

BIOCHEMICAL AND LASER-POLARIMETRIC MARKERS OF HEPATOCYTE CYTOLYSIS SYNDROME UNDER CONDITIONS OF TOXIC DAMAGE AND PROTEIN DEFICIENCY

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Abstract

Limited intake of exogenous proteins under the conditions of irrational diet, essential amino acids deficiency and an enhanced loss of endogenous protein with a negative nitrogen balance led to the development of acute or chronic liver disease. In the literature, numerous examples of acute and chronic injuries experimental models using toxic damage factors with various mechanisms of prolonged action are given. The model of acetaminophen-induced injury is one of the most common because the nature of changes in the body depends on the dose and duration of action of the toxin.

At the same time, the search for new screening methods for early diagnosis of hepatopathology which could ensure the accuracy, minimally invasiveness and budget of the procedure, seems relevant. Because of this, the advantage of the obtained results compared to the existing ones is to develop an algorithm of combined use of biochemical and optical methods to predict metabolic changes and selection of key biochemical markers for early diagnosis of nutrient-associated pathological conditions.

The aim of the study is to investigate biochemical and laser-polarimetric markers of hepatocyte cytolysis syndrome under the conditions of toxic damage and protein deficiency.

Objects and materials

Studies were carried out on white adolescent outbred rats aged 2.5-3 months and weighing 110-130 g.

The animals were divided into four groups:

- 1 – intact rats kept on the complete semi-synthetic AIN-93 diet developed by the American Institute of Nutrition (C);
- 2 – animals fed a low-protein diet containing 1/3 of the normal daily protein requirements for 28 days (LPD);
- 3 – rats in which toxic injury was modelled by acetaminophen at the rate of 1250 mg/kg of body weight in 2% starch gel suspension administered orally once a day for two days (TI);
- 4 – animals administered toxic doses of the agent against the background of alimentary protein deficiency (LPD+TI).

The study material is represented by serum samples. For optical studies, native blood smears were used, which were stored at a temperature of 4 ° C.

Indicators of hepatocyte cytolysis – the level of ALT, AST, LDH, the concentration of total and direct bilirubin was determined on automated biochemical analyser HUMASTAR 200 SR.

Results of biochemical research

It was established, that the administration of acetaminophen toxic doses is the key factor in increasing ALT and AST activities in rats' serum compared with the control indicators (Fig. 1). It should be noted that the level of studied enzymes reaches maximum values under the conditions of xenobiotic administration against the background of protein deficiency (> 3 times compared with the control) (Fig. 1).

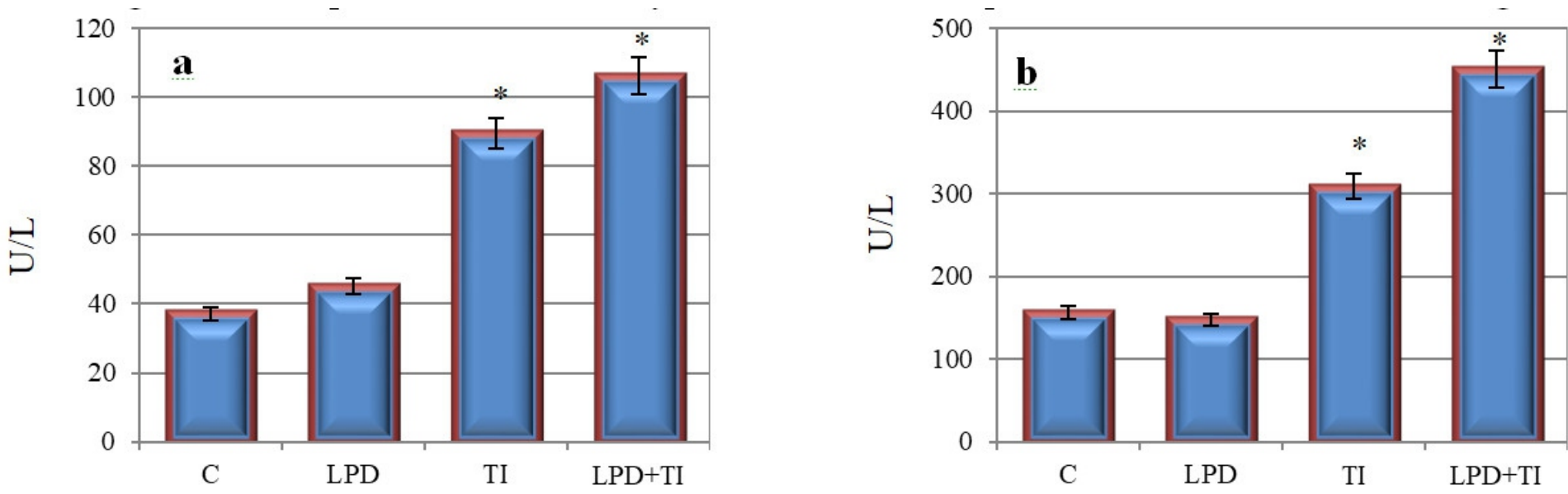


Fig. 1. ALT (a) and AST (b) activities in rats' serum under the conditions of acetaminophen-induced toxic injury against the background of protein deficiency C – control; LPD – rats kept on a low-protein diet; TI – rats with acetaminophen-induced toxicity; LPD + IT – rats with acetaminophen-induced toxicity against a background of alimentary protein deficiency; * – statistically significant difference as compared to control values (P ≤ 0.05).

POLARIZATION-MICROSCOPIC RESEARCH METHODS

Polarization measurements were carried out with a polarizing microscope.

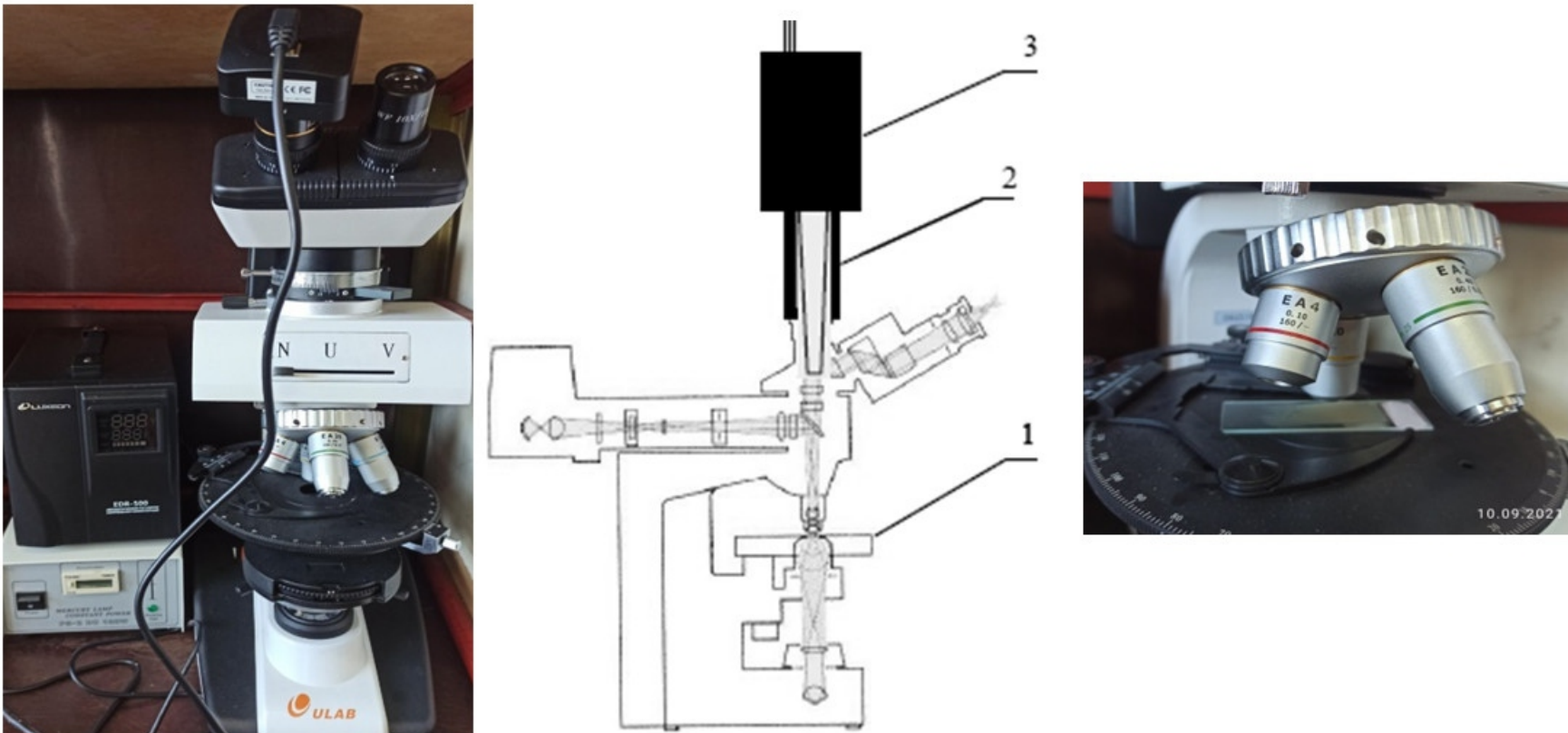


Fig. 4. Images and optical layout of a polarizing microscope

$$\begin{aligned} S_{i=1}^{0;45;90;\odot} &= I_0^{0;45;90;\odot} + I_{90}^{0;45;90;\odot}; \\ S_{i=2}^{0;45;90;\odot} &= I_0^{0;45;90;\odot} - I_{90}^{0;45;90;\odot}; \\ S_{i=3}^{0;45;90;\odot} &= I_{45}^{0;45;90;\odot} - I_{135}^{0;45;90;\odot}; \\ S_{i=4}^{0;45;90;\odot} &= I_{\odot}^{0;45;90;\odot} + I_{\oplus}^{0;45;90;\odot}. \end{aligned} \quad (1)$$

$$\begin{aligned} \alpha^* &= 0,5 \arctg \frac{S_{i=3}}{S_{i=2}}; \\ \beta^* &= 0,5 \arcsin \frac{S_{i=4}}{S_{i=1}}. \end{aligned} \quad (2)$$

Discuss the results of biochemical research

Alanine aminotransferase and aspartate aminotransferase is the intracellular enzymes and their concentration in cells is much higher than in serum. So, normally only a small amount is found in the blood. As a result of plasma membranes damage under the pathologies conditions, cellular enzymes are released and enter the bloodstream, as resulted in pronounced hyperenzymemia may be observed. Therefore, the enzymatic activity of ALT and AST is a reliable biochemical criterion for damage of plasma membranes.

The increase in the ALT and AST levels indicate the inflammatory nature of the disease and the development of acute liver injury, which quickly develops and is a consequence of the destruction of parenchymal cells.

POLARIZATION MEASUREMENT RESULTS

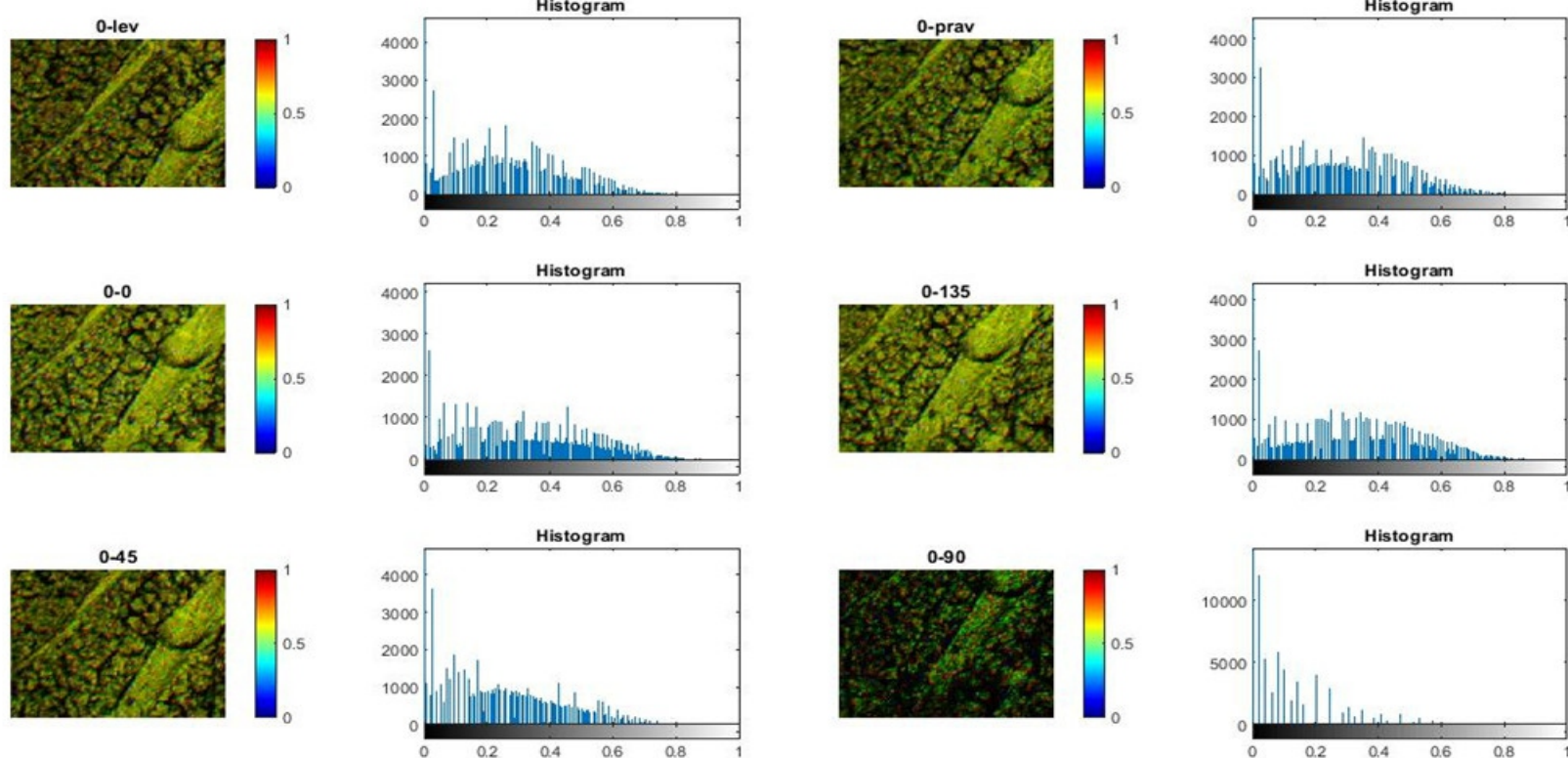


Fig. 5. 2 - D polarized images of the pancreas.
Table 1 With the collection of statistical moments of the first-fourth orders

Statistical moments of 3-4 orders (Asymmetry (A), Kurtosis (E)) n polarization images of histological sections of biological tissues		
Color polarized image through green filter		
Statistical moments	Azimuth (α)	Ellipticity (β)
Asymmetry (A)	0.1223185	-0.0944514
Excess (E)	-0.6504864	-12.5252979

Conclusions

- Thus, the acetaminophen-induced toxic injury against the background of protein deficiency is accompanied by the development of cytolytic syndrome, which is based on a disorder of the cell membrane permeability, destruction of membrane structures, hepatocytes necrosis, release into the blood of intracellular enzymes and accumulation of bile pigments.
- Analyzing the results of polarization measurements, namely the statistics of 2 - D distributions of azimuth and ellipticity, a diagnostic criterion is revealed in a sharp increase in the statistical characteristic of kurtosis for distributions of ellipticity.

LITERATURE

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