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Research Article

The Study of Acute and Subchronic Toxicity of Therapeutic Undory Clay

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ABSTRACT

One of the most promising methods of sorption detoxification is enterosorption. The detoxification effect of enterosorption is largely determined by the physico-chemical properties of the sorbents, the nature of the pathological process and the way the sorbents are introduced. In Russia there have been registered 14 pharmaceutical substances, on the basis of which 32 medicinal preparations with adsorption activity were developed. Nevertheless, not all of them have been widely applied in clinical practice, so the problem of inventing new drugs with enterosorption properties is topical. In this respect, natural mineral raw materials, in particular therapeutic clays, are promising. The introduction of modern enterosorbents into clinical practice presupposes compulsory detailed study of their specific pharmacological activity and safety at the stage of experimental preclinical studies. The authors consider the therapeutic Undory clay as a promising source of enterosorbents. The article gives the results of studying the acute and subchronic toxicity of the medical clay Undorovskaya, proving the safety of its further use as an enterosorbent.

Keyword: Therapeutic; undory clay; acute toxicity; subchronic toxicity

INTRODUCTION

Detoxication of the body with the use of the enterosorption method is currently an independent method of treatment of enteric intoxications, and is also included in complex therapy for various diseases (acute respiratory viral infections, digestive disorders, intestinal infections, poisoning with toxic substances, allergic diseases, etc.) [1].Unlike invasive methods, enterosorptionis based on oral intake of medications that can adsorb toxic substances of endogenous and exogenous origin in the digestive canal. It is one of the safest methods, which has almost no contraindications, and highly effective as well [2].Quite promising in this respect there turn out to be mineral raw materials, in particular clays. They have a good adsorption effect, and they also have a lot of direct and mediated therapeutic and prophylactic effects, which are caused by the physico-chemical properties and structure of clay-forming minerals which can bind and remove toxic substances from the body [3].

The aim of the study is to study the acute and subchronic toxicity of the therapeutic Undory

clay to solve the issue of safety of its further use as an enterosorbent.

MATERIALS AND METHODS

The therapeutic Undory clay (TU 9369-002-02590678-2006) was obtained from the unique powerful Kimmeridgian clay deposit of the Undory resort of the Ulyanovsk region. The samples represented extremely thin fine powder of gray color. Animal experiments were conducted in accordance with the rules adopted by the European Convention for the Protection of Vertebrates used for experimental and other scientific purposes (European Convention for the Protection of Animals Vertebrate Animals for Experimental and Other Scientific Purposes (ETS 123) .Strasbourg, 1986) [4].

The study of acute toxicity was conducted on 80 mature white noninbred mice of both sexes with a body weight of 20-24 g, which were randomized to control and four test groups. Each group consisted of an equal number of males and females. All the test animals were kept in identical conditions, with the standard temperature and light conditions and diet. Access to water and food was free. Therapeutic Undory clay was injected in the form of 12%, 24%, 32%, 40% suspension to the experimental animals intragastrically once in doses of 3,000 mg / kg, 6,000 mg / kg, 8,000 mg / kg, 10,000 mg / kg, respectively. The control mice received equivalent volumes of purified water.

Observations of the animals were carried out for two weeks. At the same time, the results of intoxication and the number of dead animals were noted. Subchronic toxicity was studied in 80 mature white noninbred mice of both sexes with a body weight of 20-24 g, which were randomized to control and four experimental groups (males and females).

The therapeutic clay was injected into the stomach of the animals at doses of 3,000 mg / kg, 6,000 mg / kg, 8,000 mg / kg, 10,000 mg / kg for 14 days. The choice of experimental doses is determined by the fact that the minimum dose under study(3000 mg / kg) corresponds to a double daily therapeutic dose recommended in clinical practice for enterosorbents of this group. As a standard, a similar enterosorbent on the basis of dioctahedralsmectite was chosen, taking into account the recommendations of GOST 12.1.007-76, the modified OECD classification and the GHS classification.

The maximum dose - 10,000 mg / kg - was a 7-fold daily therapeutic dose, and was maximum possible in volume for introduction into the stomach of mice. The remaining doses (6,000 mg / kg, 8,000 mg / kg) were intermediate. [6].

During the subchronic experiment, we observed daily the general condition in the experimental animals. The dynamics of body weight was assessed relatively to the baseline at 3, 7, 10, 14 days.

The evaluation of the emotional-behavioral reactivity of the mice was carried out using the Open Field test (OP). The test was performed before the injection of the drug (background indices) and after the injection: on day 1 and on day 14 [7,8].

The study of hematological parameters included counting the number of erythrocytes and leukocytes in a semi-automatic hematological analyzer ("HOSPITEX DIAGNOSTICS", Italy). Determination of biochemical blood indices (ALT, AST, LDH, alkaline phosphatase, urea, creatinine) was carried out with the help of a biochemical analyzer ("HOSPITEX DIAGNOSTICS", Italy) using standard biochemical sets produced by Diakon-DS (Russia).

Euthanasia of animals was carried out in a CO_2 - chamber.

At the end of the suchronic experiment, the absolute and relative masses of the internal organs of the experimental animals were determined.

Pathomorphological studies were conducted with a macro- and microscopic examination of internal organs - the liver, the fundus of the stomach, the lean and colon of the mice. For this purpose they were fixed in a 10% formalin solution, and then poured into paraffin. Sectionsof 5 microns thick were coloured with hematoxylin-eosin and examined with a light microscope (Carl Zeiss Primo Star, Germany) with an increase of x150.

The data were processed with the use of the IBM SPSS program, Statistics Version 20. The methods employed are descriptive statistics, Student's t-test and one-way analysis of variance with a posteriori criteria. The differences were considered significant at $p < 0,05$.

RESULTS

The results of the studies showed that intragastric injection of the drug in all the tested doses (from 3 000 to 10 000 mg / kg), with a single injection, did not lead to the death of mice (table 1).

Table 1: Acute toxicity of therapeutic Undory clay upon injection into the stomach of mice

Dose (mg / kg)	Number of animals in the group	Number of death
3000	16	0
6000	16	0
8000	16	0
10000	16	0
Control, water	16	0

With a single injection of the drug into the stomach, the behavioral and physiological responses of the animals were within the normal range of the tested doses in the first 8 hours of continuous observation: satisfactory appearance, the condition of the coat and visible mucous membranes, normal coordination of movements, frequency of urination and urine staining. There was an adynamia and increased grooming in mice, which received the suspension at doses of 8,000 mg / kg and 10,000 mg/ kg.

On the following day, in mice, which received suspension in the maximum dosage (10,000 mg / kg), there was marked fecal matter. Boluses for some time did not depart from the anus. These symptoms lasted for 2 days on their own. It should be noted that there were no signs of pain reaction.

During the subchronic experiment, the drug did not affect the behavioral responses of the animals receiving clay at doses of 3,000 mg / kg, 6,000 mg / kg, 8,000 mg / kg, 10,000 mg / kg. They were within the physiological norm: normal coordination of movements, satisfactory frequency of urination and urine staining were noted. There was marked a slight compaction of the consistency of the faecal mass. During the whole subchronic experiment the drug did not influence the dynamics of the body weight of mice receiving the drug at doses of 3,000 mg / kg, 6,000 mg / kg, 8,000 mg / kg, 10,000 mg / kg compared to controller ($p < 0,001$) (table 2)

Table 2: Dynamics of the body weight of mice (d) during the subchronic experiment

Doses of injected suspension, mg / kg	Terms of study, days				
	1	3	7	10	14
3000	22.0±0.26	22.2±0.27	22.3±0.32	22.8±0.31	23.6±0.29
6000	21.7±0.35	21.9±0.36	22.7±0.29	23.0±0.30	23.6±0.29
8000	21.9±0.84	22.2±0.60	22.7±0.20	23.2±0.19	23.7±0.16
10000	21.6±0.95	21.8±0.96	22.4±0.29	22.8±0.28	23.4±0.29
Control, water	21.7±0.36	21.8±0.36	23.9±0.44	22.8±0.31	23.4±0.30

While studying the behavior of animals in the "Open field" test, under the conditions of the subchronic experiment, on the first day before and after the injection of the drug, a similar pattern was observed: after placement in the chamber immediately there was a short fading reaction followed by active orienting and research behavior. Besides, they tried to leave the central part of the chamber, where the animals had been initially placed. Then, along the periphery, there were short vertical stands without support to the

side of the chamber, there should be also noted "mink reflex" and moderate emotionality, characterized by small episodes of short grooming, infrequent acts of defecation. On the 14thday of observation, the "Open field" test was repeated.

Hematologic blood indices were evaluated in mice injected with clay in doses of 6,000 mg / kg, 8,000 mg / kg, 10 000 mg / kg and the control group(table 3). Injection of the drug for 14 days in high doses did not cause significant changes in the biochemical parameters of the mice (table 4).

Table 3: Hematologic indices of animals in the subchronic experiment with the introduction of therapeutic Undory clay in doses of 8000 and 10,000 mg / kg, (n = 80)

Group of Animals	With the injection of a dose of 8000 mg / kg				With the injection of a dose of 10,000 mg / kg			
	The absolute number of leukocytes * 10^9 / l		The absolute number of red blood cells, 10^{12} / l		The absolute number of leukocytes * 10^9 / l		The absolute number of red blood cells, 10^{12} / l	
	Before the injection of the drug	At the end of the experiment	Before the injection of the drug	At the end of the experiment	Before the injection of the drug	At the end of the experiment	Before the injection of the drug	At the end of the experiment
Controlfemales	5.0±0.2	5.0±0.3	5.7±0.2	5.7±0.1	5.1±0.3	5.1±0.4	5.7±0.1	5.6 ±0.2
Experimentalfemale	5.1±0.3	5.1±0.4	5.7±0.5	5.7±0.4	5.0±0.3	5.1±0.3	5.6±0.4	5.6±0.5
Controlmales	5.2±0.2	5.2±0.1	6.0±0.1	6.0±0.2	5.2±0.3	5.2 ±0.2	6.0±0.3	6.0±0.5
Experimentalmales	5.2±0.3	5.2±0.2	6.0±0.2	6.0±0.2	5.2±0.3	5.2±0.2	5.9±0.3	5.9±0.3

Table 4: Biochemical indices of blood serum of the mice receiving therapeutic Undory clay in doses of 6000, 8000 and 10,000 mg / kg in a subchronic experiment

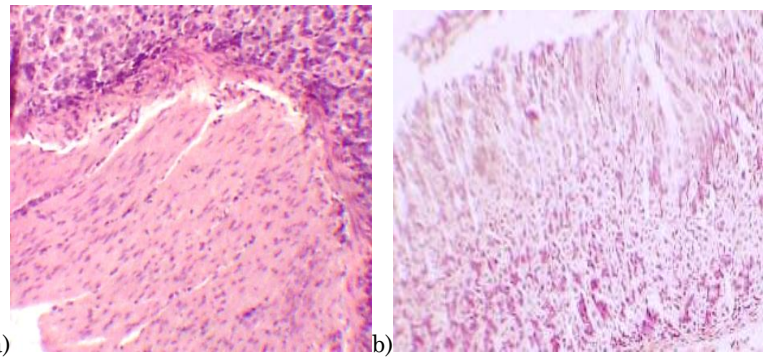
Indicators	Groups / doses (mg / kg)							
	6000		8000		10000		Control	
	Males	Females	Males	Females	Males	Females	Males	Females
ALT/GPT, u/ l	51.0±5.0	48.0±2.4	50.0±4.0	49.0±3.7	51.0±5.0	49.0±4.4	51.0±5.2	49.0±2.1
AST/GOT, u/ l	115.0±10.0	120.0±11.3	117.0±15.6	120.0±13.3	117.0±11.3	121.0±12.0	118.0±14.3	120.0±13.2
LD/LDH, U/ l	1796± 25.4	1967±23.8	1789±19.0	1899±24.2	1795±21.5	1900±21.7	1807±19.0	1978±20.0
ALP, U/ l	329.0±9.0	323.0±8.8	331,0±7.9	329.0±9.3	330.0±9.0	327,0±7.8	330.0±8.3	326.0±7.9
UREA-UV, mkmol / l	7.1±0.9	7.0±0.4	7.1±0.5	7.1±0.6	7.0±0.3	7.1±0.5	7.1±0.4	7.1±0.2
Creatinine, mkmol / l	65.0±2.4	69.3±3.0	68.6±2.9	71.0±3.1	56.4±2.5*	58.7±2.1*	70.7±4.2	71.4±3.4

The values of the mass coefficients [9] of the liver, stomach (fundus), small intestine (jejunum) and colon (colon) of mice in experimental groups were calculated (table 5).

Table 5: The absolute mass of the internal organs of mice (n = 80)

Investigated Organs	Groups / doses, mg / kg			
	6000	8000	10000	Control
Liver	1,959±0,3	1,964±0,3	1,962±0,2	1,959±0,1
Stomach (fundus)	0,666±0,07	0,667±0,06	0,669±0,09	0,667±0,09
Smallintestine (jejunum)	2,161±0,1	2,162±0,3	2,161±0,1	2,163±0,3
Largeintestine (colon)	1,318±0,3	1,316±0,2	1,317±0,2	1,316±0,3

A histological examination of the internal organs of the animals of the control and all experimental groups was carried out. Thus, the structure of the stomach wall of animals corresponds to the norm: the mucosa has a typical structure lined with glandular prismatic epithelium, with well-defined gastric pits. Native stomach glands are well developed. In the mucosa and submucosa, signs of inflammation, vascular reactions, or dystrophic changes are absent. The muscular membrane is three-layered, without features (figure 1).



a) b)

Fig. 1: Microscopic structure of the muscular membrane of the stomach (a, b) of animals whichreceived therapeutic Undory clay at a concentration of 10 000 mg / kg for 14 days. Stained with haematoxylin-eosin , increase X150.

In the jejunum, the villi of the mucosa are high, covered with a crimped epithelium without visible defects. The number of goblet cells is insignificant. The crypts are narrow, deep, at the bottom in the epithelium of the figure of mitosis. In a submucosal base, single lymphoid follicles are found. The muscular membrane is relatively thin, including the inner circular and outer longitudinal layers, separated by a thin layer of connective tissue.

The mucosa of the large intestine is lined with a single-row prismatic limbic epithelium; the number of goblet cells reaches 50% of all available cell types, which corresponds to the norm. Crypts are well expressed, at the bottom there can be determined figures of mitosis, indicating the presence of physiological regeneration. Vessels of connective tissue stroma are not dilated. In the submucosa there are lymphoid follicles, the germinal centers in which are not expressed, since there is no acute phase of inflammation. The muscle shell is represented by two layers, with no visible changes. Morphological manifestations of pathological processes in the thick and thin sections of the intestine are absent.

On animal liver preparations of all studied groups there can be observed a characteristic lobate structure. Interlobular connective tissue partitions are not expressed, which corresponds to the specific features of the structure of the liver of mice. Microscopic signs of edema, fullness, venous congestion, inflammatory and dystrophic changes are absent. Vessels of portal and caval systems without features, perisinusoidal spaces are not expanded. In hepatocytes, uneven deposition of glycogen inclusions is determined. Intrahepatic bile ducts are lined with cubic epithelium, not dilated. The microscopic structure of the liver corresponds to the norm (Figure 2).

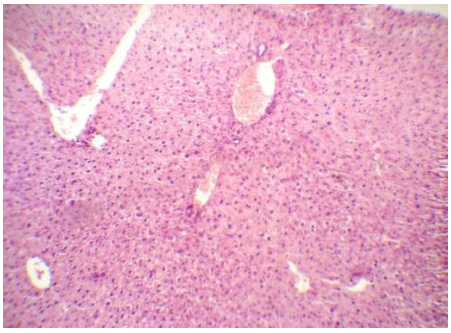


Fig. 2: Microscopic structure of the liver of the animals which receivedUndory clay in a concentration of 10 000 mg / kg for 14 days, increase X150

DISCUSSION

Since the intragastric administration of the drug in all tested doses (from 3 000 to 10 000 mg / kg), with a single injection, did not lead to the death of mice, it was not possible to calculate the parameters of acute toxicity. Thus, with a single-dose injection into the stomach, the dose that did not cause death of the experimental animals was 10 000 mg / kg. In accordance with GOST 12.1.007-76, the drug can be attributed to class IV of toxicity and danger, which is a low-toxic substance. There were no differences in the symptoms of acute drug poisoning between male mice and female mice.

During the subchronic experiment the drug did not affect the behavioral responses of the animals receiving clay at doses of 3,000 mg / kg, 6,000 mg / kg, 8,000 mg / kg, 10,000 mg / kg, which implies good tolerability of the study object.

While studying the behavior of animals in the "Open field" test, under the conditions of the subchronic experiment, on the first day before and after the injection of the drug, a similar pattern was observed. On the 14thday of observation, the "Open field" test was repeated. There was a predominance of horizontal motor activity along the periphery of the chamber, short episodes of movement to the center of the chamber and peering into the "mink". Emotional condition of the animals was also moderate. The indices of the animals of the control and experimental groups did not differ significantly (p = 0,796) (Figure 3, 4).

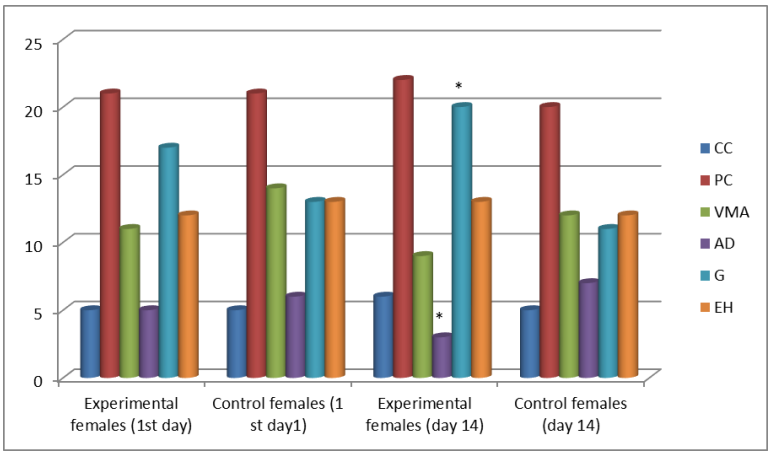


Fig. 3: The number of forms of behavior of female mice in a subchronic experiment with the intragastric injection of therapeutic Undory clayin a dose of 10,000 mg / g, (sc-intersection of sectors in the central sector of the chamber, ps-intersection of sectors in the peripheral part, vma- vertical motor activity, ad- acts of defecation, g-grooming, eh- examination of holes)

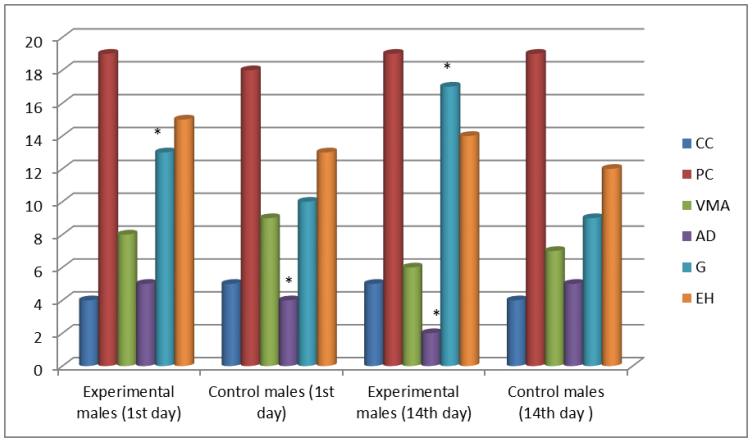


Fig. 4: The number of forms of behavior of male mice in a subchronic experiment with the intragastric injection of therapeutic Undory clayin a dose 10,000 mg / g, (sc-intersection of sectors in the central sector of the chamber, ps-intersection of sectors in the peripheral part, vma- vertical motor activity, ad- acts of defecation, g-grooming, eh- examination of holes)

Comparing the indices of vertical motor activity by groups on day 1 and day 14, there were found statistically significant differences (p = 0,029). The changes in vertical motor activity can be explained by a decrease of interest of the animals to the familiar object. In the females and males of experimental groups, the number of large grooming episodes is significantly higher in comparison with the control group (p <0,001). Presumably, this is due to the method of injection and the desire to remove the residues of the introduced suspension. In the males and females of experimental groups, on the 14th day of injection of the drug, there was a significant decrease in defecation acts (p <0,001). The number of boluses and acts of defecation refer to the emotional behavior of the animal. In our case, this fact is also one of the signs of a gastrointestinal disorder in the form of constipation. There were found boluses kept for some time under the tail of the animal. This phenomenon disappeared on its own after the injection of the drug was stopped.

Hematologic blood indices were evaluated in mice injected with clay in doses of 6,000 mg / kg, 8,000 mg / kg, 10 000 mg / kg and the control group. There were no statistically significant differences within the groups at the beginning and at the end of the experiment (p> 0, 05).

Injection of the drug for 14 days in high doses did not cause significant changes in the biochemical parameters of the mice.

When considering indicators which characterize the function of the kidneys, there was only a statistically significant decrease in serum creatinine in the mice receiving a clay suspension at a maximum dose of 10,000 mg / kg (p <0,001). In our case, the decrease in the index is due to the physicochemical properties and ion exchange capacity of clays (correction of biologically important cations Na +, K + and Mg 2+)

The calculated values of the mass coefficients [9] of the liver, stomach (fundus), small intestine (jejunum) and colon (colon) of mice in the experimental groups did not differ significantly from the control ones (p> 0,05) (Table 5).

On histological examination of the internal organs of the control animals and all experimental groups there were revealed no pathological changes. The histological structure of the animal organsunder study, which received intragastrically therapeutic Undory clay in doses of 3000 mg / kg, 6000 mg / kg, 8000 mg / kg, 10,000 mg / kg for 14 days, does not have pathological changes and corresponds to the norm for this species of animals.

CONCLUSION

The presented results of the study show that withsingle and long-term injection (subchronic experiment)Undory clay in doses of 3 000 mg / kg, 6 000 mg / kg, 8 000 mg / kg, 10 000 mg / kg does not have toxic effects on the body of mice (males and females), and does not cause pathological changes in the internal organs (liver, fundus of the stomach, jejunum and colon). Injection of a dose of 10 000 mg / kg causes a temporary increase in acts of grooming, compaction of stool and worsens their defecation,

which is associated with the physicochemical and sorption properties of the object under study.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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