Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 20s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Influence Of Ultrasonic Waves on The Activity of Above and Nadp - Dependent Liver Isocytedehydrogenase in Rats and Ways of Their Correction

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Cite this paper as: Gafurdjon Mukhamedov, Dilnoza Babakhanova, Parida Mirkhamidova, Rano Alimova, Gulnara Shakhmurova, Otabek Eshonkulov, (2025) Influence Of Ultrasonic Waves on The Activity of Above and Nadp - Dependent Liver Isocytedehydrogenase In Rats and Ways of Their Correction. *Journal of Neonatal Surgery*, 14 (20s), 443-449.

ABSTRACT

Isocitrate dehydrogenase is a key enzyme in the tyrcarboxylic acid cycle and is involved in the oxidative decarboxylation of isocitrate (IC) to form alpha-ketoglutarate (α-ketoglutarate) and CO_2. The goal was to investigate the effect of ultrasonic waves on the activity of lipid peroxidation, isocitrate dehydrogenase in rat liver mitochondria, as well as the effect of mulberry leaf extract and biosep oil extract. The experiment involved white lab rats weighing 180-200 grams each. The ultrasound device used was Mindrey DP-50 Vet. The work is based on the following methods: Schneider differential centrifugation, spectrophotometry, chromatography, pH-metry, photometry. The article presents data on the suppression of the activity of NAD and NADP-dependent isocitrate dehydrogenase (IDH) enzymes in liver mitochondria in rats irradiated with ultrasound for 5 minutes. Deep inhibition of enzymes was observed on the 1st and 3rd days after exposure to ultrasonic waves (ultrasound), which in turn led to disruption of membrane structures and changes in lipid peroxidation processes in rat liver mitochondria. In the groups of rats, in which the correction was carried out with mulberry extract and biosep oil extract, restoration of the activity of NAD and NADP-IDH enzymes in liver mitochondria was observed.

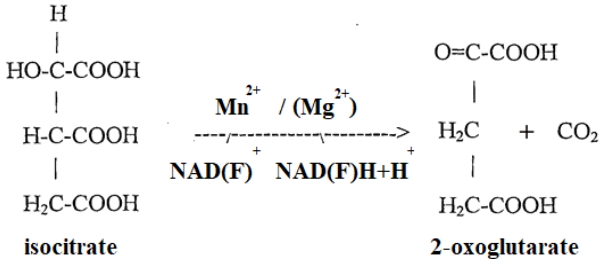
The obtained data show that the correction effects of mulberry extract were more effective than those of biosep oil extract

Keyword: *: hepatocyte, mitochondria, isocitrate dehydrogenase, extract, biosep, ultrasound wave.*

1. INTRODUCTION:

The key enzymes of the tricarboxylic acid cycle are citrate synthetase and IDH. IDH is an enzyme that catalyzes the oxidative decarboxylation of isocitrate (IC) with the formation of alpha-ketoglutarate (α-ketoglutarate) and CO_2 [1, 366].

IDH catalyze the oxidative decarboxylation of D,L — threo-Ds-IC to 2-oxoglutarate(2-OG) (Picture. 1) [2, 277; 3,1861-1879].



Picture. 1. Reaction catalyzed by isocitrate dehydrogenase

The enzyme has two forms of coenzyme specificity: NAD-dependent IDH (EC 1.1.1.41, NAD-IDH) and NADP-dependent IDH (EC 1.1.1.42, NADP-IDH).

It is believed that IDH plays an important role in the functioning of the antioxidant system (AOS) of the body [4, 1185-1196; 5, 1057-1065; 6, 635-642; 7, 33946-33957].

Thus, a number of authors noted that mitochondrial NADP-IDH(mNADF-IDH) plays a significant role in the formation of reducing equivalents for glutathione peroxidase / glutathione reductase - the mitochondrial system, which is of great importance for the work of AOS [8, 16168-16176; 9, 441-448; 10, 44-51; 11, 1053-1061; 12, 13385-13394; 13, 1012-1018].

The reaction catalyzed by NAD-IDH is irreversible [14, 729-734], is a step in the tricarboxylic acid (TCA) cycle and represents an important reaction of oxidative catabolism. The NADH formed during the reaction is transferred to the respiratory chain of mitochondria, and IDH can limit the total rate of metabolite flow through the TCA [15, 272; 16, 3745-3750]. It is believed that NAD-IDH is localized around NADH, oxidoreductase on the inner membrane of mitochondria [17, 9509-9514; 18, 10800-10805].

The conversion of IC to 2-OG catalyzed by NADP-dependent IDH is reversible [19, 338]. Oxalosuccinate is formed as an intermediate in the NADP-IDH reaction [20, 311]. NADP-IDH is present in many tissues, with the highest content observed in the heart, liver and skeletal muscles [21, 1861-1879].

The activity of NADP-IDH in rat hepatocytes is localized by 70-80% in the cytoplasm of the cell, and by 20-30% in mitochondria.

A number of researchers have shown that with the development of oxidative stress, the enzymes of antioxidant defense of hepatocytes are activated [22, 169-176; 23, 23; 24, 23] and enzymes that contribute to the functioning of AOS [25, 23; 26, 22].

It is believed that mNADP-IDH plays a major role in the biosynthesis of fatty acids and cholesterol, and regulation of the enzyme can be used to lower lipid levels [27, 3169-2180].

Recently, ultrasound has been widely used in biology and medicine [28, 4-17; 29, 102-106]. However, little has been described about the impact of ultrasound on the human body and animals [30, 9813-9816; 31, 116-118]. Currently, significant material has been accumulated on the biological effect of ultrasound, however, these are the results of experimental studies and observations of the effects of ultrasound for therapeutic purposes [32, 57-60].

In this work, we studied the effect of ultrasound on the activity of rat liver enzymes and carried out search work on the way of correction with plant antioxidants.

The aim of the study was to study the effect of ultrasound on the activity of NAD and NADP-dependent IDH in rat liver mitochondria

2. MATERIALS AND METHODS

The research was conducted on white lab rats, each weighing between 180-200 grams (permission No. 6/14-1697 dated September 27, 2022 from the Bioethics Committee of the Ministry of Health of the Republic of Uzbekistan). The Mindrey DP-50 Vet, an ultrasound device specifically designed for animals, was employed in the study. The rats were subjected to ultrasound waves at a frequency of 7.5 MHz for 5 minutes.

For the experiment, the rats were categorized into distinct groups to analyze the effects of ultrasound waves and their subsequent mitigation:

Group I: Healthy control (n=5)

Group II: Subjected to 5 minutes of ultrasound exposure (n=5-6)

Group III: Ultrasound exposure followed by mulberry extract treatment (n=5-6)

Group IV: Ultrasound exposure followed by biosep treatment (n=5-6)

In the experiment, rats of group III after 5 minutes of exposure to ultrasound were injected with 1 ml of mulberry extract once a day for 5 days in relation to body weight, and rats of group IV were orally injected with 1 ml of biosep for 5 days.

The activity of mitochondrial enzymes in their liver was studied 1, 3, 5, 10 and 15 days after the administration of mulberry (*Morus nigra L*) and biosep(*Júglans régia*, *Matricária*, *Méntha piperíta*) extracts to rats subjected to ultrasound.

Rat liver mitochondria were isolated by differential centrifugation proposed by W.C. Schneider [33, 619-635] and by the modification method of Kuzmin et al. [34, 1684-1697]. IDH activity was determined on a UV/VIS spectrophotometer in the range of 340 nm [35, 351-353]. 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, where nuclei and subcellular samples were deposited. After separation of the nuclei, the supernatant was centrifuged at 12,000 rpm for 10 minutes. The precipitate obtained (the crude fraction of mitochondria) was suspended in 0.25 M sucrose solution in TCM

buffer and centrifuged under the same conditions. This washing of the mitochondrial fraction was repeated two more times. The sediment of mitochondria was suspended in 0.25 M sucrose in TCM buffer and used in experiments.

The method for determining the activity of NADP-dependent IDH is based on the oxidation of isocitrate and the formation of NADPH. To determine the activity of the enzyme, the following incubation medium was prepared: 2.6 ml of 0.1 M Tris-HCl buffer solution (pH-7.4), 0.1 ml of 4 mM NADP solution, 0.1 ml of 0.1 M solution of $MnCl_2$ and 0.1 ml of 0.1 M solution of sodium isocitrate. After stirring, the reaction was initiated by the introduction of 0.1 ml of mitochondria. Enzyme activity was determined by the rate of isocitrate oxidation and NADPH formation at 340 nm (ε = 6.22 mM–1 cm–1) [35, 351–353].

The activity of NAD-IDH is judged by the rate of oxidation of isocitric acid, which is accompanied by the restoration of equimolar amounts of NAD+. An increase in the amount of the reduced form of NAD in the incubation medium is recorded spectrophotometrically at a wavelength of 340 nm.

To determine the activity of NAD-IDH, a somobilization medium (0.6% Triton X-100 in 30 mM Na_2HPO_4 solution, 22.5% $(NH_4)_2SO_4$, 1.5 mM $MnCl_2$ on 30% glycerol) is added to the mitochondrial precipitate. The mitochondrial pellet, together with the comobilization medium, is quantitatively transferred to a small glass homogenizer and suspended by hand. To destroy mitochondria, the sample is left on ice for 25-30 minutes. The presence in the mixture along with the usual detergent Triton X-100 of additional components (ammonium sulfate, glycerol) stabilizes the structure of NAD-IDH after the destruction of mitochondria. To carry out the enzymatic reaction, 2.9 ml of incubation medium (2.5 ml of 0.1 M Tris-HCl buffer solution (pH-7.2), 0.1 ml of 1 mM NAD solution, 0.1 ml of 0 .4 M $MnCl_2$ solution, 0.1 ml of 0.2 M ADP solution and 0.1 ml of 0.16 M sodium isocitrate solution) and 0.1 ml of the mitochondrial preparation. The first reading of the spectrophotometer readings at a wavelength of 340 nm is carried out exactly 20 s after the introduction of the drug, then the readings of the device are recorded for 1 min every 15 s [35, 351-353].

The activity of NADP-IDH and NAD-IDH is expressed in μmol/min/mg of protein. The amount of protein in mitochondria was determined by the Lowry method [36, 265-275]. The difference between the results obtained in the control groups, ultrasound and ultrasound + mulberry, ultrasound + biosep was calculated by t-test, where the P<0.05 value represents statistical significance.

3. RESULT AND DISCUSSION

According to the results of the study, when ultrasound on the liver of rats in the range of 7.5 MHz using the Mindrey DP-50 Vet UZI apparatus for 5 minutes, the activity of the NAD-IDH enzyme in the mitochondria of hepatocytes by 1, 3, 5 , 10 and 15 days compared with the control groups, respectively, was 41.8±7.4%, 37±1.1%, 30.6±0.7%, 22.8±0.5%, 16±0, 3% (Table 1).

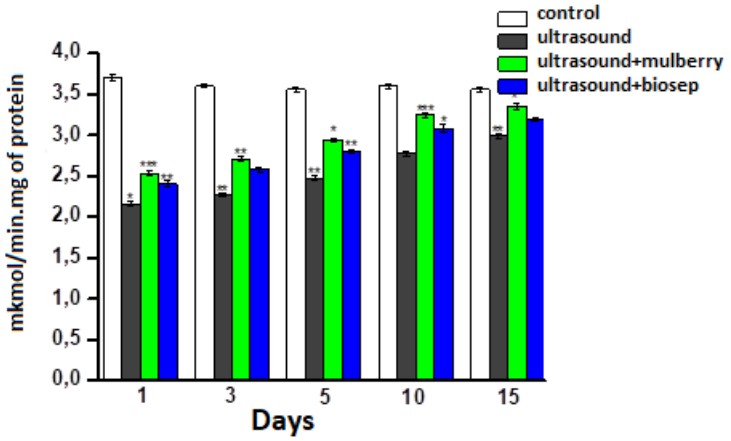
The data obtained indicate a violation of the activity of the enzyme NAD-IDH in the mitochondria of the liver of rats under the influence of ultrasound (table 1). A sharp decrease in the activity of the NAD-IDH enzyme in the liver mitochondria of this group of rats compared with the control was detected on the 1st and 3rd days after exposure to ultrasound, i.e. 41.8±7.4% and 37±1.1% , respectively (Fig. 1).

Table 1Effects of mulberry and biosep extracts on the activity of NAD-IDH under the influence of ultrasound on the mitochondria of rat hepatocytes (1, 3, 5, 10 and 15-day dynamics) (μmol/min/mg of protein) (*P<0.05, n=5-6)

№	Experience group	n	1 day	3 day	5 day	10 day	15 day
I	Control	5	3,71±0,039	3,60±0,017	3,56±0,024	3,60±0,025	3,56±0,031
II	ultrasound	6	2,16±0,028*	2,27±0,023**	2,47±0,029**	2,78±0,028	2,99±0,028**
III	ultrasound + mulberry	5	2,53±0,030***	2,71±0,027**	2,94±0,029*	3,25±0,028***	3,36±0,041
IV	ultrasound + biosep	5	2,41±0,026**	2,58±0,025	2,81±0,021**	3,08±0,051*	3,20±0,022

In experiments, the effect of correcting mulberry extract on the activity of the NAD-IDH enzyme in the liver mitochondria of group III rats was obtained (Table 1). On days 1, 3, 5, 10, 15, its activity was 10±0.2%, 12.3±0.3%, 13.2±0.3%, 13±2.6% and 10±1.7% respectively compared with group II. The enzymatic activity of NAD-IDH in the mitochondria of hepatocytes of this group of rats recovered organically by the 10th and 15th days.

The activity of the NAD-IDH enzyme in the mitochondria of hepatocytes of group IV rats, which were corrected with biosep extract, was 6.7±1.5%, 8.7±2.1%, 9.5±0.2%, 8.4 ±1.1%, 5.9±0.1%, respectively, compared with group II (Picture. 2).



Picture.2. Influence of extracts of mulberry and biosep on the activity of NAD-IDH when exposed to ultrasound on the mitochondria of rat hepatocytes (1, 3, 5, 10 and 15-day dynamics) (µmol/min/mg of protein) (*P<0.05, n=5 -6)

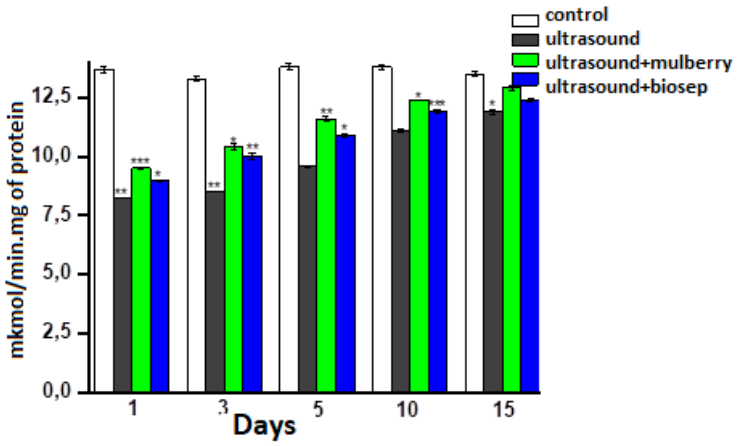
A similar picture was observed when studying the activity of NADP-IDH. After exposure to ultrasound on the liver of rats in the range of 7.5 MHz using the Mindrey DP-50 Vet UZI apparatus for 5 minutes, the activity of the NADP-IDH enzyme in the mitochondria of hepatocytes on days 1, 3, 5, 10 and 15 compared with the control groups respectively amounted to 39.7±0.9%, 36±1%, 30.5±0.6%, 19.6±0.5%, 11.9±0.3% (Table 2).

Table 2 Effects of mulberry and biosep extracts on the activity of NADP-IDH when exposed to ultrasound on the mitochondria of rat hepatocytes (1, 3, 5, 10 and 15-day dynamics) (µmol/min 1 mg protein) (*P<0.05, n=5 -6)

№	Experience group	n	1 day	3 day	5 day	10 day	15 day
I	Control	5	13,7±0,135	13,3±0,105	13,8±0,151	13,8±0,088	13,5±0,086
II	ultrasound	6	8,26±0,022**	8,51±0,023**	9,59±0,040	11,1±0,093	11,9±0,11*
III	Ultrasound + mulberry	5	9,51±0,024***	10,4±0,131*	11,6±0,112**	12,4±0,112*	12,9±0,105
IV	Ultrasound + biosep	5	8,97±0,025*	10,0±0,129**	10,9±0,088*	11,9±0,082**	12,4±0,088

The data obtained indicate a violation of the activity of the enzyme NADP-IDH in the mitochondria of the liver of rats under the influence of ultrasound (table 2). A sharp decrease in the activity of the NADP-IDH enzyme in the liver mitochondria of this group of rats compared with the control was detected on the 1st and 3rd days after exposure to ultrasound, i.e. 39.7±0.9% and 36±1%, respectively .

A significant effect of the mulberry extract correction on the activity of the NADP-IDH enzyme in the liver mitochondria of group III rats was found (Table 1). On days 1, 3, 5, 10, 15, its activity was 9.1±1%, 14.2±0.3%, 14.6±0.4%, 9.4±2% and 7.5± 1.5%, respectively, compared with group II. The enzymatic activity in the mitochondria of hepatocytes of this group of rats was moderately restored by the 10th and 15th days (Picture. 3).



Picture.3. Effects of extracts of mulberry and biosep on the activity of NADP-IDH when exposed to ultrasound on the mitochondria of rat hepatocytes (1, 3, 5, 10 and 15-day dynamics) (µmol/min 1 mg protein) (*P<0.05, n=5 -6)

The activity of the NAD-IDH enzyme in the mitochondria of hepatocytes of group IV rats, which were corrected with biosep extract, was 5.2±0.5%, 11.2±0.3%, 10.5±0.3%, 5.8 ±1.5%, 3.4±1%, respectively, compared with group II (Picture. 3).

4. CONCLUSION

From the results obtained, it was concluded that the restorative effect of mulberry extract is more pronounced than that of Biosep. Consequently, the effect of ultrasonic waves on rat liver mitochondria led to increased lipid peroxidation and a noticeable decrease in the activity of isocitrate dehydrogenases. These changes in lipid peroxidation disrupted the functional activity of membranes, which in turn affected the Krebs cycle. The study showed that oil extracts of mulberry leaves and biosep have antioxidant properties, helping to partially restore their activity.

Since the leaves of the medicinal mulberry contain substances with antioxidant properties such as vitamin C, flavonoids and general antioxidants, their extract can be used to correct the effects of ultrasound on liver cells. Changes in lipid peroxidation led to disruption of the functional activity of mitochondrial membrane enzymes - NAD and NADP isocitrate dehydrogenase. Such changes entailed changes in the antioxidant defense system. The results of the study explain the elucidation of the mechanisms of damage to liver tissue by ultrasonic waves and the correction of mitochondrial dysfunction caused by it using mulberry leaf extract and Biosep oil extract. The practical significance of the study results is that mulberry leaf extract and Biosep oil can be used as a means of correcting liver mitochondrial dysfunction caused by ultrasonic waves. It has also been established that mulberry leaf extract and biosep oil extract are biologically active compounds with antioxidant and antiradical properties.

Authors' contribution

Conceptualization - Parida Mirkhamidova

Methodology -Dilnoza Babakhanova, Parida Mirkhamidova

Formal Analysis - Dilnoza Babakhanova, Parida Mirkhamidova, Rano Alimova,

Gulnara Shakhmurova

Investigation - Dilnoza Babakhanova

Resources - Gafurdjon Mukhamedov, Otabek Eshonkulov

Writing – Original Draft Preparation - Dilnoza Babakhanova, Parida Mirkhamidova, Rano Alimova, Gulnara Shakhmurova, Gafurdjon Mukhamedov, Otabek Eshonkulov

Writing – Review & Editing, - Parida Mirkhamidova, Gulnara Shakhmurova

Supervision - Gafurdjon Mukhamedov

Project Administration - Parida Mirkhamidova

Funding Acquisition - Parida Mirkhamidova, Gulnara Shakhmurova, Gafurdjon Mukhamedov

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