



**INFLUENCE OF ULTRASONIC WAVES ON THE ACTIVITY OF NAD-DEPENDENT
MALATE DEHYDROGENASE ENZYME IN RAT LIVER MITOCHONDRIA AND WAYS
OF THEIR CORRECTION**

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Annotation

After a 5-minute ultrasonic exposure in rats on the 1st and 3rd days, a sharp decrease in the activity of the NAD-MDG enzyme in the mitochondria of their liver was observed, which in turn leads to disruption of membrane structures and changes in lipid peroxidation processes in rat liver mitochondria. It was noted that the corrective action of shotut extract was more effective than that of biosep oil extract.

Keywords: liver, mitochondria, extract, biosep, ultrasound, malate dehydrogenase.

INTRODUCTION

Ultrasound waves are widely used in modern medicine. In studying the structure and functional state of the body, USG ranks high in terms of its diagnostic convenience and level of safety. However, research conducted by scientists to study the effect of ultrasound on the human body revealed that a number of harmful effects and changes occur in the human body as a result of its use for diagnostic purposes [1].

Also, in a number of literatures, polymorphous and complex changes occur in animal and human tissues and organs under the influence of ultrasound [2; 3; 4].

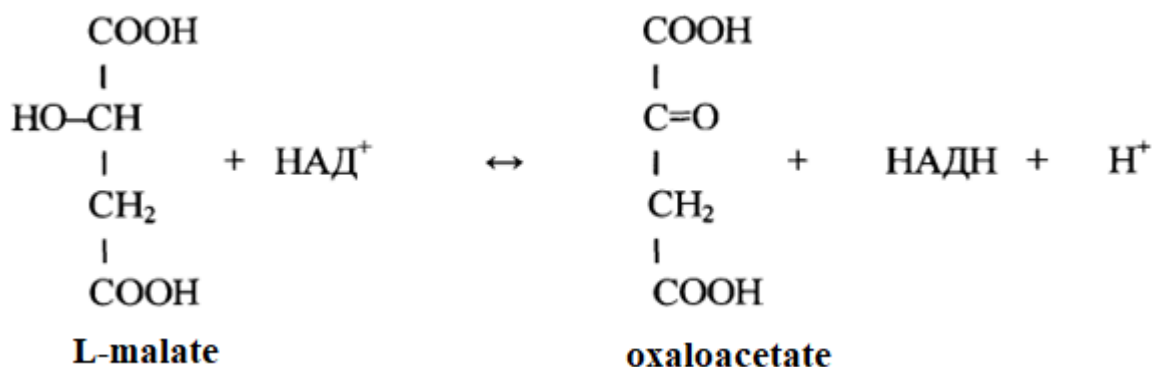
Taking into account the above, the aim of the work is to study the effect of ULTRASOUND on rat hepatocytes at the molecular level. For this purpose, it is necessary to study the effect of ultrasound on



lipid peroxide oxidation, some membrane-bound enzymes, and the activity of enzymes of the antioxidant system in liver mitochondria. [5; 6; 7; 8]

LITERATURE ANALYSIS.

Malate dehydrogenases (MDG, L-malate: NAD-oxidoreductase, K.F.1.1.1.37) catalyze the formation of oxaloacetate from L-malate by controlling the NAD/NADN dependent changes of malate and oxaloacetate substrates[9,10]:



This reaction plays a key role in the passage of malate/aspartate across the mitochondrial membrane and the tricarboxylic acid cycle in the mitochondrial matrix. In higher plants and animals, 2 malate dehydrogenases are distinguished, one of them is located in the mitochondria and the other in the soluble fraction of the cell [11]. According to the distribution in animal tissue, this enzyme is found in large quantities in the heart, then in the kidneys, and in the third place in the brain, liver and skeletal muscles [11, 12]. Therefore, it is very important to study the effect of ultrasound waves on the activity of the Nad-dependent malate dehydrogenase enzyme of rat liver mitochondria and their correction.

RESEARCH METHODS AND MATERIALS

Purebred white female laboratory rats weighing 150-220 g were used in the research. Subjects were irradiated using a Mindrey DP-50 Vet ultrasound device designed for animals. The effects of USG in 7.5 mHz mode for 5 minutes on rats were studied.

For the experiment, the rats were divided into separate model groups for the effect of ultrasound and their correction:

Group I healthy (control) (n=5)

II group exposed to ultrasound for 5 minutes (n=5-6)

Group III ultrasound + rosehip extract (n=5-6)

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

In the experiment, after a 5-minute exposure to ultrasound, group III rats were given 1 ml per body weight once a day for 5 days through a special probe, and group IV rats were given 1 ml of Biosep drug pre-orally.



1, 3, 5, 10, and 15 days after the administration of the extracts of sorghum and biosep to the rats exposed to ultrasound, the activity of their liver mitochondria enzymes was studied.

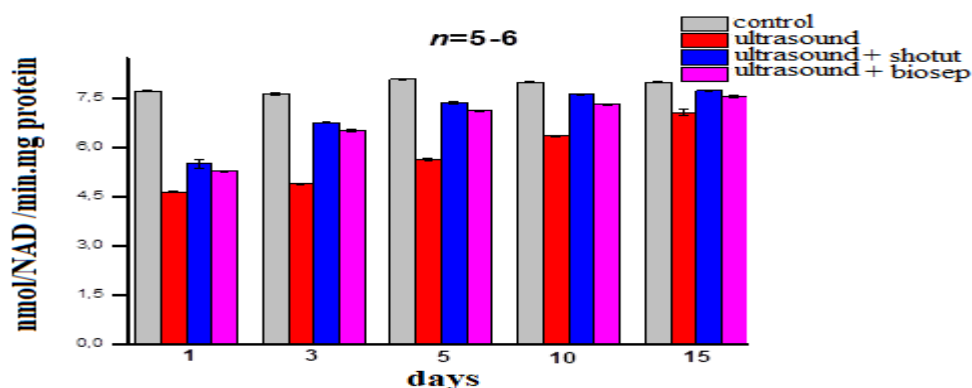
Mitochondria of rat liver were isolated by differential centrifugation using a modified method of W.C.Schneider[13] and Kuzmina et al.[14]. 0.25 M sucrose - TKM buffer solution was used to isolate mitochondria from liver tissue. A 1:10 homogenate of tissues was prepared and centrifuged at 1000 rpm for 10 minutes. The sediment was discarded and the liquid part was centrifuged at 12000 rpm for 10 minutes. The resulting precipitate was washed twice with 0.25 M sucrose solution and used to determine NAD-MDG enzyme activity. NAD-MDG enzyme activity was measured in a UV/VIS spectrophotometer at a wavelength of 340 nm[15].

To determine NAD-MDG enzyme activity, mitochondrial membranes were disrupted with the detergent triton X-100, and the following incubation medium was prepared: 1 ml of 85 mM NaOH-glycine buffer (pH 10.0), 0.1 ml of 0.25 M NAD solution, 1 ml 85 mM D, L-malate, 0.8 ml distilled water. The solutions were mixed and 0.1 ml of mitochondrial extract was added. Enzyme activity was measured at a wavelength of 340 nm ($\epsilon = 6.22 \text{ mM}^{-1}\text{cm}^{-1}$) for 3-5 minutes at 1-minute intervals. NAD-MDG enzyme activity is expressed as nmol/NAD/min 1 mg of protein[15].

The amount of protein in mitochondria was determined according to the Lowry method [16]. The difference between the results obtained from the control, experimental and experimental+shotut, experimental+biosep groups was calculated by t-test, where the value of $r < 0.005$ represents statistical reliability.

RESULTS OBTAINED AND THEIR ANALYSIS

The results of the study show that when rats' livers were exposed to 7.5 mHz range for 5 minutes through the Mindrey DP-50 Vet ultrasound device, the activity of NAD-MDG enzyme in hepatocyte mitochondria in rats of this group on days 1, 3, 5, 10 and 15 was correspondingly higher than that of the control. decreased by $40 \pm 2.7\%$, $36 \pm 1.2\%$, $30.2 \pm 0.4\%$, $20.4 \pm 1.3\%$, $11.6 \pm 1.1\%$ (Picture. 1).



Picture 1. Effects of shotut i and biosep extracts on mitochondrial NAD-MDG enzyme activity of UV-exposed rat hepatocytes (1, 3, 5, 10, and 15 days dependent on dynamics) (nmol/NADF/min 1 mg protein) (*P<0,05, n=5-6)



This, in turn, indicates that the activity of the enzyme NAD-MDG, which is a mitochondrial enzyme, is impaired under the influence of ultrasound (Table 1). A sharp decrease in the activity of NAD-MDG enzyme in the liver mitochondria of rats of this group was observed on the 1st and 3rd days after UVZ exposure, and it was found that it decreased by $40 \pm 2.7\%$ and $36 \pm 1.2\%$, respectively.

Table 1 Effects of shotut i and biosep extracts on mitochondrial NAD-MDG enzyme activity of UV-exposed rat hepatocytes (1, 3, 5, 10 and 15 days dependent on dynamics) (nmol/NADF/min 1 mg protein)

No	Experience groups	n	1-day	3-day	5-day	10-day	15-day
I	Control	5	$7,71 \pm 0,024$	$7,62 \pm 0,044$	$8,06 \pm 0,013$	$7,98 \pm 0,021$	$7,98 \pm 0,017$
II	Ultrasound	6	$4,64 \pm 0,010^*$	$4,88 \pm 0,030^*$	$5,63 \pm 0,032^*$	$6,35 \pm 0,021^*$	$7,06 \pm 0,008^*$
III	Ultrasound +shotut	5	$5,49 \pm 0,118^*$	$6,75 \pm 0,028^*$	$7,35 \pm 0,033^*$	$7,60 \pm 0,021^*$	$7,72 \pm 0,024^*$
IV	Ultrasound +biosep	5	$5,27 \pm 0,014^*$	$6,51 \pm 0,047^*$	$7,11 \pm 0,013^*$	$7,30 \pm 0,010^*$	$7,54 \pm 0,036^*$

Explanation: (* $P < 0,05$, $n = 5-6$)

A significant effect of the extract on the activity of NAD-MDG enzyme in liver mitochondria of group III rats corrected with barley extract was revealed (Picture. 1). On days 1, 3, 5, 10, 15, its activity was $11.2 \pm 0.1\%$, $24.5 \pm 1.2\%$, $22.1 \pm 0.9\%$, 15.6 ± 1 , It was found that it increased by 0% and $8.3 \pm 0.6\%$. It was observed that the enzyme activity in the mitochondria of the hepatocytes of this group was significantly restored by the 15th day (Picture. 1).

The activity of NAD-MDG enzyme in the mitochondria of rat hepatocytes of group IV corrected with fatty extract of Biosep was $8.3 \pm 0.8\%$, $21.4 \pm 0.8\%$, $18.4 \pm 0.2\%$, 11 It was $9 \pm 1.03\%$, $6 \pm 0.3\%$ (Picture. 1).

CONCLUSION

Thus, a sharp decrease in the activity of the NAD-MDG enzyme in the mitochondria of the liver was detected in the first 1 and 3 days after exposure to ultrasound for 5 minutes. Profound inhibition of the enzyme was observed on days 1 and 3 after UV exposure, which in turn led to disruption of membrane structures and changes in lipid peroxide oxidation in rat liver mitochondria. Also, in the experiments, a certain degree of restoration of NAD-MDG enzyme activity in liver mitochondria of groups of rats corrected with horsetail extract and biosep oil extract was observed.

According to the obtained results, it has been shown that the extract of milk thistle has a more effective corrective effect than the oil extract of biosep.



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