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APPLICATION OF ENTEROSORBENT ON THE BASIS OF MONTMORILLONITE IN ACUTE DIARRHEA (experimental study)

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Aim. **To study the antidiarrheal activity of enterosorbent on the basis of montmorillonite on the model of serotonin-induced diarrhea.** ***Materials and Methods.*** **The study was conducted on laboratory mice of both sexes, acute diarrhea was induced by intraperitoneal injection of serotonin hydrochloride at the dose of 0.32 mg/kg, enterosorbent was introduced orally in the form of aqueous suspension in a wide range of doses 30 minutes before introduction of serotonin. Within 4 hours, the time of onset of diarrhea, the number of defecations, the fluid content in fecal masses were taken into account. Morphological examination of the small intestine was carried out.** ***Results.*** **introduction of serotonin led to diarrhea in 100% of animals within 12.8±1.2 minutes, the number of defecations increased 2.5 times as compared to the intact animals and was 19.5±0.5 times in 4 hours, the fecal masses were predominantly semi-liquid and liquid. Use of enterosorbent coded Crim_04 at the maximum dose led to a delay in diarrhea up to 73.6±4.1 minutes, the number of defecations decreased to 9.4±0.1 with a significant solidification of feces. The antidiarrheal effect of enterosorbent was confirmed morphologically.** ***Conclusions*****. Enterosorbent on the basis of montmorillonite with laboratory code Crim_04 possesses a dose-dependent antidiarrheal effect in modeling of serotonin-induced diarrhea.**

Keywords: *enterosorbent, montmorillonite, diarrhea, antidiarrheal activity.*

Diarrhea is one of commonest diseases of water-related or food-related origin. According to WHO data, diarrhea is one of the leading causes of death in the world. Despite reduction in mortality rate of diarrhea, in 2015 1.39 million people, mostly children, died from diarrheal diseases. Every year more than 1.7 billion of cases of diarrhea are reregistered in the world [1,2].

Adjuvant therapy of acute diarrheas that can also be used in children, is enterosorption [3,4]. Of special interest here are mineral raw materials, in particular, montmorillonite. Montmorillonite is a layered mineral of smectite group. Dioctahedral smectite having similar structure with montmorillonite is in increased demand among the population of Russia [5]. Minerals of smectite group possess antidiarrheal and cytomucoprotective effect and a high sorption activity towards bacterial toxins in peroral use [6-11].

At present there is no mineral enterosorbent on the basis of domestic raw materials on the Russian pharmaceutical market [12]. Development of medical drugs on the basis of domestic substances is an important task for healthcare and for pharmaceutical industry that complies with "Pharma-2020 Strategy" [13].

Aim of research to carry our preclinical study of antidiarrheal activity of enterosorbent with laboratory code Crim_04 based of montmorillonite on the model of serotonin-induced diarrhea.

Materials and Methods

The work was carried out on the base of FSAEI "Belgorod State University" in the laboratory of preclinical studies. All stages of the work were conducted with observance of "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" [Directive 2010/63/EU].

Test object

Test object was an experimental sample of enterosorbent with laboratory code Crim_04 on the basis of montmorillonite from Crimean deposit provided by firm Crimfarmamed. The experimental sample Crim_04 is a powder ranging in color from yellowish- or grayish-white to grayish- or brownish-yellow with a smell of vanillin. Mass fraction of montmorillonite makes 62.4%, silver – 0.15%. The size of the majority of particles in the suspension is 7.08 μm.

Drugs for comparison: enterosorbent Smecta (Beaufuor Ipsen Industrie, France) and loperamide (Janssen-Cilag, France).

Laboratory animals, experimental groups

The study was conducted on 140 laboratory mices of both genders with 25±2 g mass. The animals were obtained from the vivarium of FSAEI "Belgorod State University".

The diarrhea was induced by intraperitoneal introduction of serotonin hydrochloride (5-hydroxyrtyptamine – 5-HT) (H9523, Sigma-Aldrich, USA) at the dose of 0.32 mg/kg (n=20 animals) [14]. Serotonin hydrochloride was introduced in 30 minutes after intragastric introduction of enterosorbents and loperamide. In the group of intact animals 0.9% sodium chloride solution was introduced intraperitoneally at the dose of 10 ml/kg (n=20 animals).

Animals of experimental groups were intragastrically introduced aqueous suspension of Crim_04 enterosorbents at one-time doses of 880 mg/kg, 1660 mg/kg, 3320 mg/kg and Smecta at the dose of 1660 mg/kg, loperamide at the effective dose 10 μg/kg, the doses were recalculated on the basis of the average therapeutic daily doses for humans. Animals of the control group received equivalent quantities of 0.9% sodium chloride solution.

After modelling of the pathology mice were placed onto white sheets of paper (one on a sheet) to evaluate the time of onset of diarrhea and count the number of defecations within 4 hours. The paper was changed every hour. The evidence of inhibition (EI) of diarrhea was calculated using the formula:

$$EI\ (\%)=[(Dc-De)/Dc] \times 100\%,$$

where Dc – the quantity of semi-liquid and liquid defecations in control group, De – the quantity of semiliquid and liquid defecations in studied groups. The coefficient of evidence of diarrhea (CED) was calculated using point scale of evaluation of consistence of fecal masses: 1 point – normal feces, 2 – semiliquid feces, 3 points – liquid feces. The coefficient was calculated from the formula:

$$CED=(N \times 1+S \times 2+L \times 3)/\Delta D,$$

where N – quantity of defecations with normal feces, S – quantity of defecations with

semiliquid feces, *L* – quantity of defecations with liquid feces, *ΔD* – total quantity of defecations during the observation period. Stress-related defecations at the beginning of the experiment were ignored in counting the total quantity of defecations in the experiment.

After 4 hours of observation the animals were withdrawn from the experiment under ether narcosis.

Morphological examination

For histological examination portions of small intestinal tissue were taken from animals. The portions of tissue were fixed in 10% solution of neutral formalin with subsequent paraffin coating. From the obtained blocks cuts were prepared of 5-7 μm thickness and stained with hematoxylin-eosin. Microscopic examination was conducted using microscope Micromed-6 (LOMO, Saint-Petersburg), pictures were analyzed using Micro-Analysis Pro program (OOO LOMO-Microsystems, Saint-Petersburg).

Statistical analysis

Statistical processing of the data was conducted using program pack Microsoft Excel 2010 and STATISTIKA 6.0 for Windows. Average values of the studied parameters were presented in the form (M±m) where M is the arithmetic mean, and m is standard error of the mean. Differences in parameters between the groups were analyzed using Student criterion. The difference between compared parameters with $p<0.05$ was considered reliable.

Results and Discussion

It was found that intraperitoneal introduction of serotonin hydrochloride at the dose of 0.32mg/kg caused diarrhea in 100% of animals within 15 minutes with evident increase in the number of defecations with significant predomination of watery fecal masses as compared to the intact animals (Fig. 1).

As it is seen from Figure 1 A, application of enterosorbent coded Crim_04 significantly delayed the onset of diarrhea. Here, diarrhea began 5.5 times later than in the control group. The effect was most evident with application of enterosorbent at the dose of 3320 mg/kg.

Application of enterosorbent coded Crim_04 reduced the number of defecations in modeling of serotonin-induced diarrhea in mice. Here, it was ascertained that application of enterosorbent Crim_04 at the dose of 3320 mg/kg inhibited development of diarrhea by 52.1% manifested by reduction in the amount of semi-liquid and liquid defecations compared to the control group. This result was reliably higher than in groups with introduction of enterosorbent at the doses 880 mg/kg and 1660 mg/kg (26.7% and 35.9%, respectively). For Smecta and loperamide this parameter was 34.8% и 73.8%, respectively (Fig. 1 B).

In intragastric introduction of enterosorbent coded Crim_04 reduction in the content of fluid in fecal masses was observed, defecations were mostly with semi-fluid feces. This effect was most expressed in groups of animals that received the enterosorbent Crim_04 at the dose 3320 mg/kg (Fig. 1 C).

The parameters for enterosorbent coded Crim_04 in the average therapeutic dose 1660 mg/kg did not show a reliable difference from the effect of dioctahedral smectitis at the same dose. With this, enterosorbent coded Crim_04 and Smecta were significantly inferior to loperamide in terms of time of the onset of diarrhea, total quantity of defecations and content of fluid in fecal masses.

Morphologic examination

In animals of control group the intestinal mucosa was macroscopically swollen, edematous, of pink-gray color, with separate hyperemized areas. The surface of the mucosa was covered with slightly turbid semi-liquid mucus readily washed off with water.

Microscopically, the mucosa was edematous. Shortening and deformation of villi were determined. On the end of some villi scaling off of the epithelium was seen with exposure of the plate of mucosa proper. Evident hypertrophy of crypts was determined. Blood vessels of mucosa and of submucosal layer were hyperemic. Muscular and serous membranes of the intestine were without changes. In the gut lumen a high amount of mucus was present (Fig. 2 B). These changes were not characteristic of the intact animals (Fig. 2 A).

In groups of animals that received enterosorbent with laboratory code Crim_04 at the doses of 3320 mg/kg pathological alterations in the small intestine consisted in a mild edema of the mucous membrane. No

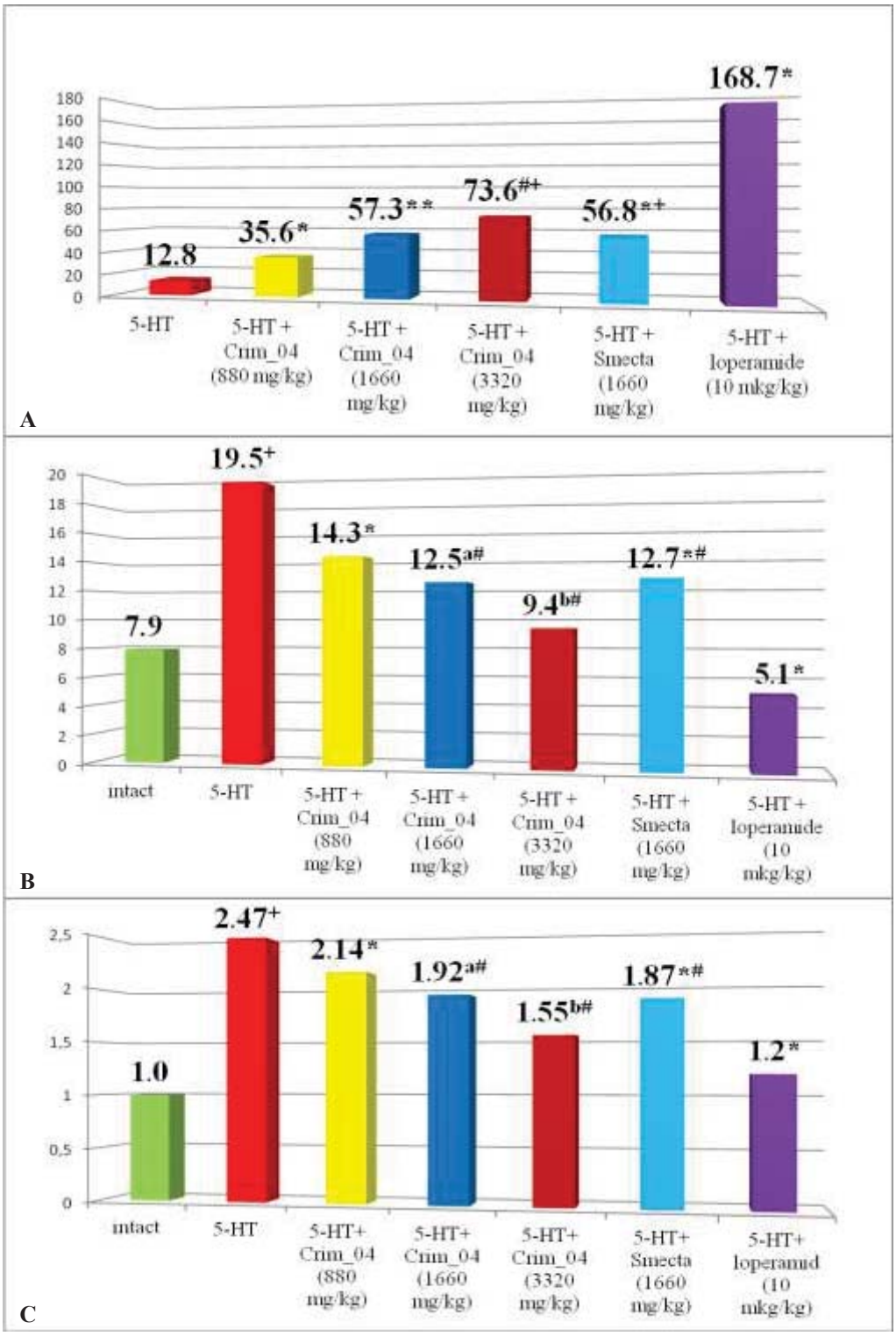

Fig. 1. Dose-dependent effect of enterosorbent with code Crim_04 on the time of onset of diarrhea (A, minutes), quantity of defecations (B, number), consistence of fecal masses (C, conventional units) in serotonin-induced diarrhea in mice.

Note:$^{+}$ – with $p<0.05$ compared to the group of intact animals; * – with $p<0.05$ compared to the control group; a – $p<0.05$ compared to the group given Crim_04 in the dose 880 mg/kg; b – $p<0.05$ compared to the group given Crim_04 at the dose of 1660 mg/kg; # – $p<0.05$ compared to the group given loperamide

hyperemized areas were seen. Scaling off of the epithelium was minimal, no exposure of the proper plate of mucous membrane was observed. Blood vessels of mucosa and of submucosal layer were moderately filled with blood. Muscle layer and serous membrane were without pathological changes (Fig. 2 E).

In the control group with modeled serotonin-induced diarrhea 1.4 times decrease in the height of villi, 1.4 times increase in the width of villi at the base and 1.2 time increase in the depth of crypts were determined as compared to the intact animals. Application of enterosorbent with code Crim_04 at the dose of 3220 mg/kg significantly improved the histological pattern of the small intestine bringing morphometric parameters up to the level of the intact animals (Fig. 3).

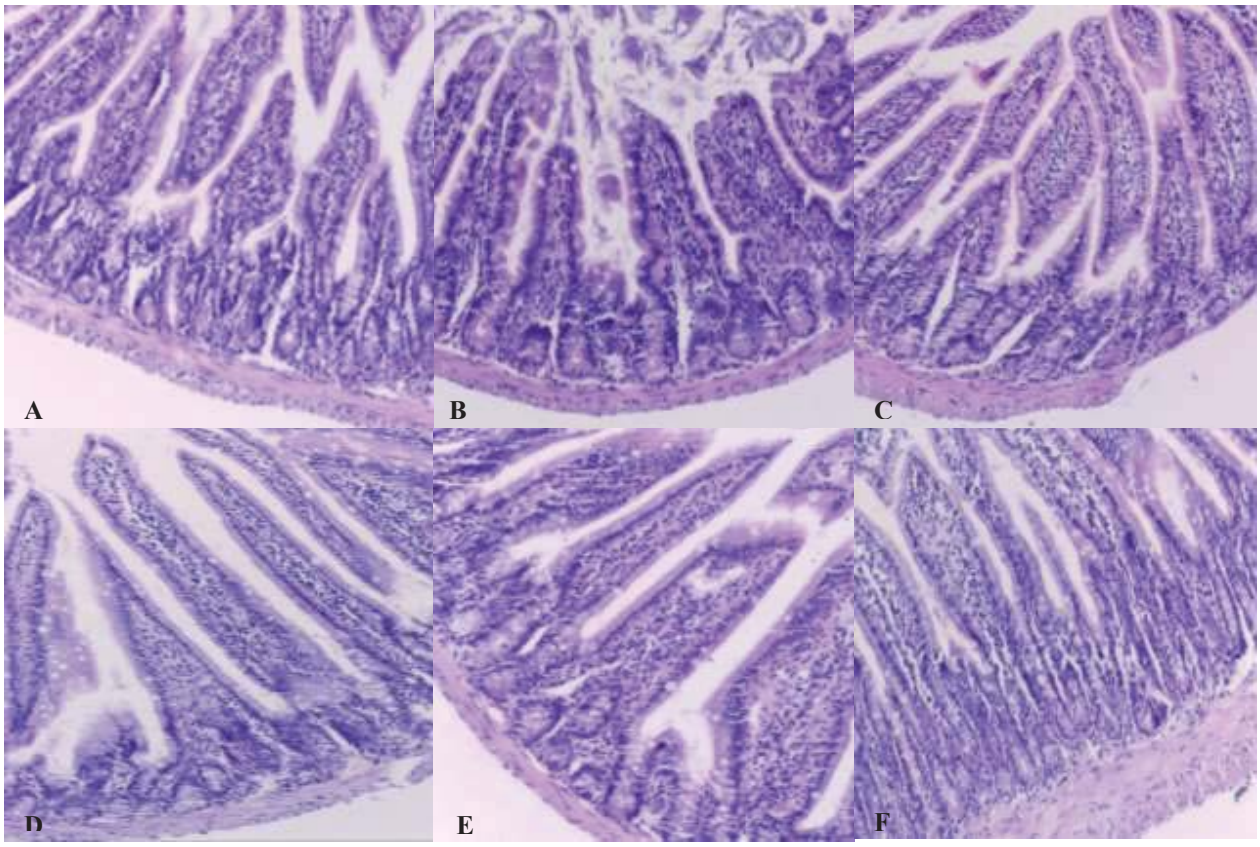

Fig. 2. Histological structure of small intestine in mice (microphoto x100):
A – intact animals; B – control group; C – enterosorbent Crim_04 at the dose of 1660 mg/kg; D – enterosorbent Crim_04 at the dose of 3220 mg/kg; E – enterosorbent Smecta at the dose of 1660 mg/kg; F – loperamide 10 μg/kg. Staining with hematoxylin and eosin

Serotonin is an important signaling molecule that controls secretion of the intestinal epithelium, motility of the walls of gastro-intestinal tract [15,16]. The mechanism of laxative effect of serotonin is likely to consist in stimulation of 5-HT_3-receptors. This causes increase in the motility of the gastro-intestinal tract, in intestinal secretion with the result of accumulation of increased amount of fluid in the gut lumen and its accelerated evacuation in the natural way [14,17]. In modeling of serotonin-induced diarrhea early development of diarrhea in mice was noted (12.8±1.2 minutes), sharp increase in the number of defecations and in the content of fluid in fecal masses (19.5±0.5 and 2.47±0.02 respectively as compared to the group of intact animals – 7.9±0.04 and 1.0±0.0 respectively).

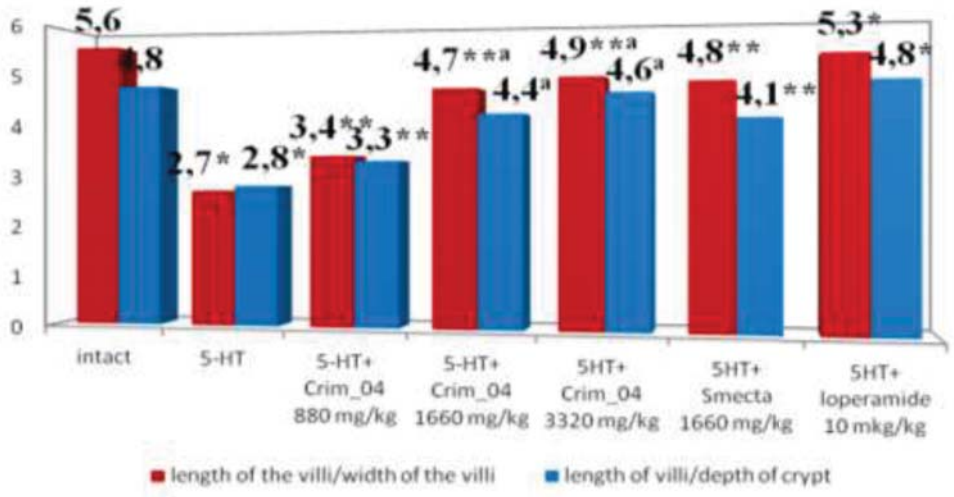

Fig. 3. Effect of enterosorbent coded Crim_04 on the ratio of the length of villi to the width and the length of villi to depth of crypts in small intestine of mice in modelling serotonin-induced diarrhea (conv. units).

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

Antidiarrheal effect of enterosorbent coded Crim_04 depended on the dose. The enterosorbent coded Crim_04 produced the highest effect at the dose of 3320 mg/kg that was manifested in the model of experimental diarrhea by prolongation of the onset of diarrhea, reduction in the total number of defecations and reduction in the amount of fluid in feces. This effect is probably associated with sorption of fluid excessively secreted into the gut lumen, with increase in the density of fecal masses, and as a result, with reduction in the speed of movement of fecal masses through the intestine [18].

Conclusions

Enterosorbent on the basis of montmorillonite with laboratory code Crim_04 possesses dose-dependent antidiarrheal activity in modeling of serotonin-induced diarrhea.

Authors have no conflict of interest to declare.

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