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**PRECLINICAL STUDY OF PHARMACOLOGICAL ACTIVITY
OF ENTEROSORBENTE ON THE BASIS OF
MONTMORILLONITE**

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Abstract

Introduction: At present, enterosorbents based on mineral raw materials are in high demand among the population. However, there are no enterosorbents on the Russian pharmaceutical market on the basis of domestic mineral raw materials.

Objectives: to study the pharmacological activity of enterosorbent based on montmorillonite of Russian origin under experimental conditions.

Methods: The methodological approach was based on the implementation of a complex of theoretical, pharmacological, toxicological, histological, biochemical, statistical methods. Models of experimental diarrhea, acute and toxic liver damage, acute experimental pancreatitis were selected.

Results and discussion: Enterosorbent based on montmorillonite Crim_04 has a dose-dependent antidiarrhoeal effect, which is manifested in an increase in the time of onset of diarrhea from 50.4% to 82.6% with various models of diarrhea, a reduction in the number of defecations from 50.4% to 64.4% liquid in them. Enterosorbent on the basis of montmorillonite has a high sorption activity to *E.coli* enterotoxin, inhibiting the outflow of fluid into the luminal cavity by 95.1%. In addition, the use of enterosorbent Crim_04 significantly improves biochemical indices in the blood serum of rats when modeling acute and chronic liver damage and acute pancreatitis.

Conclusion: The enterosorbent under the Crim_04 cipher has a dose-dependent anti-diarrhea, detoxification activity, high sorption activity for *E.coli* enterotoxin, high therapeutic efficacy in experimental pancreatitis, most pronounced at a dose of 3320 mg / kg. It can be recommended for further complex toxicological studies and clinical trials.

Keywords: enterosorbents, montmorillonite, preclinical studies, diarrhea, intoxication.

Introduction

The pathological conditions associated with the syndrome of endogenous intoxication, as well as exogenous intoxications with salts of heavy metals, toxins of fungi and bacteria are extremely common today. This is due to the growth of

atmospheric and aqueous pollutants, contamination of food and drinking water by bacterial and endotoxic agents [1, 2]. In Russia, about 90 thousand poisonings are annually registered [3]. One of the most common diseases, having water or food origin, is diarrhea.

According to WHO, diarrhea is one of the leading causes of death in the world, ranking 8th in the frequency of causes of death. In 2015, 1.39 million people died of diarrheal diseases, most of them children. In 2013, diarrhea was the second most frequent cause of death of children. Annually more than 1.7 billion cases of diarrhea are registered in the world. These statistics relate mainly to countries with low and medium-low incomes [4, 5]. The syndrome of endogenous intoxication is also accompanied by exogenous intoxications and diseases of internal organs, for example hepatitis, acute pancreatitis [6, 7, 8].

One of the most common, effective and accessible methods of detoxification of the body is the method of enterosorption [9, 10, 11, 12]. Of particular interest in this regard is mineral raw materials, in particular montmorillonite. Montmorillonite, due to its properties [13, 14, 15], has an antacid [16], antidiarrhoeal [17, 18, 19], cytomycoprotective [20], anti-inflammatory and anti-cytokine [21], high sorption activity against bacterial toxins [22, 23, 24, 25], salts of heavy metals [26], aflatoxin [24, 27], herbicides [28] for oral administration. Enriched with silver nanoparticles, montmorillonite has a bactericidal effect [22, 23, 24]. The structure, physical and chemical properties, mechanisms of sorption of toxins and xenobiotics on the surface of montmorillonite have been studied quite thoroughly [13, 29, 30].

Enterosorbent preparations based on mineral raw materials, mainly smectite dioctahedral, are in high demand among the population of Russia, far outstripping other enterosorbents [30, 31]. At the moment on the Russian pharmaceutical market there are 4 medicines with enterosorption activity on the basis of smectite minerals. However, they are all made from foreign substances. The development of medicines based on domestic substances is an urgent task for the public health and pharmaceutical industry and is consistent with the "Pharma Strategy-2020" [32].

Degree of elaboration of the research topic.

The sorption properties of clay minerals have attracted the attention of scientists for the last half-century [33, 34]. Over the past decade, many works on structure, physicochemical properties, experimental and clinical studies of the effectiveness of smectites, including montmorillonite, have been published [35, 36]. To date, there are several drugs based on smectite

clay minerals. These are mainly preparations based on smectite dioctahedral. However, the clinical efficacy of the montmorillonite calcium preparation in the treatment of aflatoxicosis (NovaSil, the manufacturer of Engelhard Chemical Corporation, USA) has been thoroughly investigated. However, there is no enterosorbent based on layered aluminosilicates produced on the basis of domestic raw materials on the Russian pharmaceutical market. To solve this problem, it is necessary to study montmorillonite of Russian origin with high sorption activity at the preclinical level.

Objectives: to study the pharmacological activity of enterosorbent on the basis of montmorillonite under experimental conditions.

Materials and methods of research

The work was performed on the basis of Belgorod State University in the preclinical research laboratory of the Center for Preclinical and Clinical Studies on 430 white mature rats, Wistar males (weight 200±20 g) and 460 laboratory mice of both sexes (weight 25±2 g). All stages of the research were carried out in accordance with the requirements of GOST ISO/IEC 17025-2009, GOST R ISO 5725-2002 and the "Rules of Laboratory Practice" approved by Order No. 708n of the Ministry of Healthcare and Social Development of the Russian Federation of August 23, 2010, in compliance with the "European Convention for the Protection of Vertebrates, used for experiments or for other scientific purposes "[Directive 2010/63 / EU]. Vivisection was carried out in accordance with the principles of the "European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes." CETS No. 123 ".

Object of study

The object of the study was a prototype of enterosorbent based on montmorillonite of the Crimean deposit under the laboratory code Crim_04, provided by the company "Krympharmamed". The experimental sample of enterosorbent Crim_04 is a powder from a yellowish- or grayish-white color to a grayish or brownish-yellow color with the smell of vanillin. The mass fraction of montmorillonite is 62.4%, silver – 0.15%. The size of the most common particles in the suspension is 7.08 µm.

Comparison preparations: Enterosorbent "Smecta" (Beaufur Ipsen Industrie, France) and loperamide ("Janssen-Cilag", France).

Methods of research

A study of the acute toxicity of montmorillonite-based enterosorbent under the laboratory code Crim_04 was carried out on laboratory mice of both sexes weighing 25 ± 2 g in accordance with the "Guidelines for preclinical drug research" [37, 38].

Calculation of doses was carried out individually for each animal in mg / kg. The calculation of the optimal therapeutic dose was carried out using tables of dose recalculation, taking into account the average therapeutic dose of the drug "Smecta" for humans, the average weight of a person is 70 kg, the average weight of the rat is 200 g, the average mouse weight is 25 g [38]. As a result, the optimal therapeutic dose for the rat was 770 mg/kg, for the mouse – 1660 mg/kg.

Laboratory mice were used to model acute diarrhea. Acute diarrhea was modeled by intraperitoneal single administration of serotonin hydrochloride (5-hydroxytryptamine hydrochloride, 5-HT) at a dose of 0.32 mg / kg (n=20 animals) [39]. In the modeling of diarrhea induced by castor oil, castor oil was injected intragastrically at a dose of 0.5 ml per individual (n=20 animals) [40], magnesium sulfate ($MgSO_4$) was used intragastrically at a dose of 2 g/kg to model $MgSO_4$ -induced diarrhea [40] (n=20 animals) [41]. Serotonin hydrochloride was used 30 minutes after intragastric administration of enterosorbents and loperamide. Castor oil and magnesium sulfate were used 30 minutes prior to administration of the investigational pharmacological agents. In the group of intact animals 0.9% sodium chloride solution 10 ml/kg (n=20 animals) was used.

In the experimental groups, the aqueous suspensions of enterosorbents Crim_04 were injected single times in animals at doses of 880 mg/kg, 1660 mg/kg, 3320 mg/kg and Smecta in a dose of 1660 mg/kg, loperamide at an effective dose of 10 μ g/kg, taking into account the recalculation of doses from the average therapeutic daily dose for humans. In the control group, the animals received an equivalent volume of 0.9% sodium chloride solution.

After modeling the pathology, the time of onset of diarrhea, the number of defecations, and

the weight of stool for 4 hours were taken into account. To calculate the severity of inhibition (SI) diarrhea used formula (1).

$$SI(\%) = \left[\frac{(D_k - D_i)}{D_k} \right] \times 100\% \quad (1),$$

where D_k – the number of wet and liquid defecations in the control group, D_i – the number of wet and liquid defecations in the study groups.

Calculation of the coefficient of severity of diarrhea (CSD) was carried out with the help of a scale scale for assessing the consistency of stool mass: 1 point – normal excrement, 2 – semi-liquid, wet defecation, 3 points – liquid defecation. The indicator was calculated using formula (2).

$$CSD = \frac{(N \times 1 + S \times 2 + L \times 3)}{\Delta D} \quad (2),$$

where N is the amount of normal excrement, S is the number of semi-liquid excrement, L is the amount of liquid excrement, and ΔD is the total number of defecations during observation. Stressed defecations at the beginning of the experiment were not taken into account when calculating the total number of fecal outcrops during the experiment.

After 4 hours of observation, the animals were anesthetized from the experiment. For pathomorphological examination, the small intestine sites were taken from animals [42].

Wistar male rats were used to model isolated gut loop. Before the experiment, the animals starved for 1 day with free access to water. Chloral hydrate 300 mg/kg was used for anesthesia intraperitoneally. After epilation of the hair on the abdomen, a laparotomy of 2.5 cm was carried out. At a distance of 5 cm from the stomach, the first ligation was applied to the intestine with a constant irrigation of 0.9% sodium chloride solution. Then, in steps of 2.5 cm, ligatures were applied to the intestine to create isolated loops, avoiding ligation of vascular feeding beams. A 0.9% sodium chloride solution was injected into the lumen of the first two loops in a volume of 0.2 ml (internal control, intact loops), the rest – thermolabile cholera-like *E.coli* enterotoxin (TCET, Sigma-Aldrich, USA) at a dose of 2 μ g/loop in a volume of 0.2 ml [43].

The aqueous suspensions of Crim_04 and Smecta[®] at a concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml were introduced into the lumen of the intestine loops with a toxin in a volume of 0.2 ml. The concentration of enterosorbents in the

suspension was determined empirically, given the permeability of the suspension through the needle of the insulin syringe. In the control group, 2 µg of enterotoxin and 0.2 ml of 0.9% sodium chloride solution. Water suspensions of enterosorbents and 0.9% solution of sodium chloride were introduced into the lumen of the gut simultaneously with *E.coli* enterotoxin. The anterior abdominal wall was sutured layer by layer. Animals were placed in individual cells. After 4 hours, the animals were removed from the experiment. The degree of toxin exposure was assessed by the dilatation index, which was calculated from formula (3).

$$ID = M/L \quad (3),$$

where M is the weight of the loop in mg, L is its length in cm.

The severity of the inhibition of fluid outflow into the lumen of the intestinal loops was determined by the formula (4).

$$SI = \left[\frac{(\Delta 2 - \Delta 1)}{\Delta 2} \right] \times 100\% \quad (4),$$

where $\Delta 1$ is the difference between the loop ID with toxin, and the control loops in the experimental animals; $\Delta 2$ – the same in the control group. At the same time, the fluid outlet level in the control loops was assumed to be 100%. For the morphological study, the sites of the small intestine were taken from the animals.

To model acute toxic damage to the liver, male rats were intraventrically injected with tetrachloromethane in an oily solution at a concentration of 1:1 at a dose of 0.5 ml / kg of active ingredient daily for six days (n=20 animals). On the seventh day, animals were intraperitoneally injected with *S.thyphi* lipopolysaccharide at a dose of 20 µg/kg [44].

To simulate chronic toxic damage to the liver intragastrically male rats were injected with carbon tetrachloride (CTC) in oily solution at a dose of 0.5 ml/kg of active ingredient daily for 20 days (n=20 animals). At 6, 13, and 20 days, animals were intraperitoneally injected with *S.thyphi* lipopolysaccharide (LPS) at a dose of 20 µg/kg [44]. Animals in intact groups received 0.9% solution of sodium chloride orally (n=20 animals).

On the third day, the animals of the experimental groups received the enterosorbent

under the Crim_04 cipher at doses of 385 mg/kg, 770 mg/kg and 1500 mg/kg and the Smecta preparation at a dose of 770 mg/kg as aqueous suspensions intragastrically 12 hours after the administration of carbon tetrachloride (n=20 animals).

In both series of experiments, the physical condition of the animals was monitored. After excretion, the animals were taken blood for biochemical studies and liver tissue for morphological investigation. The activity of alanine aminotransferase (AlAT, U/l), aspartate aminotransferase (AsAT, U/l), alkaline phosphatase (AP, U/l), urea (mmol/l), total bilirubin (µmol/L), creatinine (µmol/L) in the blood serum.

Experimental acute pancreatitis was modeled by intraperitoneal single administration of a solution of L-arginine in phosphate buffer (pH = 6.8) at a dose of 1,5 g/kg (n=20 animals) to male rats. In the intact group, the animals received a 0.9% solution of sodium chloride orally (n=20 animals). Enteric sorbent under the laboratory cipher of Crim_04 in doses of 385 mg/kg, 770 mg/kg, 1500 mg/kg in the form of aqueous suspensions were administered concomitantly with L-arginine and then every 4 hours, 4 times in total to rats intragastrically (n=20 animals). The rats in the control group were intragastrically injected with an equivalent volume of 0.9% sodium chloride solution.

The mortality and survival of animals in groups for the first 24 hours was assessed. Animals were withdrawn from the experiment under anesthesia 72 hours after the induction of acute pancreatitis. Animals were collected blood for biochemical research. The following parameters were studied: serum amylase activity (U/l), aspartate aminotransferase (AsAT, U/l), alanine aminotransferase (AlAT, U/l), glucose content (mmol/l), triglycerides (TG, mmol/l). Pancreatic tissue was taken for pathomorphological examination [45].

Biochemical research

When breeding animals from the experiment, the blood was taken into test tubes with sodium heparin as an anticoagulant for. Biochemical indicators were determined using standard reagent kits of Diakon JSC (Russia) on a biochemical analyzer URIT-800 Vet (URIT Medical Electronic Co., Ltd., China).

Morphological investigation

The tissues were fixed in a 10% solution of neutral formalin, followed by pouring into paraffin. From the resulting blocks, sections of 5-7 microns thick were prepared. The staining was performed with hematoxylin-eosin. Microscopic examination was carried out on a micrometer "Mikmed-6" (LOMO, St. Petersburg), image analysis was carried out using the program "Micro-analysis Pro" (LLC LOMO-Microsystems, St. Petersburg).

Statistical analysis

Statistical processing of data was carried out using the Microsoft Excel 2010 and STATISTIKA 6.0 software packages for Windows. The average values of the indicators studied are given in the form ($M \pm m$), where M is

the arithmetic mean and m is the standard error of the mean. To analyze the differences between the groups, the t-test of the Student was used. The difference of the compared indicators for $p < 0,05$ was considered reliable.

Results and discussion

Investigation of the dose-dependent nature of antidiarrhoeal activity of enterosorbent based montmorillonite under the laboratory code Crim_04 on various models of acute diarrhea.

It was found that an intraperitoneal injection of serotonin hydrochloride at a dose of 0.32 mg / kg causes diarrhea in 100% of the animals for 15 minutes, which is manifested by a significant increase in the number of defecations with a predominance of watery stools.

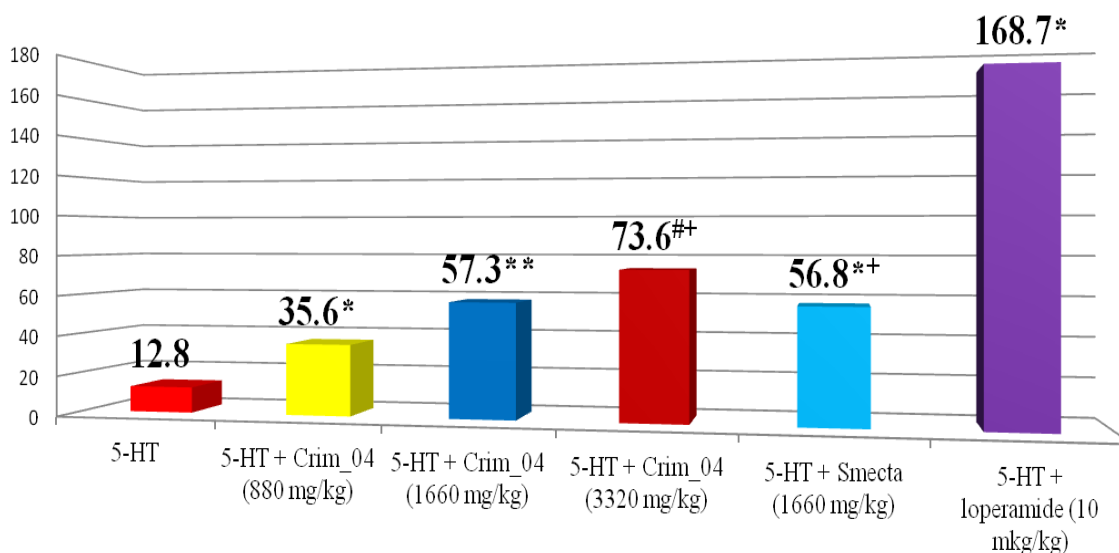


Fig. 1A. Dose-dependent influence of enterosorbent under the Crim_04 cipher for the time of onset of serotonin-induced diarrhea in mice (minutes).

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

As can be seen from Fig. 1A the use of enterosorbent under the code Crim_04 significantly increased the onset of diarrhea. Diarrhea occurred more than 5.5 times later than in the control group. This effect was most pronounced when using enterosorbent in a dose of 3320 mg/kg.

The use of enterosorbent under the Crim_04 cipher reduced the amount of defecations in mice when modeling serotonin-induced diarrhea. It was found that the use of enterosorbent Crim_04

at a dose of 3320 mg/kg caused an inhibition of the development of diarrhea by 52.1%, which is reflected in a decrease in the number of wet and liquid defecations in comparison with the control group. This result is significantly higher than in groups where the enterosorbent was used at doses of 880 mg/kg and 1660 mg/kg (26.7% and 35.9%, respectively). For Smekta and loperamide, this indicator was 34.8% and 73.8%, respectively (Fig. 1B).

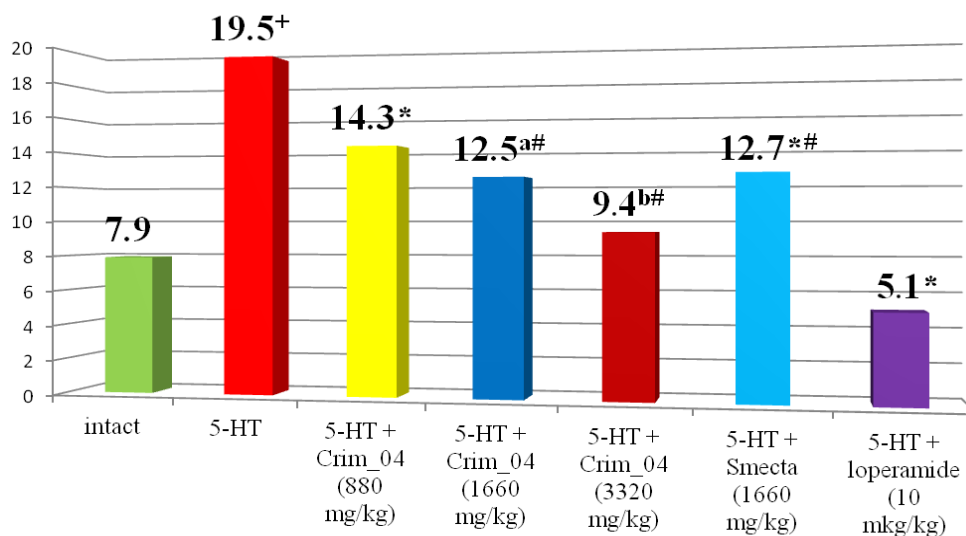


Fig. 1B. Dose-dependent influence of enterosorbent under the code Crim_04 on the number of defecations in serotonin-induced diarrhea in mice (number).

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

With intragastric administration of enterosorbent under the code Crim_04, a decrease in the fluid content in the feces was observed, defecation was predominantly moist. Most of this

effect is expressed in groups of animals receiving the enterosorbent Crim_04 at a dose of 3320 mg/kg (Figure 1C).

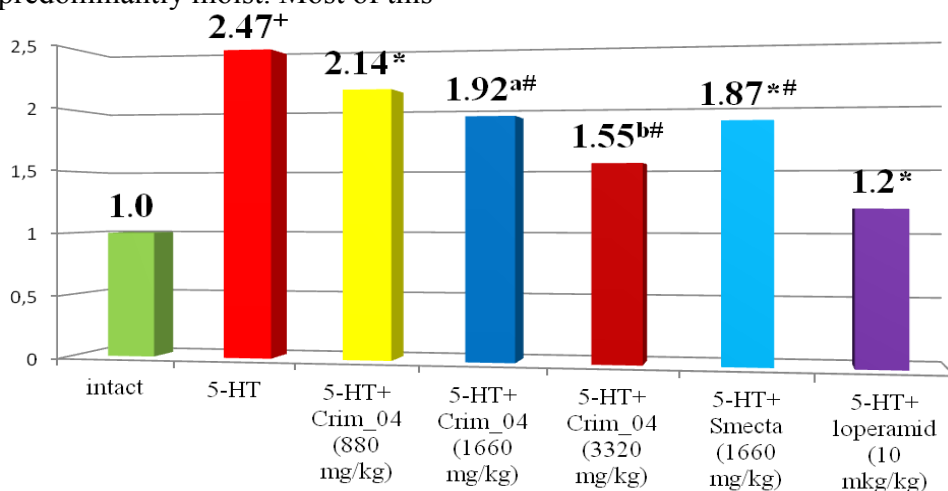


Fig. 1C. Dose-dependent influence of enterosorbent under the code Crim_04 on the consistency of feces in serotonin-induced diarrhea in mice (cond).

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

The indices of the enterosorbent under the code Crim_04 in the average therapeutic dose of 1660 mg/kg did not significantly differ from the effect of dioctahedral smectite in the same dose. At the same time, the enterosorbent under the code number Crim_04 and the "Smecta" preparation were significantly inferior to

loperamide by the time of onset of diarrhea, the total number of defecations and the fluid content in the stool.

Morphological study revealed that in the animals of the control group macroscopically the intestinal mucosa is swollen, edematous, pink-gray in color, and is hyperemic in separate areas. The

surface of the mucous membrane is covered with a slightly turbid, semi-liquid mucus, which is well washed away with water.

Microscopically the mucosa is edematous. Defined shortening and deformation of the villi. At the ends of some villi, the epithelium is squashed, exposing its own plate of mucosa. Defined hypertrophy of crypts is determined. The blood vessels of the mucosa and submucosa are full-blooded. Muscular and serous intestinal membranes are unchanged. In the lumen of the intestine, a large amount of mucus (Fig. 2B). These changes were not characteristic of intact animals (Fig. 2A).

In groups of animals that received enterosorbent under the laboratory cipher of Crim_04 at doses of 3320 mg/kg, the pathological changes in the small intestine consisted of a slight edema of the mucous membrane. Plots of hyperemia of the mucosa was not observed. The phenomena of slimming of the epithelium were minimal, there were no sites of exposure of the lamina propria of the mucous membrane. The blood supply to the vessels of the mucosa and the submucosal layer was moderate. Muscular layer and serosa without pathological changes (Fig. 2D).

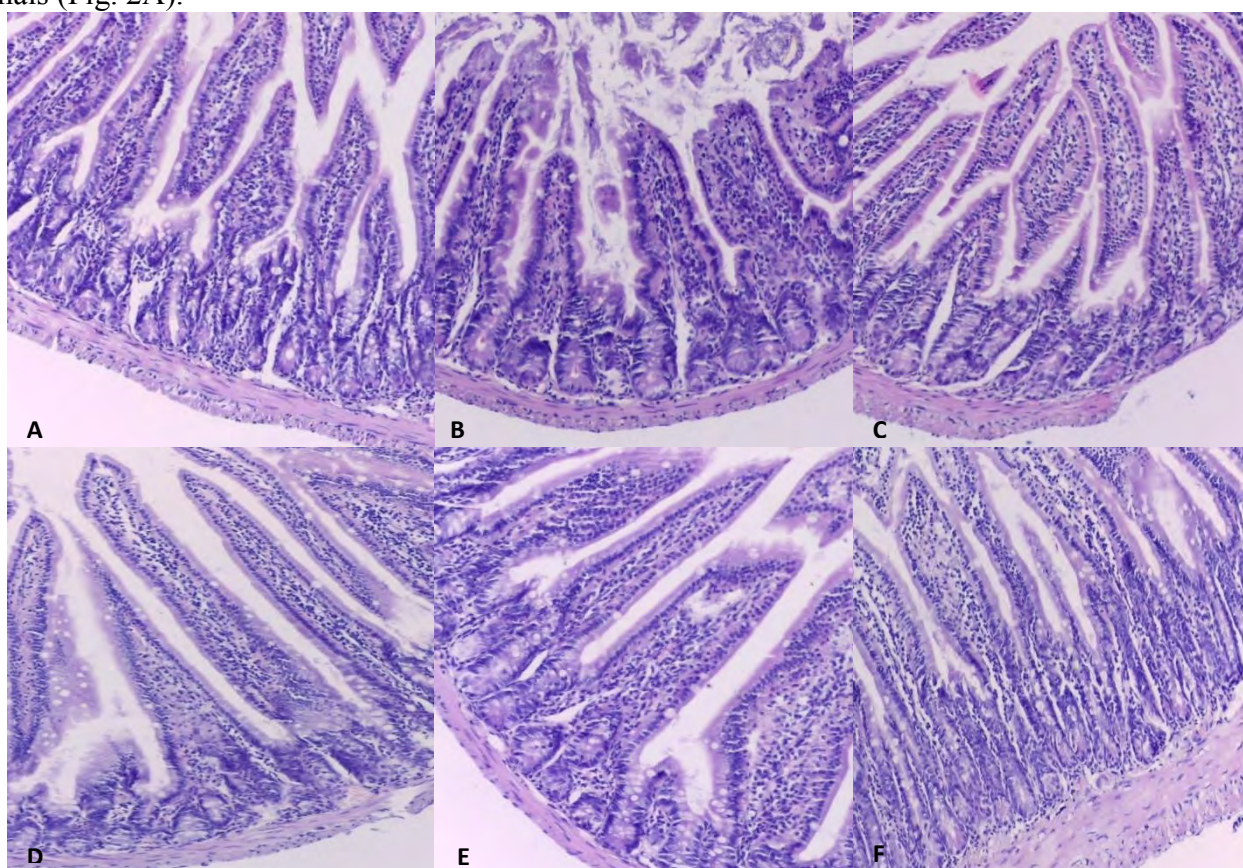


Fig. 2. Histological structure of the ileum in mice against the background of modeling serotonin-induced diarrhea (microphot $\times 100$).

Note: A – intact animals; B – control group; C – enterosorbent Crim_04 in a dose of 1660 mg/kg;

D – enterosorbent Crim_04 in a dose of 3320 mg/kg; E – Enterosorbent "Smecta" 1660 mg/kg;

F – loperamide 10 μ g/kg. Ochr. hematoxylin and eosin

In the control group with simulation of serotonin-induced diarrhea, a decrease in the height of the villi was 1.4 times, an increase in the width of the villi at the base by 1.4 times and the depth of the crypts by 1.2 times in comparison with intact animals. The use of enterosorbent

under the code Crim_04 at a dose of 3320 mg/kg significantly improved the histological picture of the small intestine, bringing the morphometric parameters closer to the level of intact animals (Fig. 3).

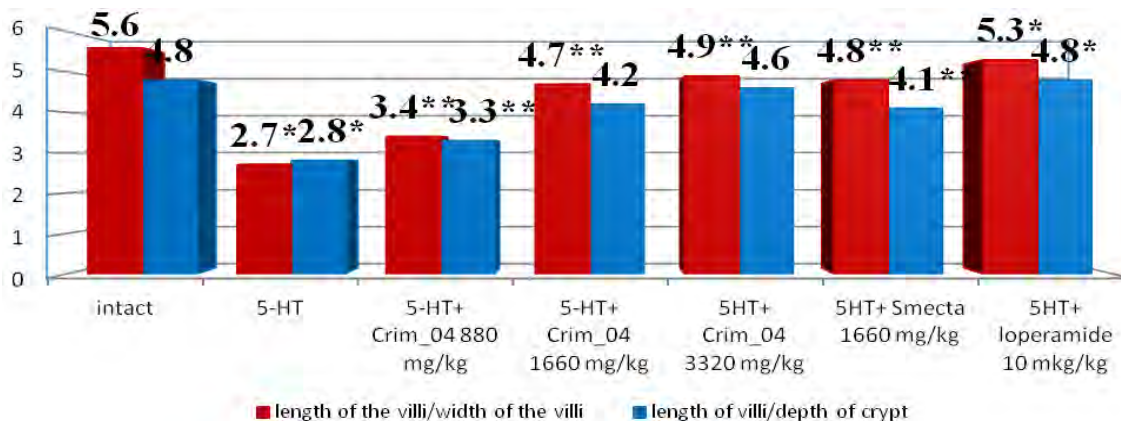


Fig. 3. Influence of the enterosorbent under the Crim_04 cipher on the ratio of the length of the villi to the width of the villi and the length of villi to the depth of crypt in the small intestine of mice when simulating serotonin-induced diarrhea (conv. Units).

Note: * – for $p < 0.05$ in comparison with the group of intact animals; ** – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg

Similar results were obtained in the modeling of castor-induced diarrhea and $MgSO_4$ -induced diarrhea. So the use of the enterosorbent Crim_04 in a dose of 3320 mg/kg on the model of diarrhea induced by castor oil increased the time of onset of diarrhea from 42.7 ± 2.9 minutes in the control group to 124.2 ± 5.4 minutes, reduced the total number of defecations by 64.4%, and significantly affected the consistency of defecations, significantly increasing their density from 2.58 ± 0.02 to 1.54 ± 0.01 points.

With the application of enterosorbent Crim_04 at a dose of 3320 mg/kg on the model of $MgSO_4$ -induced diarrhea, the time of onset of diarrhea increased from 62.8 ± 1.2 minutes in the control group to 126.8 ± 4.7 minutes, reducing the

total number of defecations by 50.5 % and significantly affected the consistency of defecations, significantly increasing their density from 2.79 ± 0.04 to 1.81 ± 0.04 points.

Investigation of the sorption activity of enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 on the model of an isolated loop of the small intestine.

The dose-dependent nature of the sorption activity of enterosorbent based on montmorillonite under the laboratory cipher of Crim_04 with respect to the thermolabile cholera-like enterotoxin *E.coli* in comparison with the "Smecta" preparation on the isolated loop model in rats is shown in Fig. 4.

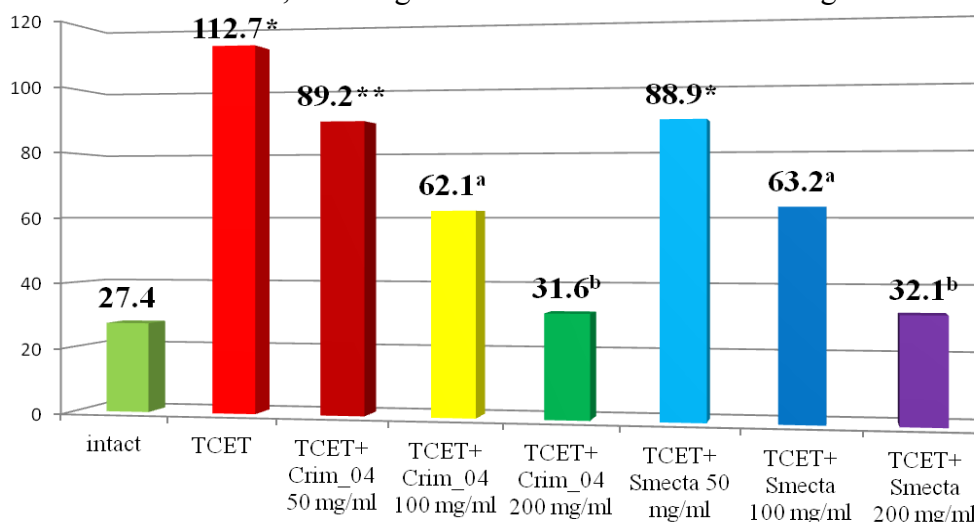


Fig. 4. Influence of the enterosorbent under the Crim_04 cipher in comparison with the Smecta preparation on the dilatation index on the isolated bowel loop model (mg/cm).

Note: * – for $p < 0.05$ in comparison with the control group; ** – at $p < 0.05$ in comparison with TCET; a – at $p < 0.05$ in comparison with enterosorbents in a dose of 50 mg/ml; b – at $p < 0.05$ in comparison with enterosorbents in a dose of 100 mg/ml

It is established that the enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 has a high sorption activity with respect to the thermolabile cholera-like

E.coli enterotoxin on the model of an isolated intestinal loop. This action is manifested by preventing the development of increased fluid formation in the lumen of the gut (Fig. 5).

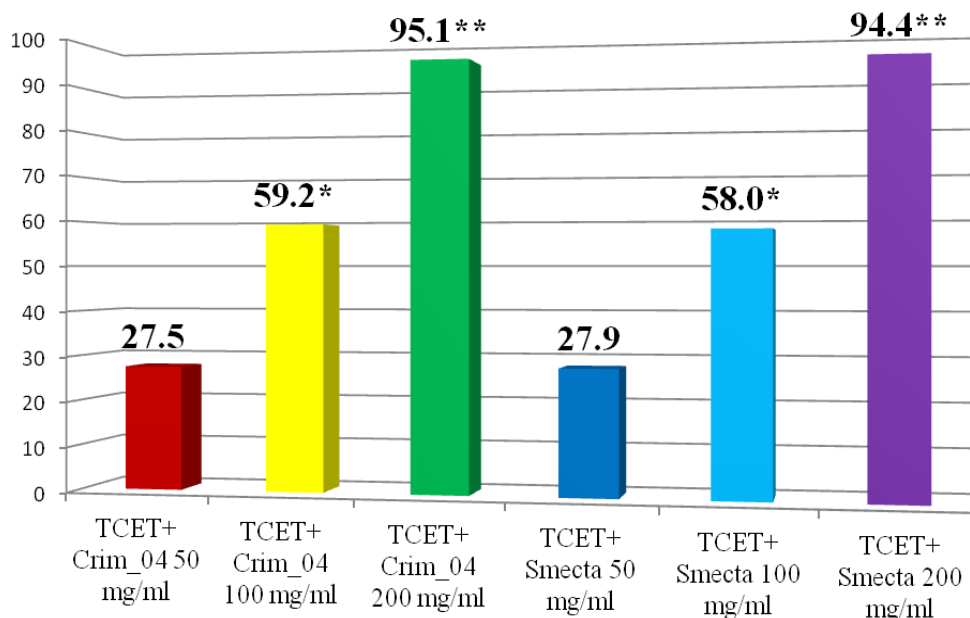


Fig. 5. The dose-dependent effect of the enterosorbent under the Crim_04 cipher in comparison with the Smecta preparation on the severity of the inhibition of fluid flow into the lumen of the gut on the model of the isolated bowel loop (%).

Note: * – at $p < 0,05$ in comparison with enterosorbents in a dose of 50 mg/ml; ** – at $p < 0,05$ in comparison with enterosorbents in a dose of 100 mg/ml

In this case, a clear dose-dependent effect is established, most pronounced when using an aqueous suspension of enterosorbent at a concentration of 200 mg/ml. This is also manifested by an improvement in the morphological pattern of the small intestine. The use of enterosorbent under the cipher of Crim_04 prevents the development of hypertrophy of crypts, swelling of the villi and increased mucosal epilation of the mucous membrane. Morphometric indices when using enterosorbent under the Crim_04 cipher are close to the level of intact loops.

The study of the detoxification activity of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 on models of acute and chronic toxic damage of the liver.

The dose-dependent character of the influence of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 on the activity of liver enzymes, biochemical parameters of blood serum in comparison with the "Smecta" preparation for modeling acute toxic damage of the liver is shown in Fig. 6A, 6B, 6C, 6D, 6E.

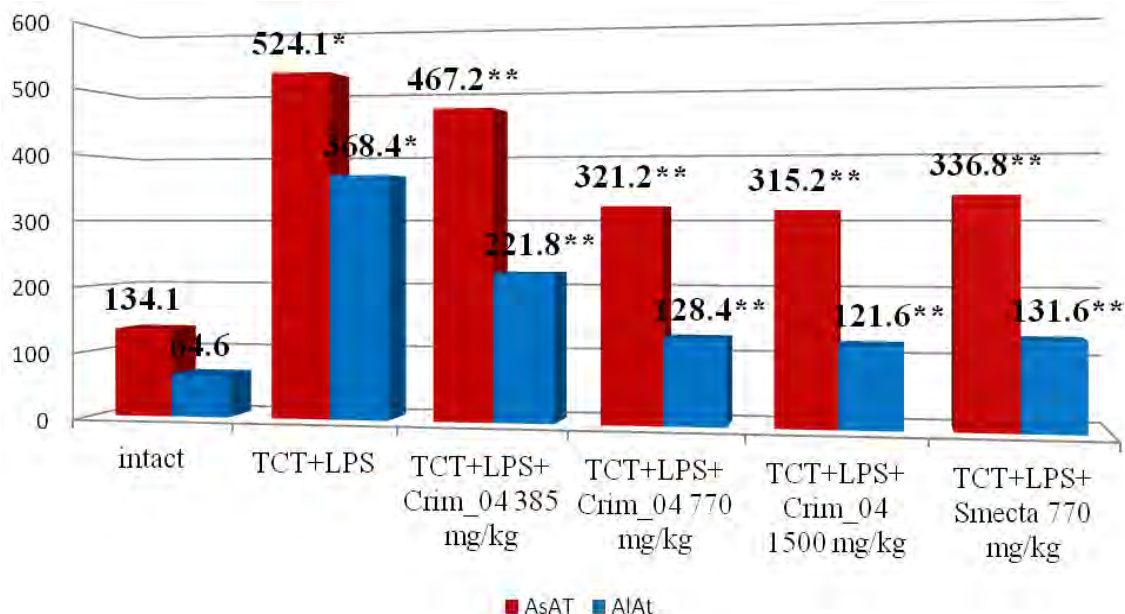


Fig. 6A. Influence of application of enterosorbent under the code Crim_04 on the activity of AsAT and AIAT in modeling acute toxic liver damage in rats (U/l).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

When using enterosorbent under the laboratory cipher of Crim_04 on the model of acute CTC-induced toxic liver damage, the activity of AsAT and AIAT in the blood plasma

of animals decreased, the de Ritis coefficient decreased from 1.4 in the negative control group to 2.5 in the group using the enterosorbent Crim_04 (Fig. 6A).

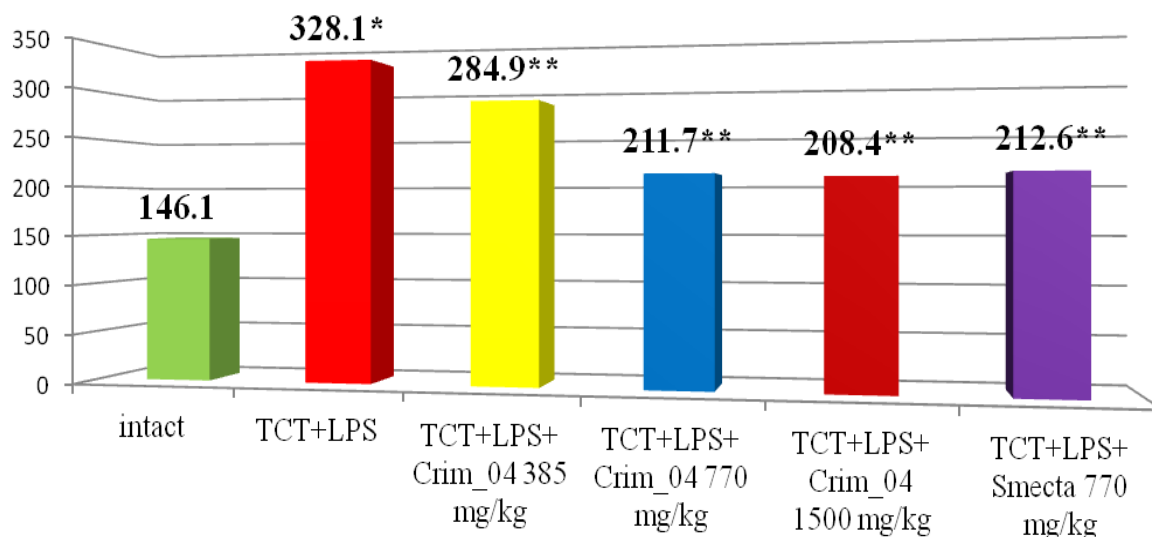


Fig. 6B. Effect of the use of enterosorbent under the code Crim_04 on the activity of alkaline phosphatase in the blood of rats when modeling acute toxic damage to the liver (U/l).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

Also, the introduction of an enterosorbent under the Crim_04 cipher caused a decrease in the activity of alkaline phosphatase (Figure 6B),

levels of urea (Figure 6C), creatinine (Figure 6D) and total bilirubin (Figure 6E) in the plasma of experimental animals.

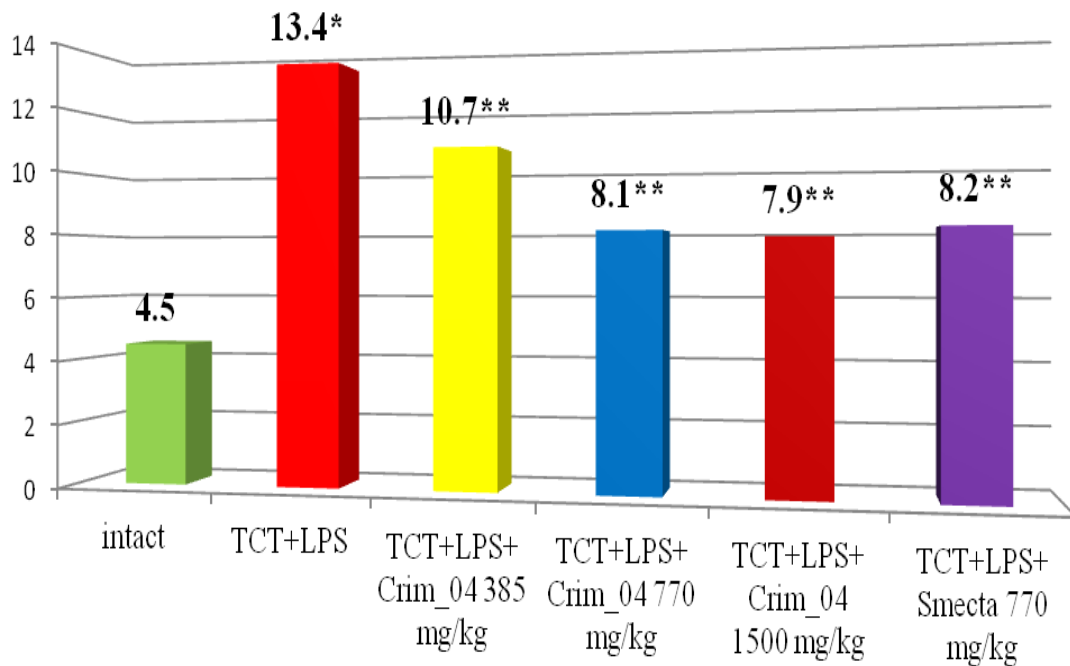


Fig. 6C. Effect of application of enterosorbent under the code Crim_04 on the level of urea in the blood of rats when modeling acute toxic damage to the liver (mmol/l).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

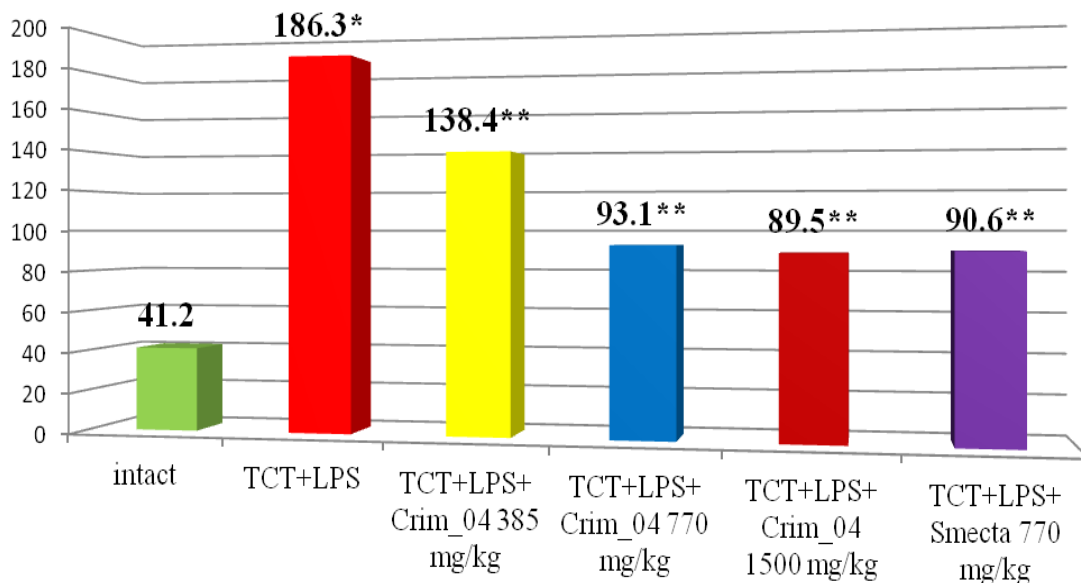


Fig. 6D. Effect of the use of enterosorbent under the code Crim_04 on the level of creatinine in the blood of rats when modeling acute toxic damage to the liver (µmol/l).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

This effect of enterosorbent Crim_04 was dose-dependent. The enterosorbent Crim_04 showed the highest result in doses of 770 mg/kg and 1500 mg/kg without increasing the effect with further dose build-up. Biochemical indices of

experimental animals when using enterosorbent under the Crim_04 cipher are not significantly different from those in the group of animals that received the Smecta preparation.

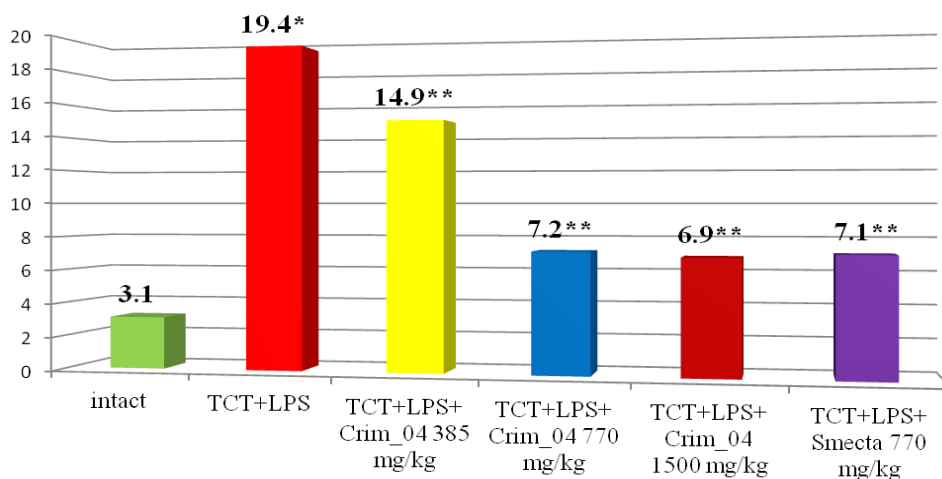


Fig. 6E. Effect of application of enterosorbent under the code Crim_04 on the level of total bilirubin in the blood of rats when modeling acute toxic damage to the liver ($\mu\text{mol/l}$).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

A normal population of hepatic cells and tissue homogeneity are shown in a typical liver sample from a group of intact animals (Fig. 7A). In liver preparations of rats receiving CTC and LPS, the presence of small-focal necrosis of hepatocytes, signs of large droplet fatty

hepatocyte, granular dystrophy of hepatocytes, some hepatocytes had the appearance of cricoid cells. The fullness of the blood vessels of the liver, moderate expansion of the veins of the portal tracts was registered (Fig. 7B).

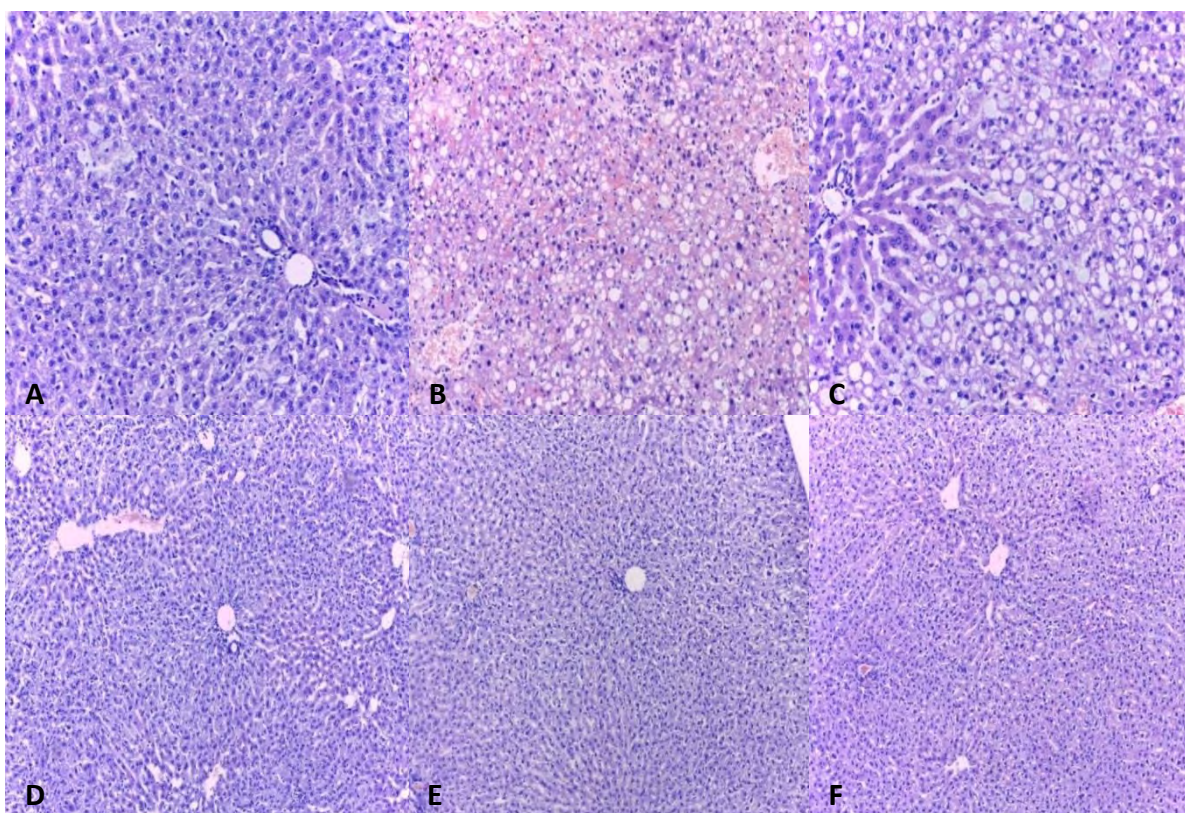


Fig. 7. Morphological examination of liver tissue (microphotography $\times 100$).

Note: A – group of intact animals; B – control group; C – enterosorbent under the cipher of Crim_04 in a dose of 385 mg/kg; D – enterosorbent under the code number Crim_04 in a dose of 770 mg/kg; E – enterosorbent under the cipher of Crim_04 in a dose of 1500 mg/kg; F – the drug "Smecta" in a dose of 770 mg/kg

In groups of animals treated with montmorillonite-based enterosorbent under the laboratory code Crim_04 at doses of 770 mg/kg and 1500 mg/kg, necrotic changes were not revealed. The structure of the liver retained normal histoarchitectonics. Hepatocytes are located typically. In some preparations, signs of small droplet fatty degeneration of hepatocytes. Determined moderate plethora of blood vessels of the liver. Portal tracts are not expanded (Fig. 7D, 7E).

In the morphometric analysis of liver tissue, it was found that when modeling acute toxic damage to the liver with 50% oil carbon tetrachloride, the volume of hepatocytes increased due to large-droplet fatty degeneration, as a result of which the nuclear-cytoplasmic index (NCI) in the control group decreased significantly. Thus, in the control group, the NCI was 0.14 ± 0.001 vs. 0.32 ± 0.001 for the group of intact animals. When the enterosorbent is used under the Crim_04 cipher, there is an increase in the parameters of the NCI, reaching a level of 0.28 ± 0.001 in the group of animals taking Crim_04 at a dose of 1500 mg/kg.

When using enterosorbent under the laboratory code Crim_04 on the model of chronic CTC-induced toxic liver damage, the activity of AsAT and AlAT in the blood plasma of animals decreased, the de Ritis coefficient increased from 1.6 to 2.3. Similarly, the introduction of enterosorbent under the code Crim_04 caused a

decrease in the activity of alkaline phosphatase, levels of urea, creatinine and total bilirubin in the blood plasma of experimental animals. This effect of enterosorbent Crim_04 was dose-dependent. The enterosorbent Crim_04 showed the highest result in doses of 770 mg/kg and 1500 mg/kg without increasing the effect with further dose build-up. Biochemical indices of experimental animals when using enterosorbent under the Crim_04 cipher are not significantly different from those in the group of animals that received the Smecta preparation.

The study of the dose-dependent nature of the effectiveness of the use of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 in the simulation of acute L-arginine-induced pancreatitis.

The dose-dependent nature of the influence of enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 on the biochemical parameters of blood serum of rats in comparison with the "Smecta" preparation against the background of modeling of acute L-arginine-induced pancreatitis is presented in the table 1. It has been established that the montmorillonite-based enterosorbent under the laboratory cipher Crim_04 has a dose-dependent effect when modeling acute L-arginine-induced pancreatitis in rats. This is expressed in a decrease in the activity of amylase, AsAt, AlAT, a decrease in serum triglyceride levels and glucose in rats.

Table 1

Influence of enterosorbent under the cipher of Crim_04 in comparison with the "Smecta" preparation on the biochemical parameters of blood of rats against the background of modeling of acute L-arginine-induced pancreatitis (M±m; n=10)

A drug	Amylase (U/l)	AsAt (U/l)	AlAt (U/l)	TG (mmol/l)	Glucose (mmol/l)
Intact	215±3	121±1	60±0,9	1.0±0.1	5.3±0.7
L-arginine	1834±12*	521±6*	115±2*	2.9±0.3*	13.5±1.0*
L-arginine + Crim_04 385 mg / kg	1320±14**	325±5**	94±4**	2.1±0.1**	10.9±2.2**
L-arginine + Crim_04 770 mg / kg	836±4 ^a	182±4 ^a	79±2 ^a	1.4±0.2 ^a	8.5±1.1 ^a
L-arginine + Crim_04 1500 mg / kg	801±3 ^a	171±2 ^a	76±2 ^a	1.2±0.1 ^a	7.9±1.2 ^a
L-arginine + Smecta 770 mg / kg	852±5**	189±4**	77±3**	1.3±0.1**	8.8±1.4**

Note: * – for p < 0.05 in comparison with the intact group; ** – at p < 0.05 in comparison with the control group; a – at p < 0.05 in comparison with Crim_04 in a dose of 880 mg/kg.

This indicates a decrease in inflammation of the pancreas. In this case, the effect of enterosorbent based on montmorillonite under the Crim_04 cipher was dose-dependent, most

pronounced at a dose of 770 mg/kg without improvement in the parameters with further dose build-up. This is also confirmed by histological examination (Fig. 8).

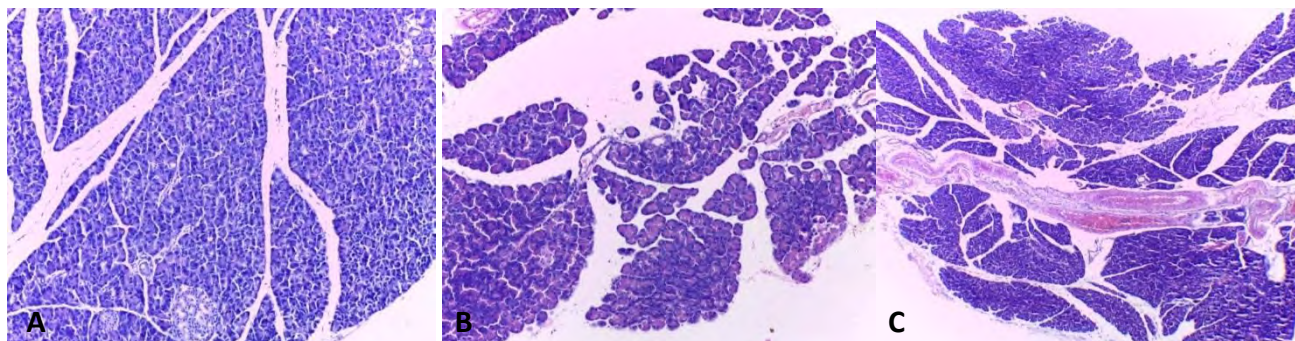


Fig. 8. Morphological examination of pancreatic tissue (microphoto $\times 100$).

Note: A – group of intact animals; B – control group; C – enterosorbent under the cipher of Crim_04 in a dose of 1500 mg/kg

In histological study, it was found that with the induction of acute pancreatitis by intraperitoneal administration of L-arginine at a dose of 1.5 mg / kg in pancreatic tissue, pronounced edema, focal necrosis of the acinocytes, islets of Langerhans of different calibers (Fig. 8B). In the group of intact animals, the pancreas retained a typical structure (Fig. 8A).

When using enterosorbent under the code Crim_04 at a dose of 385 mg / kg pancreas was moderately edematous, small foci of necrosis of acinocytes, islets of Langerhans of different calibers are determined.

In doses of 770 mg / kg and 1500 mg / kg enterosorbent under the cipher of Crim_04 prevented necrosis of the acinocytes, small puffiness of the pancreatic tissue, the islets of Langerhans of the usual structure is determined (Fig. 8C).

Discussion

In carrying out complex studies of the pharmacological activity of enterosorbent under the laboratory cipher of Crim_04, in the modeling of serotonin-induced diarrhea, $MgSO_4$ -induced diarrhea, diarrhea induced by castor oil, isolated gut loop, acute and chronic liver damage, and L-arginine induced acute pancreatitis, that the enterosorbent under the cipher Crim_04 has a dose-dependent antidiarrheal, detoxification activity, a high sorption for enterotoxin of *E.coli* activity w, high therapeutic efficacy in experimental pancreatitis, most pronounced at a dose of 3320 mg/kg.

Taking into account the results obtained in the work, it is possible to determine the recommendations and outline the prospects for further work in this area.

In the course of the study, it was shown that the enterosorbent based on montmorillonite Crim_04 has a high antidiarrheal, detoxification activity, therapeutic efficacy in experimental acute pancreatitis, showing a clear dose-dependent effect.

The data obtained in the course of the study on the pharmacological activity of the enterosorbent under the laboratory cipher of Crim_04 gives broad prospects for further study of this pharmacological agent. Enterosorbent based on montmorillonite Crim_04 can be recommended for further clinical effectiveness and safety for the treatment of acute diarrhea of various etiologies, diseases accompanied by endotoxemia, exogenous intoxications.

The data obtained in the course of the study on the pharmacological activity of the enterosorbent under the laboratory cipher of Crim_04 gives broad prospects for further study of this pharmacological agent. Enterosorbent Crim_04 can be recommended for further preclinical study of pharmacological activity in models of acute gastric ulcers, Crohn's disease, acute and chronic intoxications with salts of heavy metals, intoxications with mycotoxins and bacterial enterotoxins. Enterosorbent based on montmorillonite Crim_04 can be recommended for further clinical effectiveness and safety for the

treatment of acute diarrhea of various etiologies, diseases accompanied by endotoxiosis, exogenous intoxications.

It is promising to conduct further preclinical studies of enterosorbent based on montmorillonite under the laboratory code Crim_04 in modeling acute gastric ulcers, Crohn's disease, acute intoxications with heavy metal salts, mycotoxins, bacterial toxins, as well as complex preclinical toxicological safety tests and clinical studies of enterosorbent based on montmorillonite under the laboratory code Crim_04.

Conclusions

1. Enterosorbent based on montmorillonite under laboratory code Crim_04 on the results of the study of acute toxicity is low toxic pharmacological agent and refers to substances hazard class IV. At a dose of 8500 mg/kg, the enterosorbent does not cause lethality and changes in the functional state in mice.

2. Enterosorbent based on montmorillonite under laboratory code Crim_04 possesses a dose-dependent Antidiarrhoeal activity in the simulation serotonin-induced diarrhea, MgSO₄-induced diarrhea, diarrhea induced by castor oil. This is expressed in an increase in the onset of diarrhea, a decrease in the number of bowel movements and a decrease in the amount of fluid in the stool. At the same time, the prevention of the development of mucosal villus hypotrophy and crypt hypertrophy was detected morphometrically.

3. In the simulation, the isolated loop intestine enterosorbent based on montmorillonite possesses a cipher Crim_04 dose dependent sorption activity in relation to the heat labile enterotoxin of *E.coli* cholera, which is manifested in the prevention of exposure to toxin intestinal wall and, consequently, inhibition of fluid exudation into the gut lumen.

4. Enterosorbent based on montmorillonite under laboratory code Crim_04 disintoxicational possesses a dose-dependent effect in models of acute and chronic liver injury, which manifests itself in significant reduction in the activity of the liver enzymes, as well as levels of urea, creatinine, and total bilirubin. The activity of enterosorbent is confirmed by morphological examination of liver tissue.

5. Enterosorbent based on montmorillonite under cipher Crim_04 possesses a dose-

dependent therapeutic efficacy in experimental L-arginine-induced pancreatitis, it reduces the activity of amylase, transaminases, triglycerides, blood glucose serum. The efficacy of enterosorbent was confirmed by histological examination of pancreatic tissue.

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Conflicts of interest

The authors have no conflict of interest to declare.

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