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Physical-Chemical and Pharmacological Research on New Lignin Enterosorbent

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Abstract: The technology on obtaining enterosorbent from local feedstock—hydrolyzed lignin of cotton seed hulls named Zerotox was developed. The purpose of this research is to conduct a complex study on its physical-chemical and pharmacological properties. Samples of cotton hydrolyzed lignin based enterosorbent Zerotox were investigated, wood hydrolyzed lignin based enterosorbent Polifepan (Russia) was used as a comparison drug. The technology of new medical enterosorbent Zerotox, based on cotton hydrolyzed lignin, has been developed with its physical-chemical and pharmacological characteristics being researched. Comparative research on adsorbing capacity of sorbent Zerotox (80 mg/g) and its analogue—Polipefan (55 mg/g), which is obtained from wood hydrolyzed lignin, has displayed advantages of the new sorbent. IR-spectroscopy research on lignin sorbent Zerotox has shown presence of oxygen-containing functional groups which determine its high absorbing capacity due to its chemisorption. Atomic-adsorptive method has proved high absorbing capacity of the sorbent towards heavy metals ions. Acute and chronic toxicity and antidiarrheal activity of enterosorbent Zerotox have also been studied.

Key words: Medical sorbents, cotton hydrolyzed lignin, detoxification, absorbing properties, adsorbing capacity, functional groups, atomic-adsorptive method, pharmacological research.

1. Introduction

Pollution of environment with toxic substances and pathogenic flora causes outbreak of diseases accompanied by very strong toxicosis wherein pharmacological correction methods do not always bring positive results. According to present knowledge, internal environment of human is constantly hit by foreign communications. Over time, these substances accumulate in the body and contribute to development of endocrine and oncologic diseases, cardiovascular system and gastrointestinal tract disorders, and abnormality of embryonal development and others.

Efficient neutralization of negative impacts of all of those factors can be a solution to mentioned problems. In this regard, sorption therapy, which is aimed at detoxification of human body and is achieved via application of high-performance sorbents of medical purpose, becomes significant [1-5]. Medical sorbents are in high demand both for delivery of emergency medical care (acute toxicosis) and for nonoperative therapy of dozens of diseases. Today, up to 15% of patients are in need for sorption method treatment, while the demand for medical sorbents in environmentally adverse regions of our country alone is around 2 tons per year.

In this respect, developing technology of home based medical sorbents production and studying their efficiency by physical-chemical and pharmacological methods gain greater significance.

Previously determined absorbing properties (highly developed surface and large number of functional groups) of technical hydrolyzed lignin of cottonseed hulls served as precondition for obtaining new lignin

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enterosorbents [5]. However, technical hydrolyzed lignin, besides containing its own lignin and small amount of non-hydrolyzed cellulose, has humic, resinous and ash substances that may have an adverse impact on human body if they reach it. Alkali treatment and activation is carried out in order to clean it from unwanted adulterant [6].

It has been known that medical lignin sorbent obtained as a result of alkali treatment is unique thanks to the following functional groups it contains: methoxy, carboxylic, carbonyl, different nature hydroxylic and others [7]. This presumes an opportunity for chemical absorbing and complexing with various sorbates. On sorption capacity, cleaned *E. coli* lignin is 5-10 times better than granular carbons; it adsorbs heavy metals well and is distinguished by high efficiency during various types of intoxications. Being also soft-skeletal, it does not traumatize (compared to activated carbon and other hard-skeletal drugs) intestine mucous membrane and is capable of sorption of macromolecules and bacterial cells [8-10].

In this respect, we developed technology on obtaining enterosorbent from local feedstock—hydrolyzed lignin of cotton seed hulls named Zerotox. The purpose of this research is to conduct a complex study on its physical-chemical and pharmacological properties.

2. Materials and Methods

Samples of new cotton hydrolyzed lignin based enterosorbent Zerotox were researched. Wood hydrolyzed lignin based enterosorbent Polifepan (Russia) was used as comparison drug.

2.1 Identifying Adsorbing Capacity of Enterosorbent

Research was conducted by spectrophotometric method. Spectral characteristic of solutions were taken on spectrophotometer PYE UNICAM SP-8-500 with wave length of 668 nm. Cleaned water was used as comparison solution.

In order to carry out research, around 0.3 g of the drug (accurate weight) is put into 200 mL conical flask with a ground stopper, and 100 mL of methylene blue solution with concentration of 0.0001 g/mL is added and mixed on rotational rocking device or on shaker with oscillation number at least 120 min^{-1} in the course of an hour.

Then, contents of the flask are filtered through glass filter (#POR 40).

5 mL of both filtered material and original methylene blue solution are put into 100 mL measuring flasks, solution volumes are brought up to marked line with water and optical density of solutions is measured on spectrophotometer with wave length of 668 nm, in cuvet with a 10 mm thick layer. Adsorbing capacity of the drug in grams is figured by the following formula:

$$X = \frac{(Do - D_1)\cdot C\cdot 100}{Do\cdot m\cdot(1 - 0.01W)} \quad (1)$$

where,

Do—value for optical density of original methylene blue solution;

D_1—value for optical density of original methylene blue solution after sorption;

m—drug mass weight, g;

W—drug wetness, %;

C—concentration of methylene blue solution taken for sorption, g/mL;

100—volume of methylene blue solution taken for sorption, mL [11].

2.2 IR Spectroscopic Research of Samples

Presence of oxygen-containing functional groups on the surface of lignin sorbent is judged by detection of definite loss zones which are typical for some chemical bindings. IR spectrum of researched samples were registered on IR spectrophotometer AVATAR 360 made by Nicolet Instrument Corp., the USA. Samples in shape of "tablet" with KBr diameter of 7 mm were researched. Resolution amounted to 8 cm^{-1}, while research area to 4,000-400 cm^{-1}.

2.3 Identifying Sorption Tanks of Sorbent with Respect to Heavy Metal Ions

Researches are conducted by atomic-absorption analysis method under following conditions (Table 1):

Device—AAC Unicam-929, System "Solar";

Flame—acetylene: Air (1:1);

Method—absorption.

2.3.1 Preparing a Test sample to Identify Sorption Tank Figure (Solution B)

Around 1.0 g (accurate weight) of test sample—lignin sorbent—is weighted into 100 mL conical flask with a ground stopper and 20 mL of solution C is added. Then it is stirred for 30 minutes on mechanical stirrer and left for 24 hours. After that time, obtained solution is filtered through double decalcified paper filter.

Depending on used sorbents samples with varying wetness, two samples are prepared: sample #1 using lignin of 60% wetness, sample #2 of 20% wetness.

2.3.2 Preparing Standard Solutions of Cadmium, Plumb,Iron, Chrome, Zinc (Solution C)

1 mL of standard solution, which contains 1,000.0 mg/L ion of cadmium, plumb, iron, chrome and zinc, is put into 250 mL measuring flask. Then solution volume is brought up to marked line with water and stirred. Concentration of each heavy metal ion is 4.0 mg/L. The adsorbing capacity of sorbent is judged by the difference on composition of heavy metal ions in solutions C and B.

2.4 Research on Acute Toxicity of the Drug

Study on acute toxicity of lignin enterosorbent Zerotox was carried out on 18 white mice of both sexes weighting 18-21 g. Based on a pulvis, 6-25% suspension was prepared with Tween-20 being added as emulsifier. Prepared suspension was infused in a volume not exceeding 0.8 mL per mouse. This volume was limited due to a provision that mice are infused 0.8 mL at most. After 1-fold infusion of the drug, monitoring was held every hour on infusion day, 3 times a day on the second and third days, and once a day during the following 5 days of experiment. General behavior, wool marking, mucous condition, breath, heartbeat, motion activity and death of mice were registered [12, 13].

2.5 Methodology on Studying Specific Anti-diarrheal Activity

In order to identify specific activity of enterosorbent Zerotox, its anti-diarrheal activity was examined using 180-220 g weight male rats divided into 3 groups of 6 animals in each. Diarrhea was caused by including McClung H. J. lactose into food ration:

(1) intact group received normal laboratory ration;

(2) control group received diet with 40% lactose + adequate amount of physiological solution;

(3) experiment group received lactose + 50.0 mg/kg Zerotox;

(4) experiment group received lactose + 100.0 mg/kg Zerotox.

In all experiments, pulvis was infused in form of suspension prepared by adding emulsifier Tween 20 to water solution. Suspension had been infused into rats in form of water suspension using noninvasive catheter in 50 and 100 mL/kg dose once per day, before they were put in their individual metabolic cells for the whole feces collecting period. Mass of the feces

Table 1 Experiment conditions.

Analyte element name	Resonance line (nm)	Chink (nm)	Cathode lamp voltage (ma)
Plumb (Pb)	283.3	0.5	10
Cadmium (Cd)	228.8	0.5	12
Iron (Fe)	248.3	0.2	15
Zinc (Zn)	213.9	0.7	10
Chrome (Cr)	357.9	0.7	8

was estimated once a day applying gravimetric method and total feces mass was registered. Then feces mass was dried up until constant mass was established and wetness content of feces was registered in mg and in %.

2.6 Methodology on Identifying Chronic Toxicity of the Drug.

Chronic toxicity of lignin enterosorbent was studied on white rats. White rats of both sexes with original mass of 140-160 g were divided into 4 groups of 8 animals in each of the groups (4 male and 4 female).

Every day during 30 days water suspension of lignin enterosorbent of 50.0 mg/kg, 100 mg/kg and 500 mg/kg doses of body mass were infused intragastrically into animals. Such doses were chosen because 50-100 mg/kg is an effective therapeutic dose, so we studied 5 times and 2 times doses, i.e., 500 mg/kg and 50 mg/kg. Control group animals were infused 1-2 mL of distilled water. All experimental and control animals were contained in equal conditions and on common food ration with free access to water and food. Managing and feeding experimental animals complied with generally accepted normative standards.

Functional-metabolic activity of NADP—the dependant system of cytochrome P450 of the liver was estimated based on the following properties: content of cytochrome P450 as well as B5-terminal and middle links of this electric-transport system; activity of NADP-cytochrome of C-reductase of initial link and microsomal ferments—AH (absorption hypercalciuria) (II type oxidization substrate); content of G-6-phase ferment (microsome marker and ensuring their membranous structure by energetic compounds). Estimations of activity of microsomal ferments were done on albumin (mg/mL).

3. Results and Discussion

3.1 Comparative Study on Adsorbing Capacity of Lignin Sorbents

According to bookish data, adsorbing capacity of hydrolyzed lignin depends on its wetness as it can be clearly seen on Fig. 1.

The figure shows that as wetness of sorbents decreases, their adsorbing capacity also decreases, and it can not be restored even after wetting. Thus, at 60% wetness adsorbing capacity of Polifepan sorbent is 55 mg/g and at 10% wetness it is 23 mg/g, while for samples which were dried-up and then wetted up to 60% it is 39 mg/g.

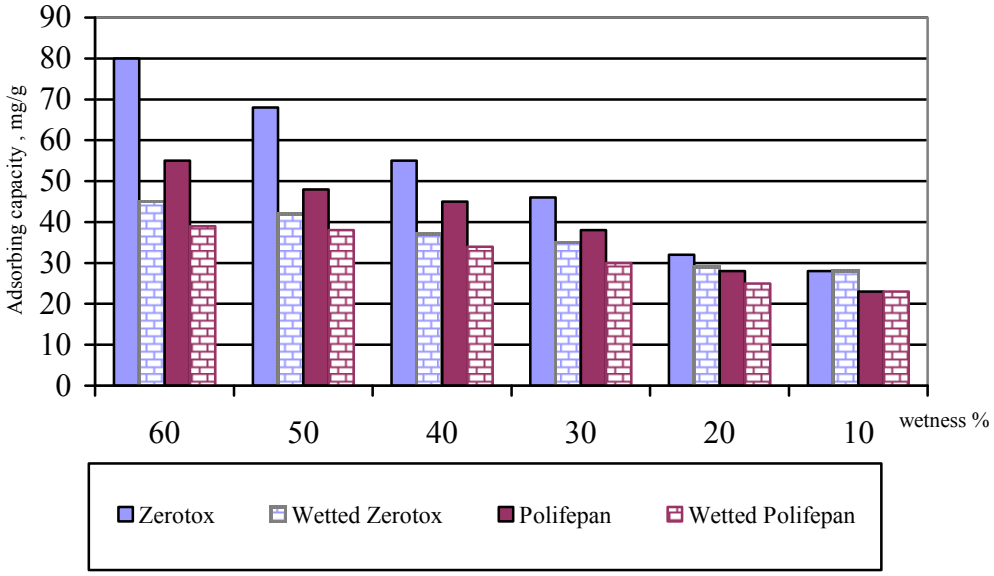


Fig. 1 Dependence of adsorbing capacity of sorbent on its wetness.

Similar condition is observed with Zerotox sorbent, which, respectively, has adsorbing capacity of 80 mg/g at 60% wetness, 28 mg/g at 10% wetness and 45 mg/g for lignin sample which was dried-up and then wetted up to 60%.

This testifies that as lignin dries, part of oxygen-containing functional groups responsible for chemisorption, which does not restore after followed wetting, vanishes. That said, adsorbing capacity of Zerotox is 45% higher than that of Polifepan.

3.2 IR Spectroscopic Research Results

In order to detect presence of functional groups on the surface of cotton hydrolyzed lignin samples, IR spectrums of original technical hydrolyzed lignin as well as samples of medical lignin with 20% and 60% wetness were taken, and the results are shown in Fig. 2.

Based on information from Refs. [14, 15], certain loss zones which are typical for some chemical bindings could be identified within the range of IR spectrum wave length. Thus, broad and intense peak in 3,600-3,000 cm^{-1} belongs to valence vibrations of hydroxyl groups (-OH) which are connected via intermolecular hydrogen bindings, and 2,950-2,800 cm^{-1} belongs to valence vibrations of C-H (CH, CH_2, CH_3 group). ν = 1,709 cm^{-1}, apparently, belongs to valence vibrations of carbonyls of carboxyl groups which are connected via hydrogen bindings with neighboring groupings, while on waves lengths of 1,697-1,698 cm^{-1} lines which are typical for carboxyl groups with internal hydrogen binding were discovered. Around 1,630 cm a line of tightly bound water was observed.

Shorter range of wave lengths of 1,600-1,500 cm^{-1} is typical for vibrations of double carbon bindings (C=C) of benzene ring. Selective loss (both deformational and vibratory) in 1,500-1,200 cm^{-1} interval is typical for such chemical bindings as carbon-to-hydrogen binding (-CH_2- and -CH).

Intensity of loss lines in 1,423-1,400 cm^{-1} area, which can belong to deformation vibrations in methoxy groups, decreases in rows so far as wetness decreases.

Approximately in the range of 1,200-1,000 cm^{-1}, it is the "oxygen bridges" (chemical bindings of C-O-C) that most actively resonate with IR radiation.

In the range of 1,000-500 cm^{-1}, deformation vibrations of O-H and C-H bindings and valence vibrations of C-O- and C-C are proved.

In the most low-frequency range of wave lengths of 500 cm^{-1} and below, it is the double oxygen bindings (C=O type) that have the most selective loss activity.

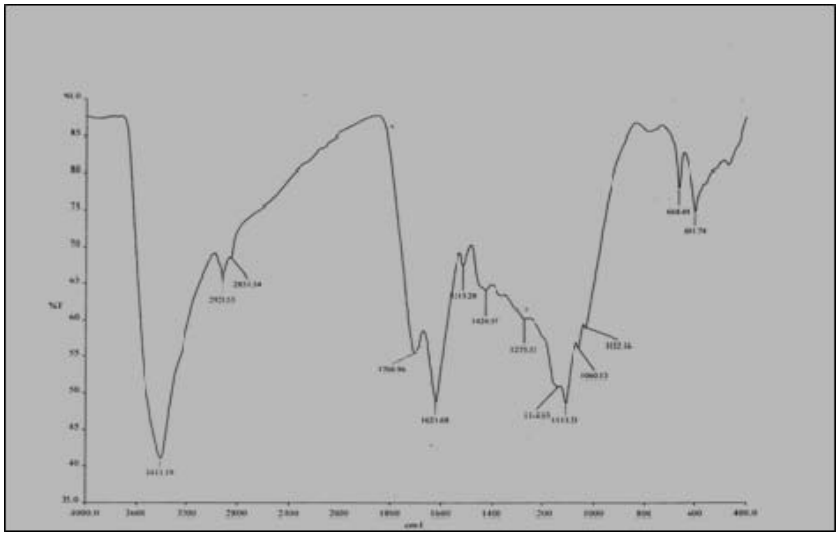
Here it becomes clear how complex, in chemical sense, the surface of researched samples is. This is one of the reasons for high absorbing activity of lignin.

3.3 Research on Sorption Capacity of Lignin Sorbent with Respect to Heavy Metals Ions

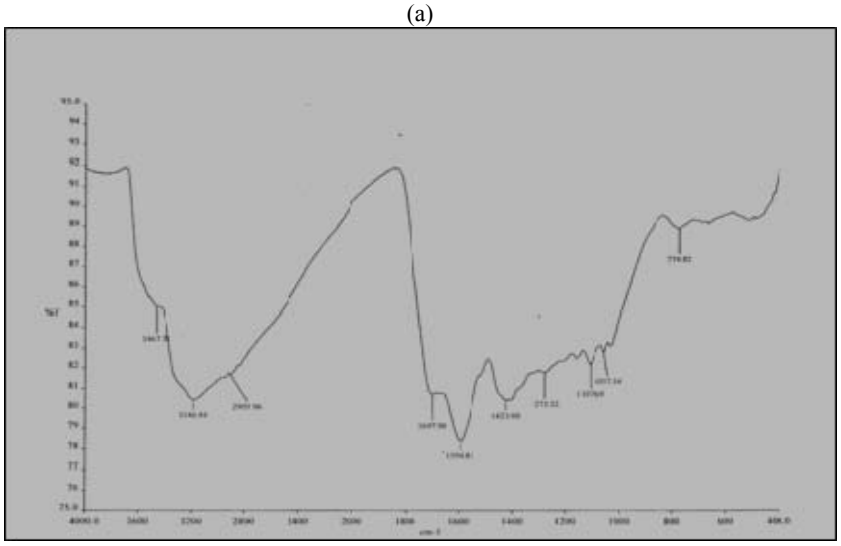
Selectiveness of a sorbent to the ions of heavy metals indicates the level of its quality [16, 17]. Samples of lignin enterosorbent Zerotox with 20% and 60% wetness were researched in order to identify their sorption capacity with respect to some ions of heavy metals. Researches were conducted by AA (atomic-adsorptive) method using Unicam 929 FF spectrometer, SOLAAR System. Results on sorption capacity with respect to Pb, Cd, Cr, Zn and Fe ions are presented in Table 2.

As it is seen from obtained results, concentration of each metal ion in original standard solutions (C) is 4.0 mg/L. After contacting with sorbents, a significant decrease of concentration of heavy metals in solutions is observed. Thus, for example, Pb (plumb) ions were not found at all in experimental solutions after a contact with sorbent.

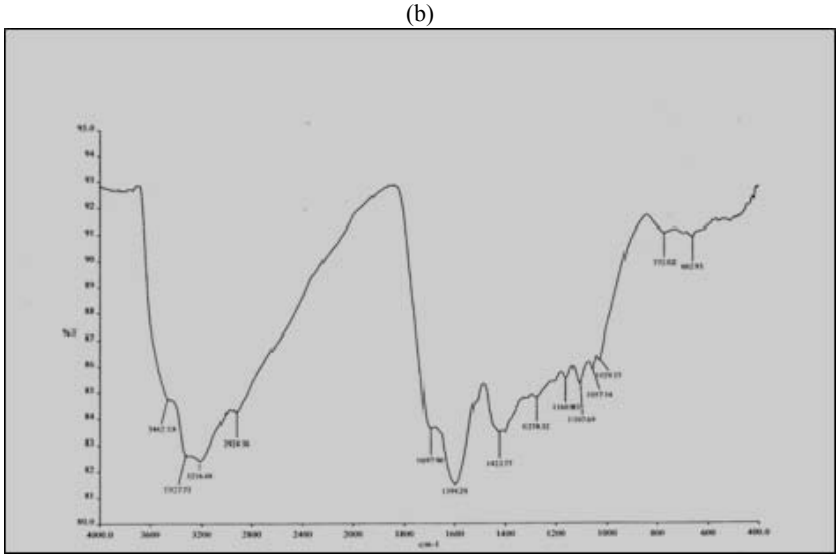
Residual concentration of cadmium in solution was 0.098 mg/L after a contact with wet sorbent, and 0.184 mg/L after a contact with lignin dried down to 20% wetness. Therefore, selectiveness of wet lignin sorbent with respect to Cd ions is higher compared with the one dried down to 20% wetness.



(a)



(b)



(c)

Fig. 2 IR spectrums: (a) original hydrolyzed lignin of cottonseed hulls; (b) Zerotox with 60% wetness and (c) Zerotox with 20% wetness.

Table 2 Results of analysis on heavy metals content in solution C and solution B.

Identified	Samples	Standard solution (solution C)	Assay #1 (solution B)	Assay #2 (solution B)
Optical density	Pb (plumb)	0.010	0.000	0.000
	Cd (cadmium)	0.141	0.005	0.008
	Cr (chrome)	0.011	0.001	0.003
	Zn (zinc)	0.241	0.004	0.007
	Fe (iron)	0.047	0.018	0.030
Concentration (mg/L)	Pb (plumb)	4.0	Non found	Non found
	Cd (cadmium)	4.0	0.098	0.184
	Cr (chrome)	4.0	0.405	1.337
	Zn (zinc)	4.0	0.065	0.112
	Fe (iron)	4.0	0.0965	0.192

Table 3 Results on acute toxicity of lignin enterosorbent pulvis when infused into mice.

Doses (mg/kg)	Number of mice: died/total
2,500.0	0/6
5,000.0	0/6
10,000.0	0/6
LD_{50} > 10,000 mg/kg	

Identical results were obtained when other metals were adsorbed. Despite the fact that almost full extraction of Cr, Zn and Fe ions by wet sorbent took place, intensity of extraction by dry sorbent of the same metals was a bit lower.

Hence, based on obtained research results, it was discovered that experimented lignin sorbents had high adsorbing capacity towards heavy metal ions. Selectiveness towards these ions decreases as sorbent dries, which testifies a loss of certain functional groups is responsible for chemisorption.

3.4 Research on Studying Acute Toxicity of Lignin Enterosorbent

Results of research on studying acute toxicity of lignin enterosorbent Zerotox show that infusion of lignin enterosorbent in 2,500 mg/kg dose did not have significant impact on animals overall condition, behavior and motion activity. After infusion of suspension of lignin enterosorbent in 5,000 mg/kg dose, no particular changes were noticed in behavior of mice. Within 14 days no death of mice were observed. With increase of dose up to 10,000 mg/kg, significant changes in behavior were not noticed and deaths of mice were not observed either (Table 3).

From results obtained from identifying acute toxicity of enterosorbent Zerotox it appears that this substance does not cause death of mice in 10,000 mg/kg dose and can be placed as the 5th class in binding toxicity classification—almost non-toxic.

3.5 Research on Specific Antidiarrheal Activity

Experiments showed that control group animals that received diet with 40% lactose in daily ration had diarrhea that was accompanied by a change of feces form, a loss of shapeliness (statefulness) of feces mass; feces of some animals became liquid in the very first day of the diet (Table 4). On the first observation day general mass of daily feces increased by 2.7 times compared with intact group. Along with increase of feces mass, the main sign of diarrhea—"watery feces"—was marked by increase of wetness by 71.8% against 53.0% compared to intact rats.

Under the effect of tested drug, depending on the dose, output of feces masses decreased down to 1,268.0 ± 35.7 mg after tested drug had been infused in 50 mg/kg dose, and down to 760.0 + 47.0 against 2,300.6 + 28.0 in controlled group, which composed 55% and 33% with respect to controlled group and went close to the level of intact animals.

Wetness in feces of experimental animals was 29.0% and 20.6% respectively, when 50 mg/kg and 100 mg/kg doses were used. Joint effect of lignin enterosorbent on feces mass and decrease of feces water

Table 4 Impact of lignin enterosorbent on feces of rats during diarrhea (M + m, n = 6).

Experiment conditions	Researched figure		
	Total mass of feces (mg)	Dry residual (mg)	Wetness (%)
Intact	850.6 ± 22.0	400.0 ± 34.9	450.0 ± 6.4 53.0 ± 1.8
Control (diarrhea)	2300 ± 28.0	650.3 ± 32.5	1650.3 ± 20.5 71.8 ± 5.0
Lignin enterosorbent, 50 mg/kg	1,268.0 ± 35.7	900.0 ± 210.0	368.0 ± 21.0 29.0 ± 2.3[xy]
Lignin enterosorbent, 100 mg/kg	760.0 ± 47.0	445.6 ± 204	157.9 ± 20.5 20.6 ± 4.1 [xy]

[xy] —Accuracy of difference compared with control when $P < 0.05$.

Table 5 Figures of functional-metabolic activity of NADP-dependent system of cytochrome P450 of the liver after 30-day infusion of lignin enterosorbent in different doses, M ± m (n = 8).

Name	Statistics	Group 1 50 mg/kg	Group 2 100 mg/kg	Group 3 500 mg/kg	Group 4 Control
Cytochrome P450, nmol/mg	M ± m % P	0.90 0.03 +4.6 $P > 0.5$	0.92 0.03 +7.0 $P > 0.25$	0.81 0.02 -5.8 $P > 0.25$	0.81 0.02
Cytochrome b5, nmol/mg	M ± m % P	0.61 0.02 +1.72 $P > 0.5$	0.65 0.02 +8.3 $P > 0.1$	0.58 0.02 -3.3 $P > 0.5$	0.60 0.01
NADP cytochrome C-reductase, nmol/min/mg	M ± m % P	84.6 1.95 +2.5 $P > 0.5$	85.9 2.30 +2.9 $P > 0.25$	80.7 2.83 -3.3 $P > 0.25$	83.5 1.98
AH nmol/min/mg	M ± m % P	0.82 0.04 +2.5 $P > 0.5$	0.84 0.02 +5.0 $P > 0.5$	0.77 0.02 -3.7 $P > 0.5$	0.80 0.03
N-AH nmol/min/mg	M ± m % P	1.60 0.04 +6.7 $P > 0.1$	1.70 0.05 +6.7 $P > 0.1$	1.40 0.03 -6.7 $P > 0.1$	1.50 0.03
G-6-phase nmol/min/mg	M ± m % P	60.3 2.20 +9.0 $P > 0.1$	61.2 3.20 +10.7 $P > 0.1$	52.7 2.70 -4.7 $P > 0.5$	55.3 1.80
Albumin m/s, mg/mL	M ± m % P	34.41 1.55 +1.8 $P > 0.5$	33.9 1.22 +1.0 $P > 0.5$	34.1 1.34 +0.8 $P > 0.5$	33.8 1.72

component points out its antidiarrheal effect. This property speaks for decrease of evacuator-emunctory function of intestines under the effect of tested drug.

3.6 Study on Chronic Impact of Lignin Enterosorbent on Functional-Metabolic Activity of the Liver

During chronic toxicity study, observation on increase of feces masses of rats showed that no difference was noticed between the group of animals that received the drug and the controlled animals group.

Analysis of obtained research results (Table 5) showed that 30-day daily intragastric infusion of lignin enterosorbent in 50 mg/kg dose into animals

contributed to the increase of content of cytochromes P 450 and b 5 by 4.6 and 1.7% ($P > 0.05$), activity of NADP cytochrome C reductase by 1.3 % ($P > 0.59$), AH by 2.5% ($P > 0.5$), N-AH by 6.7% ($P > 2.0$), G-6-phase by 9.0% ($P > 0.1$) and microsomal albumin by 1.8% ($P > 0.5$).

Infusion of lignin enterosorbent is in the same experiment conditions, but 100 mg/kg dose contributed to some bigger increase of functional-metabolic activity of the cytochrome P450 system of the liver. Thus, when compared with intact group, content of cytochrome P450 and b5 was higher by 7.0 and 8.3% ($P > 0.25$ and $P > 0.1$), activity of NADP cytochrome C reductase by 2.9 % ($P > 0.25$), AH and N-AH by 5.0% and 13.3% ($P > 0.5$ and $P > 0.1$), G-6-phase by 10.7% ($P > 0.1$) and microsomal albumin by 0.3% ($P > 0.5$).

In 500 mg/kg dose of tested drug, the activity of cytochrome P450 system of the liver decreased a little compared with previous groups. When compared with data of intact animals group, content of cytochromes P450 and b5 was lower by 5.8 and 3.3% ($P > 0.25$ and $P > 0.5$), activity of NADP cytochrome C reductase by 3.3 % ($P > 0.25$), AH and N-AH by 3.7% and 6.7% ($P > 0.5$ and $P > 0.1$) and activity of glycolysis microsome ferment by 4.7% ($P > 0.5$).

Attention should be paid to the fact that change of parameters which typify functional-metabolic activity of cytochrome P450 system, when different doses of enterosorbent was infused, had a statically non-significant increase in tested animal groups with 50 and 100 mg/kg doses and a decrease with 500 mg/kg dose of lignin enterosorbent.

Hence, obtained data indicate that during 30-day intragastric infusion, regardless of increasing 50, 100 and 500 mg/kg lignin enterosorbent into mature rats, has no impact on functional-metabolic activity of cytochrome P 450 system.

4. Conclusion

Research on study of physical-chemical, absorbing and pharmacologic features of the new lignin enterosorbent Zerotox, made based on cotton hydrolyzed lignin, showed that absorbing capacity of Zerotox is 45% higher than its analogue—Polifepan (Russia) made from wood hydrolyzed lignin. Results of IR spectroscopy proved that chemisorptive activity of sorbent Zerotox is conditioned by presence of oxygen-containing functional groups on its surface. Researched sorbent has high absorbing capacity towards heavy metals ions. Results of pharmacological tests proved non-toxicity of the drug and its specific antidiarrheal activity.

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