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Influence Of Ultrasound On Glutathione Peroxidase Enzyme Activity And Ways Of Its Correction

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Abstract

The article presents the result of the impact of ultrasound on the activity of the enzyme glutathione peroxidase and the ways of its correction by local plants. After 5 and 10 minutes of exposure to ultrasonic waves, a strong inactivation of the enzyme glutathione peroxidase of rat liver mitochondria was observed on days 1 and 3, and it was found that it did not fully recover even after 15 days. It was noted that after a 5-minute exposure to ultrasonic waves on days 1, 3, 5, 10 and 15, depending on the dynamics, the activity of glutathione peroxidase under the influence of extracts of the leaves of shotut (*Morus nigra L*) and the oil extract of biosep was restored to one degree or another and the restoration of activity enzymes was higher on the 10th and 15th day compared to the control.

Keywords: hepatocyte, mitochondria, glutathione peroxidase, extract, biosep, ultrasound.

Relevance of the topic

It is known that as a result of metabolism in living organisms, oxidation products are formed - “free radicals” and peroxide compounds of organic and inorganic substances. As a result of adverse factors, this process is developing rapidly. The resulting free radicals damage cells and destroy the immune system, leading to various infectious and degenerative diseases, including cancer and cardiovascular disease.

Reactive oxygen species are formed in all tissues, and this, in turn, leads to the development of the apoptosis process, an imbalance in the glutathione-dependent prooxidant and antioxidant defense systems and, as a result, the formation of oxidative stress in cells [1; 501-506]. Antioxidant systems neutralize the amount of free radicals in the body, i.e. bind reactive oxygen species: $^1O_2$, $\mathbf{O_2^{\bullet-}}$, H_2O_2, $HO^{\bullet}$. Free radicals are involved in three main types of reactions in the cell. First, they react with proteins, lipids or nucleic acids (DNA or RNA), causing damage or cell death [2; 97-103]. Secondly, free radicals are neutralized in reactions with low molecular weight components of the cytoplasm or membranes. And, finally, thirdly, it is neutralized by special antioxidant enzymes that catalyze the reactions of superoxide and hydroxyl radicals and lipid peroxides [3; 91-96]. Along with the enzymes of the antioxidant defense system - superoxide dismutase and catalase, the key enzymes in the catabolism of free radicals are the enzymes of the glutathione system - glutathione reductase, glutathione peroxidase, glutathione transferase. Enzymes of the glutathione system are the main component of the body's antioxidant defense system against lipid peroxidation resulting from endogenous and exogenous exposure, and not only protect cells from toxic effects, such as free radicals, but also determine the redox conditions in cells [4; 86-87].

It is known that ultrasound is widely used in biology and medicine [7, 4-17]. However, the impact of ultrasound on the human body and animals has been little studied. At present, considerable material has been accumulated on the biological effect of ultrasound, but these are the results of experimental studies and observations of the effects of ultrasound for therapeutic purposes [8, 57-60].

There are data in the literature on the effect of ultrasonic waves on the activity of antioxidant enzymes - superoxide dismutase, catalase, glutathione preoxidase on liver cells [5; 217-224; 6; 1563-1567], kidneys [7; 383-387.] and the brain [5; 217-224]. In this work, we studied the effects of correcting the action of an extract from mulberry leaves - shotut and biosep oil extracts on the activity of rat liver glutathione preoxidase after exposure to ultrasonic waves with a frequency of 7.5 MHz for 5 and 10 minutes using the Mindrey DP-50 Vet UZI apparatus.

The purpose of the study was to study the effect of ultrasound on the activity of glutathione peroxidase in rat liver mitochondria and to correct the activity of the enzyme using shotut (*Morus nigra L*) leaf extracts and biosep oil extracts.

Research methods and materials

The study was carried out on sterile laboratory white female rats weighing 150–220 g In the study, the Mindrey DP-50 Vet UZI device for animals was used with exposure to rats at a frequency of 7.5 MHz for 5 and 10 minutes.

In the experiments, rats were divided into separate model groups with the effects of ultrasound and their correction:

I group healthy (control) (n = 5)

Group II 5 (10) minute exposure to ultrasound (n = 5-6).

Ultrasound group III + shotut extract (n = 5-6)

Group IV ultrasound + biosep extract (n = 5-6)

In the experiment, after exposure to ultrasound, rats of group III were injected with 1 ml of shotut extract once a day for 5 days relative to 100 g of body weight, and rats of group IV were orally injected with 1 ml of biosep for 5 days.

The activity of mitochondrial enzymes in the liver of rats subjected to ultrasound was studied 1, 3, 5, 10 and 15 days after the injection of shotut leaf extracts and biosep oil extract.

Rat liver mitochondria were isolated by differential centrifugation proposed by W.C. Schneider [8; 619-635] and the modification method of Kuzmin et al. [9; 1684-1697]. To isolate mitochondria from the liver tissue, a 0.25 M sucrose-TKM buffer solution was used. The tissue homogenate was prepared in a ratio of 1:10 and centrifuged for 10 minutes at 1000 rpm. The precipitate obtained is washed twice with 0.25 M sucrose solution containing no EDTA and centrifuged again (12000 g, 10 min). The procedure is repeated twice more, and the resulting dense precipitate of mitochondria is carefully suspended in a 0.25 mol/l sucrose solution.

The activity of glutathione peroxidase is determined by the accumulation of oxidized glutathione: the reaction proceeds with the disappearance of oxidized glutathione and is detected on a spectrophotometer at a wavelength of 260 nm.

The decrease in optical density during the reaction is the result of the oxidation of NADPH to NADP. This situation arises due to the passage of two interrelated reactions: the formation of oxidized glutathione under the influence of glutathione peroxidase and its subsequent reduction using NADPH as a coenzyme under the influence of glutathione reductase. Control samples do not contain reduced glutathione.

Enzyme activity is determined by the appearance of glutathione in μmol per minute per 1 mg of protein (μM/min.mg) [10; 19-21]. The amount of protein in mitochondria was determined by the Lowry method [11; 265-275]. The difference between the results obtained in the control, experiment and experiment + shotut, experiment + biosep groups was calculated by t-test, where $p<0.05$, $^{**}p<0.01$; $^{***}p<0.001$ represents statistical significance.

The results obtained and their analysis

According to the results obtained, after a single 5-minute exposure to ultrasonic waves in the range of 7.5 MHz, a sharp decrease in the activity of the glutathione peroxidase enzyme in the liver mitochondria of this group of rats was observed on the 1st and 3rd days compared with the control and activity enzyme was equal to 35±2.3% and 35.6±1.3%, respectively (figure 1).

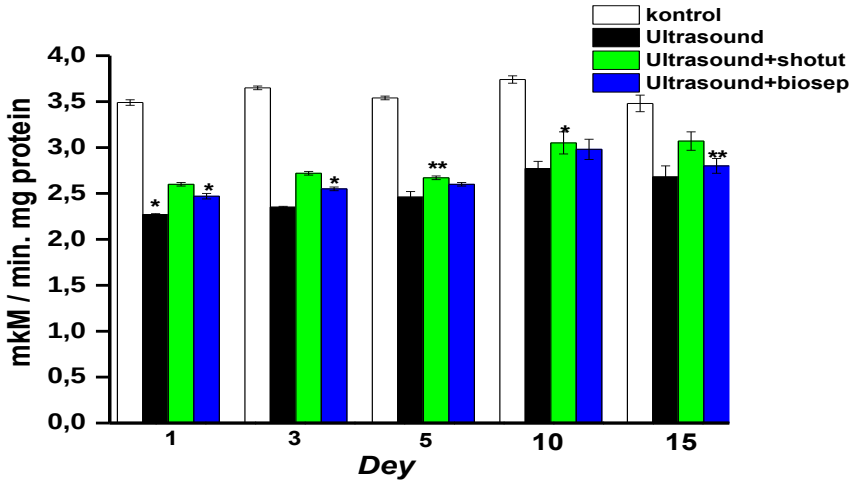

Fig.1. **Effect of shotut and biosep extracts on the activity of glutathione peroxidase during 5-minute exposure to ultrasound on the mitochondria of rat hepatocytes (1, 3, 5, 10 and 15-day dynamics) (mkM/min.mg protein)** (*p<0,05; **p<0,01; ***p<0,001, n=5-6)

A significant effect of the correction of shotut leaf extract on the activity of the glutathione peroxidase enzyme in the liver mitochondria of group III rats was found (figure 1). On days 1 and 3, the activity of the enzyme compared with group II was 9.5±1.9% and 10.1±1.6%. An increase in enzyme activity by 7.4±0.9% and 11.2±2.5% by the 10th and 15th day, respectively, was revealed. The experiments were continued on group IV rats exposed to ultrasonic waves, which were injected with biosep oil extract, and the activity of glutathione peroxidase was studied depending on the dynamics after 1, 3, 5, 10 and 15 days. According to the results of the study, the activity of glutathione peroxidase in rats of group IV on days 1, 3, 5, 10 and 15, respectively, was 5.7±2.1%, 4.3±1.7%, 4±1.9%, 5, 5±1.6% va 3.4±2.6% compared with group II (figure 1). The recovery of glutathione peroxidase activity was 8.2% higher in group III, which was corrected with shotut leaf extract, compared with group IV, which was injected with biosep oil extract.

In subsequent studies, the 10-minute effect of ultrasonic waves on the activity of the antioxidant enzyme system of rat liver mitochondria was studied. According to the results obtained, the activity of glutathione peroxidase in rat liver mitochondria under the influence of ultrasonic waves on days 1 and 3 in group II compared with the control was 47.9±2.8% and 44.6±2.1%. It was noted that by day 15, the enzyme activity was not fully restored and amounted to 35.1±2.9% compared with the control (figure 2).

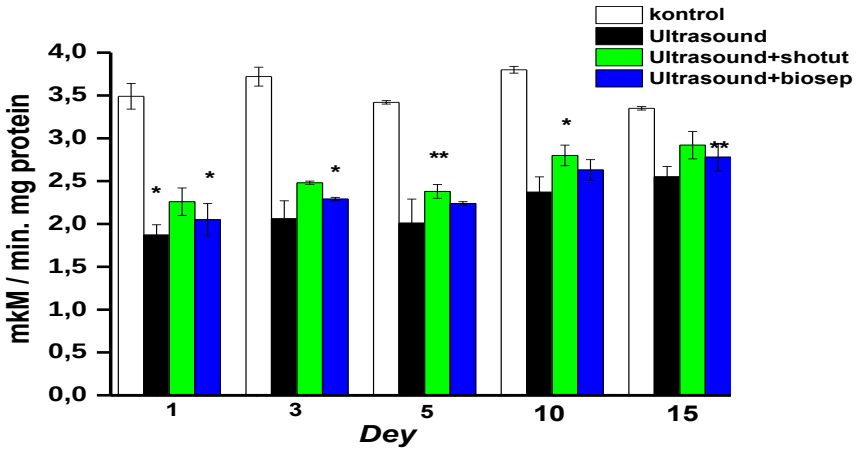

Fig.2 **Influence of shotut and biosep extracts on glutathione peroxidase activity during 10-minute exposure to ultrasound on rat hepatocyte mitochondria (1, 3, 5, 10 and 15-day dynamics) (mkM/min.mg protein)** (*p<0,05; **p<0,01; ***p<0,001, n=5-6).

In group III rats, which were given shotut leaf extract as a correction, there was a low recovery of liver mitochondrial glutathione peroxidase activity compared to group II on days 1 and 3, and by days 10 and 15, glutathione peroxidase activity significantly exceeded the activity of group II glutathione peroxidase by 11.3 ±1.9%. and 10.2±2.8%. With the continuation of experiments on rats of group IV, which, after exposure to ultrasonic waves, were orally injected with an oily extract of Biosep, the activity of glutathione preoxidase in the first days changed imperceptibly in comparison with group II, an increase in enzyme activity was found on days 10 and 15 and, respectively, amounted to 6.8± 2.4% and 6.3±2.1% (figure 2). It was found that the activity of glutaion peroxidase was 3.9% higher in animals of group III, the correction of which was carried out with shotut leaf extract, compared with group IV, which received biosep oil extract.

Summary

As a result of 5 and 10 minute exposure to ultrasonic waves, a strong inactivation of the enzyme glutathione peroxidase of rat liver mitochondria was observed on days 1 and 3, and it was found that it did not fully recover even after 15 days. It was noted that after a 5-minute exposure to ultrasonic waves on days 1, 3, 5, 10 and 15, depending on the dynamics, the activity of glutathione peroxidase under the influence of shotut leaf extracts and biosep oil extract was restored to one degree or another and the recovery of enzyme activity was higher by 10 -th and 15th days compared with the control.

Related to antioxidant enzymes, glutathione peroxidase is also one of the key enzymes in the catabolism of free radicals [12; 3217-3266; 13; 502-508]. In the body - in the mitochondria and cytoplasm, the enzyme glutathione peroxidase breaks down lipid peroxides and H_2O_2 to water and oxygen.

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