Characteristic Properties of «Enoant» Bioflavonoids in Alcoholic Liver

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Abstract

At present, the question of the effectiveness of the use of bioflavonoids in the treatment of the alcoholic liver disease is of practical interest and attention from modern medicine. Aim of our research is to analyse the antioxidant properties of Enoant in alcoholic liver and to establish the ultra morphostructural changes in liver tissue. Materials and methods - assessment of changes in alcohol-impaired rat liver were studied before and after administration of Enoant bioflavonoid concentrate. Results - The positive effect of “Enoant” is explained by the antioxidant properties of bioflavonoids, their ability to reduce peroxidation of proteins, stimulate reparative processes, activate protein synthesis and enhance the system of antioxidant protection of hepatocytes. All this allows to attribute the concentrate of bioflavonoids “Enoant” to highly active hepatoprotectors and is of practical interest at all stages of treatment of patients with different stages of liver lesions.

Keywords: Alcoholic liver damage, Liver tissue morphology, Concentrate of bioflavonoids “Enoant”, Antioxidant properties of bioflavonoids, Toxicology, Liver pathology.

Introduction

At this time, according to WHO, there is an increase in the number of liver diseases. Alcohol is one of the etiological factors that cause these diseases. Depending on the dose and duration of its use, various forms of damage occurs from alcoholic fatty infiltration to alcoholic cirrhosis [1, 4, 6, 7]. The basis of liver damage by alcohol is the activation of free-radical processes [8, 9, 10]. Medicines used in the complex therapy of these diseases have more than 1000 names. Among them are drugs that increase the resistance of the liver to pathogenic factors and restore its functional activity [5, 11]. Biological activity of grape processing products which are rich in polyphenols as well as their benefits of the human health are well known and worth of studying. It was first shown in Toulouse, France, that cardiovascular mortality stayed the lowest in Europe despite the high level of dietary saturated fats. It was named “a French paradox” [12]. It was proved later that the paradox is caused by the cardioprotective action of polyphenols in red wines that are the traditional beverages of an average Frenchman. Polyphenols are supposed to act as antioxidants that neutralize free radicals, decrease oxidative enzymes’ activity and reduce peroxide lipids’ levels in blood serum [13]. One of the richest sources of polyphenols is Vitis vinifera, and products of its processing, including flavonoids and other polyphenols of grape, wine, and grape seeds, are of a great interest due to their antioxidant properties and the ability to scavenge free radicals [14].

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Substances of plant origin, in particular, concentrate of polyphenols of grapes “Enoant”, are quite promising for clinical hepatology. Monomeric grape polyphenols have a pronounced antioxidant activity. In the concentrate of “Enoant”, it mainly consists of components of flavonoids. Their therapeutic effect is due to the ability to bind with free radicals, that stimulate the synthesis of proteins and macro-energies, which facilitates the course of biochemical reactions.

Evaluation of hepatoprotective efficacy and experimental justification for the use of bioflavonoids concentrate “Enoant” for the treatment of liver diseases is a very important issue to be discussed.

**Purpose of the Study**

Aim of our research is to analyse the antioxidant properties of Enoant in alcoholic liver and to establish the ultra morphostructural changes in liver tissue.

**Material and Method**

The model of alcoholic liver damage in rats was used in the research. The nature and extent of alcoholic damage to the liver was studied morphologically, including electron and light microscopy.

The experiments were performed on 60 non-linear white rats (mean weight 200-230 g), divided into 3 series of 20 animals each. 1st series - intact animals (control). Animals of the 2nd series were injected into the stomach with 40% ethanol for a period of 90 days at a rate of 0.016 ml of 40% ethanol per 1 g of body weight [2]. To the animals of the 3rd series, after a 90-day alcohol intoxication, the probe was injected for 21 days with a concentrate of bioflavonoids “Enoant” at a dose of 0.25 ml per kilogram of weight [3].

**Results and Discussion**

As a result of morphological examination, by the method of light microscopy, the characteristic signs of alcoholic injuries were revealed:

- dyscircularty (hyperemia of sinusoids and central venules);
- alternative, in the form of fatty and hydropic dystrophy of hepatocytes;
- Mononuclear infiltrates of portal tracts and periportal area.

Electron microscopy of hepatocytes, endothelial cells and stellate reticuloendotheliocytes in animals treated with ethanol for 90 days revealed a number of dystrophic changes.

The nuclear membrane of hepatocytes had foci of loosening and invagination, causing the contours to lose clarity. In places, the nuclear membrane was destroyed. Chromatin is condensed unevenly and dispersed as fragments throughout the nucleus area. The perinuclear space is greatly enlarged. Tanks of the granular endoplasmic reticulum were expanded and had the appearance of electron-transparent vacuoles. Hepatocyte mitochondria had edema and a rounded shape, with an electron-transparent matrix. The destruction centers of the outer membrane of the mitochondria and their cross were determined (Fig. 1). The number of ribosomes on the membranes of the granular endoplasmic reticulum was significantly reduced compared to the control group.

![Fig. 1. Round-shaped mitochondria with swollen and clarified matrix, cells of destruction of the cross and outer membrane of the mitochondria. Electronic micrograph. Magnification x 50,000.](image)

In most of the hepatocytes there was a pronounced loosening of the cytosol due to the disappearance of glycogen granules and intracellular edema, which was accompanied by a pronounced decrease in the electron-optical density of the cytoplasm (Fig. 2). In the cytoplasm, many transparent vacuoles were noted.

The cytoplasm of endothelial cells was electron-transparent, with frequent lipid inclusions. The nuclei of the endothelial cells became elongated, with deep invaginations of the nuclear membrane. Karyorrhexis was observed.

The cytoplasmic membrane, facing the sinusoid lumen, was partially destroyed and loosened. The lumen of the sinusoids revealed destructively altered
fragments of cell membranes and a structured substance of uneven electron density. There was a sharp swelling of mitochondria, some mitochondria had ruptured membranes and crosses. The cytoplasmic processes of the endothelial cells contained virtually no micropinocytosis vacuoles.

Fig. 2. Cytosol loosening in the cytoplasm of hepatocytes. Perinuclear edema. Electronic micrograph. Magnification x 2000.

Dystrophic changes in organelles were in the cytoplasm of stellar reticuloendothelial cells. The mitochondria swelled, their matrix became electron-transparent, the number of crosses decreased. The endoplasmic reticulum looked like transparent vacuoles. The Golgi lamellar cytoplasmic complex was moderately reduced. In Electron microscope, the experiments of this series, marked sharply expressed plethora of sinusoids, erythrocyte sludge, enlargement of intra acinar bile ducts and ducts.

To study the therapeutic effect of concentrate bioflavonoids “Enoant”, after 90 days of alcohol intoxication, on the background of complete alcohol deprivation, the experimental animals were administered concentrate for 21 days (3 series). In this series of experiments it was found that the trabecular structure of the liver tissue was preserved. Sinusoids were occasionally enlarged only in single preparations, the boundaries of the sinusoids were clear, the phenomena of hyperemia were absent. The dyscirculatory changes consisted only of the plethora of the interosseous veins, but not of the sinusoids. Vacualization of hepatocytes was extremely rare (Fig. 3).

Fig. 3. Minimal dystrophic changes in the liver after the use of concentrate “Enoant” on the background of previous alcohol intoxication. Painting hem-eos. Mag. x 200.

There were no alterative changes in most micropreparations. Dystrophic changes were limited to separate centers of evacuation. Hepatocytes with large nuclei and fine-grained chromatin prevailed. Duplicate hepatocytes were encountered around large portal tracts, indicating an increase in liver regenerative potency. There were many hepatocytes with hyperchromic nuclei and homogeneous light eosinophilic cytoplasm (without evidence of hydropic dystrophy). A lively histiolymphocytic response was observed in the portal tracts and the proliferation of sinusoidal cells was well expressed (Fig. 4).

Fig. 4. Proliferation of sinusoidal cells. Hematoxylin-eosin staining. Magnification x 200. [vol. 20, approx. 10]

At the ultrastructural level, the state of the cell organelles did not differ from the control: the hepatocyte nuclei were rounded, centered or eccentric. The outer and inner membranes of the nuclei were clearly observed. Chromatin was evenly distributed in the nucleoplasm. The mitochondria had a typical structure, the crosses were tightly packed. Tanks of the granular endoplasmic reticulum were slightly expanded (Fig. 5).
In the cytoplasm of hepatocytes contained many membrane-bound and free ribosomes. Submicroscopic organization of endothelial cells was not different from the norm. Activation of intracellular metabolic processes was noted in stellar macrophages. This was manifested in the moderate expansion of the granular endoplasmic reticulum tanks and the Golgi hypertrophy.

**Conclusion**

The enteral use of concentrate of bioflavonoids “Enoant” immediately after the cessation of alcoholization, reduces the damage from alcohol. After the application of “Enoant” was observed complete restoration of trabecular structure of the liver. Around the large portal tracts, in some cases, double-core hepatocytes appeared, as well as hepatocytes with hyperchromic nuclei and homogeneous light eosinophilic cytoplasm, with no evidence of hydropic dystrophy. This indicates an increase in the reparative capacity of the liver tissue.

The positive effect of “Enoant” is explained by the antioxidant properties of bioflavonoids, their ability to reduce peroxidation of proteins, stimulate reparative processes, activate protein synthesis and enhance the system of antioxidant protection of hepatocytes. All this allows to attribute the concentrate of bioflavonoids “Enoant” to highly active hepatoprotectors and is of practical interest at all stages of treatment of patients with different stages of liver lesions.

**Conflict of Interests:** None declared.

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**Ethical Clearance:** In our study involving all human participants were in accordance with ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964 and later amendments.

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