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Influence of Herbal Preparations on the Process of Lipid Peroxidation and Enzyme Activity of Rat Liver Mitochondria with Drug Damage

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Abstract: The influence of various doses (25 and 50 mg/kg) of the flavonoid quercetin, the monoammonium salt of glycyrrhizic acid (MASGA) as well as the supramolecular complex derived from them (MASGA/quercetin) on the processes of lipid peroxidation (LPO), respiration, and oxidative phosphorylation of mitochondria as well as the activity of mitochondrial respiratory chain enzymes in experimental paracetamol hepatitis were studied. In the work, methods of differential centrifugation, methods for determining the content of the LPO product - malondialdehyde (MDA) using thiobarbituric acid, polarographic methods for determining the functional state and activities of mitochondrial respiratory chain enzymes were used. It has been established that toxic hepatitis develops in experimental rats under the action of paracetamol, characterized by a more than twofold increase in MDA in mitochondria in ascorbate-dependent and NADH (nicotinamide adenine dinucleotide (NAD) + hydrogen (H))-dependent systems. Magnification of the LPO process leads to the inhibition of the respiratory and phosphorylating functions of mitochondria by 35 and 30% compared with intact animals and is accompanied by inhibition of the oxidation of NAD- and FAD-dependent substrates and inhibition of respiratory chain enzymes by approximately 40%. Correction of the identified disorders using various doses of quercetin, MASGA, and the MASGA/quercetin complex showed that all the studied compounds have the ability to restore the detected disorders in mitochondria, but the supramolecular complex MASGA/quercetin at a dose of 25 mg/kg has the greatest stimulating effect on the functional state of mitochondria. It is assumed that the supramolecular complex MASGA/quercetin can serve as the basis for the creation of hepatoprotectors for the treatment of drug-induced hepatitis. For the first time, the influence of the supramolecular complex MASGA/quercetin on the LPO process and the functional state of rat liver mitochondria in experimental toxic hepatitis caused by the administration of paracetamol was studied.

Keywords: Lipid peroxidation, glycyrrhizic acid, quercetin, paracetamol hepatitis, hepatoprotector

1. Introduction

One of the main mechanisms for the development of drug-induced liver damage is a violation of the structure and function of mitochondria. Mitochondrial disorders include functional and biochemical changes in the metabolism of proteins, fats, carbohydrates, decreased activity of respiratory chain enzymes [5]. In the treatment of liver diseases, a large group of drugs is used - hepatoprotectors that cause adequate pharmacological correction of damaged in the metabolism of the liver cell [17; 21].
Currently, in the modern pharmaceutical industry, the creation of pharmacological preparations based on plant materials is a priority due to the absence of serious side effects in the latter. From this point of view, flavonoids are considered promising substances. Most flavonoids have unique therapeutic potential but low bioavailability. In this connection, the development of modern dosage forms based on flavonoids, which makes it possible to ensure their optimal bioavailability, is relevant. One of the effective ways to increase their bioavailability is the creation of supramolecular complexes. Complexation leads to a synergistic effect of increasing their biological

activity with a simultaneous decrease in toxicity, ulcerogenic action and increases the solubility of the drug in aqueous media, i.e., the transport of the medicinal compound through the lipophilic membranes of cells, and their bioavailability are improved [4]. This helps to maintain the required concentration of the drug in the blood and faster delivery to the affected organs. Glycyrrhizic acid (GA) has long been known as a compound with anti-inflammatory activity. In particular, it has been shown that GA inhibits the production of such primary inflammatory mediators as TNF-α, interleukins IL-1β and IL-6. [6]. It is assumed that the anti-inflammatory activity of GA is associated with its antioxidant activity. The antioxidant activity of glycyrrhizic acid also largely determines its hepatoprotective activity against a number of toxic compounds [1].

2. Materials and methods of research

The aim of this study is to study the effect of the flavonoid quercetin, the monoammonium salt of glycyrrhizic acid (MASGA), as well as the supramolecular complex created on the basis of the monoammonium salt of glycyrrhizic acid with the flavonoid quercetin (MASGA/quercetin) on the process of lipid peroxidation, the functional state of mitochondria, and the activity of enzymes of the mitochondrial respiratory chain with experimental toxic hepatitis caused by paracetamol.
The experiments were carried out on outbred rats of both sexes weighing 140-200 g in accordance with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [2]. Drug-induced liver damage was reproduced by the introduction of paracetamol into the stomach at a dose of 500 mg/kg for 2 days [15]. Tested compounds: monoammonium salt of glycyrrhizic acid (MASGA), quercetin, supramolecular complex MASGA/quercetin at a dose of 25 and 50 mg/kg of animal body weight. Stronger Neo-Minophagen S (STD) (Japan) was used as a reference drug. All test compounds were administered within 12 days of hepatitis recurrence. Mitochondria from the liver of experimental and intact rats were isolated by the conventional method of differential centrifugation [9]. The rate of lipid peroxidation (LPO) was judged by the formation of malondialdehyde (MDA) in mitochondria [16]. The respiratory rate and parameters of oxidative phosphorylation were recorded by the polarographic method using a rotating platinum electrode. The activity of respiratory chain enzymes—NADH-, succinate- and cytochrome c - oxidases— was determined after a single freeze-thaw of mitochondria [19]. Protein content was determined according to the method [13].

3. Results and Discussion

The first stage of our research was the study of the content of MDA, a secondary product of peroxidation (LPO), in liver mitochondria in paracetamol hepatitis (PG) and correction with the compounds under study. It was found that the administration of paracetamol is characterized by an increase in the rate of MDA formation in liver mitochondria up to 214% in ascorbate-dependent and up to 225% in NADH-dependent systems. Initiation of a chain reaction of lipid peroxidation (LPO). Free radicals seem to lead to structural and functional rearrangement of mitochondrial membranes in hepatocytes, an increase in their permeability to ions with subsequent uncoupling of oxidative chains, and damage to the enzymatic systems of the cell [11; 12]. Similar data were obtained by I. Grattagliano and others [7]. Administration of STD to hepatitis animals caused a significant decrease in MDA in mitochondria in both systems by 45 and 69%. Figure 1 shows that all the studied compounds, after 12-day administration, inhibited the process of lipid peroxidation in liver mitochondria to varying degrees. Basically, the antioxidant effect of the compounds was quite comparable with the effect of STD. However, it was found that among the studied substances, both studied doses of the supramolecular complex MASGA/quercetin significantly exceeded the antioxidant effect of the reference drug and these doses were approximately the same in efficiency. Therefore, in subsequent experiments, we studied only the dose of the complex, equal to 50 mg/kg.

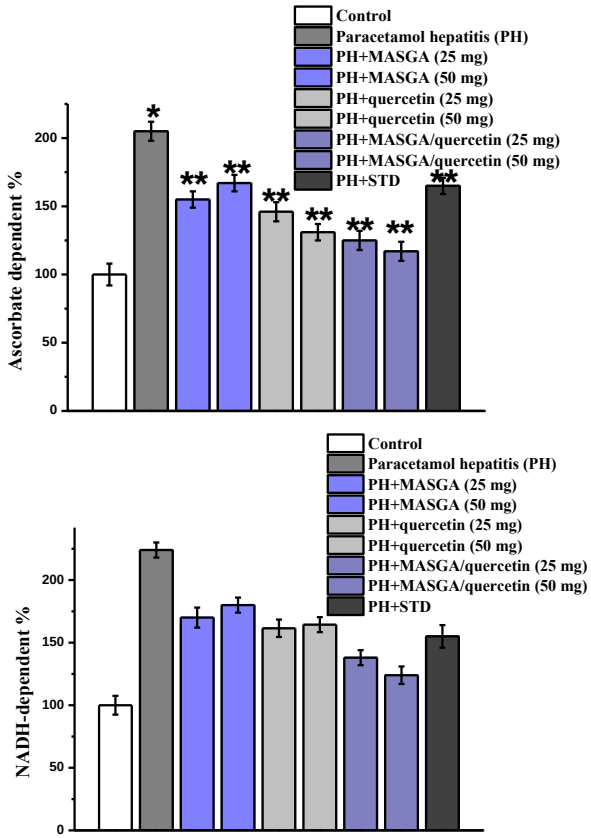

Fig.1 Effect of plant compounds on the content of MDA in rat liver mitochondria with paracetamol hepatitis (n = 7; M ± m; * p<0.05; ** p<0.01)

Source: [Compiled by the authors]

It has now been established that disturbances in the energy functions of mitochondria are a consequence of an increase in the generation of reactive oxygen species [5; 20]. Therefore, the search for compounds that, along with antioxidant properties, have the ability to directly influence some mitochondrial processes is topical.

With this in mind, the next stage of our research was to study the effect of plant compounds on respiration and oxidative phosphorylation of rat liver mitochondria in toxic paracetamol hepatitis. From the data presented in Figure 2, it can be seen that the mitochondria of intact rats are characterized by high conjugation and efficiency of oxidation and phosphorylation processes when succinate is used as a substrate for oxidation. In animals with acute liver damage, slowing of breathing, and reduction in the efficiency of phosphorylation and respiratory control were observed. Thus, the rate of oxygen consumption in the active state was significantly reduced by 44%. At the same time, a less significant decrease in oxygen consumption in the adjusted state (by 30%) was noted. The ADP/O (R/C) coefficient was also used to assess the ATP synthesis activity of mitochondria. The data obtained in this series are consistent with the results of studies by D. Pessayre and others [18].

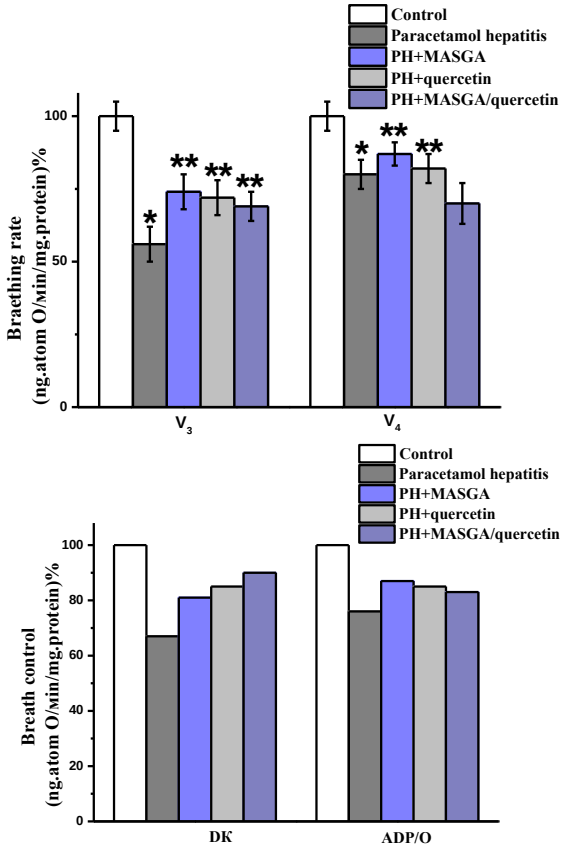

Fig. 2 Effect of plant compounds on oxidative phosphorylation and respiration of rat liver mitochondria with paracetamol hepatitis

(n = 7; M ± m; * p<0.05; ** p<0.01)
Source: [Compiled by the authors]

Introduction under these conditions to experimental animals of the investigated compounds caused various changes in the studied parameters. Among the compounds used, all studied compounds turned out to be effective: MASGA, quercetin and the supramolecular complex. Under their influence, the rate of oxygen consumption in the active and regulated states increased by more than 20 and 15%, respectively. Due to this increased respiratory control and ADP/O. The introduction of MASGA and quercetin into the animal organism separately improved the functional and metabolic parameters of the mitochondria of hepatitis rats, but the effect of these compounds on the rate of respiration and indicators of oxidative phosphorylation was less pronounced compared with the action of the MASGA/quercetin complex. We also obtained similar results when using malate oxidation as a substrate.

Thus, the studies have shown that toxic hepatitis caused by the administration of paracetamol leads to disruption of the respiratory and phosphorylating function of mitochondria and is accompanied by inhibition of the oxidation of NAD- and FAD-dependent substrates products of lipid peroxidation on the lipid matrix of mitochondrial membranes, and the indirect effect of LPO metabolites on the respiratory function of mitochondria.

In the next series of experiments, studies were carried out to determine the activity of the enzymes of the mitochondrial respiratory chain. We have chosen three enzymes of the respiratory chain. The first enzyme is the dehydrogenation of substrates controlled by enzymes containing nicotinamide coenzymes (NAD and NADP). The second enzyme is controlled by a flavin-dependent enzyme system. The third enzyme is the oxidation of reduced forms of flavin coenzymes. It is in this final enzyme of biological oxidation that hydrogen is accepted by molecular oxygen to form water. The enzymes we study: NADH dehydrogenase, succinate dehydrogenase, and cytochrome oxidase are located in the inner membrane of mitochondria and are involved in electron transfer in all three enzymes of the respiratory chain.

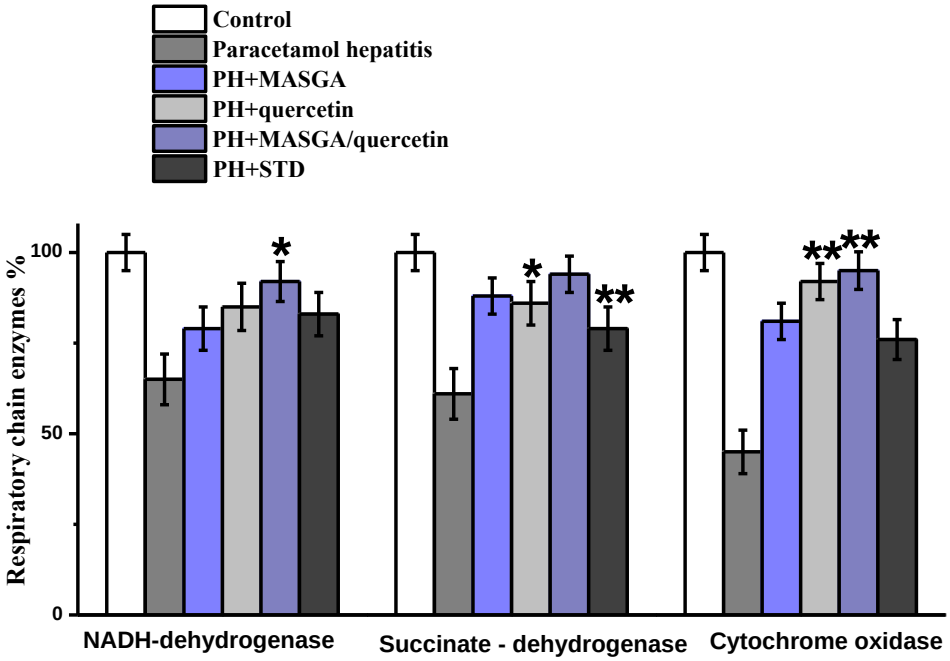

Fig. 3 Changes in the activity of respiratory chain enzymes in rat liver mitochondria with paracetamol hepatitis under the influence of plant compounds (n = 7; M ± m; * p<0.05; ** p<0.01)

Source: [Compiled by the authors]

The study of the activity of NADH - dehydrogenase of rat liver mitochondria showed that the introduction of paracetamol inhibits the activity of the enzyme by 36%. Plant compounds used as mitochondrial stabilizing agents had different effects on the activity of NADH dehydrogenase. Figure 3 shows that the 12-day administration of all studied substances increased the reduced activity of the enzyme. The most effective activators of NADH dehydrogenase are the supramolecular complex MASGA/quercetin (recovery of enzyme activity up to 83%). However, under the action of this complex and other studied compounds, the activity of NADH dehydrogenase was not completely restored.

In the next series of studies, the activity of succinate - hydrogenase in liver mitochondria of hepatitis rats was studied and it was found that in this pathology, the activity of the enzyme was significantly reduced by 39%. (Fig. 3). Inhibition of activity and restoration of this enzyme under the influence of the studied substances were approximately similar to changes in the activity of NADH dehydrogenase. Plant compounds also induced restoration of enzymatic activity to varying degrees, although the effect of MASGA/quercetin was greater than that of other substances.

Our subsequent studies were devoted to determining the activity of rat liver cytochrome oxidase with the introduction of paracetamol (Fig. 3). The figure shows that with paracetamol intoxication of the liver, the activity of this enzyme decreases by almost 48%.

Cytochrome oxidase is the final component of the respiratory enzyme chain, transferring electrons from cytochrome c to molecular oxygen. Of all the carriers of the electron transport chain, only cytochrome oxidase is able to react directly with oxygen. Cytochrome oxidase is a complex protein, the molecule of which includes two hemes, two copper atoms, and 20-30% of the lipid component. Significant inhibition of enzymatic activity found in our experiments is evidently associated with both damage to the lipid component and changes in the site containing hemes and copper atoms [8; 10]. It is known that the separation of copper from cytochrome oxidase completely inactivates the enzyme. A certain role in reducing the activity of cytochrome oxidase is played by the interaction of LPO products with the enzyme molecule, leading to the inactivation of the latter.

The supramolecular complex administered to hepatitis animals significantly restored the activity of the enzyme. The monoammonium salt of glycyrrhizic acid, the flavonoid quercetin, and the reference drug STD also significantly increased the activity of the enzyme, but compared to them, the effect of MASGA/quercetin was higher.

Thus, the results of the conducted studies show that intoxication with paracetamol leads to inhibition of the enzymes of the mitochondrial respiratory chain. It is likely that the observed inhibition of enzyme activity by hepatotoxin is associated not only with a violation of mitochondrial oxidation and inhibition of conjugated

phosphorylation, but also with the direct effect of paracetamol on the structure of respiratory chain enzymes. The compounds used had a stabilizing effect on enzymatic activities.
As is known, many flavonoids and their supramolecular complexes of natural and artificial origin are used in medical practice in the complex therapy of acute and chronic hepatitis, liver cirrhosis. One of the mechanisms of action of these compounds, in addition to the antioxidant effect, is the ability to restore the function of the respiratory chain and associated phosphorylation [3; 14]. Possibly, polyphenolic compounds are involved in the transfer of electrons along the respiratory chain. Possessing high antiradical activity, they prevent the development of free radical oxidation reactions and the formation of lipid peroxides and have the ability to bypass electron transport in the respiratory chain of mitochondria. Quercetin and its supramolecular complex with the monoammonium salt of glycyrrhizic acid, effective in our experiments, apparently cause a similar effect on the polyenzymatic complex of liver mitochondria. They have possibly the optimal redox potential and also have conformational accessibility for interaction with inhibited respiratory enzymes by performing both one- and two-electron transfer.

4. Conclusion

The results of the studies indicate that in rats with experimental toxic hepatitis caused by paracetamol in mitochondria, oxidative stress develops, accompanied by an increase in the lipid peroxidation process, and contributes to the inhibition of respiration and the process of oxidative phosphorylation. The administration of quercetin, MASGA, and their supramolecular complex to hepatitis animals caused to positive changes in the studied parameters, and the MASGA/quercetin complex at a dose of 25 mg/kg had the maximum stabilizing effect on mitochondria. Thus, the assessment of the role of mitochondrial dysfunction provides a deeper understanding of the pathogenesis of liver diseases and can serve as the basis for the development of a rational diagnostic and therapeutic strategy.

5. List used literature

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