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INTENSITY OF OXIDATIVE PROCESSES IN A RAT'S ORGANISM, DAMAGED BY TETRACHLOROMETHANE AFTER USING LANCIFOLIA HOSTA LEAVES TINCTURE

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Summary: antioxidant properties of hosta lancifolia tincture were proved in the course of experiment on animals, damaged by tetrachloromethane. These properties consist in decreased intensification of lipid peroxidation processes and protein oxidative modifications in the rat's blood serum and liver. The tincture downregulates in the damaged organism the TBA-active products, 2,4-dinitrophenylhydrazine and ceruloplasmin, intensifies catalase activity and increases the content of reduced glutathione.

Key words: hosta lancifolia leaves tincture, tetrachloromethane liver injury, oxidative processes, antioxidate system.

Introduction. Lipid peroxidation activation (LPA) is nowadays considered as one of the main pathogenic mechanism of liver injury [3]. Hepatotrophic poison tetrachloromethane (CCl_4) is used as a model of liver cells toxic injury. It intensifies free-radical processes and debilitates the organism [7].

That is why lately so much attention is paid to the problem of search and creation of medicinal products with antioxidant properties, especially, substances of natural origin.

Low toxicity and benefits of herbal remedies over synthetic drugs (wide range of therapeutically active ingredients in medicinal plants, the possibility of combined use, low frequency of side effects) contributes to their widespread use in clinical practice and makes herbal substances a valuable source for new drugs [1, 11].

Our attention has been attracted by a widely cultivated plant, which is used in folk medicine and is called hosta lancifolia. The tincture, based on this plant, is made and standardized at the department of National University of Pharmacy.

The aim of our study was to examine the effect of hosta lancifolia tincture on oxidative processes in the rat's organism, damaged by tetrachloromethane.

Materials and methods. The experiments were conducted on white outbred male rats weighing 170-180 g, which were kept on a vivarium standard diet. In order to investigate the efficacy of tincture as antioxidant agent we used the model of animals' liver injury (CCl4), which was injected twice (a day) as a 50 % oil solution at a dose of 1.0 ml/kg of body weight. The object of the study was 50 % hosta lancifolia leaves tincture at a dose of 0.15 ml/kg of body weight. As a comparator, we have chosen a phytogenic hepatoprotector silymarin (manufacturer – JSC "Sofarma), which was given to rats in the form of 1% starch slurry at a dose of 100 mg/kg of body weight.

The experimental animals were divided into four groups (18 animals per group): 1 – control; 2 – rats, damaged by tetrachloromethane; 3 – rats, damaged by tetrachloromethane and silymarin correction; 4 – rats, damaged by tetrachloromethane and hosta lancifolia tincture correctiom.

Euthanasia of rats was performed under thiopental anesthesia on the 4th, 7th and 14th day of the experiment. During the experiment all the rules of Convention for the Protection of Vertebrate Animals were followed [4].

For the investigation there were used blood serum and liver of experimental rats. The activity of oxidative processes and antioxidant system after the injection of corrective factors were evaluated by the content of TBA-active products (TBA-AP) [12], ceruloplasmin (CP) [5], reduced glutathione (RG) [13], the activity of catalase (CT) [6] and content of 2.4-dynitrofenilhidrazone (2.4 DNFH) [2].

For data statistical analysis there were used parametric (according to Student) and non-parametric (according to Wilcoxon) methods. Changes were considered as probable at $r \leq 0.05$ [8].

Results and discussions. Data received from the study of the content of TBA-AP serum and liver of animals is shown in the table 1 and 2. In rats' organism, infected with carbon tetrachloride, the content of TBA-AP significantly increased in all terms of research ($p \leq 0.05$). The maximum increase of intermediate products of free radical oxidation in blood serum was observed on the 7th day of injury (3.7 times), and in the liver it was observed on the 14th (2.2 times).

After the injection of tincture and comparator into the damaged body, there was observed reduction of TBA-AP. After applying the tincture, this indicator decreased in serum by 26 % on the 4th day of defeat, by 186 % – on the 7th day and by 37 % – on the 14th day of the experiment. Damaged by the comparator, it was lower,

than in Damaged rats' organisms: by 45 % – on the 4th day, by 203 % – on the 7th and by 75 % – on the 14th day of injury (fig.).

The research of OMP indicators showed that the content of 2.4-DNFH of neutral (370 nm) and main character (430 nm).

On the 4th day of damage the content of 2.4-DNFH of neutral character in the blood serum of experimental rats increased by 5.5 times in relation to control animals. The content of this indicator decreased significantly after using the selected drugs. In the group of animals, injected with the tincture, this indicator was higher by 4.2 times in relation to intact ones. In the group of animals, injected by silymarin, it was higher by 3.9 times. The great decrease of 2.4-DNFH of neutral character was observed on the 7th day of the experiment, when this indicator increased in relation to control animals by 2.6 times after correction by tincture and by 1.8 after injection of silymarin. This indicator increased by 6.8 times in the organisms of damaged rats (Table 1).

The maximum decrease of 2.4-DNFH of neutral character in the liver of experimental rats was observed on the 7th and 14th day after using the investigated corrective factors. The maximum decrease of 2.4-DNFH of main character was observed on the 7tt day.

As a result of radical oxidation processes, the antioxidant system in the organism of experimental animals was weakened, as evidenced by the decrease of catalase activity and content of reduced glutathione in the blood serum of rats after the injury (Table 3).

After the injury of rats' liver there was observed the increase of ceruloplasmin content in the blood serum (Table 3) during the whole experiment, which means the priority inclusion of protein in the process of neutralizing free radicals, including OH [9].

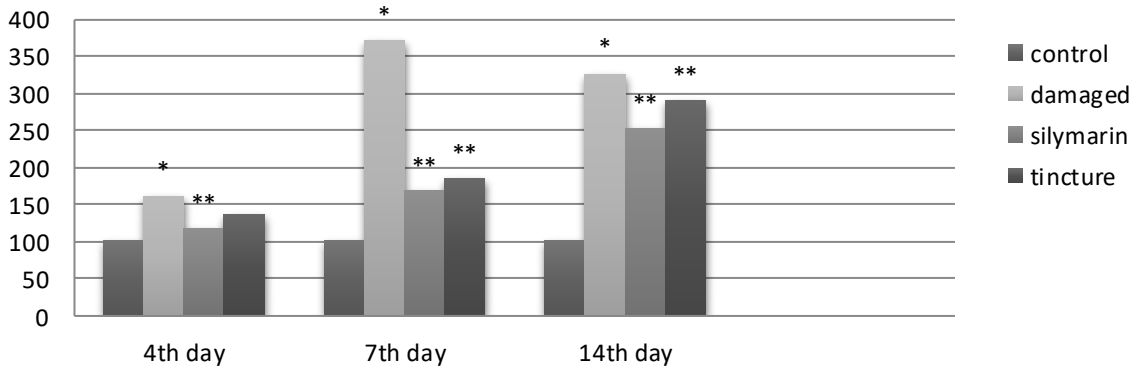

Fig. The content of TBA-active products in the blood serum of animals affected by carbon tetrachloride and after use tincture and drug comparisons, %

Table 1. The content of TBA-active products and products of proteins oxidative modification in the blood serum of rats (M±m; n=60)

Indicator	Groups of animals	Terms of the research, days		
		4th	7th	14th
TBA-AP mcmol/l	1	2.23±0.13		
	2	3.61±0.18*	8.26±0.40*	7.28±0.20*
	3	2.61±0.06**	3.73±0.16**	5.61±0.17**
	4	3.03±0.15	4.11±0.24**	6.45±0.18**
OMP (370 nm) mcmol/l	1	0.029±0.001		
	2	0.159±0.011*	0.105±0.003*	0.093±0.009*
	3	0.114±0.001**	0.078±0.0002**	0.086±0.007
	4	0.122±0.003**	0.082±0.002**	0.093±0.002
OMP (430 nm) mcmol/l	1	0.019±0.002		
	2	0.095±0.002*	0.129±0.075	0.038±0.005*
	3	0.077±0.003**	0.035±0.001	0.025±0.004
	4	0.089±0.003	0.050±0.004	0.030±0.002

Note: in the tables
* – differences between 1 and 2 groups (p≤0.05);
** – differences between 2 and 3 groups (p≤0.05);

Table 2. The content of TBA-active products and products of proteins oxidative modification in the liver of rats (M±m; n=60)

Indicator	Groups of animals	Terms of the research, days		
		4	7	14
TBA-AP mcmol/l	1	2.41±0.16		
	2	4.36±0.27*	3.20±0.07*	5.41±0.34*
	3	2.88±0.26**	2.78±0.22	3.08±0.18**
	4	3.35±0.24**	2.76±0.17	3.33±0.17**
OMP (370 nm) mcmol/l	1	0.033±0.002		
	2	0.088±0.002*	0.107±0.006*	0.123±0.009*
	3	0.068±0.002**	0.061±0.006**	0.081±0.006**
	4	0.067±0.003**	0.083±0.005**	0.095±0.005
OMP (430 nm) mcmol/l	1	0.033±0.004		
	2	0.074±0.003*	0.085±0.004*	0.096±0.004*
	3	0.046±0.003**	0.052±0.003**	0.071±0.004**
	4	0.060±0.003	0.071±0.003	0.082±0.003**

Table 3. The indicators of the antioxidant system in the blood serum of rats (M±m; n=60)

Indicator	Groups of animals	Terms of the research, days		
		4	7	14
CP (g/l)	1	17.43±0.56		
	2	28.20±1.80*	22.30±1.35*	33.95±0.93*
	3	20.01±0.97**	19.38±0.68	20.55±1.50**
	4	21.93±1.43**	19.51±0.90	21.81±0.38**
CT mcat/l	1	0.97±0.01		
	2	0.52±0.04*	0.35±0.02*	0.52±0.02*
	3	0.68±0.01**	0.42±0.02	0.64±0.04
	4	0.64±0.03	0.42±0.03	0.56±0.02
RG mcmol/l	1	14.36±0.88		
	2	12.56±0.37	9.35±0.25*	9.57±0.24*
	3	14.24±0.75	10.39±0.29**	10.74±0.15**
	4	15.38±0.45**	10.11±0.36	10.62±0.11**

According to results showing at Table 3, the effect of silymarin on the CP content in the blood serum on the 4th and 14th days was bigger, than of tincture. On the 7th day of the experiment, the efficiency of the tincture turned out to be on the same level with comparator.

There was observed the decrease of CT activity and RG content in the liver of animals, affected by tetrachloromethane (Table 4). Due to the indicators, shown in Table 4, there was increase of catalase activity and reduced glutathione content during three periods of experiment after hosta tincture and phytogenic hepatoprotector correction. Besides, the tincture turned out to be as much effective as silymarin. On the 14th day of the experiment the CT activity increased by 1.3 times in comparison with the damaged animals after using either silymarin or hosta tincture.

It is naturally caused by high content of hydroxycinamic and organic acids, which demonstrate antioxidant properties. It is approved by investigation of bioactive substances in the tincture, conducted in the National University of Pharmacy [10].

In consequence of the research conducted there was determined a positive effect of hosta lancifolia tincture on free radical oxidation process (it decreases the content of TBA-active products and products of proteins oxidative modification), activated after damaging the rats by tetrachloromethane. Using the tincture at dose of 0.15 ml/kg led to the increase of catalase activity, the content of reduced glutathione and the decrease of ceruloplasmin content in the damaged organism, showing its positive effect on the indicators of antioxidant system.

Table 4. The indicators of the antioxidant system in the liver of rats (M±m; n=60)

Indicator	Groups of animals	Terms of the research, days		
		4	7	14
CT mcat/l	1	0.44±0.04		
	2	0.30±0.01*	0.24±0.01*	0.27±0.01*
	3	0.36±0.02	0.30±0.01**	0.35±0.01**
	4	0.42±0.02**	0.31±0.01**	0.35±0.01**
RG mcmol/l	1	6.60±0.52		
	2	2.82±0.16*	3.17±0.22*	2.02±0.19*
	3	3.22±0.13	5.35±0.20**	5.71±0.23**
	4	3.13±0.19	3.89±0.11**	5.48±0.08**

This suggests the prospects of further study of pharmacological of hosta lancifolia leaves tincture.

Conclusion. Result of investigation: a positive effect of hosta lancifolia leaves tincture on the processes of free radical oxidation (decrease in the content of TBA-active products and products of proteins oxidative modification), activated after damaging the rats by tetrachloromethane. Activity of catalase, glutathione content and reduction of ceruloplasmin in the affected body is increased at application of tincture at a dose of 0.15 ml/kg, and indicated on its positive effect on indexs of antioxidant system.

This suggests about the prospects of further investigation of the pharmacologic properties of hosta lancifolia leaves tincture.

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ІНТЕНСИВНІСТЬ ОКИСНЮВАЛЬНИХ ПРОЦЕСІВ В ОРГАНІЗМІ ЩУРІВ, УРАЖЕНИХ ТЕТРАХЛОРМЕТАНОМ, ПІСЛЯ ВИКОРИСТАННЯ НАСТОЙКИ З ХОСТИ ЛАНЦЕТОЛИСТОЇ

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Резюме: в експерименті на тваринах, уражених тетрахлорметаном, доведено антиоксидантні властивості настойки з листя хости ланцетолистої, що проявляється зниженням інтенсифікації процесів перекисного окиснення ліпідів та окиснювальної модифікації білків у сироватці крові та печінці щурів. Під її впливом відбувається зниження вмісту ТБК-активних продуктів та 2,4-динітрофенілгідразонів, підвищення активності каталази, вмісту відновленого глутатіону та зниження вмісту церулоплазміну в ураженому організмі.

Ключові слова: настойка з листя хости ланцетолистої, тетрахлорметанове ураження печінки, окиснювальні процеси, антиоксидантна система.

ИНТЕНСИВНОСТЬ ОКИСЛИТЕЛЬНЫХ ПРОЦЕССОВ В ОРГАНИЗМЕ КРЫС, ПОРАЖЕННЫХ ТЕТРАХЛОРМЕТАНОМ, ПОСЛЕ ИСПОЛЬЗОВАНИЯ НАСТОЙКИ ИЗ ХОСТЫ ЛАНЦЕТОЛИСТНОЙ

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Резюме: в эксперименте на животных, пораженных тетрахлоретаном, доказаны антиоксидантные свойства настойки из листьев хосты ланцетолистной, что проявляется снижением интенсификации процессов перекисного окисления липидов и окислительной модификации белков в сыворотке крови и печени крыс. Под влиянием настойки происходит снижение содержания ТБК-активных продуктов и 2,4-динитрофенилгидразонов, повышение активности каталазы, содержания восстановленного глутатиона и снижение содержания церулоплазмина в пораженном организме.

Ключевые слова: настойка из листьев хосты ланцетолистной, тетрахлорметановое поражение печени, окислительные процессы, антиоксидантная система.

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