

Hepatoprotective Action of Phytopreparation from *Limonium gmelinii*

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Abstract

Effects of phytopreparation from a plant of *Limonium gmelinii* were studied on livers of white breedless rats at chronic impact of cadmium sulfate and carbon tetrachloride. It was established the administration of cadmium sulfate during six months caused chronic hepatitis, while the simultaneous administration of preparation from *Limonium gmelinii* and cadmium sulfate did not result of destructive changes in a liver. Chronic intoxication of the animals by carbon tetrachloride during six months caused progressive postnecrotic cirrhosis. The simultaneous administration of phytopreparation and carbon tetrachloride caused postnecrotic fibrosis in the central part of hepatic lobule only. Not only dystrophic and necrobiotic processes also numerous inflammatory cell infiltrates were observed in hepatic parenchyma of animals which were treated by cadmium sulfate and carbon tetrachloride, while it was not observed at animals which were medicated with phytopreparation. Thus, on the basis of the data was concluded about hepatoprotective action of phytopreparation from *Limonium gmelinii*.

Introduction

Industrial pollution of an environment, wide application of chemicals and drugs has resulted to increase number of chronic liver diseases are caused by xenobiotics [1]. There are about 80000 of the hepatotoxic agents now, to which a person contacts during all life [2]. Therefore, the development of drugs having hepatoprotective properties is urgent. There are such hepatoprotectors in clinical practice now:

- 1) antioxidants;
- 2) inhibitors of microsomal enzymes that reduce the injuring metabolites formation;
- 3) the preparations which recover membranous;
- 4) inductors of metabolic processes raising regenerative potential of hepatic parenchyma [3].

The majority of hepatoprotective drugs are import expensive and inaccessible for the population. The development of effective domestic medicines is carried out very insufficiently. However rich and various floras of Kazakhstan which numbers more than 100 medicinal plants, can be a source of accessible and cheap raw material for manufacture of the vital medicinal preparations with hepatoprotective action. For introduction in medical practice the most perspective plants

are selected in view of their bioactivity, resources in the territory of Kazakhstan, conditions of cultivation, complexity of technological reception of phytopreparations on their basis. Results of regular and multiplane research have shown such valuable medicinal plant is *Limonium gmelinii* [4-6].

Phytopreparation from *Limonium gmelinii* is crystalline substance of brown color with bitter taste, good soluble in water and water- alcohol solutions obtained by the water- acetone extraction with consequent concentration of solution in soft conditions. Its biologically active complex is presented by flavonoids of oxidated and reduced types (7-14%), tannins (40-60%), aminoacids (2-3%), carbohydrates (1-2%), vitamin C, microelements (0.59%), carotinoids, highest carboxylic acids. The main monomeric flavane is (-) -epigallocatechingallate. Flavonoids of oxidated type are represented by isoramnetin and also by quercetin, myricetin, their mono- and diglucosides (myricitrin, galactopyranozides of quercetin and myricetin, rhamnoglucoside of myricetin, rutin). 2-o- β -D-galloil and 2,3-o- β -D-digalloilglucose were isolated from hydrolised tannins.

Monosaccharides are represented by glucose, rhamnose, the disaccharide by rutinose, oligosaccharides consist of residual groups of xylose and galactose. Gallic, lilac, isovanilline phenol acids are identi-

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fied. Phytopreparate contains all known 20 natural α -aminoacids, but among them proline, tyrosine, tryptophane, cystein and lysine are dominating.

Thus, the biological active compounds of the preparation are polyphenols, which, as it is known, have antioxidant action. [2,3,7-9]. It is known, in a basis of toxic damage of a liver the activation of lipid peroxide oxidation processes (LPO) lays. The increase of the primary and secondary LPO product contents and decrease of antioxidant ferments activity characterize these processes [10]. Therefore in the present research the action of phytopreparation from *Limonium gmelinii* at experimental chronic toxic damage of a liver was studied.

Experimental

Experiments for the *Limonium* preparation influence were conducted on livers of white breedless rats at chronic intoxication of cadmium sulfate and carbon tetrachloride. Sixty rats of reproductive age were divided into six experimental groups till ten animals. In the first group animals received 15 mg/kg cadmium sulfate (CdSO_4) with food daily within 6 months. In the second groups the animals received CdSO_4 and 2 ml of 2% water solution of the phytopreparation daily during the same time. This concentration of phytopreparation (2% solution) was the most effective and was detected in experiments. It was established that low concentration (1% solution) did not have any medicine effects. The more high concentration caused the digestive disease (snap). In the third group the rats inhaled carbon tetrachloride (CCl_4) three times a week for six months. In the fourth group the rats received CCl_4 three times a week and 2 ml perorale of 2% water solution of the phytopreparation daily. In the fifth group the rats received only a 2%-solution of the phytopreparation peroral the same time. The sixth group was made up of intact rats.

After six months the animals were decapitated and samples of the liver were fixed in 10% buffered formaldehyde solution for light microscopic examination. The fixed samples of the liver were dehydrated in a graded series of ethanol and embedded in paraffin. Section 6 μm thick were stained with hematoxylin and eosin. The histological preparations were examined and photographed by light microscope MBI-15.

The results of our morphological research do not need by statistical analysis. We observed the same structural changes in each of ten rats in six experimental groups.

Results and discussion

Analysis of the histological preparations has shown that the rats in both groups (intact and those which received the phytopreparation) had not revealed any pathological changes in the liver (Figs. 1,2).

In comparison with control rats and animals received only phytopreparation, in hepatic parenchyma of CdSO_4 -administrated animals were observed such pathologies like interstitial oedema, sharp expansion of sinusoids, activation of reticuloendotelial system cells, extensive necrosis of hepatocytes, numerous inflammatory cell infiltrates (Fig. 3).

In hepatic parenchyma of animals, received both cadmium sulfate and phytopreparation the hydropic dystrophy of individual hepatocytes and insignificant expansion of sinusoids were observed. We were not observing necrosis of hepatocytes and inflammatory infiltrates at rats of this group (Fig. 4). This fact testifies to positive action of phytopreparation at cadmium sulfate administration. Probably, orto-disposed phenol hydroxyl groups of polyphenols which include in the structure of phytopreparation, form the complex connections with cadmium ions and neutralize toxic action. On the other hand, as it was shown

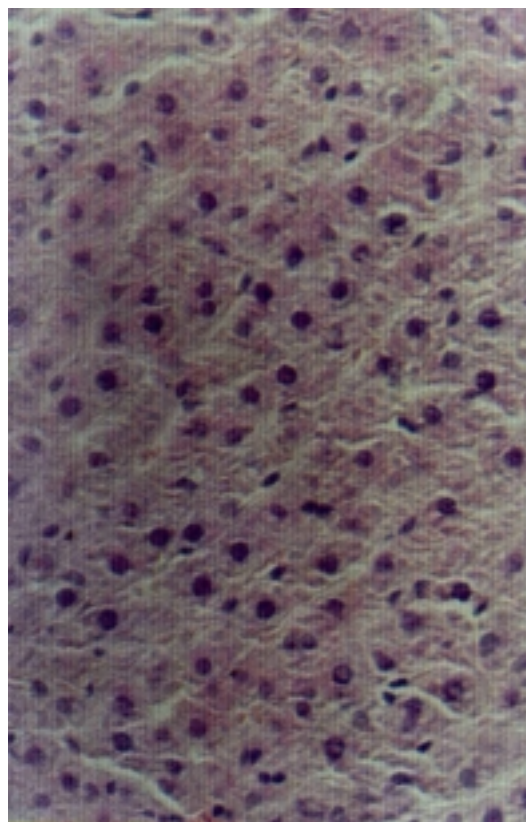


Fig. 1. Liver of a control animals.

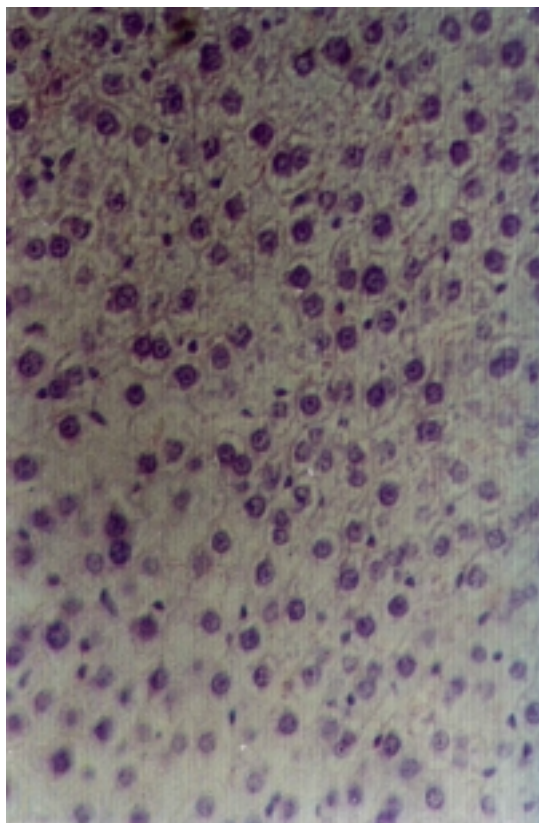


Fig. 2. Liver of a phytopreparation medicated rats.

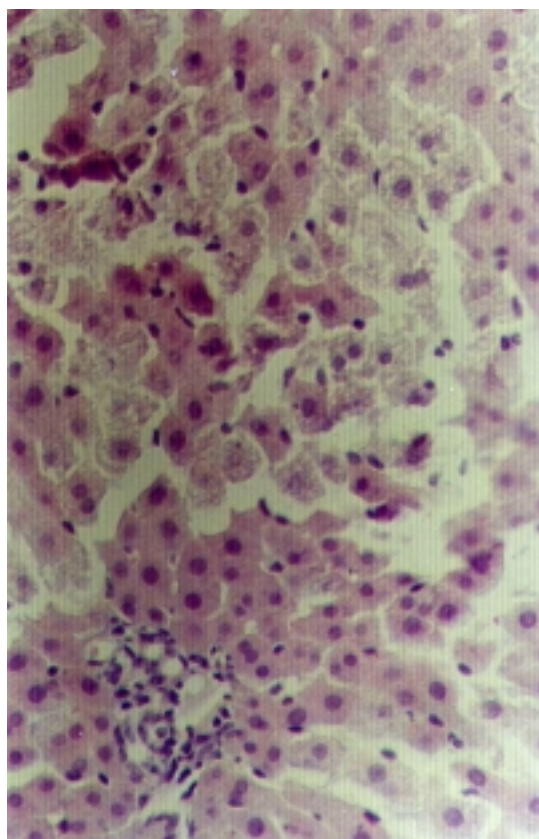


Fig. 3. Liver of a cadmium sulfate administrated rats.

in our previous researches, the hepatotoxic action of cadmium sulfate is connected to activation of a LPO processes and oppression of antioxidant system enzymes [10]. It is possible the hepatoprotector action of researched phytopreparation connects with antioxidant properties of polyphenols include in its structure.

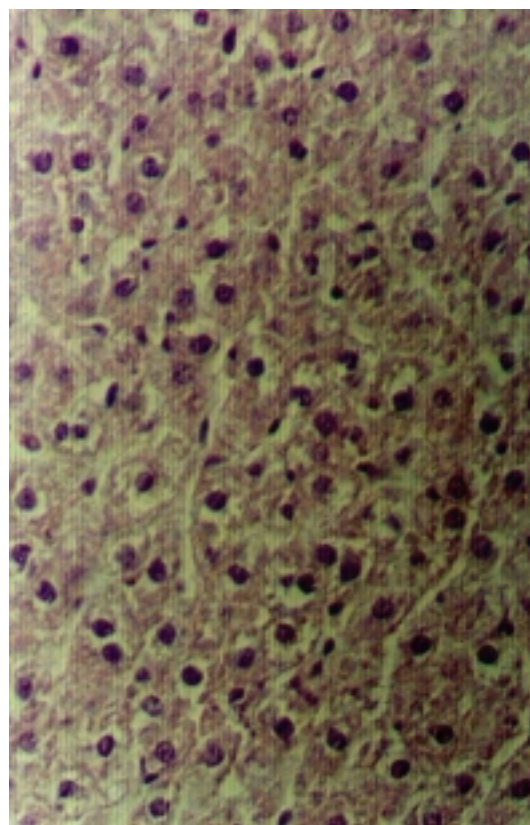


Fig. 4. Liver of rats administrated both cadmium sulfate and phytopreparation.

Chronic intoxication of the animals by CCl_4 caused progressive postnecrotic liver cirrhosis. Along with dystrophic and necrobiotic processes with prevailing fat degeneration of centerlobular hepatocytes and local colliquation necrosis, fibrous transformations were observed. There were widely revealed in the liver parenchyma the tissue-joining septa, both inside the liver lobules and on its periphery. Thus, so-called false lobules formed in the liver parenchyma of the rats intoxicated with CCl_4 for six months. The destructive processes were accompanied by chronic inflammation; infiltrates presented by polymorphous-nucleus leucocytes, lymphocytes and macrophages localized around the necrosis sites (Fig. 5).

At animal from the thought experimental group after 6 months developed postnecrotic fibrosis of a

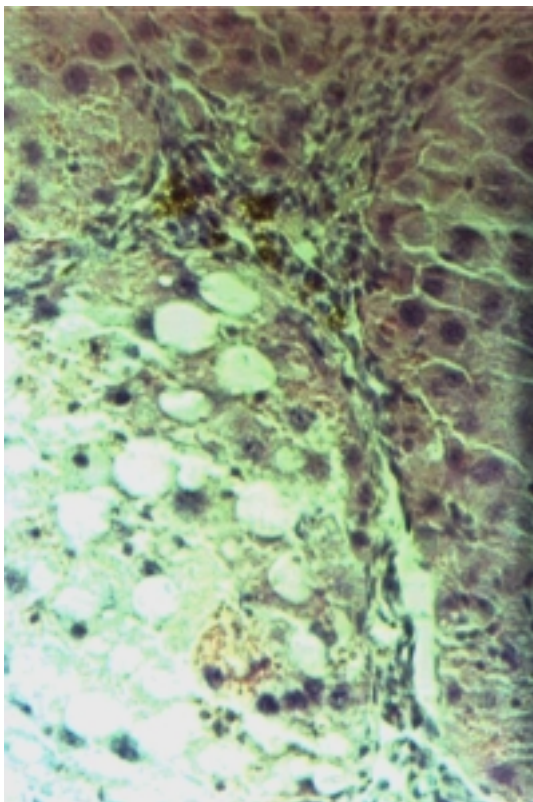


Fig. 5. Liver of a carbon tetrachloride intoxicated rats.

liver. There were no false lobules; the fibrosis was actual in the central part of the liver lobule only. The hepatocytes were mostly subjected to hydropic dystrophy, and the fat degeneration of liver was not observed. Moreover, in comparison with the rats from the third experimental group, these rats did not have inflammation-cell infiltrates, which demonstrates anti-inflammation action of the investigated preparation. In the degeneration nodes there were observed hypertrophied hepatocytes with large hyperchromic nuclei, which demonstrated more active reparation processes in the animals' liver that received the phytopreparation (Fig. 6).

As it is known, the mechanism of toxic action carbon tetrachloride is connected with formation of a free radical CCl_3 in system of cytochrome P-450. The CCl_3 radicals interact with endogene lipids, first of all by fat acids of bio membranous and cause a reaction of peroxide oxidation [11]. The modern hepatology is proved that a determinative factor of functional activity of hepatocytes membranes is the condition of lipid peroxide oxidation process (LPO). An exhaustion of mechanisms the LPO regulation conducts to destruction of membranous, complete loss of specific functions and its lysis [7]. Apparently, pathological changes in rats' liver at chronic inhalation

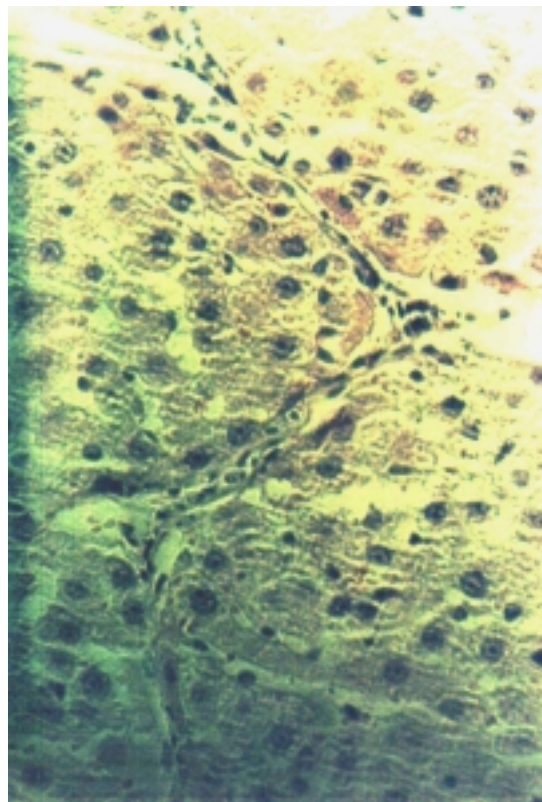


Fig. 6. Liver of a carbon tetrachloride and phytopreparation administrated rats.

influence of CCl_4 also are connected with activation of LPO processes. Absence of cirrhotic changes at rats of the fourth group are treated both CCl_4 and phytopreparation from *Limonium gmelinii*, probably, are connected with its antioxidant properties, as it is established, that one of components of a preparation are flavonoids which concern to a class of polyphenols [6]. As it is known, polyphenols directly accept free electrons and protons and extinguish a free radical stage of a LPO process [8].

Maybe the absence of inflammatory cell infiltrates in a liver of phytopreparation-medicated rats is connected with anti inflammatory action of included in its structure polyphenols [9].

Conclusion

It has been demonstrated that cadmium sulfate causes chronic toxic hepatitis after chronic administration for six months, while the simultaneous influence of phytopreparation from *Limonium gmelinii* and CdSO_4 does not result to destructive changes in a liver. Chronic intoxication of the animals by CCl_4 after six months caused progressive postnecrotic cirrhosis. The simultaneous reception of

phytopreparation and CCl_4 results in development postnecrotic fibrosis in the central part of the liver lobule only. In this case, as other authors have shown, changes can have convertible character if the influence of pathogenetic factor will be cancel [12]. Apparently, the long influence of phytopreparation will promote this process. Besides the destructive processes in liver of cadmium sulfate and carbon tetrachloride intoxicated animals were accompanied by chronic inflammation. While it was not observed at animals were medicated with phytopreparation. Thus, on the basis of the received data it is possible to conclude, that phytopreparation from *Limonium gmelinii* has hepatoprotective action, that is apparently connects with anti oxidative and anti inflammatory properties of components, included in its structure.

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