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Endogenous intoxication and morpho-functional changes in the liver during experimental acute generalized peritonitis in diabetic rats

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Objective. The study aims to evaluate the severity of endogenous intoxication and characterize morpho-functional liver changes during experimental acute generalized peritonitis (AGP) in diabetic rats.

Methods. Fifty-six adult male Wistar rats were used, including 8 controls and 48 males with experimental pathology. Diabetes mellitus was induced by an intraperitoneal (i.p.) injection of streptozotocin (60 mg/kg). On day 14, AGP was induced by i.p. injection of a 10% filtered fecal suspension. Endogenous intoxication was assessed by measuring hydrophilic and hydrophobic molecular products in the blood. Liver function was evaluated by serum aminotransferase activity, total protein, and protein fractions. Histological analysis of liver tissue was performed using standard hematoxylin-eosin staining.

Results. A progressive increase in endogenous intoxication was observed peaking on day 7. This was marked by a significant elevation in middle molecular weight molecule (MMWM) concentrations at wavelengths of 254 nm and 280 nm by 103.0% ($p<0.001$) and 340.0% ($p<0.001$), respectively. The erythrocyte intoxication index (EII) increased by 148.8% ($p<0.001$) compared to controls. Concurrently, aminotransferase activity increased, while serum total protein and albumin levels decreased. Histologically, inflammatory infiltration and vascular congestion were evident on day 1 progressing to hepatocellular dystrophy and necrosis by day 3. By day 7, signs of hepatic failure were present including disruption of trabecular architecture, hydropic degeneration, intracellular cholestasis, and portal tract expansion due to vascular hyperemia.

Conclusions. Experimental acute generalized peritonitis in diabetic rats resulted in a pronounced endogenous intoxication accompanied by progressive morpho-functional liver damage culminating in hepatic insufficiency by day 7.

Keywords: acute generalized peritonitis, diabetes mellitus, endogenous intoxication, hepatic cytolysis, liver dysfunction, rats

It is widely recognized that endogenous intoxication according to most clinical, biochemical, and immunological manifestations is considered to be a nonspecific process caused by an imbalance between the synthesis and elimination of products of "normal" and pathological metabolism (Filip and Skrypinets 2019; Savytskyi et al. 2021; Verveha et al. 2023). One of the main causes of endogenous intoxication in acute generalized peritonitis (AGP) is bacterial toxins, which trigger the release of a large amount of endogenous inflammatory mediators leading to systemic pathological reactions (Mammadova 2021). Activation of

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proteolytic enzymes, one of the sources of which is the liver, causes tissue damage and increased production of autolysis products, resulting in the accumulation of toxic metabolites (Ahmed et al. 2011).

The severity of endogenous intoxication is determined not only by the intensity of toxic substances entering the bloodstream, but also the effectiveness of the main detoxification mechanisms responsible for toxin elimination. Liver failure, which develops during progressive endotoxicosis, is marked by a decrease in detoxification capacity, leading to a generalized toxic effect on the body and the development of multiple organ failure, ultimately the main cause of death in AGP (Recknagel et al. 2012; Kumar et al. 2020; Li and Chen 2021).

It is an undeniable fact that the primary strategy for combating endogenous intoxication in AGP is focused on its source, the inflammatory process in the peritoneum, the severity, of which under comorbid conditions, is the subject of investigation in this section. This study aims to evaluate the severity of endogenous intoxication and characterize morpho-functional liver changes during experimental AGP in diabetic rats.

Material and Methods

Animals. The study was conducted on 56 adult male white laboratory rats of the Wistar line with a body weight of 180–220 g. The rats were kept in standard conditions of the vivarium of the State Non-Profit Enterprise «Danylo Halytsky Lviv National Medical University».

Laboratory animals were maintained under standard conditions (room temperature 22±2°C, and relative humidity 55±10%) on a light:dark cycle of 12:12 h of artificial light (lights on from 6:00 AM to 6:00 PM). They had free access to food and water. Handling and care of animals were conducted according to the positions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986); European Council Directive 86/609/EEC (1986); Law of Ukraine No 3447 – IV On Protection of Animals from Abuse; the General Ethical Principles of Experiments on Animals, approved by the First National Congress of Bioethics of Ukraine (2001), as confirmed by the conclusions of the Committee of the Danylo Halytskyi Lviv National Medical University (Protocol No8 as of November 23, 2020, Protocol No6 as of June 22, 2021).

Design of the experiment. Animals were divided into three groups: group 1 – controls (rats injected subcutaneously with 0.9% sodium chloride solution); group 2 – peritonitis rats (rats with simulated acute peritonitis); and group 3 – diabetic peritonitis rats (diabetic rats with simulated acute peritonitis).

Induction of experimental diabetes and experimental acute generalized peritonitis. Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ) (Sigma-Aldrich) (60 mg/kg body weight) freshly diluted in citrate buffer (10 mM, Na citrate pH 4.5) (Ramos-Lobo et al. 2015). Prior to the injection, 10% betadine solution was three times applied into the right iliac area. The needle was injected at 45° angle into the surface of the frontal abdominal wall until it felt "sinking". After the injection, the animals received solution of glucose per os for the first 24 h in order to prevent transitory hypoglycemia. The blood glucose concentration was measured by the glucose oxidase method. On day 14 of the development of diabetes mellitus, the level of glucose was determined in the blood of the rats and performed further modeling of acute inflammation of the peritoneum (combined pathology) in the animals with hyperglycemia level that exceeded 11.1 mmol/L.

Acute generalized peritonitis was modeled by injecting 10% filtered-feces suspension into the abdominal cavity in the dose of 0.5 mL per 100 g of body weight. The feces suspension was obtained by mixing isotonic solution and contents of the cecum of three intact animals and twice filtrated through a double layer of etamine. The feces suspension was injected not later than 20 min after its preparation. In order to prevent damage to the internal organs, the animals were held in a vertical position by the caudal end to the top. We injected the necessary amount of feces suspension by puncture of the ventral wall in the center of the midline of the front abdominal wall by directing the needle tip into the area of the right and then the left hypochondrium into the right and left iliac areas.

Biochemical analysis. To determine the content of middle molecular weight molecule (MMWM), the acid-soluble fraction was isolated from the serum. It was obtained by adding 1.8 ml of 10% solution of trichloroacetic acid to 0.2 ml of serum. Subsequent centrifugation was performed at 3000 rpm for 30 min. The isolated 0.5 ml fraction was diluted 1:10 with distilled water and the optical density was determined at 254 nm (chain amino acids) and 280 nm (aromatic amino acids) against distilled water on a spectrophotometer. The results were expressed in conventional units numerically equal to the extinction.

To diagnose hepatic insufficiency, the level of cytolytic enzymes, alanine aminotransferase

(ALT) and aspartate aminotransferase (AST), was determined using the colorimetric method described by Reitman and Frankel (1957). The protein-synthesizing function of the liver was evaluated by measuring total serum protein concentration using the biuret reaction followed by fractionation of serum proteins via polyacrylamide gel electrophoresis (Vlizlo et al. 2008). The experiment was conducted on days 1, 3, and 7 following the administration of fecal suspension. These time points correspond to the reactive, toxemic, and terminal phases of the peritonitis progression.

Histopathological examinations. At the end of experiment, all rats were decapitated. Liver of all rats was thoroughly examined for the gross lesions and liver tissue specimens were collected in 10% neutral buffer formalin. After fixation of the liver specimens in 10% neutral buffer formalin, dehydration in ascending grades of alcohol and clearance in xylene were done. Finally, the liver specimens were embedded into paraffin. Paraffin sections of 5 μm thickness were cut and stained with hematoxylin and eosin as outlined by (Bancroft and Gamble 2008).

Statistical analysis. Statistical analysis of the obtained data was performed using Microsoft Excel and Statistica 6.0 software. The mean value (Mean) and standard error of the mean (SEM) were calculated. The significance of differences between independent quantitative variables with normal distribution was assessed using Student's t-test, with differences considered statistically significant at $p<0.001$.

To identify and evaluate relationships between quantitative indicators, correlation analysis was conducted using the parametric method with calculation of the Pearson correlation coefficient (r). Correlation strength was interpreted as strong when $r>0.7$, moderate when $r>0.3–0.7$, and weak when $r\leq0.3$. A positive correlation coefficient indicated a direct relationship between variables, while a negative value indicated an inverse relationship.

Results

In rats with AGP, a statistically significant increase in the level of $MMWM_{254}$ was observed on days 1, 3, and 7 by 81.8%, 78.8%, and 72.7%, respectively, compared to the control group (Table 1) indicating pronounced endogenous intoxication. The concentration of $MMWM_{280}$ increased by 140.0% ($p<0.001$) on day 1, by 120.0% ($p<0.001$) on day 3, and by 100.0% ($p<0.01$) on day 7 relative to control animals. We also recorded an elevation in the EII on days 1, 3, and 7 of acute peritoneal inflammation by 124.2% ($p<0.001$), 113.0%, and 107.1% ($p<0.001$), respectively, compared to the control group.

In rats with experimentally induced AGP against the background of diabetes mellitus, the concentration of $MMWM_{254}$ increased significantly on days 1, 3, and 7 of the study by 93.9% ($p<0.001$), 97.0% ($p<0.001$), and 103.0% ($p<0.001$), respectively, compared to the control group (Table 2). On the first day of the experimental combined pathology, $MMWM_{280}$ levels were 3.8 times higher than in the control group. On days 3 and 7, $MMWM_{280}$ concentrations did not differ significantly from each other, but remained elevated at 4.4 times the control values ($p<0.001$), which may indicate a substantial accumulation of water-soluble toxic metabolites as a result of the simulated combined pathology.

Table 1

The content of middle molecular weight molecules (MMWM) in the serum and erythrocyte index of intoxication of experimental animals in different periods of development of experimental acute generalized peritonitis

Parameter	Control group	Experimental group		
		Day 1	Day 3	Day 7
$MMWM_{254}$ (condit. units)	0.33±0.01	0.60±0.07 p<0.01	0.59±0.02 p<0.001	0.57±0.03 p<0.001
$MMWM_{280}$ (condit. units)	0.05±0.01	0.12±0.01 p<0.001	0.11±0.01 p<0.001	0.10±0.01 p<0.01
EII (%)	32.2±1.5	72.2±1.6 p<0.001	68.6±2.4 p<0.001	66.7±2.3 p<0.001

Abbreviations: EII – erythrocyte intoxication index; $MMWM_{254}$ – middle molecular weight molecules at 240 nm; $MMWM_{280}$ – middle molecular weight molecules at 280 nm. Data are presented as mean±SEM (n=8); p – significance of differences relative to control group of animals.

Table 2

The content of middle molecular weight molecules (MMWM) in the serum and erythrocyte index of intoxication of experimental animals in different periods of development of experimental acute generalized peritonitis against the background of diabetes mellitus

Parameter	Control group	Experimental group		
		Day 1	Day 3	Day 7
$MMWM_{254}$ (condit. units)	0.33±0.01	0.64±0.05 p<0.001	0.65±0.01 p<0.001	0.67±0.04 p<0.001
$MMWM_{280}$ (condit. units)	0.05±0.01	0.19±0.02 p<0,001	0.22±0.01 p<0,001	0.22±0.02 p<0.001
EII (%)	32.2±1.5	73.20±1.70 p<0.001	78.80±2.60 p<0.001	80.10±3.40 p<0.001

Abbreviations: EII – erythrocyte intoxication index; $MMWM_{254}$ – middle molecular weight molecules at 240 nm; $MMWM_{280}$ – middle molecular weight molecules at 280 nm. Data are presented as mean±SEM (n=8); p – significance of differences relative to control group of animals.

The development of endogenous intoxication was associated with increased structural destabilization of biomembranes, as evidenced by the elevated adsorptive capacity of erythrocytes, which reflects enhanced membrane permeability. The EII, similar to the MMWM levels, increased markedly during the course of peritonitis in diabetic rats. On days 1 and 3, the index increased by 127.3% and 144.7%, respectively, compared to the control. The most pronounced change was observed on day 7, with a 148.8% increase ($p<0.001$) relative to baseline. These findings suggest a more sustained presence of toxic metabolites in the terminal phase of peritonitis under diabetic conditions. The analysis of dynamic changes in serum levels of various MMWM fractions and the EII revealed a consistent upward trend, indicating a progressive intensification of endogenous intoxication in diabetic rats with AGP across all stages of acute peritoneal inflammation.

We found that the activity of ALT, a cytosolic liver-specific enzyme, increased linearly in the blood serum of animals with AGP throughout all stages of the disease (Figure 1). On day 1 of AGP, ALT activity significantly increased by 143.3%, on day 3 by 176.7%, and on day 7 by 196.7% compared to the control group. In animals with AGP combined with diabetes mellitus, ALT activity exceeded control values by 146.7% on day 1, 200.0% on day 3, and 230.0% on day 7. A statistically significant difference in ALT activity between the group with combined pathology and the AGP-only group was observed on day 7 (11.2%; $p<0.05$). Given that ALT is a liver-specific enzyme, its elevated plasma levels should be interpreted as a consequence of endotoxin release into the bloodstream due to the modeling of severe fecal peritonitis, as well as intensified catabolic processes under simulated STZ-induced diabetes, which are accompanied by increased erythrocyte permeability and plasma membrane damage.

A similar dynamic was observed when analyzing AST. Its activity increased by 140.5% on day 1 and by 171.4% on day 3 of AGP compared to the control. On day 7, AST levels reached 1.2±0.02 U/L exceeding the control value by 185.7%. In animals with AGP combined with diabetes mellitus, AST activity increased by 154.8%, 176.2%, and 192.9% on days 1, 3, and 7, respectively ($p<0.001$) and exceeded the corresponding values in the AGP-only group by 5.9%, 1.8%, and 2.5%, respectively.

The progressive increase in aminotransferase activity in the blood of diabetic rats with AGP indicates an intensification of hepatic cytolysis and impaired liver function. Notably, the highest enzyme activity recorded at the terminal stage of peritonitis in the presence of diabetes mellitus is likely associated with a depletion of protective reserves in the affected rats and the accumulation of toxic metabolites. This leads to increased permeability of the plasmalemma and to some extent, intracellular membranes of hepatocytes, further indicating the progression of hepatic cytolysis, and liver dysfunction.

To assess the relationship between the intensity of hepatic cytolysis and the severity of endogenous intoxication during the progression of experimental AGP against the background of diabetes mellitus, correlation coefficients (r) were calculated between ALT levels and the concentrations of $MMWM_{254}$,

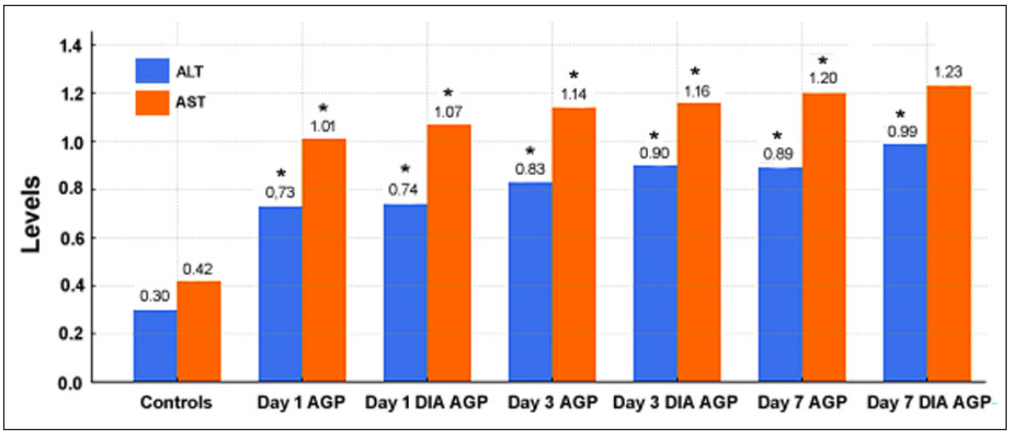

Figure 1. Dynamics of the serum levels (U/L) of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the experimental animals. Statistically significant changes were taken into account compared to the controls (*p<0.05).

$MMWM_{280}$ fractions, and the EII on days 1, 3, and 7 of the study.

The correlation analysis between markers of endogenous intoxication and the intensity of hepatic cytolysis in diabetic rats with AGP revealed a direct correlation between ALT activity and the levels of $MMWM_{254}$ and $MMWM_{280}$ fractions (r=0.85 and r=0.75; $p<0.05$) on day 1 of the study.

On day 3 of the combined pathology progression, the correlation between ALT and $MMWM_{254}$ was r=0.89, while the correlation between ALT and $MMWM_{280}$ was r=0.87 ($p<0.05$). The strongest associations between MMWM fractions and ALT activity were observed on day 7 of the study.

Analysis of the relationship between the EII and ALT activity in the blood of diabetic rats with AGP also revealed strong direct correlations.

A direct and statistically significant correlation was established between ALT activity and the EII on day 1 of AGP against the background of diabetes mellitus (r=0.76; $p<0.05$). A similar correlation was observed on day 3 of the study, with a slightly stronger coefficient of r=0.83 ($p<0.05$). The strongest correlation between ALT levels and EII was verified on day 7, reaching r=0.89 ($p<0.05$).

Thus, the obtained data on the correlation in diabetic rats with AGP confirm the dependence of the severity of endogenous intoxication on the duration and activity of the combined pathological processes. This is evidenced by the increasing strength of the correlation depending on the observation period of acute peritonitis under conditions of concomitant hyperglycemia.

The established linear positive correlation between the levels of MMWM, the EII value, and ALT activity indicates that the severity of endogenous intoxication is accompanied by an increase in hepatic cytolysis and vice versa.

Determining the fractions of MMW peptides is a method for assessing endogenous intoxication allowing interpretation of the hydrophilic component of toxicity. The hydrophobic component can be assessed based on the level of albumin, which binds and transports fat-soluble toxins.

It was found that in the blood of rats with AGP, the albumin level decreased by 18.5% ($p<0.05$) on day 1. On days 3 and 7, the reduction compared to the control group reached 32.2% ($p<0.01$) and 39.6% ($p<0.01$), respectively (Table 3). In the group with experimentally induced AGP combined with diabetes mellitus, albumin levels significantly declined throughout the study by 28.6%, 36.1%, and 45.4%, respectively, relative to the control.

The study of indicators of the liver's protein-synthesizing function revealed a significant decrease in total protein levels in animals with experimental AGP at all stages of the study. On day 7, the lowest value was recorded at 57.1±1.42 g/L, whereas in the control group this parameter ranged within 65.2±1.70 g/L. Alongside, the decrease in serum albumin levels in rats with acute peritoneal inflammation, a slight increase in the globulin fraction was observed: on day 3, this indicator rose to 43.1±1.65 g/L, and on day 7, to 43.4±1.95 g/L. The conducted studies indicate an imbalance in the albumin-to-globulin (A/G) ratio in the blood serum of rats with experimental AGP. As a result, the A/G ratio in the animals was 0.45±0.02 on day 1, 0.36±0.04 on day 3, and 0.32±0.03 on day 7 compared to 0.53±0.01 in the control group. Such a value of the ratio indicates suppression of the protein-synthesizing function of the liver during the development of experimental AGP.

Table 3
Total protein content and its fractions in the blood of rats with AGP

Parameter	Control group	Experimental group		
		Day 1	Day 3	Day 7
Total protein (g/L)	65.20±1.70	59.70±1.65 p<0.05	58.50±1.80 p<0.05	57.10±1.42 p<0.01
Albumin (g/L)	22.70±1.22	18.50±1.15 p<0.05	15.40±1.40 p<0.01	13.70±2.15 p<0.01
Globulins (g/L)	42.50±1.37	41.20±1.10	43.10±1.65	43.40±1.95
A/G ratio	0.53±0.01	0.45±0.02 p<0.01	0.36±0.04 p<0.001	0.32±0.03 p<0.001

Abbreviations: AGP – acute generalized peritonitis; A/G – albumin-to-globulin ratio. Data are presented as mean±SEM (n=8); p – significance of differences relative to control group of animals.

Table 4
Total protein content and its fractions in the blood of diabetic rats with AGP

Parameter	Control group	Experimental group		
		Day 1	Day 3	Day 7
Total protein (g/L)	65.20±1.70	59.00±1.60 p<0.05	57.70±1.82 p<0.01	56.40±1.45 p<0.01
Albumin (g/L)	22.70±1.22	16.20±1.16 p<0.01	14.50±1.23 p<0.001	12.40±2.10 p<0.001
Globulins (g/L)	42.50±1.37	42.80±1.15	43.20±1.56	44.00±1.84
A/G ratio	0.53±0.01	0.38±0.03 p<0.001	0.34±0.02 p<0.001	0.28±0.02 p<0.001

Abbreviations: AGP – acute generalized peritonitis; A/G – albumin-to-globulin ratio. Data are presented as mean±SEM (n=8); p – significance of differences relative to control group of animals.

When studying the state of the liver's protein-synthesizing function in animals with AGP against the background of concomitant diabetes mellitus, similar changes in total protein content were observed as in animals with AGP without comorbid pathology. Thus, this parameter decreased significantly on days 1, 3, and 7 by 9.5%, 11.5%, and 13.5%, respectively, compared to the control group (Table 4).

A slight increase in blood globulin levels was observed throughout all study periods, with the highest value relative to the control group recorded on day 7. The A/G ratio, as a characteristic of protein metabolism, is a comprehensive indicator of the overall activity of catabolic and anabolic processes. It was found that in diabetic rats with AGP, this indicator significantly decreased. Notably, on days 3 and 7, the A/G ratio was 0.34±0.02 and 0.28±0.02, respectively.

Thus, in the case of experimental acute peritonitis combined with diabetes mellitus, the reduction in total protein, albumin, globulin, and the A/G ratio on days 1, 3, and 7 of the experiment indicates impaired protein-synthesizing function of the liver throughout all stages of peritonitis development. The lowest level of total protein observed on day 7 points to severe disruption of the liver's protein-synthesizing function during the terminal stage of acute peritoneal inflammation in the setting of combined pathology.

Histological examination of the liver on day 1 of AGP development showed mild disruption of the lobular architecture. Central veins were clearly visualized, moderately dilated, and contained a small number of erythrocytes. Sinusoids were outlined primarily in pericentral zones and exhibited sparse macrophage presence alongside occasional erythrocytes within their lumens. The portal tracts showed intensified lymphohistiocytic infiltration.

By day 3, the hepatic structure demonstrated marked degenerative changes. Hepatocellular dystrophy had progressed predominantly manifesting as hyaline droplet degeneration with an increased number of necrotic cells. Central veins remained dilated and contained few erythrocytes. In several regions, sinusoidal spaces appeared poorly defined or were absent altogether. The trabecular organization of hepatocytes was disrupted. Macrophage activity remained low.

On day 7, histological analysis revealed extensive destruction of hepatic lobular structure (Figure 2). Key features included pronounced fatty degeneration of hepatocytes, loss of intercellular cohesion, and complete disintegration of trabecular architecture. Central veins were markedly dilated and filled with numerous erythrocytes. Sinusoids were not observed in most microscopic fields, while the few visible areas exhibited signs of venous congestion.

The cumulative morphological analysis confirmed the presence of both inflammatory-infiltrative changes (lymphohistiocytic infiltration of portal tracts, hyaline droplet degeneration), progressive degenerative-dystrophic, and necrotic processes (fatty degeneration and hepatocyte necrosis) that intensified throughout the course of experimental peritonitis development.

On day 1 of the experimental peritonitis in diabetic rats, histological examination revealed disruption of the trabecular organization of hepatocytes throughout the entire hepatic lobule. The cytoplasm of cells in the centrilobular zone, middle third of the lobule, and periportal areas appeared granular, and in some visual fields, pale and vacuolated. The majority of hepatocytes contained nuclei. Cellular contours were moderately distorted and the hepatocyte structure appeared heterogeneous. Although most cells retained nuclei, there was noticeable variability in nuclear size across different hepatocyte populations. The portal tracts were enlarged primarily due to vascular dilation and congestion along with mild expansion of bile ducts. Bile pigments were absent within the ductal lumens. No perivascular edema was observed. Moderate lymphohistiocytic infiltration was present in the portal areas.

By day 3, the trabecular organization of hepatocytes remained disrupted across the entire lobule. Cytoplasmic granularity persisted in the centrilobular, midlobular and periportal regions with areas of cytoplasmic rarefaction and vacuolation also noted (Figure 3). Intracellular cholestasis was observed, while most hepatocytes retained nuclei, anucleated hepatocytes were also present. Cell contours were markedly altered and the hepatocyte morphology was heterogeneous indicating dystrophic and necrotic changes. The portal tracts were expanded primarily due to vascular congestion and mild bile duct dilation. No bile pigments were observed. Perivascular edema was absent. Moderate lymphohistiocytic infiltration persisted.

On day 7, histological analysis revealed a complete loss of the hepatocellular trabecular organization. The cytoplasm of hepatocytes across the lobule was pale and showed signs of hydropic degeneration (Figure 4). Intracellular cholestasis was still present. Most hepatocytes retained their nuclei, though anucleated cells were occasionally seen. Cell boundaries were severely distorted and hepatocyte morphology remained heterogeneous corresponding to the progressive dystrophic and necrotic changes.

The portal tracts appeared markedly dilated due to vascular congestion and mild bile duct expansion. No bile pigment accumulation was detected in the ducts. No signs of perivascular edema were noted. Lymphocytic and histiocytic infiltration remained moderate.

Discussion

The results obtained in this study confirm that under the conditions of experimental peritonitis against the background of diabetes mellitus, rats develop a pronounced endogenous intoxication syndrome accompanied by a significant hepatic dysfunction. The dynamics of biochemical markers, including elevated levels of MMWM, the EII and the transaminase activity, indicate profound metabolic disturbances and hepatocellular damage. These changes are particularly pronounced on day 7 of the combined pathology modeling, when the parameters peak, reflecting the inability of the liver to effectively biotransform toxic metabolites. These findings are consistent with the study by Mammadova (2021) who has demonstrated that the concentration of diene conjugates, malonic dialdehyde, and MMW peptides in the blood of patients with advanced peritonitis increased significantly in accordance with the clinical stage of the disease.

A critical factor in the pathophysiological mechanisms of intoxication is hypoalbuminemia, a decrease in albumin, the main transport protein in plasma. Albumin binds a wide range of hydrophobic molecules including lipid peroxidation products, free fatty acids, bilirubin, and other endogenous toxins. Research by Barbeiro et al. (2024) have revealed that hypoalbuminemia in rats increases the mortality from endotoxemia associated with severe cardiovascular dysfunction and oxidative stress. Our findings demonstrate that experimental AGP in STZ-induced diabetic rats disrupts multiple hepatic functions, particularly protein synthesis.

In this context, morphological confirmation of hepatic dysfunction becomes especially pertinent. Histological analysis revealed characteristic pathological alterations in diabetic rats with AGP including marked nuclear polymorphism of hepatocytes, cytoplasmic vacuolization with signs of hydropic degeneration, intracellular cholestasis, vascular hyperemia, and lymphohistiocytic infiltration within the portal tract regions. These findings align with those of Hnativ and Plytka (2023) who have reported significant disruption of lobular architecture and acute disturbances in portal vein circulation

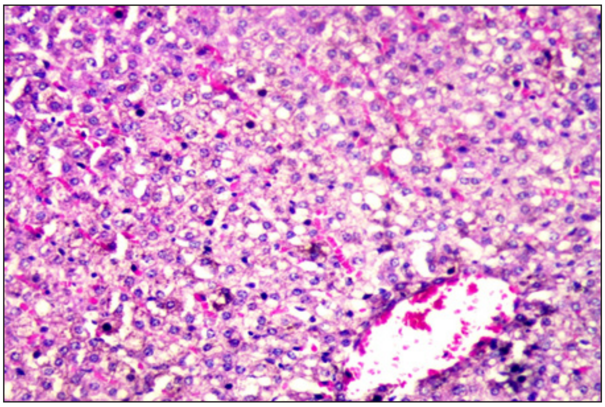

Figure 2. Histology of the liver structure on day 7 of acute generalized peritonitis (AGP) in non-diabetic rats (HE, ×400). Progressive dystrophic changes in hepatocytes are observed, accompanied by a disruption of trabecular organization.

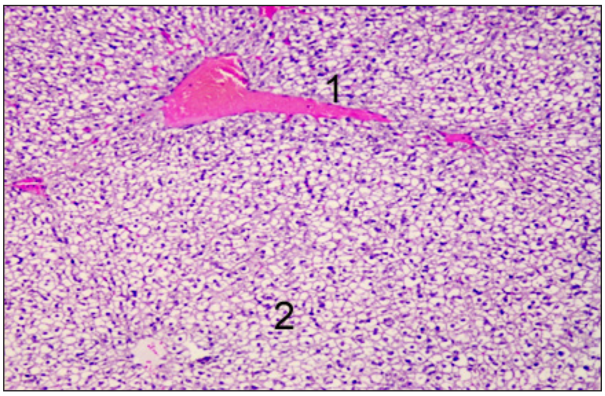

Figure 3. Histology of the liver structure on day 3 of acute generalized peritonitis (AGP) in diabetic rats (HE, ×200). Moderate dilation and congestion of portal vessels (1) and fatty and hydropic degeneration are observed (2).

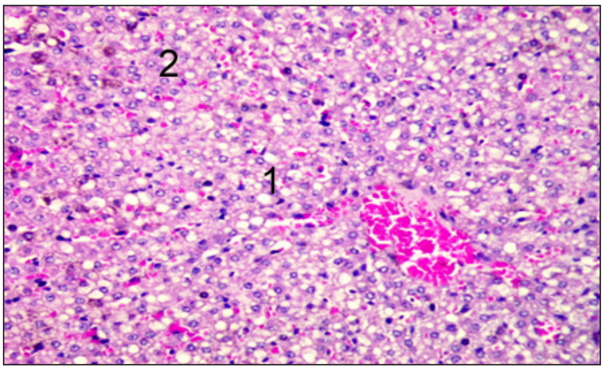

Figure 4. Histology of the liver structure on day 7 of acute generalized peritonitis (AGP) in diabetic rats (HE, ×400). Hepatocytes exhibit widespread fatty and hydropic degeneration characterized by cytoplasmic vacuolization and pallor (1). In some of the cells, intracellular cholestasis is present (2).

accompanied by marked fatty degeneration in the livers of guinea pigs with modeled peritoneal sepsis.

Collectively, the morphological and biochemical results reflect profound structural and functional disintegration of hepatic architecture. The data demonstrate a synergistic impact of metabolic dysregulation in the setting of peritonitis and diabetes mellitus mediated through mechanisms of endogenous intoxication and hepatic insufficiency. The reduction in protein-synthetic function, especially of albumin, emerges as a pivotal contributor to hepatic deterioration by facilitating the accumulation of free hydrophobic toxic compounds, further aggravating hepatocyte damage, and driving progressive liver dysfunction.

Conclusion. Experimental AGP in diabetic rats resulted in a pronounced endogenous intoxication accompanied by progressive morpho-functional liver damage culminating in hepatic insufficiency by day 7.

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References

Ahmed MME, Wang T, Luo Y, Ye S, Wu Q, Guo Z, Roebuck BD, Sutter TR, Yang JY. Aldo-Keto Reductase-7A protects liver cells and tissues from acetaminophen-induced oxidative stress and hepatotoxicity. Hepatology 54, 1322–1332, 2011.

Bancroft JD, Gamble M. (Eds.) Theory and practice of histological techniques, 6th Edition. Churchill Livingstone Elsevier, Philadelphia, PA, Elsevier Health Sciences 2008.

Barbeiro HV, Catanozi S, Barbeiro DF, Lorigados CB, Passarelli M, Soriano FG. Albumin replacement reduces mortality in hypoalbuminemic rats with LPS-induced endotoxemia, 2024. PREPRINT (Version 1) available at Research Square.

Filip SS, Skrypinets YP. Dynamics of endogenic intoxication findings and non-specific resistance in complex treatment of peritonitis by using endolymphatic combine therapy. Sci Bull Uzhhorod Univ Ser Med 1, 56–59, 2019.

Hnativ VV, Plytka OV. Simulation of peritoneal sepsis and its treatment with serum in an experiment: peculiarities of morphological disorders of liver, spleen and kidney tissues. Rep Morphol 29, 38–44, 2023.

Kumar A, Verma S, Gupta R. Endotoxemia and liver failure in secondary peritonitis: clinical correlations and prognostic implications. World J Gastroenterol 26, 1423–1435, 2020.

Li Y, Chen Z. Hepatic dysfunction in sepsis and peritonitis: role of inflammatory mediators and microcirculatory disturbances. J Hepatol 75, 894–906, 2021.

Mammadova ET. Endogenous intoxication indicators in advanced peritonitis and their correction. Klin Khir 88, 39–44, 2021.

Ramos-Lobo AM, Buonfiglio DC, Cipolla-Neto J. Streptozotocin-induced diabetes disrupts the body temperature daily rhythm in rats. Diabetol Metab Syndr 7, 39, 2015.

Recknagel P, Gonnert FA, Westermann M, Lambeck S, Lupp A, Rudiger A, Dyson A, Carré JE, Kortgen A, Krafft C, Popp J, Sponholz C, Fuhrmann V, Hilger I, Claus RA, Riedemann NC, Wetzker R, Singer M, Trauner M, Bauer M. Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis. PLoS Med 9, e1001338, 2012.

Reitman S, Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. Am J Clin Pathol 28, 56–63, 1957.

Savytskyi IV, Mukhin OM, Tsypoviaz SV, Merza YM, Zashchuk RG, Znamerovsky SG, Badiuk NS. Oxidative stress and lipid peroxidation in experimental peritonitis. Pharmacologyonline 1, 125–129, 2021.

Verveha BM, Gutyj BV, Lishchuk SH, Holubiev MI, Mylostyvyi RV. Oxidative modification of proteins and antioxidant status in blood of the rats with experimental acute generalized peritonitis against the background of streptozotocin-induced diabetes. Regul Mech Biosyst 14, 260–265, 2023.

Vlizlo VV, Maksymovych IA, Halyas VL, et al. Laboratory diagnostics in veterinary medicine (reference book). Lviv, 92, 2008.