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DYNAMICS OF ANTIOXIDANT SYSTEM ACTIVITY AFTER APPLYING ENTEROSGEL IN SIMULTANEOUS AFFECTION OF RATS WITH CARBOFOS AND CARBON TETRACHLORIDE

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Abstract

Organophosphorus pesticides carbofos, are widely used in agriculture, industry and even at home to control insect pests. However, organophosphorus pesticides are highly toxic. Organochlorine compounds, which are represented by carbon tetrachloride, a classic hepatotropic poison, also lead to poisoning. In real life, often there is a combined effect of several toxic factors, which, depending on the conditions, can disturb the prooxidant and antioxidant balance in the body and lead to severe diseases.

Antioxidants are most often used to suppress activated oxidative processes and restore body compensatory forces. But in conditions that are accompanied by development of endogenous intoxication, sorption therapy is necessary. Today, Enterosgel is widely used; it is an organosilicon enterosorbent with a specific spectrum of absorption action, which shows the ability to effectively reduce endogenous intoxication.

The aim of this study was to investigate the effectiveness of Enterosgel and its effect on the antioxidant system in cases of simultaneous affection of rats with carbofos and carbon tetrachloride.

In the experiment on rats affected by carbofos and carbon tetrachloride, a progressive increase in the content of lipoperoxidation products during the experiment (on the 30^{th} day of carbofos poisoning and the 7^{th} day of tetrachloromethane hepatitis, this index in the serum increased in 3.3 times, in the liver – in 1.8 times). With underlying activation of oxidative processes, the changes in the antioxidant system were observed: catalase activity decreased in the liver and myocardium of the affected rats, as well as the content of reduced glutathione in the serum and liver during the whole period of research. An increase in the content of ceruloplasmin in the serum was evidenced, which by the end of the experiment was by 48% higher than normal.

The Enterosgel sorbent used reduced the activated oxidative processes and restored the body protective and compensatory forces, which was confirmed by a decrease of lipoperoxidation products in all tissues as well as increased indicators of the antioxidant system in the liver and myocardium of the affected animals.

Key words: carbofos, carbon tetrachloride, Enterosgel, antioxidant system, reduced glutathione, catalase, ceruloplasmin

Introduction

According to the WHO estimates, 3 million people suffer from acute FOS poisoning each year, and for 200,000 people poisoning is fatal [1]. For the last two decades, organophosphorus pesticides have been one of the main causes of acute and chronic poisoning among agricultural workers, among the population in household use of insecticides as well as in accidental or intentional use that requires emergency care, accurate diagnosis, effective treatment and prognosis for intoxication consequences [2,3,4,5].

Organophosphorus compounds (OPC) are the most widely used pesticides worldwide, so their residues and metabolites are widely distributed in the environment, increasing the overall burden of adverse effects of chemical factors on human health and ecological systems [6,7,8].

The WHO notes that humans are exposed to OPC through consumption of food, beverages and inhalation of polluted air [2].

Toxic properties of these substances are initial, intermediate and final products of the chemical industry, which include carbon tetrachloride. These are the substances that are accumulated in the liver, brain, muscles, adipose tissue [9,10].

Getting into the human body, these xenobiotics lead to oxidative stress, enhance endogenous intoxication and reduce body defences, including the antioxidant system. To eliminate the above disorders, the drugs that limit the activity of free radical oxidation are used. Recently, enterosorbents have been introduced into complex treatment regimens for intoxications of various origins. Enterosgel is one of such drugs; it has significant sorbent properties due to a large absorption surface that facilitates reducing the amount of endogenous toxins in the body and eliminating them out of it [11]. This leads to a decrease of oxidative processes and restoration of the body defences.

The aim of this research was to study the effectiveness of Enterosgel and its effect on the antioxidant system in cases of simultaneous affection of rats with carbofos and carbon tetrachloride.

Methods

The research was performed on 54 white male rats weighing 175 -200 g, which were kept on a standard diet at the vivarium of I. Horbachevsky Ternopil National Medical University. All the studies were performed following the General Principles of Experiments on Animals approved by the National Congress of Bioethics (Kyiv, Ukraine 2001) [12].

The animals were divided into 9 groups: group 1 involved the intact control; group 2 – the animals affected by carbofos for 10 days and tetrachloromethane for 4 days; group 3 – the animals with the same affection as group 2 and Enterosgel management; group 4 – 10 days of carbofos and 7 days of CCI_4 poisoning; group 5 – the rats affected by carbofos for 10 days and 7 days of toxic hepatitis development (animals of this group were managed with Enterosgel for 10 days); group 6 – the rats affected by carbofos for 30 days and 4 days of tetrachloromethane hepatitis development; group 7 – the rats affected by toxicants as in group 6 and management with Enterosgel throughout the experiment; group 8 – 30 days of carbofos affection and 7 days of CCI4; group 9 – the affected rats as in group 8 and Enterosgel management for 30 days.

Carbofos was administered intragastrically daily as an aqueous solution at a rate of 20 mg/kg of body weight of the animal, which is 1/10 of the LD_{50} [4]. Tetrachloromethane was administered intraperitoneally twice a day in the form of 50% oil solution at a dose of 1.0 ml/kg of animal weight [11]. The animals were administered with Enterosgel intragastrally daily at a dose of 120 mg/kg of body weight. The doses were defined according to the average therapeutic dose for humans and converted to animals [13] taking into account body temperature and species sensitivity.

The rats were euthanized using sodium thiopental. Serum, myocardium and animal liver were selected for studying.

The activity of oxidative processes and the state of the antioxidant system after the introduction of corrective factors was assessed by the content of TBA-active products (TBA-AP) [12]. The method is based on the reaction of interaction (in an acidic environment at high temperature) of malonic dialdehyde with thiobarbituric acid with the formation of a coloured red complex with a maximum absorption at a wavelength of 532 nm [14].

To determine the content of reduced glutathione (RG) as one of the main components of a non-enzymatic part of the antioxidant system, we used the method [15]; its principle is interaction of 5,5-dithiobis (2-nitrobenzoic) acid (Elman's reagent) with free SH groups of RG and formation of thionitrophenyl anion of yellow colour, the amount of which is directly proportional to the content of SH groups. The activity of catalase (CT) was determined by the method [16]; its principle is based on the ability of hydrogen peroxide to form with ammonium molybdate a stable coloured complex of yellow colour. The content of ceruloplasmin was determined by the method [16]; it is based on the ability of p-phenylenediamine to oxidize with formation of coloured compounds of pink colour in the presence of ceruloplasmin. The content of ceruloplasmin is proportional to the intensity of the colour.

The results were statistically processed by means of the Statistics 6.0 method. The data distribution was analysed according to the Kolmogorov-Smirnov normality criterion. The obtained indicators were of a parametric distribution, so the difference between the groups was analysed according to the Student's t-test and non-parametric Wilcoxon test for related samples. The $\chi 2$ criterion was used to estimate the difference between the categorical data. The difference in probability indices was $p \geq 0.95$ (significant difference p). The differences were considered statistically significant at $p \leq 0.05$ [17].

Results

It is established that xenobiotics develop oxidative stress, in which the generation of reactive oxygen intermediates exceeds their elimination by enzymatic and non-enzymatic systems of antioxidant defence of cells [18]. The main biochemical mechanism of oxidative processes activation during body affection by toxicants is the formation of free radical products.

After administration of carbon tetrachloride and carbofos to the rats, a progressive increase in TB-AP content in serum, liver and myocardial was observed throughout the experiment. On the 30th and 7th days of toxicant administration, the content of TBA-AP increased in serum by 234%, in the liver – by 84%, in the myocardium – by 48% compare to the intact animals (Table 1).

The results obtained prove a significant activation of lipoperoxidation processes in the rats in cases of simultaneous action of carbofos and carbon tetrachloride. The introduction of each toxicant enhances the toxic effects of another one.

To correct the detected disorders, Enterosgel enterosorbent (the active substance of methyl silicic acid hydrogel) was used.

The studied enterosorbent showed an effective influence on this indicator throughout the experiment, undoubtedly reducing it (at the end of the study on the 30th and 7th days the content of TBA-AP in the serum decreased by 31% (Fig. 1), in the liver – by 14% and in the myocardium – by 5% compare to the affected rats).

We studied the effect of enterosgel on the enzyme link of the antioxidant system, in particular on the activity of catalase, which breaks down toxic peroxide toxic to the body. Table 2 shows the results of the study of the effect of xenobiotics on the activity of catalase in the serum, liver and myocardium of the affected animals.

Decreased CT activity in the liver and myocardium of rats contributes to accumulation of toxic disputation product of superoxide anion radical – hydrogen peroxide that evidences of rapid depletion of the antioxidant defence system in cases of toxic affection of the body that leads to damage to enzyme molecules by peroxidation products. One of the reasons for the decreased CT activity may be degradation of free and endoplasmic reticulum-bound ribosomes, responsible for enzyme synthesis, caused by prolonged exposure to toxins. Enterosgel had a positive effect on CT activity in both serum and liver of the affected animals, probably ($p<0.05$) increasing it at all times of the research.

In the groups of the affected animals receiving the corrective factor, on the 10th and 4th days of the toxicant exposure, CT activity increased slightly, on the 7th day of toxic hepatitis with underlying carbofos affection, CT activity increased by 8% after administration of Enterosgel into the liver and myocardium of the animals. In cases of a 30-day affection with carbofos and on the 4th day of tetrachloromethane affection, under the enterosorbent action, this indicator increased by 8%

in the liver and by 11% in the myocardium of the affected rats. On the 30th and 7th day of the toxicant administration, a 14% increase in CT activity was observed in the liver and a 13% increase in the myocardium after Enterosgel administration.

In response to introduction of the toxic factors into the animals' body the defensive and compensatory forces of the body activate that is manifested by changes in the content of Cu-depositing protein of the acute phase – CP-antioxidant (Table 3).

An increase in the CP content caused by affection by the toxicants was evidenced at all times of the research. In cases of a ten-day affection with carbofos and on the 4th day of CCl4 affection, the content of ceruloplasmin in the serum increased in 1.6 times, after the use of enterosgel, this indicator decreased by 20% compare to the affected animals. On the 7th day of toxic hepatitis caused by a 10-day administration of carbofos this indicator increased in 1.4 times, and after administration of the sorbent it decreased by 18%.

An increase in the content of CP in the serum of the affected animals, which progressively increased with longer study duration, was also evidenced. On the 30th and 7th seventh days of introduction of toxicants, the content of CP increased in 1.5 times; after administration of Enterosgel it decreased by 23%.

The functional source of the antioxidant defence system is the glutathione system, which is involved in inactivation of hydrogen peroxide and lipoperoxides, performs a protective function for SH groups in the membrane proteins [15,19]. Glutathione is one of the components of this system.

It was found out that the content of RG significantly decreased in the serum and liver of the rats affected by xenobiotics compare to the intact control (Table 4). On the 30th and 7th day of affection with the toxicants, the content of RG decreased in the serum by 35%, in the liver – by 15%. After administration of Enterosgel, an increase in the RG content in the serum and liver of the affected rats was evidenced throughout the whole study period. At the end of the experiment, this indicator increased by 28% in the serum, by 8% – in the liver (Fig. 2).

The changes in RG content (i.e., decrease in all follow-up periods) may be associated with both direct and enzymatic binding of individual substrates to the thiol group of glutathione and to the disulfide binding of oxidized glutathione. On the other hand, the decrease in RG can also be associated with the previously detected intensification of lipoperoxidation processes in the organs of rats affected by the studied toxicants that leads to accumulation of a significant number of toxic intermediates. The latter have a damaging effect on biomembranes and biomolecules that leads to their destruction, which enhances endogenous intoxication of the organism under these conditions [20].

Enterosgel, which has a significant absorption surface, absorbs endogenous toxins and removes them from the body that contributes to restoring the body defences, and the antioxidant system in particular. Obviously, the effect of this sorbent on the antioxidant system is manifested indirectly due to reduction of endogenous intoxication in the animals affected by carbofos and carbon tetrachloridethat was established previously [11,21].

Conclusions

It has been established that the simultaneous affection of rats with carbofos and tetrachloromethane is accompanied by activation of lipoperoxidation processes that leads to development of oxidative stress in the body. Under these conditions, a decrease in the activity of the body defences, including antioxidant defence system was evidenced – a decrease in reduced glutathione content in the serum and liver, increased catalase activity and ceruloplasmin content in the serum and decreased catalase activity in the liver and myocardium of the affected raanimalsts. The Enterosgel sorbent led to a decrease in activated oxidative processes and restoration of the body defensive and compensatory forces that was confirmed by our research.

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Table 1. The content of TB-AP in the serum (µmol / l), liver (µmol / kg) and myocardium (µmol / kg) of animals under the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel (M ± m; n = 54)

Groups of animals	Term of research, days			
	10+4CCl_4	10+7CCl_4	30+4CCl_4	30+7CCl_4
Serum				
Intact control	0,92±0,03			
affected by toxicants	1,31±0,02*	1,70±0,01*	2,62±0,02*	3,07±0,01*
affected +enterosgel	1,14±0,01**	1,45±0,01**	2,36±0,01**	2,79±0,01**
Liver				
Intact control	8,65±0,27			
affected by toxicants	11,66±0,03*	12,21±0,04*	13,82±0,03*	15,92±0,04*
affected +enterosgel	10,32±0,02**	11,50±0,02**	12,46±0.01**	14,75±0,02**
Myocardium				
Intact control	11,25±0,09			
affected by toxicants	12,85±0,02*	13,63±0,11*	15,64±0,05*	16,67±0,06*
affected +enterosgel	11,92±0,02**	12,71±0,02**	14,94±0,01**	16,07±0,02**

Note: Here and in the following tables * - probable changes between the indicators of intact control animals and affected toxicants, $p < 0,05$; ** - probable changes between the indicators of animals affected by toxicants and animals that were corrected with enterosgel, $p < 0.05$.

Table 2. Catalase activity in serum (µcat / l), liver (µcat / kg) and myocardium (µcat / kg) of animals under the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel (M ± m; n = 54)

Groups of animals	Term of research, days			
	10+4CCl_4	10+7CCl_4	30+4CCl_4	30+7CCl_4
Serum				
Intact control	91,83±2,18			
affected by toxicants	113,17±5,05*	128,33±3,03*	137,50±2,75*	134,67±6,19*
affected +enterosgel	98,33±3,20	100,17±2,99**	111,00±4,08**	107,00±5,58**
Liver				
Intact control	104,67±4,66			
affected by toxicants	89,67±2,27	87,00±2,11*	89,00±2,41*	86,33±2,50*
affected +enterosgel	97,67±2,97	95,83±4,21	97,50±1,96**	100,33±3,51**
Myocardium				
Intact control	87,33±1,91			
affected by toxicants	77,00±2,05*	76,00±2,92*	74,00±2,73*	77,00±2,35*
affected +enterosgel	77,17±1,97**	83,67±2,27	81,67±1,74	82,83±0,65**

Table 3. The content of ceruloplasmin in the serum of animals (mg / l), with the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel (M ± m; n = 54)

Groups of animals	Term of research, days			
	10+4CCl_4	10+7CCl_4	30+4CCl_4	30+7CCl_4
Intact control	3,35±0,11			
affected by toxicants	5,27±0,26*	4,83±0,16*	5,57±0,24*	4,95±0,19*
affected +enterosgel	4,60±0,30**	4,23±0,19**	4,83±0,17**	4,18±0,21**

Table 4. The content of reduced glutathione in the serum (µmol / l) and liver (µmol / kg) of animals under the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel (M ± m; n = 54)

Material research	Groups of animals	Term of research, days			
		10+4CCl_4	10+7CCl_4	30+4CCl_4	30+7CCl_4
Serum	Intact control	0,263±0,020			
	affected by toxicants	0,190±0,015*	0,182±0,017*	0,186±0,018*	0,173±0,018*
	affected +enterosgel	0,220±0,023	0,236±0,016	0,236±0,012	0,248±0,026
Liver	Intact control	0,338±0,016			
	affected by toxicants	0,260±0,010*	0,250±0,019*	0,266±0,020*	0,286±0,011*
	affected +enterosgel	0,277±0,026	0,293±0,016	0,318±0,032	0,315±0,015**

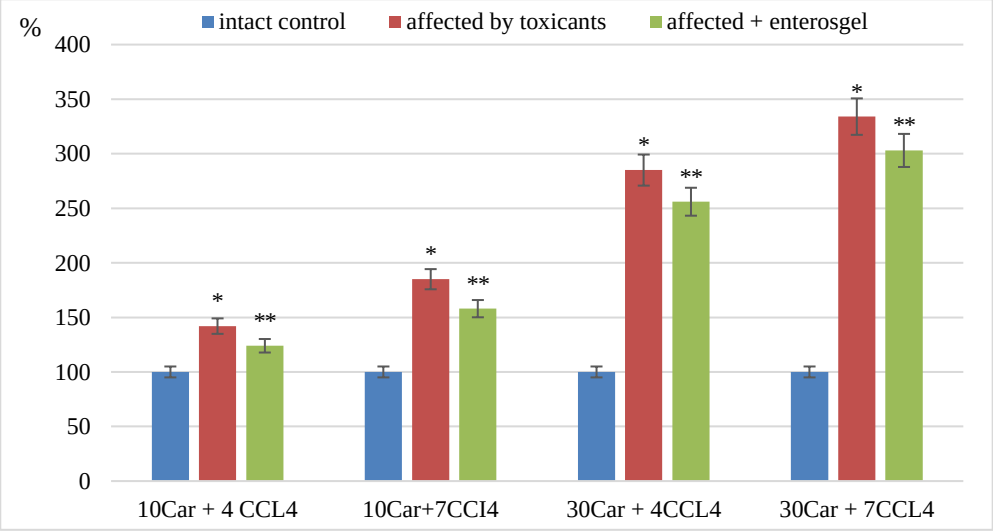

Figure 1. The content of TB-AP in the serum of animals under the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel, %

Note: Here and in the following figures * - probable changes between the indicators of intact control animals and affected toxicants, p < 0,05; ** - probable changes between the indicators of animals affected by toxicants and animals that were corrected with enterosgel, p < 0.05.

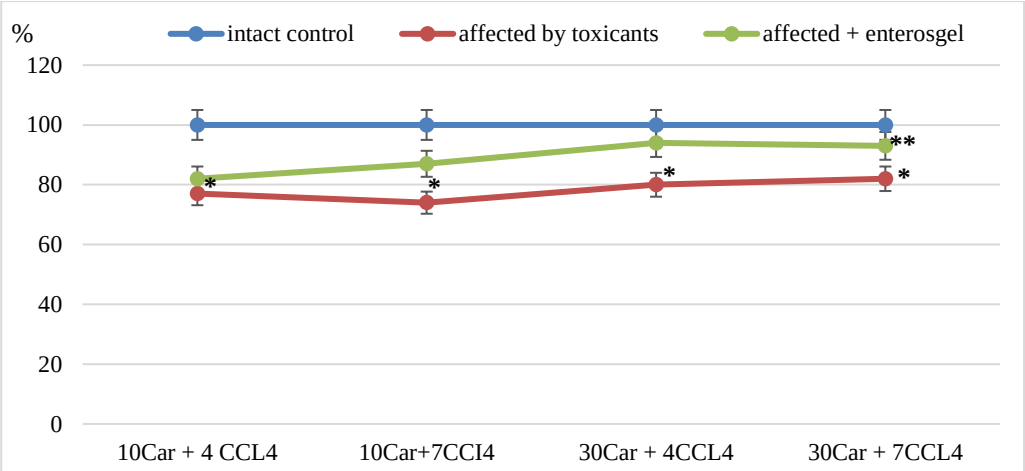

Figure 2. The content of reduced glutathione in the liver of animals under the simultaneous action of carbon tetrachloride and carbophos after application of enterosgel, %