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EFFECT OF APPLE PECTIN ON THE HISTROSTRUCTURE OF THE LIVER UNDER ACUTE AND CHRONIC ALCOHOL INTOXICATION

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Abstract

Alcohol is perhaps the oldest of all known psycho-active substances, which is widely used by the population of the globe in large quantities. Treatment of acute poisoning in all countries is carried out according to standardized protocols of medical care, which, depending on the stage and severity of intoxication, include drugs of different pharmacological groups: plasma substitutes, tranquilizers, opiate receptor antagonists, drugs that accelerate alcohol metabolism and its metabolism, enterosorbents.

The global nature of the problem of treatment of alcohol poisoning, pharmacological properties (including sorption and prebiotic), virtually no toxicity and side effects, the ability to form a gel-like protective layer between the contents and mucous membranes of the gastrointestinal tract and the potential of domestic production of apple pectin.

The aim of the research was - experimental study of the effect of pectin on the histrostructure of the liver in acute and chronic alcohol intoxication.

Morphological researches of liver tissue in acute and subchronic alcohol intoxication confirm the detoxifying and organoprotective effect of apple pectin, as its use in the treatment regimen reduces the intensity of dystrophic changes and regression of fatty liver, restores reparative changes in polycystic ovary disease.

Keywords: *apple pectin, acute alcohol intoxication, chronic alcohol intoxication, liver*

Introduction Alcohol is perhaps the oldest of all known psycho-active substances, which is widely used by the population of the globe in large quantities. Ukraine is no exception. According to the WHO in 2016, the average consumption of pure alcohol per capita in the world was 6.4 liters, in Europe - 11.2 liters, and in Ukraine - 13.8 liters. Mortality from acute poisoning and alcohol-related diseases (cirrhosis of the liver, cancer and injuries) in 2016 according to world statistics was 3 million people, including in Ukraine - more than 18,500. young people from 20 to 34 years. Acute alcohol poisoning occurs infrequently and is mainly caused by surrogate alcohol. Chronic poisoning, alcohol dependence or alcoholism are the main causes of diseases such as alcoholic liver disease, liver cirrhosis and cancer [1]. In addition, prolonged alcohol consumption significantly alters the metabolism of proteins, carbohydrates and lipids, which leads to the development of metabolic syndrome, complications of diabetes, diseases of the cardiovascular, nervous, digestive systems. [2].

Treatment of acute poisoning in all countries is carried out according to standardized protocols of medical care, which, depending on the stage and severity of intoxication, include drugs of different pharmacological groups: plasma substitutes, tranquilizers, opiate receptor antagonists, drugs that accelerate alcohol metabolism and its metabolism, enterosorbents.

In chronic alcoholism, treatment is aimed at two ways: first, to reduce cravings for alcohol, and secondly, to correct disorders of the nervous, cardiovascular, hepato-biliary, endocrine systems [3]. Enterosorbents are indicated for both acute and chronic poisoning. According to the ATC classification - this group A07BC - intestinal adsorbents, which include pectin.

Pectin - heteropolysaccharide of plant origin, which has the properties of a sorbent and prebiotic [4]. Pectins are used as enterosorbents for intoxication with heavy metal salts [5], influence of ionizing radiation [6], intestinal infections [7]. The daily requirement of pectin in the diet of an adult is 5–6 g. [8]. Dosage of pectin for these indications, according to the literature, is for adults 12-15 g a day.

Pectin is highly effective due to the large area of the active surface and removes toxins, heavy metal ions from the body; is safe, without traumatic effect on the mucous membrane of the gastrointestinal tract (GIT) in contrast to activated charcoal; does not cause constipation, does not affect the intestinal microbiocenosis and has prebiotic properties.

However, in the available literature there are no data on systemic experimental and clinical studies of the use of apple pectin powder as a sorbent for alcohol intoxication. The raw material base for the production of pectin in Ukraine is sufficient for the production of 5 thousand tons per year, which is planned after the opening of the plant of the group of companies T.V. Fruit in Gorodok, Lviv region in 2020.

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It is known that the pathogenetic causes of toxic effects of alcohol and its metabolites on the liver and the development of alcoholic liver disease are the accumulation of acetaldehyde and induction due to oxidative stress, the formation of stable complexes with proteins - acetaldehyde adducts, oxidation of membrane phospholipids, peroxide increase in the synthesis of lactate, acetate and the development of lactic acidosis, disorders of lipid metabolism, hypoxia, increased collagenogenesis, fibrogenesis, carcinogenesis. Acetaldehyde adducts have antigenic properties and cause the formation of antibodies. Prolonged chronic alcohol intoxication leads to irreversible changes in the liver parenchyma and may be complicated by liver fibrosis and cirrhosis and / or transform into cancer [9, 10].

Histological changes in alcoholic liver disease are accompanied by hydropic dystrophy, the formation of Mallory cells, the development of edema and centrilobular necrosis of hepatocytes, followed by infiltration of polymorphonuclear leukocytes and lymphocytes. Increased activity free radical oxidation and increase production reactive oxygen species lead to system suppression antioxidant protection and accumulation thiobarbituric acid - active products that enhance immune inflammation

in the liver parenchyma and contribute to the development of steatohepatosis [11, 12, 13].

The aim of the research was - experimental study of the effect of pectin on the histrostructure of the liver in acute and chronic alcohol intoxication.

Materials and methods.

In the first stage, acute alcohol intoxication was simulated on 65 white nonlinear rats of both sexes weighing (180-200) g, which were kept on a standard diet and with unrestricted access to water. Animals were injected with a 40% solution of ethanol in the stomach using a probe with oil at the rate of 2 ml/ 100 g body weight for 3 days [14]. Apple pectin powder was used 30 min after the introduction of ethanol in the amount of 200 mg / 100 g body weight, and the comparison drugs - activated carbon and silicon dioxide 0.25 and 0.05 g, respectively, dose calculations were performed according to the method [15]. Euthanasia of animals was performed for 3 days.

In the second stage, chronic alcohol intoxication was reproduced. The study was performed on 35 white early-bred rats (males) weighing (180–220) g, which were kept on a standard diet of vivarium with free access to water and food. Experimental animals were injected with ethanol (30%) in the stomach for 28 days after 1 hour. after feeding with a probe with oil at the rate of 2 ml / 100 g of body weight 1 time per day according to standard methods [16, 17, 18]. Apple pectin powder and reference drugs were administered in the same manner as described above. Euthanasia of animals was performed on the 28th day of the experiment.

Euthanasia of animals in the model of acute and chronic alcohol intoxication was carried out by introducing a solution of sodium thiopental at a rate of 40 mg / kg body weight [19]. Pieces of rat liver were used as material for histological examination. Preparations were performed to gain access and prepare the organ to take histological material.

For general histological examination, the liver was fixed in a 10% solution of neutral formalin (Ph-7,0). The fixation time was 24 hours. Subsequently, pieces of liver were placed in an ascending battery of alcohols for dehydration, then in chloroform, a mixture of chloroform-paraffin (1:1), paraffin (at temperature 37℃). After paraffin preparation, the pieces were poured into paraffin. The production of serial paraffin sections with a thickness of 4-6 μm was performed on a sled microtome.

Histological examinations were performed under a light optical microscope Leica DME (Germany) on histological specimens stained with hematoxylin and eosin, with the comprehension of perivasal, periportal and intermediate lobes.

In order to objectify quantitative studies, computer morphometry of objects in histological specimens was performed. In the first stage, digital copies of the optical image of areas of microscopic specimens were obtained using a digital camera Nikon Coolpix 4500 (Japan) when using different microscope lenses (×4, ×10, ×20, ×40, ×100). Digital copies of the images were then analyzed using a computer program Image Tool 3,0 for Windows (free license).

The calculation of morphometric parameters was performed at least in 10 digital copies (lens magnification ×40) optical image of areas of microscopic preparations in each animal.

Morphometric analysis of the liver was performed taking into account the following indicators:

1.The share of parenchyma in percentage.

2. The number of mononuclear normal cells per 100 hepatocytes.

3. The number of dinuclear normal cells per 100 hepatocytes.

4. The number of dystrophically altered cells per 100 hepatocytes.

5. The number of necrotic-apoptically altered cells per 100 hepatocytes.

6. The average area of the hepatocyte

7. The middle perimeter of the hepatocyte

8. The average area of the nucleus of the hepatocyte

9. The middle perimeter of the hepatocyte nucleus

10. Nuclear cytoplasmic index.

11. The share of sinusoidal capillaries in percentage.

12. The share of sinusoidal capillaries without blood in percentage.

13. Percentage of sinusoidal capillaries with blood.

14. The proportion of connective tissue in percentage.

For a semi-quantitative assessment of lipid content used a five-point scale: 1 degree (minimum)

- hepatocytes with fatty inclusions are only on the periphery of the lobe in the triad, 2 degree (weak) - hepatocytes containing lipids occupy approximately 1/4 – 1/3 length of hepatic plates in the periportal zone, grade 3 (moderate) - such hepatocytes occupy 1/3 – 1/4 length of hepatic plates on the periphery of the lobe, 4 degree (high) - hepatocytes with fat droplets occupy 1/2 – 2/3 length of hepatic plates, 5 degree (maximum) - hepatocytes with fatty inclusions are in all lobe.

Semi-quantitative assessment of the intensity of the necrotic-apoptotic process conducted according to a five-point system, according to which: 1 point - weak changes, 2 points - medium changes, 3 points - clear changes and 4 points - changes are very pronounced.

Statistical analysis of the results was performed using computer programs Microsoft Exel using the methods of variation statistics. The arithmetic mean value was determined (M), standard error (m), Student's criterion (t), probability factor (p). Values were taken as probable $p<0,05$.

Results and Discussion

Under conditions of acute alcohol intoxication, the trabecular radial structural organization of the liver is disrupted mainly due to dystrophic changes of hepatocytes in different parts of the classical liver lobes: both in the peripheral and intermediate and central zones. (fig. 1, fig. 2).

The severity of fatty degeneration was 4.3 when the lipid content was quantified on a five-point scale. The metric size of hepatocytes is increased: the average area of a liver cell makes 412,47±36,32 mkm2 (in the control group – 325,73±22,18 mkm2), middle perimeter – 71,98±4,87 mkm (control group – 63,96±3,41 mkm). Part of the parenchyma was 92,58±7,38 % (control group – 84,49±5,93 %). The increase in cell size is mainly due to the accumulation of transparent fat vacuoles in their cytoplasm - from small size with the development of microvesicular dystrophy to vacuoles of medium caliber (medium vacuolar dystrophy) and large vacuoles (large vacuolar dystrophy). In large-scale fatty dystrophy, the nuclei were displaced to the plasmolemma, and the cytoplasm was visualized optically empty with a narrow eosinophilic rim when stained with hematoxylin and eosin. The contours of dystrophically altered hepatocytes of irregular polygonal shape, fuzzy, veiled, especially in moderate and large-scale dystrophy.

The majority of hepatic parenchymal cells were mononuclear normal cells (61.25 cells per 100 hepatocytes), but their number was significantly lower than in the control group (82.47%). The proportion of dystrophically altered cells in acute ethanol intoxication averaged 21.1 cells per 100 hepatocytes (comparison group - 4.5%). In some experimental animals, fatty degeneration of liver cells reached 74%, dramatically disrupting the trabecular structure of the organ and causing compression of the surrounding sinusoidal hemocapillaries (fig. 3).

The nuclei of hepatocytes are mostly round, medium area 71,59±5,22 mkm2 (in the control group, the average area of the hepatocyte nucleus was 52,12±3,24 mkm2), with an average perimeter 32,84±2,53 mkm (control group – 25,59±1,19 mkm). When calculating the nuclear cytoplasmic index, it was found that this indicator is 0.17 (control group - 0.16). The contours of the nuclei are mostly clear. Heterogeneous chromatin: light areas of euchromatin with dark hematoxylin lumps of heterochromatin. In most nuclei of liver cells, small rounded nucleoli were visualized.

In the group of untreated rats, the number of dinuclear hepatocytes was sharply reduced - 4.9 dinuclear cells per 100 liver cells (intact control group - dinuclear cells were 12.31%). At the same time, in acute ethanol intoxication, the number of hepatocytes with irreversible alternative changes increased sharply - 12.75 cells per 100 hepatocytes (control group - 0.72% of cells with irreversible degenerative changes) (fig. 4).

Most of these cells with signs of necrosis, in which there was a granular eosinophilic cytoplasm, often with vacuoles of different sizes in it, sharply blurred contours of plasmolemma, irregular cell boundaries, nuclei mostly veiled, pale purple, in part of the cells in the nucleus sizes with slightly compacted chromatin.

In cases of karyolysis, rexis of the cytoplasm of cells with its disintegration into separate eosinophilic lumps was also noted. In single hepatocytes, the size of which is slightly reduced, the cytoplasm is saturated eosinophilic, mostly

homogeneous, the nuclei are also slightly reduced and with homogeneous chromatin, with clear contours of the karyolema. Plasmorexis was also observed in single such cells with the formation of homogeneous eosinophilic apoptotic cells.

The lumens of sinusoidal hemocapillaries are mostly narrowed by enlarged dystrophically altered liver cells. In acute ethanol intoxication, the proportion of sinusoidal hemocapillaries was 3,18±0,17 % against 12,31±0,9% in the group of intact animals. Bloodless capillaries predominated, their share – 2,26±0,17 % against 0,92±0,05 % capillaries with blood in the lumen (control group – 9,48±0,36 % and 2,83±0,19 %). However, the analysis of the percentage showed an increase in acute ethanol intoxication the number of capillaries that are filled with erythrocytes – 29 % against 23 % in the comparison group and, accordingly, the reduction in acute ethanol intoxication of the number of capillaries that are not filled with erythrocytes – 71 % against 77 % control group. In acute ethanol intoxication, single lymphocytes and macrophages were also observed in the lumen of sinusoidal hemocapillaries.

In several cases, the dominance of exudative changes over alternative ones was noted, which was accompanied by leukocyte-macrophage infiltration both in the portal tracts and around the central veins (fig. 5), as well as by visualization of leukocyte groups in the lumen of sinusoidal hemocapillaries with individual signs. Degenerative changes of hepatocytes were less pronounced.

Connective tissue of the portal tracts with signs of edema, accompanied by defibering of connective tissue fibers and an increase in its percentage to 2,53±0,17 % (control group –1,19±0,09 %). Vascular wall with signs of plasma permeation and in some - with fibrinoid necrosis, which is manifested by homogenization of the wall with its rich eosinophilic color (pic.5). In portal tracts, especially at fibrinoid changes of a vascular wall, along with hypostasis lymphocytic and macrophage infiltration with single neutrophilic leukocytes, with an exit of single cells outside of a boundary plate is noted. (fig. 5, fig. 6).

The use of apple pectin in acute ethanol intoxication was accompanied by regression of degenerative changes in liver cells with a marked restoration of the trabecular structure of the organ (fig. 7).

Regression of degenerative changes was characterized by a decrease in the accumulation of lipid vacuoles in the cytoplasm of hepatocytes, especially large lipid droplets, to a lesser extent medium droplets. In the cytoplasm of liver cells were noted mostly small transparent vacuoles in the peripheral parts of the classical liver lobes, less often in their intermediate zone (fig. 8).

The degree of lipid accumulation was estimated to be 2.2, which is twice less than in acute ethanol intoxication without the use of apple pectin, but still remained higher than in the group of intact animals. The number of dystrophically altered cells was 9.25 per 100 hepatocytes, which is about 2.5 times less than in the control group without treatment. Despite a significant reduction in the accumulation of lipids in the cytoplasm of hepatocytes, their contours remained blurred, the cytoplasm granular.

The number of mononuclear normal cells increased to 78.2 cells per 100 hepatocytes. This was also accompanied by the restoration of the lamellar structure of the liver, especially in the central parts of the classical hepatic lobules, where the degree of fatty degeneration was minimal or absent.

In several cases, a pronounced regression of dystrophic changes in hepatocytes to 0.6 by semi-quantitative assessment of lipid content with complete restoration of the trabecular structure of the organ and reactive hyperemia of sinusoidal hemocapillaries in the central and intermediate zones of the liver lobes (fig. 9).

Simultaneously with the decrease in lipid accumulation in the cytoplasm of hepatocytes, the number of dinuclear liver cells doubled to 8.94 cells per 100 hepatocytes, which indicates an increase in reparative processes in the liver under the influence of apple pectin (fig. 10).

In parallel with the growth of the regenerative potential of the liver with the use of apple pectin, irreversible alternative processes decreased, which was manifested by three times fewer cells with signs of necrosis and apoptosis - 3.61 cells per 100 hepatocytes. Not only the number of these cells decreased, but also the intensity of this pathological process - 0.4 points according to the semi-quantitative assessment of intensity.

The decrease in lipid accumulation in the cytoplasm of hepatocytes was accompanied by a decrease in the proportion of parenchyma – 86,38±5,64 % and, accordingly, a decrease in the area and perimeter of hepatocytes (the average area of the hepatocyte is 367,34±24,32 мкм2, middle perimeter of the hepatocyte – 67,84±5,31 mkm).

The nuclei of liver cells are round, with heterogeneous chromatin, with a clear nucleolemma, with the presence of nucleoli. The nuclei of hepatocytes were located mostly in the central part of the cells. According to the metric study, the average area of the hepatocyte nucleus was 62,45±4,61 mkm2, middle perimeter of the hepatocyte nucleus – 27,85±1,84 mkm. When calculating the nuclear cytoplasmic index, it was found that this figure is 0.16, which corresponds to that in the group of intact animals.

The decrease in the area of hepatocytes and the share of parenchyma in general against the background of regressive degenerative processes was accompanied by an increase in the share of sinusoidal hemocapillaries – 7,36±0,45 %, which is twice as much as with the acute use of ethanol without pectin. However, a similar tendency to increase the proportion of sinusoidal hemocapillaries, which are filled with blood, was observed with the use of apple pectin. So the share of these capillaries was 2,35±0,15%, that is, when recalculating 32 % relative to the total area of sinusoidal hemocapillaries. The proportion of sinusoidal hemocapillaries that are not filled with blood was 5,01±0,37 %, which in recalculation is 68 %. These changes in the slight increase in the proportion of sinusoids that are filled with blood, most likely due to reactive hyperemia of the capillary bed of the liver with an increasing decrease in the area of liver cells [12].

Significant changes under the condition of QoS were observed in the connective tissue of the portal tracts, where the edema of connective tissue fibers and the intensity of inflammatory infiltration decreased sharply. (fig. 11).

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