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The apple pectin influence upon the liver histological structure and the activity of lipid peroxidation in experimental acute alcohol intoxication

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Abstract

Alcohol acute intoxication is a worldwide problem because of increasing morbidity and mortality. Different medications are used in treatment, but we still look for something new effective and accessible. By ATC classification pectin belongs to A07BC - intestinal adsorbents, is a natural nontoxic product, has the prebiotic properties and is well tolerated by patients. The aim of this study was to clear up the influence of apple pectin on survival, histological structure of the liver and the activity of the lipid peroxidation in rats with acute alcohol intoxication. Alcoholic animals (40% ethanol 2 ml / 100 g body weight p.o.) were classified into 4 groups without treatment and using pectin or reference preparations (activated charcoal and silicon dioxide). Apple pectin is reliably effective when use before the introduction of ethanol and 1 hour after it, that was proved by histological and biochemical investigation. The indexes obtained showed the effectiveness of pectin, which equates to reference medications with similar mechanism of action.

Keywords: Apple pectin, acute alcohol intoxication

Introduction

Excessive alcohol consumption and related intoxication is a worldwide problem because of its ability to increase morbidity, mortality and criminality. Alcohol absorption takes place mainly in the stomach (70%) and in the duodenum (25%), while only a small percentage occurs in the large intestine ^[1]. The metabolism of alcohol happens by gastric alcohol dehydrogenase (ADH) - 10%, liver ADH - 80%, microsomal ethanol oxidizing system (MEOS, CYP2E1) - 6-8%, and catalase - 2-4% ^[2,3]. ADH oxidizes ethanol to acetaldehyde. Acetaldehyde then enters the mitochondria where it is oxidized to acetate by mitochondrial aldehyde dehydrogenase (ALDH). Oxidation of ethanol can also occur in peroxisomes via the activity of catalase, but this oxidation pathway requires the presence of a hydrogen peroxide (H₂O₂) generating system and plays no major role in alcohol metabolism ^[4]. Additional acute consequences of alcohol metabolism include hypoxia in the liver and the formation of highly reactive oxygen species (ROS) that is associated with cancer development, atherosclerosis, diabetes, inflammation, aging, and other harmful processes. During ethanol oxidation ROS production increases dramatically due to induction of CYP2E1 and by activation of Kupffer cells in the liver. Both acute and chronic alcohol consumption can increase ROS production and lead to oxidative stress ^[5].

Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. The annual production of pectin is 40 000 t in the world and about 4000 t in Ukraine. Pectin's therapeutic properties and indications for use in medical practice are due its ability absorb and thus deactivate some endo- and exogeneous substances ^[6]. By ATC classification pectin is classified as A07BC - intestinal adsorbents and has the prebiotic properties ^[7]. But we didn't find any data about possible therapeutic effect of pectin in alcohol intoxication.

The aim of study was to clear up the influence of apple pectin on survival, histological structure of the liver and the activity of the lipid peroxidation in rats with acute alcohol intoxication.

Materials and methods. The study was performed on 60 white male outbred rats with a body weight of 170-195 g, which injected 40% ethanol into the stomach with a probe at a rate of

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2 ml / 100 g body weight. The investigated powder of apple pectin (PE "Company" Dana, I ", Kyiv, Ukraine) was used in the amount of 0.2 g / 100 g of body weight, and the comparison preparations - activated charcoal (250 mg tablets, Borschagovsky CPhP, Ukraine) and silicon dioxide ("white coal" 210 mg tablets, Omnifarm LLC, Kyiv, Ukraine) 0.25 and 0.05 g per 100 g of body weight, respectively. The investigated and reference substances were used prior to the introduction of ethanol and 1 hour after it. Animals were divided into groups: intact; alcoholized: 1- without treatment; 2- with the introduction of pectin; 3- with the introduction of activated carbon; 4-injected with silicon dioxide. Euthanasia of animals was conducted by the thiopental anaesthesia in dose 40 mg/kg body weight [8] after 3 days of the trial, then we examined the histological structure of liver using a light-optical microscope of Leica DME (Germany) on histological preparations stained with hematoxylin and eosin, with the perivascular peri-vascular, peripartrial, and intermediate sections. At the first stage, digital copies of the optical image of microscopic parts were obtained using a Nikon Coolpix 4500 digital camera (Japan). Subsequently, digital copies of the image were analyzed with the computer program Image Tool 3.0 for Windows (free license). The activity of lipid peroxidation (MDA and DC), catalase activity in blood serum determined according to [9]. Experiments with animals were carried out according to the Geneva Convention (1986) and approved by the bioethics commission of IFNMU. The statistical analysis was performed by Microsoft Exel and Statistica 5.5 (Multiple Regression) software using variation statistics methods.

Results and discussion: The survival in groups of alcoholic animals without treatment was 70%, all treated and intact rats survived. That means all medications with absorbent properties used in our trial decreased the toxic effect of alcohol. The histological specimens investigation showed a pronounced changes in liver tissue.

In acute intoxication with ethanol, the trabecular radial structural organization of the liver is primarily due to degenerative changes in hepatocytes in different parts of the classical hepatic lobules: both in the peripheral and intermediate and central zones (fig. 1, 2).

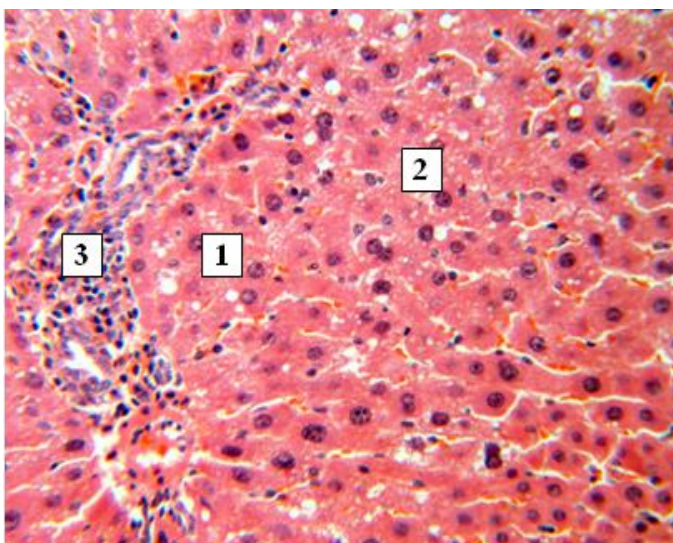


Fig 1: Acute ethanol intoxication. Fatty dystrophy of hepatocytes in the peripheral (1) and intermediate (2) zones of the classical hepatic lobe. Leukocyte-macrophage infiltration in the portal path (3). Stain: Hematoxylin and eosin. Magnification: 10X/40X

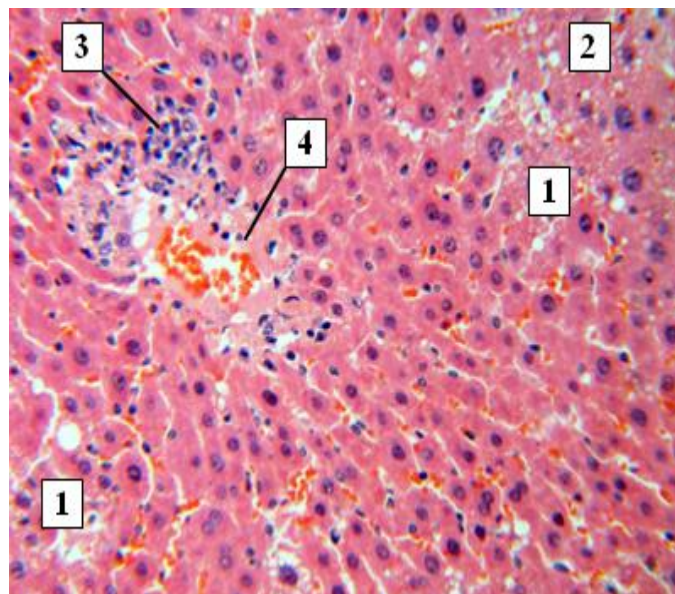


Fig 2: Acute ethanol intoxication. Fatty dystrophy of hepatocytes in the intermediate (1) and central (2) zones of the classic hepatic lobules. Leukocyte-macrophage infiltration in the portal path (3). Plasma edema of the vein wall (4). Stain: Hematoxylin and eosin. Magnification: 10X/40X

Most hepatic parenchymal cells are represented by single nucleus normal cells (61,25 cells per 100 hepatocytes), but their number is significantly lower than in the intact group (82.47%). The proportion of dystrophically altered cells in acute ethanol intoxication is an average of 21.1 cells per 100 hepatocytes (a intact group of 4.5%). In some alcoholic animals fatty dystrophy of liver cells reaches 74%, abruptly violating the trabecular structure of the organ and causing compression of surrounding sinusoidal hemocapillars (Figure 3)

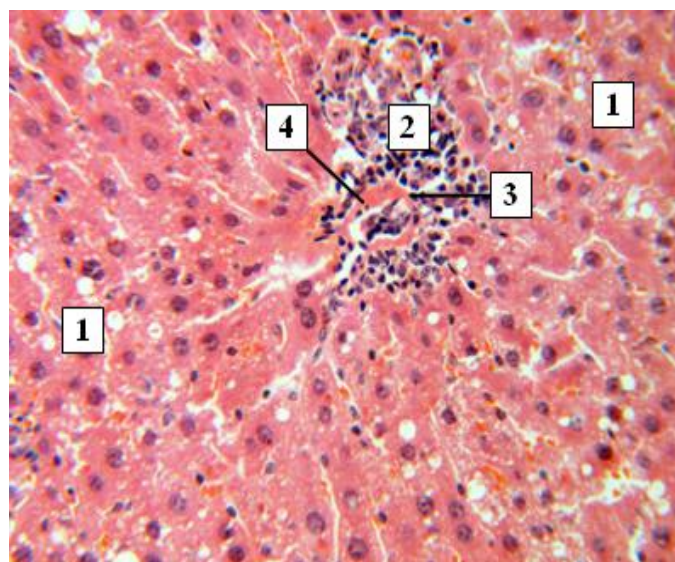


Fig 3: Acute ethanol intoxication. Disseminated fatty dystrophy of hepatocytes with vacuoles of different caliber (1). Leukocyte-macrophage infiltration in the portal path (2). Plasma edema (3) and focal fibrinoid necrosis (4) of the vessel wall of the portal tract. Stain: Hematoxylin and eosin. Magnification: 10X/40X

The use of pectin in acute intoxication with ethanol is accompanied by regression of degenerative changes in the liver cells with marked restoration of the trabecular structure of the organ (Fig. 4).

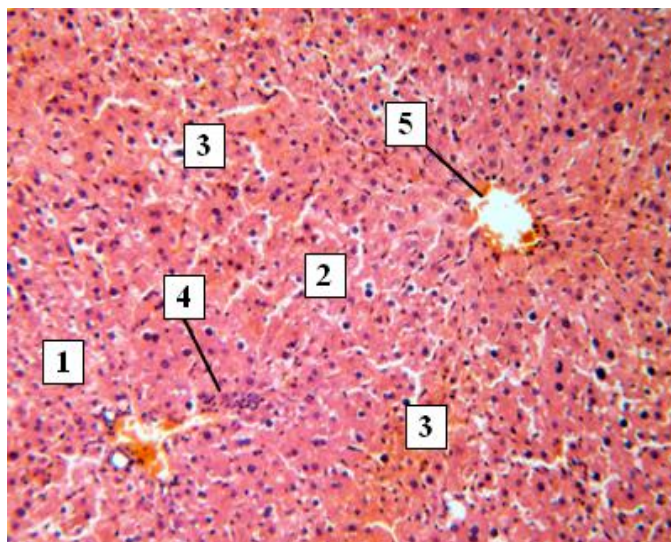


Fig 4: Acute ethanol intoxication in use of pectin. Fracturing fatty degeneration of hepatocytes in peripheral (1) and intermediate (2) zones of liver lobules. Focal fusion of sinusoidal hemocapillars (3). Groups of leukocytes and macrophages in the portal path (4). 5 - central vein. Stain: Hematoxylin and eosin. Magnification: 10X/40X

The degree of lipids accumulation in a semi-quantitative estimate is 2.2, which is twice less than with acute ethanol intoxication without use of pectin, but still remains greater than in the control group. Simultaneously with the decrease in the lipids accumulation in the hepatocyte cytoplasm, doubling the number of bipolar liver cells - 8.94 cells per 100 hepatocytes - is increasing, indicating an amplification of reparative processes in the liver under the influence of pectin (Figure 5).

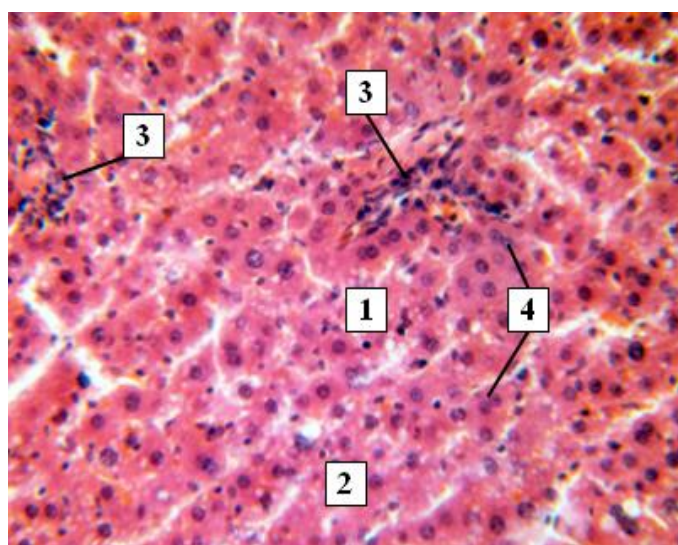


Fig 5: Acute ethanol intoxication in use of pectin. Fine and middle-grained fatty degeneration of hepatocytes in the peripheral (1) and intermediate (2) zones of portal lobes. Single leukocytes and macrophages in portal tracts (3). Dual-core hepatocytes (4). Stain: Hematoxylin and eosin. Magnification: 10X/40X

The number of dystrophically altered cells is 9.25 per 100 hepatocytes, which is about 2.5 times less than in the group of alcoholic rats. Despite the significant decrease in the accumulation of lipids in the hepatocyte cytoplasm, their contours remain blurred, and the cytoplasm is granular. The number of single nuclei of normal cells is increasing - 78.2 cells per 100 hepatocytes. The swelling of connective tissue fibers and the intensity of inflammatory infiltration are sharply reduced and the fraction of connective tissue in this case, according to the metric study, is $1.35 \pm 0.11\%$.

The pronounced changes in the use of pectin are noted in hepatocytes and the connective tissue of portal tracts, where sharply decreases the edema of connective tissue fibers and the intensity of inflammatory infiltration.

The activity of lipid peroxidation we investigated due to malonic dialdehyde (MDA), diene conjugates (DC) and catalase contents in blood serum. The results we obtained in this part of our trial showed the significant increase of all three studied indicators in non-treated alcoholic animals: MDA increased to $4,11 \mu\text{mol} / \text{l}$ (105% higher), DC - $4,58 \mu\text{mol} / \text{l}$ (33% higher) and catalase grow up to $3,93 \mu\text{mol} / \text{min}$ in mg protein (63%) comparing to intact. The oxidation occurs primarily in the liver via several routes, the major pathway being by the alcohol dehydrogenase. In addition, minor routes via catalase-dependent oxidation and oxidations by the stomach also provide its metabolism [6]. Hence increased levels of MDA and DC occurred because of oxidative stress caused by generation of reactive oxygen species in alcohol metabolism via oxidative processes. The influence of pectin and other absorbents used in comparison showed decreased activity of lipid peroxidation and catalase content in blood serum.

Table 1: The contents of MDA, DC and catalase activity in blood serum of alcoholic animals without treatment and using sorbents ($M \pm m$, $n=5-7$)

Experimental groups	MDA, $\mu\text{mol} / \text{l}$	DC, $\mu\text{mol} / \text{l}$	Catalase, $\mu\text{mol} / \text{min}$ in mg protein
Intact	$2,01 \pm 0,12$	$3,44 \pm 0,06$	$2,41 \pm 0,12$
Alcohol	$4,11 \pm 0,15^1$	$4,58 \pm 0,08^1$	$3,92 \pm 0,11^1$
Alcohol+Pectin	$2,77 \pm 0,12^{1,2}$	$3,50 \pm 0,05^2$	$2,97 \pm 0,08^{1,2}$
Alcohol+Charcoal	$2,91 \pm 0,12^{1,2}$	$3,67 \pm 0,11^{1,2}$	$3,18 \pm 0,07^{1,2}$
Alcohol+Silicon Dioxide	$2,74 \pm 0,1^{1,2}$	$3,73 \pm 0,11^{1,2}$	$3,08 \pm 0,14^{1,2}$

Statistical analysis was done using variation statistics methods. Results are expressed as meaning of contents MDA and DC ($M \pm m$) and Catalase activity: ¹- significantly different from intact; ²- significantly different from alcoholic animals.

The levels of MDA and DC and catalase activity were reliably smaller from alcoholic animals in all treated groups but significantly higher from intact.

Conclusions: Apple pectin used prior to the introduction of ethanol and 1 hour after it showed decrease of liver lesion and significant inhibition of lipid peroxidation in acute alcohol intoxication in rats. According to the studied parameters, pectin is equile to standard sorbents. The obtained results testify to the possibility of using pectin as a sorbent for the treatment of acute alcohol intoxication, and considering its prebiotic properties it is likely to have a positive effect on the gastrointestinal tract functions restoration during the period of rehabilitation after acute alcohol poisoning.

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