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**THE APPLICATION OF SORBEX IN CONDITIONS OF
SIMULTANEOUS AFFECTION OF ANIMALS BY THE ISONIAZID,
RIFAMPICIN AND HEXAVALENT CHROMIUM COMPOUNDS**

In the researches on rats of different age groups were revealed that using of enterosorbent sorbex (150 mg/kg) had expressed decrease processes of free radical oxidation and oxidative modification of proteins in animals of an immature age and an old age compared to the affected rats. It contributed to the decrease of the permeability of cell membranes and endogenous intoxication in the affected organism.

Key words: free radical processes; endogenous intoxication; isoniazid; rifampicin; hexavalent chromium compounds; sorbex

INTRODUCTION. In recent years, there is an increasing of the loading on the human body by various toxic factors that is caused by the environmental pollution from these, including heavy metals [5, 14]. Under the most interest there are the metals that are widely used in the production of human activities, such as lead, mercury, nickel, chromium, cadmium, zinc, cobalt, copper, etc. [14]. The influence on the body of chromium compounds (VI) is accompanied by a wide range of toxic effects and serious damages to internal organs (kidneys, lungs, liver) [15].

At the same time the scientists are continuing the researches of the mechanisms of drug poisoning of an organism [3, 17]. One of the many issues is the study of the biochemical mechanisms of liver injuries with isoniazid and rifampicin, which are widely used to treat tuberculosis. It is known that the above

mentioned anti-TB drugs violate the structure of the liver, reduce the activity of enzymes of energy in lymphocytes, which is leading to the disruption of mitochondrial membrane stability with activating its peroxidal oxidation of lipids and reducing antioxidant defenses [6, 8, 9, 16]. Thus, the risk of hepatitis is increased in the patients who are simultaneously receiving both the isoniazid and rifampicin. In addition to that hepatitis occur in 5-8% of cases, while under the isoniazid monotherapy the appearing of hepatitis is observed in 1.2% of cases, and under the rifampicin monotherapy - 0.3% [8].

Toxic liver damages that are caused by the above mentioned xenobiotics are accompanied with the endogenous intoxication syndrome, for the removing of which some eliminate chelators are used [1, 3, 7]. Sorbents remove toxins from the intestinal lumen, clean digestive juices of the gastrointestinal tract, perform reverse passage of toxins and metabolites from the blood, and modify the lipid and amino acid spectrum of the intestinal contents. According to Y. I. Honskoho and co-authors [4], enterosorption is not symptomatic, but pathogenetic treatment for liver injuries.

At the same time in the modern literature a differentiated approach to the use of absorbents for this pathology is not covered enough. Therefore, there is the topicality of an urgent search and study of the impact of new correction factors on metabolic disorders, which are caused by hexavalent chromium compounds against a background of the isoniazid-ryfampitsynovoho liver damage.

The materials and approaches of research.

Experiments, which were conducted on outbred white male rats of three age periods: puberty (3-month animals weighing 90-110 g) - 1st age group, sexual maturity (6-month animals, weighing 150-170 g) – 2nd aging and aging group (animals 18 months of age, weighing 280-300 g) - 3rd age group were kept on a standard diet vivarium of the Ternopil State medical University. The experimental destruction of the animals was carried out under conditions of combined administration of isoniazid, rifampicin and hexavalent chromium compounds.

Isoniazid and rifampicin the animals received intragastric administration daily by using metallic probe in an aqueous solution at a dose of 0.05 g / kg and 0.25 g / kg, respectively, for 7 and 14 days. Compounds of hexavalent chromium the animals received in the same way - in a solution of potassium dichromate in a dose of 3 mg / kg.

Euthanasia was performed by using the thiopental natrium on the 7th and 14th day from the beginning of enteral dosing of xenobiotics. Blood was taken from the heart of animals. The homogenates of liver and blood serum were studied.

All experimental animals of each age period were divided into the following groups: 1 - control rats (injected by Phys. solution) 2 - animals that were injected simultaneously with $K_2Cr_2O_7$, isoniazid and rifampicin, 3 - infected animals that succumbed to correction by the sorbex.

The animals received sorbex daily intragastrally as starch suspension in a dose of 150 mg / kg of the body weight throughout the experiment. The dose of sorbex was selected empirically, based on an average therapeutical dose for humans.

Keeping of pets and experiments were conducted in accordance with the regulations of the "European Convention for the protection of spinal animals that are used for experimental and other scientific purposes" [13].

The activity of free radical processes were evaluated by determining the content of foods oxidative modifications of proteins (2.4 - DNFH) [2] and TBC-active products (TBC-AP) [12]. The expression of endogenous intoxication syndrome was determined by the contents of the middle mass molecules (MMM) [11] and the percentage of the damage of erythrocyte membranes (erythrocyte indices) (EII) [10].

The results were subjected to statistical analysis in the Excel using Stjudent's t-criterion [5]. Results were considered authentic at $p \leq 0,05$.

Results and discussions. After the researches it was found that MDA-AP in blood serum of all age groups significantly increased while being influenced with xenobiotics, especially in immature rats on the 14th day from the beginning of the experiment. Thus, in the 1th age group it increased by 6 times when being compared with intact animals, and 2nd and 3rd groups - by 4.8 and 6.5 times accordingly. The same tendency was observed in the liver of affected animals. This shows the most marked development of the free radical processes in the organisms of aging animals, which may cause membrane-destructive processes in their cells [4].

Table 1

**THE CONTENTS OF TBC-ACTIVE PRODUCTS
IN THE SERUM (mmol / l) AND LIVER (mmol / kg) OF RATS,
AFFECTED BY TOXINS
AND AFTER USING SORBEX (M ± m; n = 6)**

The materials of the research	The groups of animals	The age groups of animals					
		I		II		III	
		The period of the research, 24 hours					
		7-a	14-a	7-a	14-a	7-a	14-a
Blood serum	Intact	0,89±0,10		0,74±0,05		0,62±0,02	
	Affected	5.31± 0.32*	5.48± 0.48*	3.44± 0.09*	3.57± 0.14*	3.60± 0.12*	4.05± 0.15*
	Affected+ Sorbex	2.24± 0.09**	2.42± 0.08**	2.36± 0.14**	2.61± 0.10**	2.25± 0.09**	2.62± 0.08**
Liver	Intact	16.66±2.25		5.88±0.45		6.94±0.20	
	Affected	53.20± 2.82*	58.08± 2.78*	15.71± 0.72*	17.52± 1.23*	19.21± 0.81*	25.26± 0.54*
	Affected+ Sorbex	28.85± 1.85**	31.41± 1.53**	12.18± 0.52**	14.43± 0.84**	15.42± 0.72**	18.96± 0.56**

Note: Here and in the following tables * - significant changes between intact animals and affected, $p \leq 0,05$;

** - Significant changes between affected animals and animals that underwent by the correction of sorbex, $p \leq 0,05$.

As we can see from the data in Table 1, when using the enterosorbent sorbex there is the decrease of the MDA-AP products as in the blood serum as in the liver of animals in all age groups. Significant impact on the above mentioned index enterosorbent caused in the 1st age group. The contents of the MDA-AP products significantly decreased in blood serum by 345% and 343% below the level of the affected animals on the 7th and 14th day, respectively, in the liver – by 160% on the 14th day from the beginning of the experiment.

**THE INDICATORS OF THE OXIDATIVE MODIFICATION OF
PROTEINS (370_{HM}) (mmol / g protein) IN THE SERUM
AND LIVER OF THE RATS, AFFECTED BY TOXINS
AND AFTER USING SORBEX (M ± m; n = 6)**

The materials of the research	The groups of animals	The age groups of animals					
		I		II		III	
		The period of the research, 24 hours					
		7-a	14-a	7-a	14-a	7-a	14-a
Blood serum	Intact	0.09±0.005		0.073±0.002		0.120±0.004	
	Affected	0.202± 0.014*	0.393± 0.002*	0.137± 0.002*	0.148± 0.009*	0.272± 0.004*	0.361± 0.004*
	Affected+ Sorbex	0.083± 0.003**	0.084± 0.003**	0.099± 0.002**	0.100± 0.002**	0.111± 0.003**	0.113± 0.001**
Liver	Intact	0.091±0.003		0.066±0.002		0.124±0.006	
	Affected	0.191± 0.010*	0.237± 0.002*	0.158± 0.003*	0.162± 0.003*	0.364± 0.005*	0.483± 0.004*
	Affected+ Sorbex	0.096± 0.001**	0.097± 0.002**	0.080± 0.004**	0.081± 0.003**	0.144± 0.007**	0.155± 0.005**

In the serum of animals of the 1st age group was observed more significant number of products of peroxidation of proteins than in the liver, which may be the result of the damage of the membranes of hepatocytes with xenobiotics and arrivals of toxic factors in the blood.

We investigated the indices of the OMB in the animals' organisms after application of the enterosorbent sorbex. Thus, when being injected into the body of

toxins in the aging rats there is the decrease of OMB products of the main character in the liver by 177% on the 7th day from the beginning of the experiment and to 264% - on the 14th day. A similar tendency was observed in the serum of the infected rats. The smallest corrective influence sorbex showed in the serum of the mature rats where the activity of processes of the oxidative modification of proteins decreased only by 52% on the 7th day in comparison with the infected animals.

We investigated the products contents of the oxidative modification of proteins at 430 nm (2.4 DNFH neutral) (Table 3). In comparison of the affected animals of all ages with the intact ones it was revealed that oxidative processes in the serum and liver on the 14th day from the beginning of the experiment had increased in the 1st age group at 483% and 248%, respectively, in the 2nd age group - at 113 % and 174% in the 3rd age group - at 428% and 315%.

Table 3

**THE INDICATORS OF THE OXIDATIVE MODIFICATION OF
PROTEINS_(430 HM) (mmol / g protein) IN THE SERUM
AND LIVER OF THE RATS, AFFECTED BY TOXINS
AND AFTER USING SORBEX (M ± m; n = 6)**

The materials of the research	The groups of animals	The age groups of animals					
		II		II		III	
		The period of the research, 24 hours					
		7-a	14-a	7-a	14-a	7-a	14-a
Blood serum	Intact	0.024±0.001		0.030±0.002		0.053±0.02	
	Affected	0.048± 0.002*	0.140± 0.002*	0.047± 0.001*	0.064± 0.003*	0.261± 0.005*	0.280± 0.005*
	Affected+ Sorbex	0.037± 0.002**	0.046± 0.002**	0.036± 0.002**	0.038± 0.002**	0.087± 0.001**	0.091± 0.001**
Liver	Intact	0.027±0.002		0.023±0.002		0.094±0.006	
	Affected	0.069± 0.003*	0.094± 0.001*	0.055± 0.002*	0.063± 0.004*	0.267± 0.003*	0.390± 0.005*
	Affected+ Sorbex	0.027± 0.002**	0.029± 0.001**	0.031± 0.002**	0.031± 0.001**	0.112± 0.002**	0.112± 0.002**

This tendency to increase the activity of oxidative processes in the affected animals can be explained by the increase of the toxicity, which is caused by the combined action of both isoniazid, rifampicin and hexavalent chromium on formation in the body of the reactive oxygen. The last ones interact with cellular biopolymers, participating in the reactions peroxidation and damage of biomolecules.

The greatest positive impact of the sorbex on the indices of OMB₄₃₀ was observed in serum of the 1st and 3rd age groups, which during the last study period was significantly decreased.

Thus, we found out that the poisoning of the organism with Cr⁶⁺ against a background of the isoniazid-rifampicinal liver damage leads to the activation not only lipid peroxidation processes, but also to intensification of the processes of oxidative modification of proteins. This is accompanied by the deepening of endogenous intoxication of the organism, the markers of which are the average weight molecules.

Table 4 shows that on the seventh day of the research in the serum of the affected rats there was the increase of the contents of MSM₁ (dominated by the chain amino acids) and set 154% in the 1st age group, 39% - in the 2nd age group, 51% - in 3rd age group (from the level of the intact animals).

**THE CONTENTS OF MSM₁ IN THE SERUM (equivalent unit\ I)
AND LIVER OF THE RATS, AFFECTED BY TOXINS
AND AFTER USING SORBEX (M ± m; n = 6)**

The materials of the research	The groups of animals	The age groups of animals					
		II		II		III	
		The period of the research, 24 hours					
		7-a	14-a	7-a	14-a	7-a	14-a
Blood serum	Intact	0.63±0.05		1.31±0.05		1.41±0.02	
	Affected	0.97± 0.04*	1.09± 0.04*	2.17± 0.06*	2.29± 0.06*	2.87± 0.06*	3.12± 0.03*
	Affected+ Sorbex	0.77± 0.04**	0.80± 0.04**	1.93± 0.04**	1.87± 0.06**	2.43± 0.03**	2.44± 0.07**
Liver	Intact	0.46±0.05		1.35±0.06		1.34±0.02	
	Affected	0.87± 0.03*	0.90± 0.04*	2.30± 0.07*	2.37± 0.05*	2.06± 0.12*	2.25± 0.05*
	Affected+ Sorbex	0.48± 0.04**	0.53± 0.05**	1.58± 0.27**	1.79± 0.17**	1.72± 0.09	1.93± 0.01**

A similar tendency was observed in the liver of the affected animals.

Using sorbex the contents of MSM₁ significantly decreased in the serum and liver of all age groups and almost reached the level of the intact animals, showing the elimination from blood the products of metabolism of xenobiotics.

We studied the contents MSM₂ (dominated by aromatic amino acids) and found out the significant growth of them in all studied tissues after simultaneous lesion of both isoniazid, rifampicin and hexavalent chromium compounds (Table 5).

Table 5

**THE CONTENTS OF MSM₂ IN THE SERUM (equivalent unit\ I)
AND LIVER OF THE RATS, AFFECTED BY TOXINS
AND AFTER USING SORBEX (M ± m; n = 6)**

The materials of the research	The groups of animals	The age groups of animals					
		I		II		III	
		The period of the research, 24 hours					
		7-a	14-a	7-a	14-a	7-a	14-a
Blood serum	Intact	0.61±0.05		1.02±0.06		1.35±0.05	
	Affected	0.93± 0.04*	0.94± 0.04*	2.00± 0.06*	2.03± 0.06*	2.94± 0.07*	3.25± 0.03*
	Affected+ Sorbex	0.72± 0.02**	0.76± 0.02**	1.91± 0.08**	1.89± 0.05**	2.49± 0.06**	2.50± 0.09**
Liver	Intact	0.45±0.05		1.15±0.05		1.39±0.05	
	Affected	0.85± 0.03*	0.87± 0.04*	2.13± 0.09*	2.17± 0.03*	2.07± 0.15*	2.32± 0.04*
	Affected+ Sorbex	0.51± 0.04**	0.55± 0.05**	1.79± 0.11**	1.84± 0.11**	1.99± 0.01**	1.95± 0.09**

Thus, in the liver of the affected animals the contents of MSM₂ increased in the immature rats on the 14th day to 93%, in the mature animals – to 88%, in the aging ones - to 66%. In the animals that got sorbex the contents of MSM₂ decreased during the research in all age groups and were significantly different from such contents in the infected rats.

A similar tendency to the increase of the contents of MSM₂ was observed in the serum of all groups of animals after lesion. The usage of sorbex led to normalization of this index in the serum of the animals of the 1st, 2nd and 3rd researching groups.

This reduction of MSM in the organs of the rats after using sorbex can be explained by the fact that after the biotransformation and conjugation of xenobiotics in the liver, there is happening its elimination by biliary system in the intestinal lumen, where they are absorbed again by the enterosorbent.

Thus, there is a decrease of the concentration of toxic compounds in the blood and reduction of systemic endogenous intoxication, there is normalization of free radical oxidation.

Another indicator of endogenous intoxication of the animals is the erythrocyte index, which indicates the degree of damage of the erythrocyte membrane (Pic. 1).

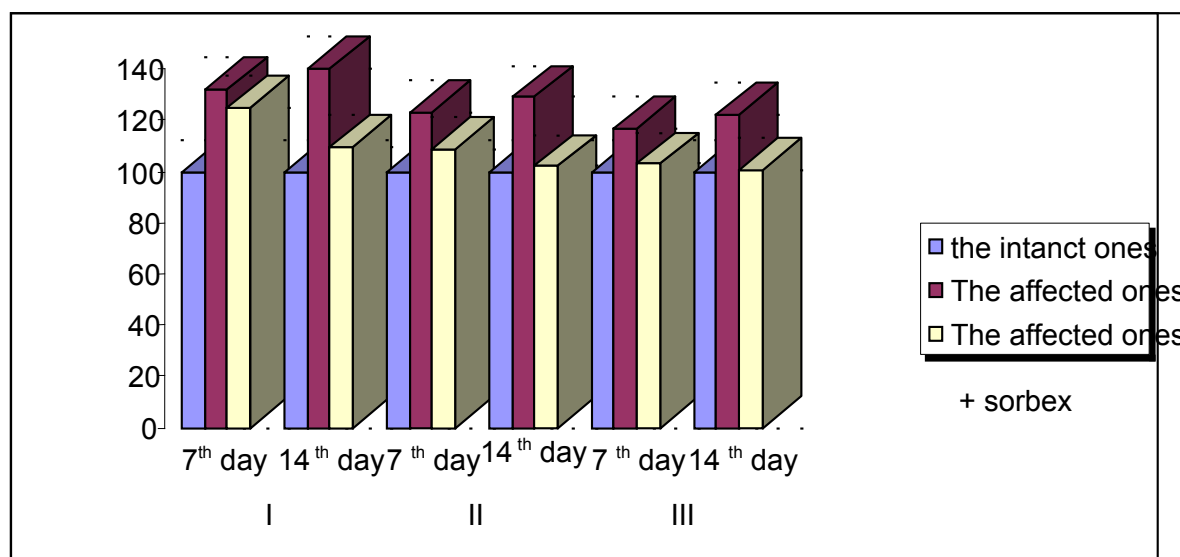


Fig. 1. The erythrocyte index of the intoxication in the blood of the affected animals after the usage of sorbex, %

Already on the 7th day from the start of toxins functioning was noted a significant increase of the erythrocyte intoxication index: in the immature animals by 32%, in the mature ones – by 23% and in the aging ones - by 17%, in comparison with the intact animals. These changes can be explained by the fact that the permeability of the erythrocyte membrane had increased as a result of the affecting on it by the active oxygen species that are formed during the metabolism of xenobiotics on monooxygenase system.

The most effective impact of sorbex was resulted in all age groups on the 14th day of the experiment. According to our data, the erythrocytic intoxication index had decreased in in the immature rats by 30%, in the mature ones – by 27% and in the aging ones – by 21% when being compared with affected animals.

Thus, the usage of sorbex leads to the reduction of the endogenous intoxication, that can be manifested by a decrease of the contents of MSM in the serum of all age groups of animals and reduction of the percentage of erythrocyte permeability membrane.

CONCLUSIONS. It was found that lesion of the animals by the hexavalent chromium compounds and antituberculous drugs was accompanied by the activation of lipid peroxidation and oxidative modification of proteins, resulting in the change of the cell membrane permeability, as evidenced by the increase of the erythrocyte index of intoxication. The usage of enterosorbent sorbex has resulted in the depressing processes of free radical oxidation in the rats' organisms which were affected by xenobiotics. This was accompanied by the decrease of the endogenous intoxication and decrease of the permeability of cell membranes. Taking into consideration the correspondence of the given model to people's lesion under conditions of anthropogenic pollution, it will allow us to recommend the methods which were used by us to assess the severity of chemical toxicity against a background of the drug-induced hepatitis.

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