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Research Article

Evaluation of gastroprotective activity of a chitosan-based gel containing dexpanthenol

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Abstract

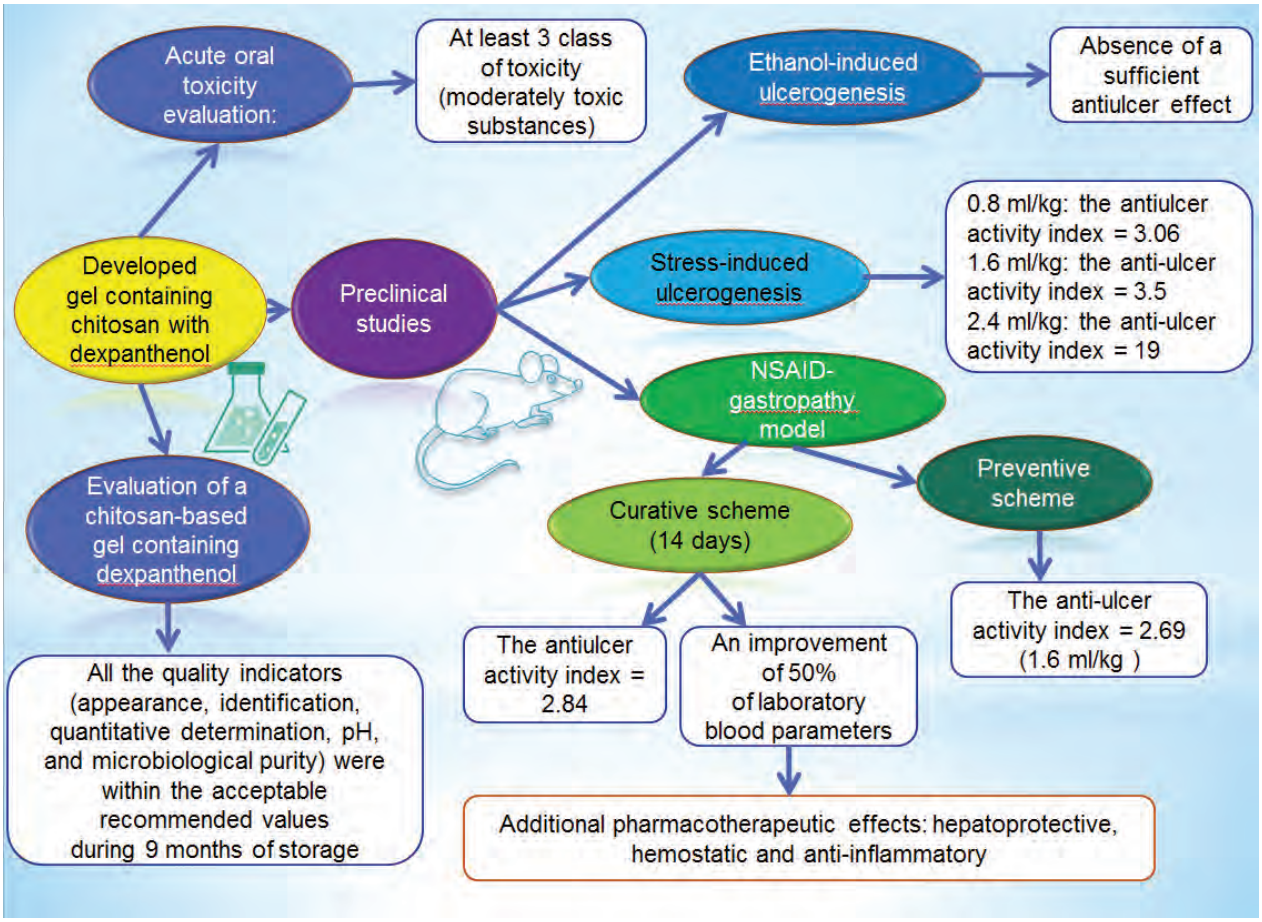
Introduction: Development of new gastroprotectants for treatment of acid-related digestive disorders remains an urgent task of gastroenterology and pharmacology, due to the wide prevalence of this category of diseases, as well as the problem of insufficient efficacy and safety of the existing regimens. The aim of this study is to develop a chitosan-based gel containing dexpanthenol, and experimentally evaluate its gastroprotective activity in preclinical studies.

Materials and methods: Preclinical studies were carried out on 4 different models of ulcer formation: NSAID gastropathy, 2 schemes (preventive or curative), ethanol-induced ulcerogenesis, and stress-induced ulcerogenesis. The gel under study containing chitosan and dexpanthenol was used in 3 doses (0.08, 0.16 and 0.24 ml/100 g of body weight).

Results and discussion: The chitosan-based gel containing dexpanthenol in models of NSAID gastropathy and stress-induced ulcerogenesis (preventive scheme) has a pronounced gastroprotective effect, exceeding the effect of sucralfate. In NSAID-gastropathy model (curative scheme), chitosan-based gel containing dexpanthenol has a gastroprotective effect exceeding that of omeprazole, and also reduces the manifestations of organotropic toxicity of diclofenac sodium, exhibiting, in addition to the gastroprotective effect, anti-inflammatory, hepatoprotective and hemostatic properties according to laboratory and histological studies.

Conclusion: The chitosan-based gel containing dexpanthenol is low toxic and has a pronounced gastroprotective effect in models of NSAID gastropathy (preventive and curative schemes) and stress-induced ulcerogenesis, which makes it promising for the prevention and treatment of ulcer formation in NSAID gastropathy and stress ulcers, in order to reduce the number and area of ulcerative defects, and to reduce the manifestations of organotropic toxicity of NSAIDs.

Graphical abstract:



Keywords

ethanol-induced ulcerogenesis, gastroprotectants, NSAID gastropathy, oral gel, pharmacology, preclinical studies, stress-induced ulcerogenesis.

Introduction

It is known that the existing groups of antiulcer drugs mainly suppress the secretory function of the gastric mucosa without having a gastroprotective effect, and also cause a number of serious side effects. Therefore, further development of new gastroprotective agents is relevant. It is promising to study the gastroprotective effect of such substances as: chitosan – a natural polysaccharide, and dexpanthenol – an alcoholic analogue of D-pantothenic acid. In the Russian Federation, chitosan is not registered as a pharmaceutical substance (State Pharmacopoeia of the Russian Federation, 14th edition), it is only used in cosmeceutics, dentistry, drug delivery systems and as a component of dietary supplements for general strengthening, hypocholesterolemic effects and improving the functional state of the gastrointestinal tract (Dutta et al. 2004; Subhapradha et al. 2013; Shehriar et al. 2017).

Depending on the physicochemical properties, chitosan has wound healing, antiulcer, anti-inflammatory, antioxidant, immunostimulatory, antibacterial, hemostatic, hepatoprotective, hypolipidemic, enterosorbent, and antitoxic effects (Fedosov 2017; Buzlama et al. 2020a; Buzlama et al. 2021).

Dexpanthenol having a significant list of pharmacological effects, such as wound healing, anti-inflammatory, antioxidant, hepatoprotective effects, can become a promising component for the development of new gastroprotective drugs (Li-Mei et al. 2016; Uysal et al. 2017).

The ability of chitosan to prevent ulcer formation was obtained only in some experimental studies (Ito et al. 2000); however, in these studies, certain dosage forms were not proposed, and only high doses were applied, which were not quite convenient for clinical use. In another study, in an NSAID-gastropathy model, the gastroprotective activity of a gel containing 1% high-viscosity chitosan at 3 different doses (0.8, 1.6, and 2.4 ml/kg) was

studied with a single oral prophylactic application, and as a results for this experiment, for the first time it was proved that chitosan-based gel at doses of 1.6 and 2.4 ml/kg has a high gastroprotective activity (the calculated value of the antiulcer activity index is 2.4 and 4.69, respectively), exceeding the effectiveness of the well-known reference drug (bismuth tripotassium dicitrate) (Buzlama et al. 2021).

The antiulcer activity of dexpanthenol has not been practically studied. On the other hand, the international application US 2008/0214619A1 (Wolfe et al. 2007) proposes the combined use of proton pump inhibitors such as omeprazole and dexpanthenol in order to induce a rapid onset of action, to increase the duration and to optimize the clinical efficacy of proton pump inhibitors. The protective effect of dexpanthenol in acetic acid-induced colitis in rats is also known; biochemical and histopathological improvements were revealed, probably due to its antioxidant effect (Cagin et al. 2016). So far, no studies have been conducted on the development of a gel containing chitosan in combination with dexpanthenol for the purpose of prevention and treatment of ulcer formation.

It is important that such a modern dosage form as gel has a number of advantages, including convenience, painlessness, ease of use, and the ability to form a thin film on the surface, providing mechanical protection and preventing microbial contamination (Anurova et al. 2016). The use of chitosan as a gel-forming base is promising.

Materials and methods

Experimental animals

The studies were carried out on the basis of the Faculty of Pharmacy of Voronezh State University (VSU); preclinical studies were carried out at the laboratory of the Department of Pharmacology and Clinical Pharmacology. The studies were carried out on 195 laboratory animals: 161 white outbred conventional male rats, weighing 200–220 g, and 34 white outbred conventional male mice, weighing 18–25 g, obtained from the Stolbovaya Branch of the Scientific Center of Biomedical Technologies of the Federal Medical Biological Agency. The studies were carried out in compliance with the ethical principles of animal experiments, taking into account the requirements of the European Convention for the Protection of Vertebrate Animals, and minutes №. 42-04 of 10.12.2018 of the Ethics Committee for Expertise in Biomedical Research of Voronezh State University.

Pharmaceutical substances and reagents

1. High-viscosity chitosan (Sigma-Aldrich, Japan) from crab shells with a deacetylation degree of 80% and a weight average molecular weight of 600 kDa.
2. Chemically pure pharmaceutical substances: dexpanthenol (JSC Vekton, Russia), glacial acetic acid (JSC Vekton, Russia).
3. Reference drugs: omeprazole, medicinal drug “Omeprazole-Akrikhin” (JSC Akrikhin, Russia), the contents of a 20 mg capsule were crushed and suspended in water and administered at the chosen doses; sucralfate, medicinal drug ”Venter” (JSC Krka, d.d., Novo mesto, Slovenia), tablets were crushed, suspended in water and administered at the chosen dose.

Development methods and physico-chemical research methods

At the stage of pharmaceutical development, quality standards for chitosan-based gel containing dexpanthenol were established for the following indicators: appearance, identification, quantitative determination, pH, and microbiological purity. The identification of the gel components (chitosan, dexpanthenol) was carried out using the IR spectroscopy method (on the basis of the Voronezh State University Centre for the Collective Use of Scientific Equipment), according to the method described in (Buzlama et al. 2020b). Quantitative determination of chitosan in the gel was studied by the gravimetric method, dexpanthenol – by high performance liquid chromatography (HPLC), on the basis of the Voronezh State University Centre for the Collective Use of Scientific Equipment, according to the method described in (Doba et al. 2021).

Acute toxicity study

The study of acute toxicity of the chitosan-based gel containing dexpanthenol was carried out in two series of experiments: single (once) and multiple (daily for 14 days) oral intragastric administration of the maximum allowable dose, which was 1.0 ml/each animal, according to the recommendations of the Preclinical Drug Research Guide (Mironov, 2012). In the single administration group, observations were made daily until day 14. In the multiple daily administration group, observations were made daily until the end of the experiment.

Gastroprotective effect study

The preclinical studies were carried out on 4 different models of ulcer formation: the NSAID-gastropathy model, 2 schemes (prevention, curative), ethanol-induced ulcerogenesis, and stress-induced ulcerogenesis.

Experimental design for NSAID-gastropathy

In the model of NSAID-gastropathy, 2 schemes were used – ”prevention” and ”curative”, depending on the scheme of administration of the studied drugs and the purpose of the application. In both schemes, gastric ulcers were induced by a single oral administration of sodium diclofenac (Diclofenac-Solopharm drug in the form of an ampoules of 25 mg/ml, Grotex, Russia) at a known ulcerogenic dose of 50 mg/kg (Buzlama et al. 2013). Prior to the experiment, the animals were deprived of food for 18 hours

with free access to water. The degree of ulceration of the gastric mucosa was assessed 3 hours after the administration of diclofenac sodium; euthanasia was carried out by an overdose of chloroform anesthesia. Animal stomachs were removed for histological examination immediately after dissection.

Scheme 1"prevention": screening for preventive gastroprotective action

In this scheme, 35 white outbred conventional male rats, weighing 220±20 g, were divided into 5 groups of 7 rats each. The studied chitosan-based gel containing dexpanthenol was administered once orally at doses of 0.8, 1.6 and 2.4 ml/kg of animal body weight 1 hour before the administration of diclofenac sodium. Sucralfate was used as a reference drug at a dose of 0.014 g/kg, equivalent to the therapeutic dose for humans.

Scheme 2 "curative": study of the pharmacotherapeutic effect (assessment of the gastroprotective effect and the prevention of manifestations of organotropic toxicity)

In this scheme, 70 white outbred conventional male rats, weighing 220±20 g, were divided into 4 groups: control, an intact group of 10 rats, and 2 experimental groups of 20 rats each. To study the pharmacotherapeutic activity of chitosan-based gel containing dexpanthenol in this model, a dose of 1.6 ml/kg of body weight was selected, which showed the greatest efficiency among the studied doses in all the other studied models. One of the experimental groups of animals was injected with the reference drug omeprazole at a dose of 3 mg/kg (Chatterjee et al. 2012). The studied drugs (omeprazole and the chitosan-based gel containing dexpanthenol) were administered to the animals of the experimental groups 1 hour before the administration of diclofenac sodium and then daily once a day for 14 days. Registration of mortality was carried out for 14 days. In the intact group (healthy animals), blood samples for laboratory tests and organs (stomach, liver, intestines) were taken on the first day for histological studies. In the control group, blood samples were taken for laboratory tests and organs (stomach, liver, intestines) for histological studies on days 1, 7, and 14. In the experimental groups (omeprazole, chitosan-based gel with dexpanthenol), blood samples were taken for laboratory tests and organs (stomach, liver, intestines) for histological studies on days 7 and 14.

Experimental design for ethanol-induced ulcerogenesis

This model was reproduced according to the well-known method described in (Hajrezaie et al. 2015) by oral administration of absolute ethanol 96% purity, in a volume of 0.5 ml/100 g of animal body weight (5 ml/kg). The study was conducted on 21 white outbred conventional male rats, weighing 220±20 g, divided into 3 groups of 7 animals each. Animals were subjected to food deprivation 24 h before the experiment with free access to water. An average dose of chitosan-based gel containing dexpanthenl was used, which showed gastroprotective activity in the model of NSAID-gastropathy: 1.6 ml/kg of body weight. The studied gel containing chitosan and dexpanthenol was administered intragastrically once 1 hour before ethanol. The reference drug omeprazole was administered once orally 1 hour before ethanol at a dose of 20 mg/kg of animal body weight (Segawa et al. 1987). The degree of ulceration of the gastric mucosa was assessed 1 hour after the administration of ethanol; euthanasia was carried out by an overdose of chloroform anesthesia.

Experimental design for stress-induced ulcerogenesis

Stress ulcers of the gastric mucosa were caused by a combination of a stress factor and hypothermia; for that, immobilization device was used in the dorsal position with isolation from extraneous noise at an air temperature of 3–7 °C in a refrigerator for 4 hours (Thabrew and Arawwawala 2016). This model was carried out on 35 white outbred conventional male rats, weighing 220±20 g, divided into 5 groups of 7 individuals each. Animals were subjected to food deprivation 18 hours before the experiment with free access to water. The studied gel containing chitosan with dexpanthenol was administered orally once at doses of 0.8, 1.6 and 2.4 ml/kg of animal body weight. The reference drug omeprazole was administered orally once at a dose of 20 mg/kg of animal body weight (Segawa et al. 1987). The studied gel and the reference drug were administered 1 hour before immobilization. The degree of ulceration of the mucous membrane was assessed immediately after the end of the immobilization period; euthanasia was carried out by an overdose of chloroform anesthesia. After euthanasia, pathological and anatomical studies were carried out, and the relative weight of the stomach, adrenal glands, thymus and spleen (g/kg of body weight) was determined.

Planimetric method for determining the area of ulcers on the gastric mucosa

In all the studied models, counting the number of ulcerative defects on the gastric mucosa of animals and determining the area of ulcers were carried out by the planimetric method using palettes with a scale-coordinate grid (Buzlama et al. 2012). The Paul's index was calculated based on the area of ulcers using the well-known formula (1) (Buzlama et al. 2017):

$$IP_S = \frac{S \times F\%}{100\%}, \quad (1)$$

where IP_S is the Paul's index by the ulcer area criterion, S is the total area of ulcers on average per animal, mm^2, F is the percentage of animals with ulcers in the group, %.

Based on the obtained values of the Paul's index in the control and experimental groups, the value of the antiulcer activity index was determined according to the well-known formula (2) (Buzlama et al. 2017):

$$AU = \frac{IP_k}{IP_e}, \quad (2)$$

where *AU* is the antiulcer activity index of the studied drug, IP_k is the Paul's index in the control group, IP_e is the Paul's index in the experimental group. An *AU* value over 2.0 indicates sufficient antiulcer activity of the studied drug.

Laboratory research methods

Blood sampling was carried out by heart puncture under chloroform anesthesia. Laboratory studies were carried out in an independent private laboratory, using a semi-automatic biochemical analyzer Clima MC-15 (RAL, Spain), an automatic hematology analyzer Drew-3 (Biocode Hycel Holdings SAS, France), and a coagulometer ACKa 2-01/"Astra" (Notice Group, Russia), by using diagnostic reagents of SPD Renam (Russia).

Pathoanatomical and histological research methods

The histological studies were carried out on the basis of Voronezh Regional Pathological and Anatomy Bureau. Necropsy, complete evisceration, and sampling of internal organs were performed to assess pathological changes, measure organ weights, and take samples for histological studies using well-known methods (Korzhevskiy and Gilyarov 2010; Suleymanov et al. 2012; Krivolapova 2019). After the measurement of the absolute mass of the internal organs, the calculation of the relative mass (g/kg) was carried out using the following formula (3):

$$W = \frac{a \times 1000}{b}, \quad (3)$$

where W – is the relative organ weight, g/kg, a – is the absolute organ weight, g, b – body weight, g.

Statistical analysis

The obtained primary data were subjected to statistical processing by generally accepted methods of mathematical statistics (Prozorovskiy 2007). Differences were considered statistically significant at a significance level of $P<0.05$; $P<0.01$; $P<0.001$. Statistical data processing was carried out using licensed programs Microsoft Office Excel 2019, and Statistica 10.0.

Results and discussion

Development and evaluation of a chitosan-based gel containing dexpanthenol

As a result of pharmaceutical development of a chitosan-based gel containing dexpanthenol, for the first time a homogeneous transparent gel with pH≥5 was prepared, stable during storage for at least 9 months, containing 1% chitosan, 0.43% dexpanthenol and 0.25% of glacial acetic acid.

The quality standards of the chitosan-based gel containing dexpanthenol were established for the following indicators: appearance, identification, quantitative determination, pH, and microbiological purity, all these quality indicators were within the acceptable recommended values over 9 months of storage (Table 1).

Acute oral toxicity evaluation:

The study of acute toxicity of chitosan-based gel containing dexpanthenol was carried out by single and multiple oral intragastric administration of the maximum allowable dose (1 ml/each animal). In both groups, mortality and changes in the clinical condition of the animals were not observed during the observation period. According to pathological and anatomical studies, no significant changes in the mass of internal organs were found (Table 2). The calculation of the maximum tolerated doses of chitosan and dexpanthenol was carried out based on their concentration in the gel formulation (chitosan 1%, which is 10 mg/ml and dexpanthenol 0.43% – 4.3 mg/ml) and the maximum tolerated value of the gel 50.76 ml/kg. Maximum tolerated dose of chitosan: 507.6 mg/kg. Maximum tolerated dose of dexpanthenol: 218.2 mg/kg. The estimated therapeutic dose (once) of the chitosan-based gel containing dexpanthenol: 1.6 ml/kg. Suggested therapeutic dose of chitosan: 16 mg/kg. Suggested therapeutic dose of dexpanthenol: 6.88 mg/kg. Considering that lethal doses could not be reached, the estimated single therapeutic dose of the gel is 1.6 ml/kg (chitosan 16 mg/kg, dexpanthenol 6.88 mg/kg), and the maximum tolerated value of the gel is 50.76 ml/kg, the estimated range values of the therapeutic index can be: estimated Therapeutic Index (TI): 50.76/1.6=31.75. Thus, chitosan-based gel containing dexpanthenol belongs to at least 3rd class of toxicity (moderately toxic substances), exceeding the expected therapeutic dose by 31.75 times is safe.

Screening of the preventive gastroprotective effect of chitosan-based gel containing dexpanthenol in NSAID-gastropathy model

For pharmacological studies, the estimated average therapeutic dose of the chitosan-based gel containing dexpanthenol was chosen – 1.6 ml/kg of animal body weight; this dose was calculated based on the known LD_{50} of chitosan and it is equivalent to 1/1000 LD_{50}. Based on the average therapeutic dose, the minimum and maximum therapeutic doses of the studied gel were selected as the following: 0.8 and 2.4 ml/kg of animal body weight. In NSAID-gastropathy model, the gastroprotective activity of chitosan-based gel containing dexpanthenol was administered orally once at 3 different doses – 0.8, 1.6, 2.4 ml/kg (Table 3). It was proved for the first time that chitosan-based gel containing dexpanthenol at the studied dose of 1.6 ml/kg exhibits the maximum gastroprotective activity (anti-ulcer activity index was 2.69), a decrease in the number of ulcers by

Table 1. Evaluation of the quality standards of the developed chitosan-based gel containing dexpanthenol

Shelf life, months	Appearance	Identification	Quantitative determination		pH	Microbiological purity
	Homogeneous transparent gel	IR spectroscopy 4000–1000 cm^{-1}	Dexpanthenol 0.408–0.451 g	Chitosan 0.95–1.05 g	4.0–6.0	In 1 g no more than 100 bacteria and fungi
0	+	+	0.435±0.02	1.00±0.01	5.26±0.004	+
3	+	+	0.433±0.03	1.00±0.01	5.30±0.008	+
6	+	+	0.433±0.02	1.01±0.02	5.28±0.006	+
9	+	+	0.431±0.04	0.99±0.04	5.32±0.002	+

Table 2. Relative mass of internal organs after a single and multiple administration of the studied chitosan-based gel containing dexpanthenol

Weight of organs, g/kg	Intact	CD, single administration		CD, multiple administration	
	Average value	Average value	Difference with intact, %	Average value	Difference with intact, %
Stomach	23.68±2.812	19.63±1.444	-17.1%	20.44±1.681	-13.7%
Liver	62.16±2.378	67.39±3.490	8.4%	57.53±2.469	-7.4%
Kidneys	19.47±1.035	19.08±0.780	-2.0%	17.00±0.763	-12.7%
Heart	7.15±0.327	7.02±0.283	-1.9%	8.01±0.309	11.9%
Lungs	8.79±0.476	8.46±0.506	-3.7%	9.54±0.347	8.5%
Spleen	4.09±0.512	3.73±0.256	-8.8%	3.48±0.227	-14.9%
Brain	9.78±0.528	10.31±0.423	5.4%	10.74±0.403	9.8%
Testicles	5.67±0.344	6.00±0.330	5.8%	6.17±0.360	8.8%

Note: CD – chitosan-based gel containing dexpanthenol.

Table 3. Evaluation of the gastroprotective activity of chitosan-based gel containing dexpanthenol in NSAID-gastropathy model

Group	Number of ulcers, pieces	Area of ulcers, mm^2	Number of animals with ulcers, %	Paul's index	The antiulcer activity index
Control	14.82±1.225	46.94±5.792	100	46.94	–
Reference drug – sucralfate	9.64±1.323**	36.09±12.645	100	36.09	1.3
Difference from control, %	-35.0	-23.1	–	–	–
CD at 0.8 ml/kg	13.40±1.860	26.80±4.779*	100	26.80	1.75
Difference from control, %	-9.6	-42.9	–	–	–
CD at 1.6 ml/kg	12.60±1.6	17.40±4.02***	100	17.40	2.69
Difference from control, %	-15	-62.9	–	–	–
CD at 2.4 ml/kg	12.8±2.922	27.4±7.061*	100	27.40	1.7
Difference from control, %	-13.7	-41.6	–	–	–

Note: * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, CD – chitosan-based gel containing dexpanthenol.

15.0%, and a significant (P<0.001) decrease in the area of ulcers by 62.9% were found, exceeding the effectiveness of the well-known reference drug sucralfate (the value of the antiulcer activity index–1.3).

According to the pathological and anatomical studies in the experimental groups, a significant decrease in the number and area of ulcerative defects, as well as a decrease in hyperemia of the gastric mucosa were revealed (Fig. 1). According to histological studies, the oral administration of chitosan-based gel containing dexpanthenol resulted in a decrease in the number and depth of ulcerative defects, and also exhibited signs of a moderate decrease in the intensity of inflammation, which confirms the data of the conducted pathological and anatomical studies.

Comparative evaluation of the gastroprotective effect of chitosan-based gel containing dexpanthenol in ethanol-induced ulcerogenesis

In this model, the antiulcer activity index for the studied gel, administered orally once at a dose of 1.6 ml/kg of body weight, did not exceed 2, which characterizes the absence of a sufficient antiulcer effect (Table 4).

In the model of an ethanol-induced ulcerogenesis, the developed chitosan-based gel containing dexpanthenol does not have sufficient gastroprotective activity. Probably, the lack of a gastroprotective effect in this model is due to the pharmacokinetic interaction between the studied gel containing chitosan with dexpanthenol and ethanol in the stomach. It is possible that mixing with ethanol leads to the dissolution of primary associates in the form of liquid micro- and nanocrystals of chitosan. This effect can cause a decrease in viscosity and changes in the physicochemical properties of the chitosan-based gel (Uspenskii et al. 2010). Thus, the importance of maintaining the viscosity of the developed gel for the implementation of the mechanism to prevent the ulcer formation was revealed. Probably, the combined use of chitosan gel and ethyl alcohol should be avoided, including recommending that patients refrain from drinking alcoholic beverages during treatment.

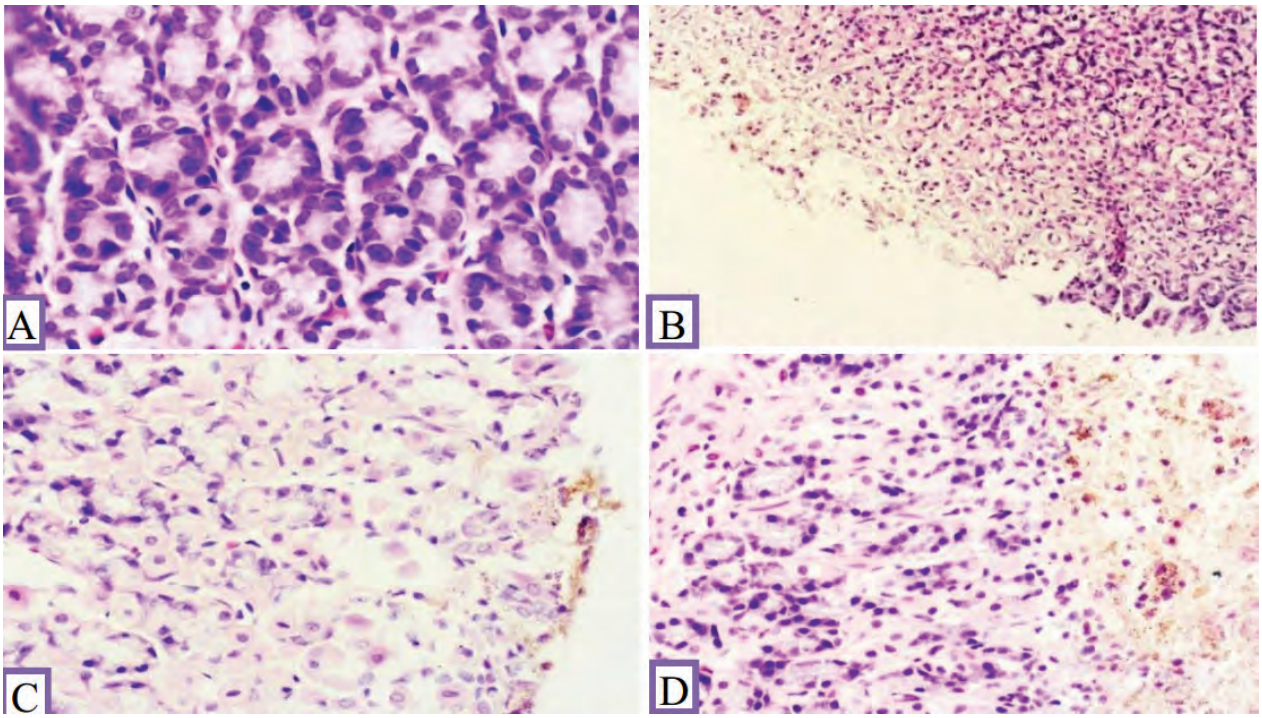


Figure 1. Histological Study Of The Gastric Mucosa In Nsaid-Gastropathy Model. Hematoxylin-Eosin Stain: Intact Group (**A**), Control Group (**B**), Group Of Chitosan-Based Gel Containing Dexpanthenol (**C**), Group Of The Reference Drug Sucralfate (**D**).

Table 4. Evaluation of the gastroprotective activity of chitosan-based gel containing dexpanthenol in ethanol-induced ulcerogenesis

Group	Number of ulcers, pieces	Area of ulcers, mm²	Number of animals with ulcers, %	Paul's index	Antiulcer activity index
Control	13.83±1.327	105.67±14.163	100%	105.67	–
Omeprazole	8.00±2.098	46±18.482*	100%	46	2.18
Difference from control, %	72.9	-56.4	–	–	–
CD at 1.6 ml/kg	10.17±1.167	65.67±13.928	100%	65.67	1.6
Difference from control, %	-26.5	-37.9	–	–	–

Note: * – P<0.05 – significant difference versus control, CD – chitosan-based gel containing dexpanthenol.

Comparative evaluation of the gastroprotective effect of chitosan-based gel containing dexpanthenol in stress-induced ulcerogenesis

It was found that in this model, the reference drug omeprazole showed highly pronounced gastroprotective activity (the calculated value of the antiulcer activity index was 209.09) (Table 5). After the administration of the chitosan-based gel containing dexpanthenol at a dose of 0.8 ml/kg of body weight, the calculated value of the antiulcer activity index was 3.06, which confirms a pronounced gastroprotective effect. For a dose of 1.6 ml/kg, the calculated value of the anti-ulcer activity index was 3.5, and for a dose of 2.4 ml/kg, the calculated value of the anti-ulcer activity index was 19, which indicates a high gastroprotective activity and allows us to discern the presence of a direct proportional relationship between an increase in the dose of chitosan-based gel containing dexpanthenol and the degree of the gastroprotective effect. It is important to notice that although the value of the antiulcer activity index for the group treated with omeprazole was 209.09, and for the group treated with chitosan-based gel containing dexpanthenol at a dose of 2.4 ml/kg, it was 19, according to the ulcer area criterion, the effectiveness was comparable, for the group of omeprazole, the decrease in the ulcer area was by 97.08% and for the group of chitosan-based gel with dexpanthenol – by 92% smaller than in the control group; a large value of the index of antiulcer activity for the omeprazole group was due to the significant decrease in the number of animals with ulcers. According to pathological and anatomical studies in the groups treated with omeprazole and chitosan-based gel containing dexpanthenol, a significant decrease in the number and area of ulcers was revealed. Chitosan-based gel containing dexpanthenol at a dose of 0.16 ml was the most effective, helping to prevent stress involution of the adrenal glands and reciprocal hypertrophy of the thymus comparing with the control group.

Study of the pharmacotherapeutic effect of chitosan-based gel containing dexpanthenol in NSAID-gastropathy model (curative scheme)

Due to the fact that in all the studied models, the dose of 1.6 ml/kg had enough efficiency among the studied

Table 5. Evaluation of the gastroprotective activity of chitosan-based gel containing dexpanthenol in stress-induced ulcerogenesis

Group	Number of ulcers, pieces	Area of ulcers, mm²	Number of animals with ulcers, %	Paul's index	Antiulcer activity index
Control	9.89±1.559	23.00±4.472	100%	23	–
Omeprazole	1.00±1.00**	0.67±0.667***	16.6%	0.11	209.09
Difference from control, %	-89.88	-97.08	–	–	–
CD at 0.8 ml/kg	4.17±1.447*	7.50±3.585*	100%	7.50	3.06
Difference from control, %	-57.9	-67.4	–	–	–
CD at 1,6 ml/kg	6.29±1.948	6.43±1.325**	100%	6.43	3.5
Difference from control, %	-36.4	-72.0	–	–	–
CD at 2.4 ml/kg	2.00±1.238**	1.83±1.078***	66.6%	1.21	19
Difference from control, %	-79.8	-92	–	–	–

Note: * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, CD – chitosan-based gel containing dexpanthenol.

doses of the chitosan-based gel containing dexpanthenol, it was chosen to study its pharmacotherapeutic effect by a daily short-term course of treatment against NSAID-gastropathy, to assess the gastroprotective effect and the prevention of manifestations of organotropic toxicity of diclofenac sodium. In the model of NSAID gastropathy in the curative scheme, administration of chitosan-based gel containing dexpanthenol reduced mortality by 21.6% comparing with the control group. Treatment with chitosan-based gel containing dexpanthenol once daily for 14 days provided a significant decrease in both the area of ulcers by 64.8% (P<0.05) and the number of ulcers by 50.4% comparing with the control group; the calculated value of the antiulcer activity index was 2.84, which indicates a pronounced gastroprotective activity and exceeds the effectiveness of omeprazole at a dose of 3 mg/kg in this model (Table 6).

An important effect on the biochemical signs of organotropic toxicity of diclofenac sodium was exerted by the chitosan-based gel containing dexpanthenol, which contributed to the normalization of 50% of biochemical parameters, namely alanine transaminase ALT (P<0,001) and aspartate aminotransferase AST (P<0.05) (Table 7), which confirms the well-known hepatoprotective effect of chitosan and dexpanthenol (Ozcelik et al. 2014; Uysal et al. 2017).

Chitosan-based gel containing dexpanthenol provided a significant important increase in the reduced content of erythrocytes RBC (P<0.05) and the width of the distribution of erythrocytes RDW (Day 14, P<0.01), which indicates a decrease in signs of ulcerative bleeding. It seems to be an important advantage that the effectiveness of chitosan as a hemostatic agent can contribute to the relief of ulcerative bleeding (Pogorielov and Sikora 2015). At the same time, chitosan-based gel containing dexpanthenol significantly reduced the number of leukocytes WBC (P<0.001) and normalized the erythrocyte sedimentation rate ESR (P<0.001), which indicates a decrease in nonspecific signs of inflammation, which is known for the both components of the gel – chitosan and dexpanthenol (Friedman et al. 2013; Davydova et al. 2016; Li-Mei et al. 2016). There was no significant effect of chitosan-based gel containing dexpanthenol on hemoglobin Hgb, hematocrit Hct, mean corpuscular hemoglobin MCH, mean corpuscular volume MCV, mean corpuscular hemoglobin concentration MCHC, Color index, platelets, segmentonuclear neutrophils, lymphocytes, and the mean platelet volume MPV. In general, it was found that chitosan-based gel containing dexpanthenol improved and normalized the values of 4 out of 14 indicators of the general blood test, which is more than a quarter – 28.5% (Table 8).

The results of the studies of the hemostatic system showed that chitosan-based gel containing dexpanthenol normalized all the 6 out of 6 parameters (international normalized ratio INR, prothrombin index PTI, partial thromboplastin time PTT, activated partial thromboplastin time aPTT, fibrinogen, and thrombin time), confirming the decrease in the signs of ulcerative bleeding (Table 9). As a result, chitosan-based gel containing dexpanthenol provided an improvement in 12 out of 24 parameters, which is 50% of laboratory blood parameters, confirming the decrease in the manifestations of organotropic toxicity.

According to histological studies, chitosan-based gel containing dexpanthenol after 14 days of daily administration reduced the signs of ulceration, infiltration and periulciric gastritis, edema and inflammatory infiltration in the tissues of the small intestine, as well as signs of necrosis, dystrophic and necrobiotic changes, abscesses in the liver tissue, which in general indicates a gastroprotective and hepatoprotective effect, confirming the data of pathological, anatomical and biochemical studies (Figs 2, 3).

In models of NSAID-gastropathy and stress-induced ulcerogenesis (preventive scheme), the leading component of the mechanism of the gastroprotective effect of chitosan, which is a polysaccharide in terms of its chemical structure; is probably that chitosan in the gel dosage form exhibits properties similar to mucus glycoproteins, providing protection of the gastric mucosa from damaging exposure to hydrochloric acid and pepsin. On the other hand, in the model of NSAID-gastropathy (curative scheme), chitosan, as a compound containing amino groups, forms polyvalent bridges between its positive charges and the negative charges of sulfated mucin or glycosaminoglycans formed on the surface of ulcers, which also provides protection for the gastric mucosa, especially in the area of ulcerative lesions (Ito et al. 2000; Buzlama et al. 2021).

Table 6. The study of the pharmacotherapeutic effect of chitosan-based gel containing dexpanthenol in the model of NSAID-gastropathy (assessment of the gastroprotective effect)

Group	Number of ulcers, pieces	Area of ulcers, mm²	Number of animals with ulcers, %	Paul's index	Antiulcer activity index
Control 1 day	14.60±2.205	57.80±7.774	100%	57.80	–
Control 7 day	9.50±1.848	46.00±5.583	100%	46.00	–
Difference from control 1 day, %	-34.9	-20.4	–	–	–
Control 14 day	6.33±1.054**	38.17±7.476	100%	38.17	–
Difference from control 1 day, %	-56.6	-34.0	–	–	–
Difference from control 7 day, %	-33.3	-17.0	–	–	–
Omeprazole 1 day	23.50±8.50	44.50±2.500	100%	44.50	1.29
Difference from control 1 day, %	61.0	-23.0	–	–	–
Omeprazole 7 day	6.50±0.847	30.33±4.402#	100%	30.33	1.51
Difference from control 7 day, %	-31.6	-34.1	–	–	–
Difference from omeprazole 1 day, %	-72.3	-31.8	–	–	–
Omeprazole 14 day	4.71±0.644	22.29±3.099##	100%	22.29	1.71
Difference from control 14 day, %	-25.6	-41.6%	–	–	–
Difference from omeprazole 1 day, %	-79.9	-49.9	–	–	–
CD 1.6 ml/kg 1 day.	13.33±2.028	26±1.155**	100%	26.00	2.22
Difference from control 1 day, %	-8.7	-55	–	–	–
CD 1.6 ml/kg 7 day.	6.83±0.946	19.50±2.247**.#	100%	19.50	2.35
Difference from control 7 day, %	-28.1	-57.6	–	–	–
Difference from CD 1 day, %	-48.8	-25.0	–	–	–
CD 1.6 ml/kg 14 day	3.14±0.261*.##	13.43±2.298*.##	100%	13.43	2.84
Difference from control 14 day, %	-50.4	-64.8	–	–	–
Difference from CD 1 day, %	-76.4	-48.4	–	–	–

Note: * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, # – P<0.05, ## – P<0.01, ### – P<0.001 – significant difference versus the value at the first day of the studied group, CD – chitosan-based gel containing dexpanthenol.

Table 7. Evaluation of the prevention of manifestations of organotropic toxicity during the pharmacotherapeutic administration of chitosan-based gel containing dexpanthenol in the model of NSAID-gastropathy (biochemical parameters)

Group	ALT, units per liter	AST, units per liter	Urea, mmol/L	Creatinine, μmol/L
Intact	53.67±4.372	72±3.786	5.41±1.463	53.67±1.33
Control 1 day	27.75±3.01+++	87.50±8.098	7.02±0.743	42.75±1.843++
Difference from intact, %	-48.3	21.5	29.7	-20.3
Control 7 day	61±14.64*	76.67±8.452	6.82±0.185	41.33±5.667
Difference from intact, %	13.7	6.5	26.1	-23
Difference from control 1 day, %	-119.8	-12.4	-2.8	-3.3
Control 14 day	83.5±2.598++	130.7±27.406*	5.25±0.401**	49.25±3.705
Difference from intact, %	55.6	81.6	-3	-8.2
Difference from control 7 day, %	36.6	76.67	-23.1	19.2
Omeprazole 7 day	53.67±3.844	75±3.606	5.31±0.450**	51±2.309
Difference from intact, %	0.0	4.2	-1.8	-5
Difference from control 7 day, %	-12.0	-2.2	-22.1	23.4
Omeprazole 14 day	58.67±4.055***	64.33±2.963*	6.44±0.491*	41±5.132
Difference from intact, %	9.3	-10.6	19	-23.6
Difference from control 14 day, %	-29.7	-50.8	22.7	-16.8
CD 1.6 ml/kg 7 day	41.0±19.0	60.33±4.33	5.17±1.175	51±2.309
Difference from intact, %	-23.6	-16.2	-4.4	-5
Difference from control 7 day, %	32.8	-21.3	-24.2	26.4
CD 1.6 ml/kg 14 day	49.50±4.406***	67±1.826*	5.08±0.081	47.00±0.00
Difference from intact, %	-7.8	-6.9	-6.1	-12.4
Difference from control 14 day, %	-40.7	-48.8	-3.1	-4.6

Note: + – P<0.05, ++ – P<0.01, +++ – P<0.001 – significant difference versus intact, * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, CD – chitosan-based gel containing dexpanthenol, ALT – alanine transaminase, AST – aspartate aminotransferase.

It is known that the use of antioxidants helps to reduce the negative effects of free radicals in the process of ulcer formation (Hassan et al. 1998). Dexpanthenol, as an antioxidant, increases the levels of reduced glutathione (GSH), coenzyme A and enhances ATP synthesis (Li-Mei et al. 2016; Gorski et al. 2020). In addition, dexpanthenol markedly inhibits lipopolysaccharide (LPS)-induced neutrophiles influx, protein leakage, and release of tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (Li-Mei et al. 2016). These processes play an important role in protecting cells from oxidative stress, which in turn affects the reduction of the inflammatory response and leads to an

Table 8. Evaluation of the prevention of manifestations of organotropic toxicity during the pharmacotherapeutic administration of chitosan-based gel containing dexpanthenol in the model of NSAID-gastropathy (general blood test)

Group	RBC, $\times 10^{12}$/L	Hgb, g/L	Hct, %	RDW, %	WBC, $\times 10^{9}$/L	ESR, mm/hr	Lymphocytes, %
Intact	6.28± 0.773	117.67±14.38	35.13±4.116	17.37±0.636	10.97±3.862	9.33±0.882	82.63±4.074
Control 1 day	4.71± 0.44^{+}	90.5±9.64	25.73±2.65^{+}	18.63±0.515	9.08±2.338	1.5±0.289^{+++}	52.85±4.365^{+++}
Difference from intact, %	-25.0	-23.1	-26.8	7.2	-17.2	-83.9	-36
Control 7 day	5.17±0.664	94.67±8.838	29.6±1.997	22.83±1.244$^{++,*}$	24.07±5.817^{+}	1.33±0.33^{+++}	67.53±1.77^{++}
Difference from intact, %	-17.7	-19.5	-15.7	31.5	119.4	-85.7	-18.3
Difference from control 1 day, %	9.8	4.6	15.1	22.6	165	-11.1	27.8+
Control 14 day	5.84±0.712	108.0±19.218	31.6±4.803	20.73±1.129^{+}	21.33±1.084^{+}	1.17±0.441^{+++}	71.83±7.201
Difference from intact, %	-7.1	-8.2	-10.1	19.4	94.5	-87.5	-13.1
Difference from control 7 day, %	12.8	14.1	6.8	-9.2	-11.4	-12.5	6.4
Omeprazole 7 day	9.74±1.133**	176±21.079**	50.5±5.039**	16.9±0.173***	13.37±3.626	1.33±0.333^{++}	64.6±1.115^{+}
Difference from intact, %	55.1	49.6	43.7	-2.7	21.9	-85.7	-21.8
Difference from control 7 day, %	88.3	85.9	70.6	-26	-44.5	0.0	-4.3
Omeprazole 14 day	7.45±0.245*	137±4.933	40.33±1.477	17.07±0.133**	11.23±3.269**	6.67±1.453**	78.93±1.133
Difference from intact, %	18.6	16.4	14.8	-1.7	2.4	-28.6	-4.5
Difference from control 14 day, %	27.7	26.9	26.6	-17.7	-47.3	470	9.9
CD 1.6 ml/kg 7 day	6.73±1.121	124.67±24.673	36.77±6.722	21.23±1.220	15.83±3.530	3.67±1.202$^{*,+}$	70.1±3.535
Difference from intact, %	7.2	5.9	4.6	22.3	44.4	-60.7	-15.2
Difference from control 7 day, %	30.2	31.7	24.2	-7	-34.2	175.9	3.8
CD 1.6 ml/kg 14 day	7.40±0.249*	125±2.887	37.33±0.713	16.5±0.874**	11.5±0.945***	6.00±0.577***	75.87±5.741
Difference from intact, %	17.7	6.2	6.3	-5	4.9%	-35.7	-8.2
Difference from control 14 day, %	26.7	15.7	18.1	-20.4	-46.1	412.8	5.6

Note: $^{+}$ – P<0.05, $^{++}$ – P<0.01, $^{+++}$ – P<0.001 – significant difference versus intact, * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, RBC – red blood cell count, Hgb – hemoglobin, Hct – hematocrit, RDW – the width of the distribution of erythrocytes, WBC – white blood cell count, ESR – erythrocyte sedimentation rate, CD – chitosan-based gel containing dexpanthenol.

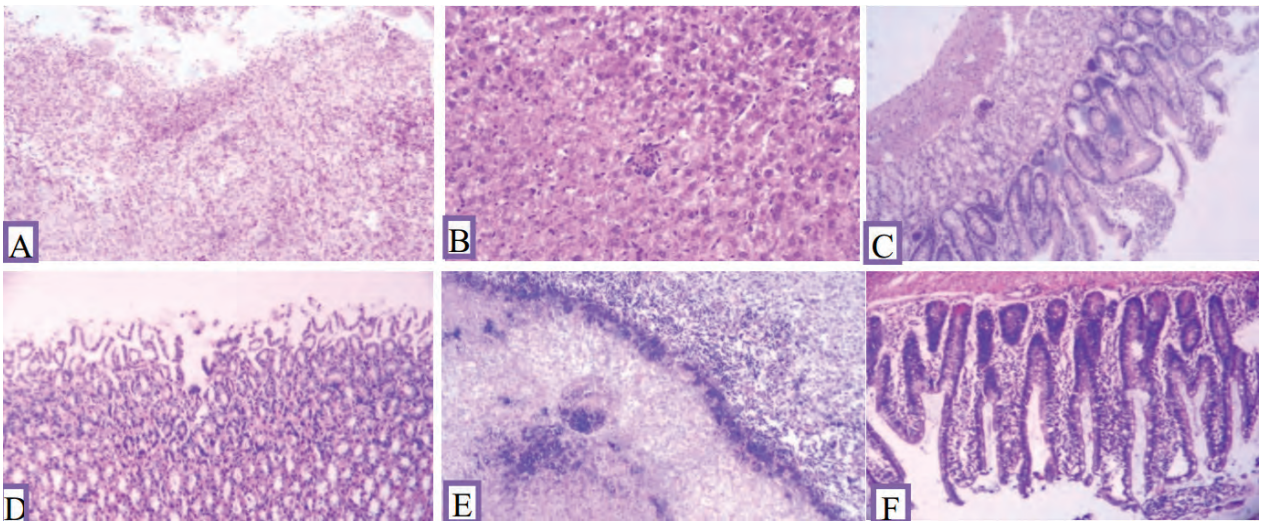


Figure 2. Control Group. **A.** Gastric Mucosa Day 7; **B.** Liver Day 7; **C.** Small Intestine Day 7; **D.** Gastric Mucosa Day 14; **E.** Liver Day 14; **F.** Small Intestine Day 14.

acceleration of the healing process of ulcers due to the synergistic antioxidant and anti-apoptotic effect. In addition, the presence of a combination gel of chitosan with dexpanthenol in the composition of the dosage form should probably provide a prolongation of the action of dexpanthenol on the gastric mucosa and increase efficiency.

It is known that the absorption of chitosan in the intestine increases by decreasing molecular weight and increasing water solubility. However, even high molecular weight chitosan is subject to be partial absorption (Zeng et al. 2008). Therefore, along with the systemic effect of dexpanthenol, a systemic effect of chitosan is possible, which explains the hepatoprotective and gastroprotective effect of the chitosan-based gel containing dexpanthenol revealed in this study for the prevention and curation of NSAID-gastropathy and stress-induced ulcerogenesis.

Table 9. Evaluation of the prevention of manifestations of organotropic toxicity during the pharmacotherapeutic administration of chitosan-based gel containing dexpanthenol in the model of NSAID-gastropathy (indicators of the blood coagulation system)

Group	PTT, sec.	PTI, %	INR	Fibrinogen, g/L	aPTT, sec.	Thrombin time, sec.
Intact	17.13±0.371	82.33±1.856	1.24±0.00	1.73±0.033	11.33±0.296	14.93±0.784
Control 1 day	16.03±0.423^{+}	88.00±2.483	1.18±0.038	1.25±0.087^{+++}	16.35±0.851^{+++}	13.05±1.063
Difference from intact, %	-6.5	6.9	-5.2	-27.9	44.3	-12.6
Control 7 day	19.8±0.709$^{++,*}$	71.33±2.848$^{*,++}$	1.50±0.075$^{*,++}$	4.27±0.186***	28.8±2.666$^{*,+++}$	12.87±0.801^{+}
Difference from intact, %	15.6	-13.4	21	146.8	154.1	-13.8
Difference from Control 1 day, %	23.6	-18.9	27.7	241.6	76.1	1.4
Control 14 day	14.53±0.923$^{*,+}$	98.00±5.817$^{*,+}$	1.03±0.075$^{*,+}$	2.80±0.191$^{**,+++}$	14.88±1.016$^{**,++}$	12.38±0.765^{+}
Difference from intact, %	-15.2	19	-16.9	61.5	31.3	-17.1
Difference from control 7 day, %	-26.6	37.4	-31.3	-34.4	-48.4	-3.8
Omeprazole 7day	17.4±0.651*	81.33±3.333*	1.28±0.087*	4.10±0.153^{+++}	14.00±0.702$^{***,+}$	14.00±1.305
Difference from intact, %	1.6	-1.2	3.5	136.9	23.5	-6.2
Difference from control 7 day, %	-12.1	14	-14.4	-3.9	-51.4	8.8
Omeprazole 14 day	16.63±0.384*	85.00±2.082*	1.20±0.043*	1.7±0.1***	9.83±0.176$^{***,+}$	15.27±0.570**
Difference from intact, %	-2.9	3.2	-3.5	-1.9	-13.2	2.2
Difference from control 14 day, %	14.5	-13.3	16.2	-39.3	-33.9	23.4
CD 1.6 ml/kg 7 day	17.50±0.529*	80.67±2.333*	1.33±0.043*	3.7±0.306^{++}	13.3±0.985***	13.53±1.213
Difference from intact, %	2.1	-2	7	113.8	17.4	-9.4
Difference from control 7 day, %	-11.6	13.1	-11.6	-13.3	-53.8	5.2
CD 1.6 ml/kg 14 day	17.63±1.195*	75.75±6.303*	1.36±0.092*	1.60±0.091***	10.18±0.873**	14.25±0.704*
Difference from intact, %	2.9	-8	9.9	-7.7	-10.2	-4.6
Difference from control 14 day, %	21.3	-22.7	32.3	-42.9	-31.6	15.2

Note: $^{+}$ – P<0.05, $^{++}$ – P<0.01, $^{+++}$ – P<0.001 – significant difference versus intact, * – P<0.05, ** – P<0.01, *** – P<0.001 – significant difference versus control, PTT – partial thromboplastin time, PTI – prothrombin index, INR – international normalized ratio, aPTT – activated partial thromboplastin time.

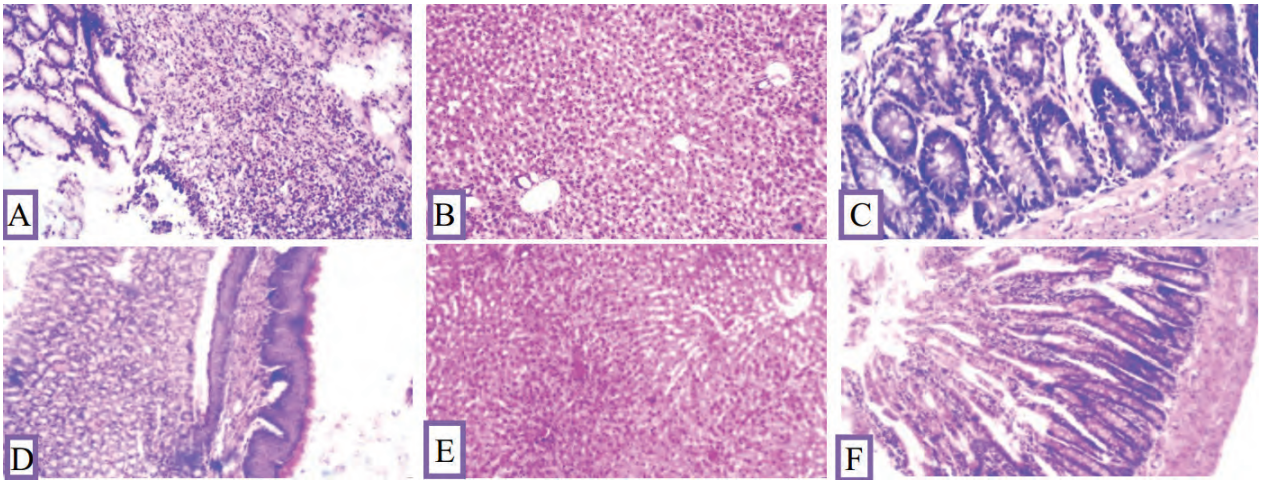


Figure 3. Group Of Chitosan-Based Gel Containing Dexpanthenol. **A.** Gastric Mucosa Day 7; **B.** Liver Day 7; **C.** Small Intestine Day 7; **D.** Gastric Mucosa Day 14; **E.** Liver Day 14; **F.** Small Intestine Day 14.

In view of the fact that chitosan-based gel containing dexpanthenol was quite effective in two (NSAID-gastropathy and stress-induced ulcerogenesis) of three different models of ulcer formation, it should be concluded that its gastroprotective activity is due not only to local gastroprotective and hemostatic effects, but also to systemic effects of dexpanthenol (and partly chitosan), implemented through anti-inflammatory, hepatoprotective, and possibly antioxidant mechanisms.

Consequently, for the first time it has been proven that the developed chitosan-based gel containing dexpanthenol is a promising pharmaceutical form for the prevention and curation of ulceration in the gastrointestinal tract, characterized by low toxicity, and in addition to the gastroprotective effect, the ability to provide additional pharmacotherapeutic effects such as hepatoprotective, hemostatic and anti-inflammatory effects.

Conclusion

For the first time, the optimal composition of a gel was developed containing 1% high-viscosity Sigma-Aldrich chitosan with 0.43% dexpanthenol for oral administration, with pH 5.47, stable during storage for 9 months at T 15–25 °C.

For the first time, the gastroprotective effect of the developed chitosan-based gel containing dexpanthenol was revealed in preclinical studies in models of NSAID-gastropathy

(prevention and curative schemes), ethanol and stress-induced ulcerogenesis; low toxicity was proved (presumed toxicity class III – moderately toxic substances). It was also proven that chitosan-based gel containing dexpanthenol has a pronounced gastroprotective activity in the model of NSAID-gastropathy with a single prophylactic use, surpassing the efficiency of the reference drug (sucralfate) and with a short-term course of administration for 14 days, surpassing the efficiency of the reference drug omeprazole, and also reduces the manifestations of organotropic toxicity of diclofenac sodium and exhibits, in addition to gastroprotective action, anti-inflammatory, hepatoprotective and hemostatic properties. A pronounced dose-dependent gastroprotective activity of the studied chitosan-based gel containing dexpanthenol was also proven with a single prophylactic use in the model of stress-induced ulcerogenesis.

The obtained results correlate with the literature data on the presence of the gastroprotective effect of chitosan; at the same time, for the first time we found that when chitosan and dexpanthenol used together as part of an oral dosage form (gel formulation) in models of NSAID-gastropathy and stress-induced ulcerogenesis exhibit a pronounced gastroprotective effect and reduce signs of organotropic toxicity of diclofenac sodium.

It is promising to use a 1% chitosan-based gel containing 0.43% dexpanthenol for the prevention and treatment of ulceration in NSAID gastropathy and stress-induced ulcerogenesis in order to reduce the number and area of ulcerative defects, and reduce the manifestations of organotropic toxicity of non-steroidal anti-inflammatory drugs NSAIDs. The suggested dosing regimen are the following:

- for planned prevention of ulcer formation: orally 1 hour before taking NSAIDs or before an expected stressful situation at a dose of 1.6 ml/kg (chitosan 16 mg/kg, dexpanthenol 6.88 mg/kg) per dose;
- in the complex treatment of NSAID-gastropathy: orally daily at a dose of 1.6 ml/kg (chitosan 16 mg/kg, dexpanthenol 6.88 mg/kg) 1–5 times/day, within 14 days.

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