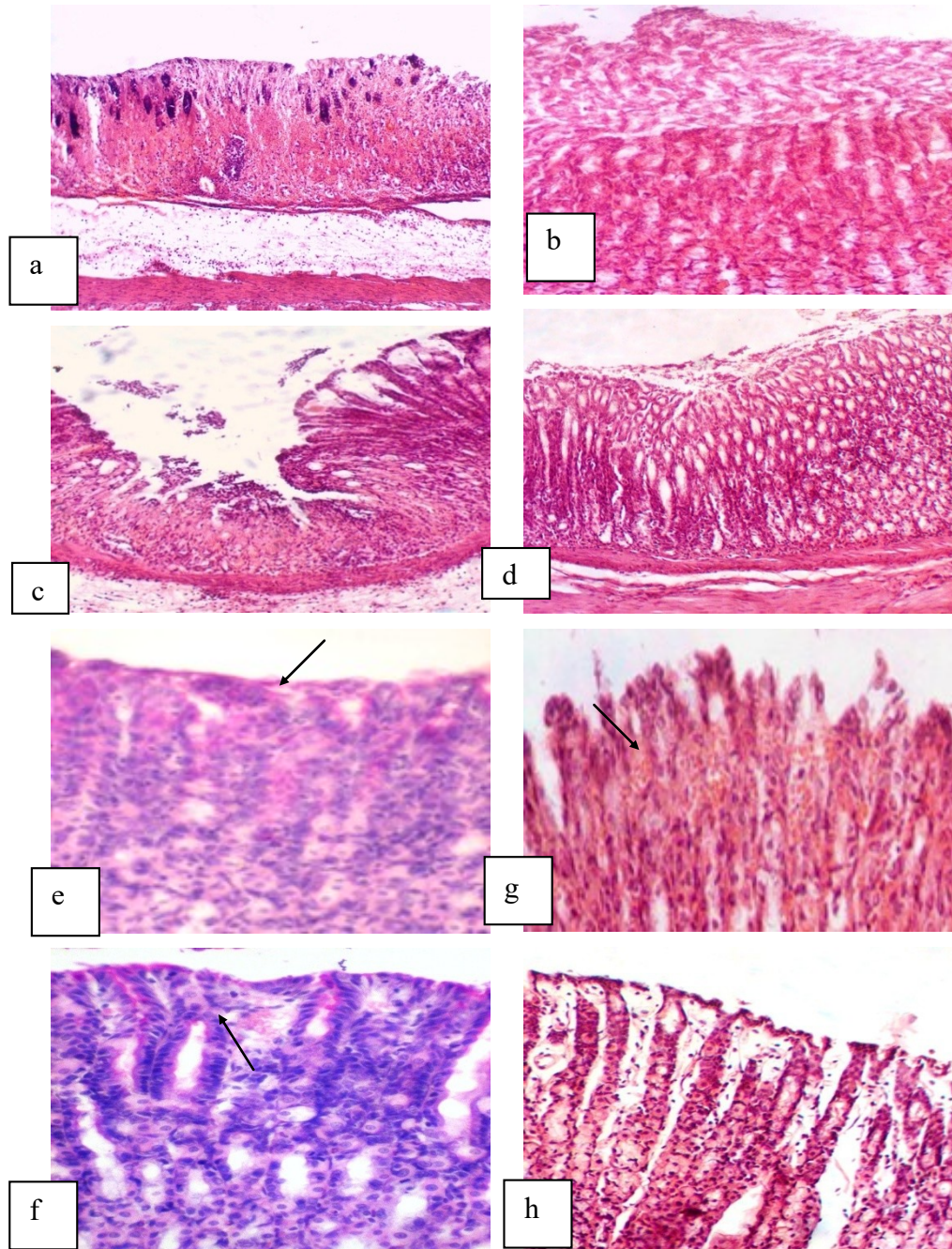
Groups of rats	Indicators, points				
	The presence of defects in the micropreparation	Depth of lesion of glandular tubes	Stromal edema	Hemocapillary disorders	The severity of mucoid secretion outside the areas of destruction
Intact control	0	0	0	0	3.0
CP	3.5	3.66	3.0	2.66	1,16
DEAL	2.0	1,33	2.0	1,33	2.5
Ranitidine	1.0	0.83	1.0	1.0	2.66



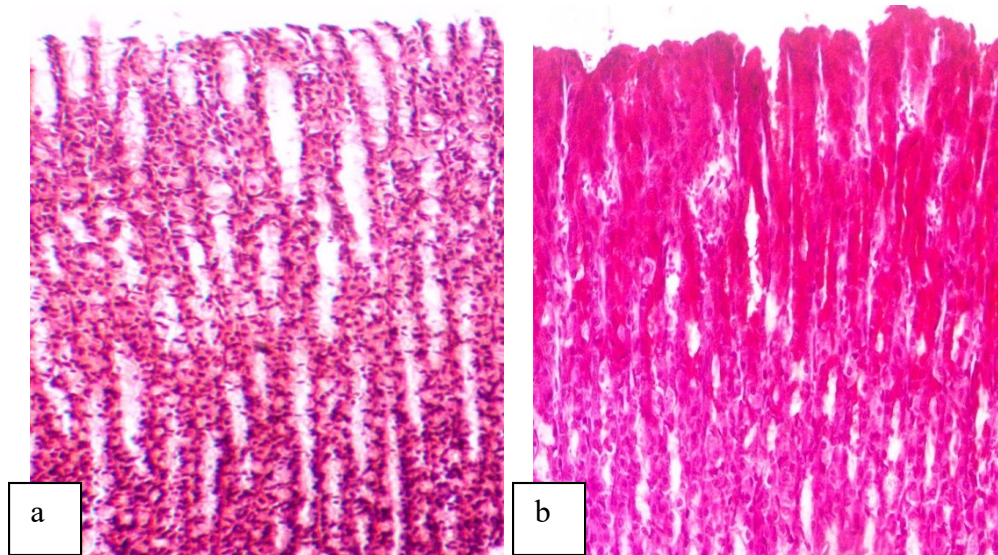
**Figure 1.** Mucous membrane of the fundal (a-b) and pyloric (c-d) part of the stomach of intact rats: a, c - normal condition of the foveolar epithelium, glandular cells of glandular tubes (hematoxylin-eosin, x100); b, d - a significant content of PAS-positive material in mucus-forming cells (PAS-reaction, x400)



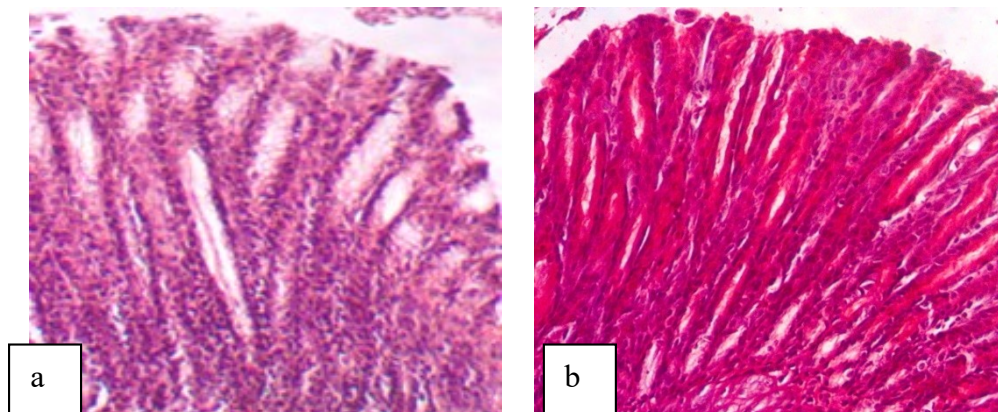
**Figure 2.** The mucous membrane of the fundal (a-b) and pyloric (c-d) part of the stomach of rats, which were administered alcohol-prednisolone mixture: acute ulcer (a, c), superficial erosion (b, d). Explicit depletion of the cytoplasm with PAS-positive material (e-f); erythrocyte stasis in the capillary network subepithelially, hemorrhage (g), subepithelial edema of the stroma (h). a-b, e-f - PAS reaction, c-d - hematoxylin-eosin. x250



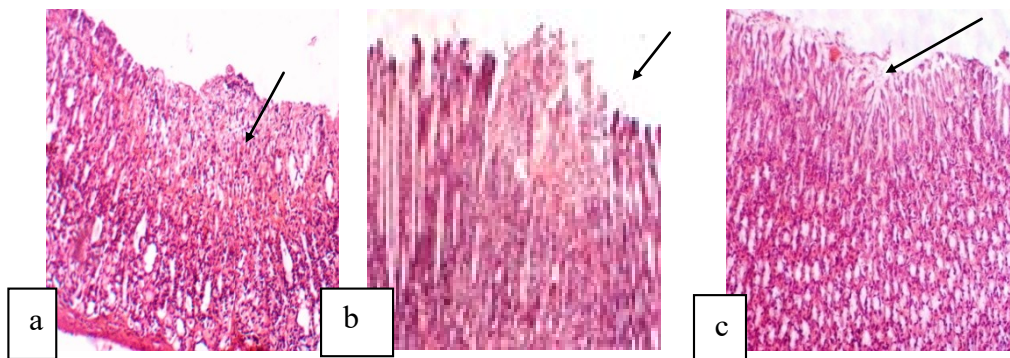
**Figure 3.** Mucous membrane of the fundus of the stomach of rats administered with DEAL. Moderate decrease in the height of the superficial epithelial cells, restoration of the histostructure of the glandular tubes (a), the content of PAS-positive material (b). a - hematoxylin-eosin, b - PAS reaction. x200



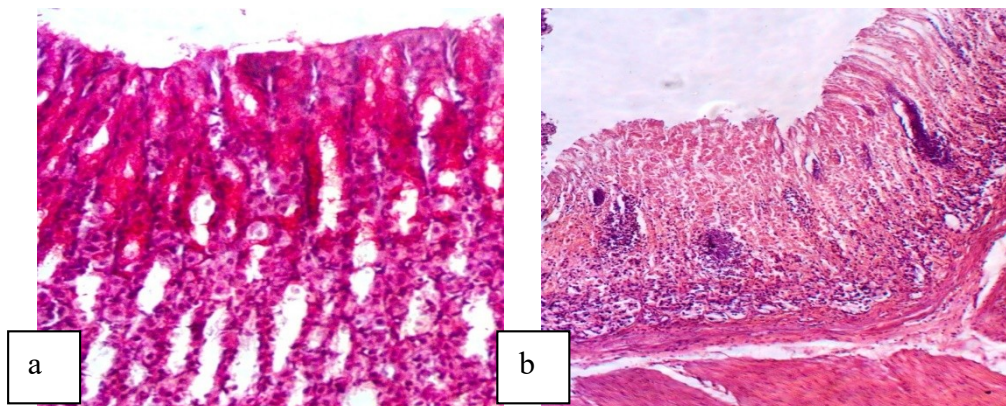
**Figure 4.** The mucous membrane of the pyloric (a-b) part of the stomach of the rat, which was administered DEAL. Normal condition of the mucosa (a, x250), the content of PAS-positive material (b, x200). a - hematoxylin-eosin, b - PAS reaction



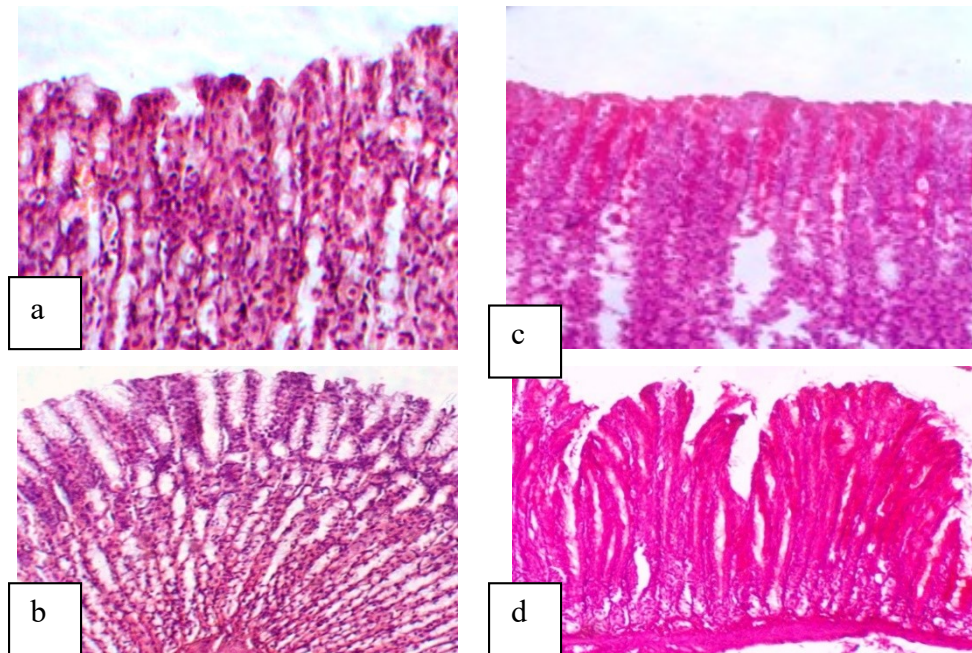
**Figure 5.** The mucous membrane of the fundal (a, c) and pyloric (b) part of the stomach of rats, which were administered DEAL: a - b - acute ulcer, plethora of capillaries, the preservation of glandular tubes under the defect; in - focal surface erosion. Hematoxylin-eosin. x200



**Figure 6.** The mucous membrane of the fundal (a) and pyloric (b) part of the stomach of rats, which were administered DEAL: a - complete recovery of the content of PAS-positive material in the superficial-foveolar epithelium; b - acute disseminated ulcer. a - PAS reaction, b - hematoxylin-eosin. x200



**Figure 7.** The mucous membrane of the fundal (a, c) and pyloric (b, d) parts of the stomach of rats, which were administered ranitidine: the normal state of the epithelium and glandular tubes (a-b), the content of PAS-positive material (c, d). a-b - hematoxylin-eosin, c-d - PAS reaction. x200



**Figure 8.** The mucous membrane of the fundus of the stomach of rats administered ranitidine: small acute ulcer (a), superficial erosion (b); edema and plethora of the capillary network (c), restoration of PAS-positive reaction outside the zones of destruction (d). a-c - hematoxylin-eosin, d - PAS reaction. x200

