# Clinical and morphological manifestations of acute hepatitis in fish

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**Abstract.** This study analyzed the modeling of acute toxic hepatitis in fish using phenol as an inducing agent under controlled experimental conditions. The effects of phenol exposure at a concentration of 4 mg/L for 48 hours on two-year-old goldfish were investigated. The formation of experimental and control groups of fish, followed by maintenance under standardized aquarium conditions, was studied. Blood sampling from the tail vein and liver collection after euthanasia for comprehensive analysis were determined. Biochemical testing of whole blood, including assessment of alanine aminotransferase and aspartate aminotransferase activity, was performed. Changes in total bile acid levels, a diagnostically significant indicator of liver function, were identified. Histomorphological analysis of the liver, including preparation and examination of tissue sections, was studied. The development of vacuolar degeneration of hepatocytes as the main morphological sign of acute hepatitis in fish was established. A combination of biochemical and morphological changes characterizing the pathological process in the liver was identified. A comprehensive description of the clinical and morphological manifestations of induced hepatitis in fish in an experimental model is presented.

## 1 Introduction

The study of liver pathologies in fish is a relevant and important task in various fields, including veterinary science, ecology, aquaculture, and biomedical research.

Fish are often used as indicators of the health of aquatic ecosystems. Liver pathologies in fish can indicate water pollution with toxic substances, such as heavy metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs). Studying these pathologies helps to assess the level of pollution and develop measures to improve water quality.

In aquaculture and fish farming, fish health directly impacts their productivity and product quality. Liver pathologies can lead to decreased growth, deterioration in meat quality, and even mass mortality. The study of these diseases allows the development of preventive and therapeutic measures, which contributes to increasing the efficiency and sustainability of fish farming.

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Liver pathologies can be caused by a variety of factors, including infections, toxins, and metabolic disorders. Studying these diseases helps veterinarians develop effective diagnostic and treatment methods, which is important for keeping fish healthy and preventing the spread of diseases.

The blood system reacts most quickly to changes in metabolic processes in the body. In addition to this, the liver is the most important histophysiological marker of the state of the body in fish. It can serve as a bioindicator by which one can judge the development of pathologies. At the same time, morphological changes that occur in fish in other organs and tissues (heart, muscle tissue, gonads, kidneys, etc.) can be considered, first of all, as a result of a decrease in the detoxification function of the liver. Thus, the diagnosis of fish diseases associated with liver damage and metabolic disorders should be based not only on biochemical markers but also on an assessment of the degree of change in the structure of liver tissue.

Taking into account the above, we set a goal - to determine the characteristics of the clinical and morphological manifestations of acute hepatitis in fish, using the example of Prussian carp.

## 2 Materials and Methods

The study was conducted using two-year-old Prussian carp purchased by the Federal State Budgetary Educational Institution of Higher Education “St. Petersburg State University of Veterinary Medicine”, with an average body length of 15.3±1.5 cm and an average weight of 52.2±3.4 g. The study used 20 fish, divided into experimental (n=10) and control groups (n=10).

Complied with the basic requirements set out in the “Rules of Laboratory Practice” in accordance with the Order of the Ministry of Health and Social Development of the Russian Federation dated August 23, 2010 No. 708n “on approval of the Rules of Laboratory Practice”. Ethical principles for the treatment of laboratory animals were observed in accordance with the “European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. cets no. 123.” All manipulations with the animals were carried out in accordance with the ethical principles approved by the Ethics Committee at the Federal State Budgetary Educational Institution of Higher Education "Saint-Petersburg State University of Veterinary Medicine".

During the 7-day acclimatization period before the experiment, adult fish were kept under optimal conditions in freshwater aquariums filled with tap water that had been filtered and oxygenated (AQUAEL FAN 1 plus internal filter, 320.0 l/h), at an average temperature of 20.0±1.0°C, a daylight period of 12 h/12 h, and twice-daily feeding with commercial dry granules (Tetra Wafer Mix, Melle, Germany). The fish in the experimental group were induced to develop acute hepatitis by inoculation with phenol at a concentration of 4.0 mg/l for 48 hours.

At the end of the experiment, blood samples were collected from the tail vein, and the fish were euthanized (using MS-222 (Tricaine Methanesulfonate), as recommended by AVMA) in order to collect liver samples for histomorphological examination (3 technical replicates were used in the experiment in order to exclude artifacts during the study, as well as for the purpose of statistical reliability).

Biochemical studies of whole blood were performed on a veterinary automated biochemical analyzer, MNCHIP Celercare V5 (MNCHIP (China)), by adding 100.0 µl of whole blood to the reagent disk of the analyzer MNCHIP Profile "Extended" Comprehensive (24). To assess the condition of the liver, the level of intracellular transaminases was determined: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the total concentration of bile acids.

From the obtained liver tissue samples, histological sections were made according to the generally accepted method, which were stained with hematoxylin and eosin. The obtained sections were studied on an Olympus BX43 microscope using Olympus cellSens Entry software.

## 3 Results

When studying histological sections, it was established that the liver of the studied fish species is covered with a thin capsule of fibrous connective tissue. The organ parenchyma is represented by liver cells - hepatocytes. The latter do not form ordered beams characteristic of mammals, but form plates anastomosing with each other, separated by sinusoids. Due to this organization of the parenchyma, there is no clear division into lobules, as a result of which, in the sections studied, it has a spongy appearance (Figure 1).

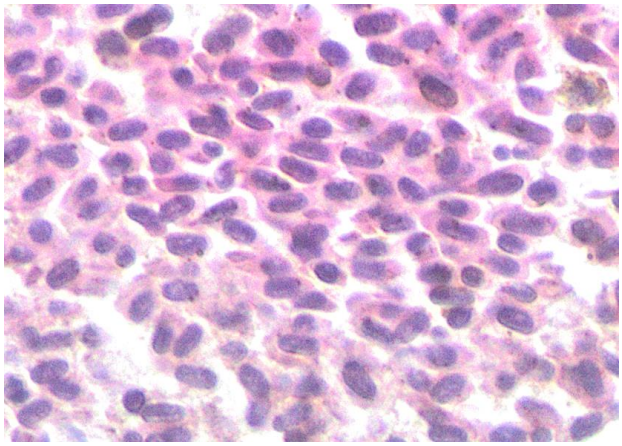

**Figure 1.** Histological section of Prussian carp liver. Hematoxylin and eosin staining. Magnification ×400

Normally, hepatocytes have a prismatic shape and contain one, rarely two, basophilic nuclei of a rounded ovoid shape, occupying a central position in the cell. In the case of fat deposits in the cytoplasm, its nuclei are displaced in clusters to the basal pole of the cell.

The sinusoids are lined with highly flattened endothelial cells that extend along the sinusoids. They have a small volume of cytoplasm and one nucleus. In the area of its location, endotheliocytes have the maximum height, so on histological sections it seems that the nuclei protrude into the lumen of the sinusoid.

Sometimes in the lumen of the sinusoids there are fat-containing cells, which, unlike hepatocytes, have a large part of the cytoplasm filled with large fat droplets.

Also in the lumen of the sinusoids there are erythrocytes (Figure 2). The latter have basophilic nuclei of an oval-elongated, sometimes cigar-shaped form, and oxyphilic cytoplasm, stained in a crimson hue. Also, accumulations of melanomacrophages were detected in the liver parenchyma (Figure 2).

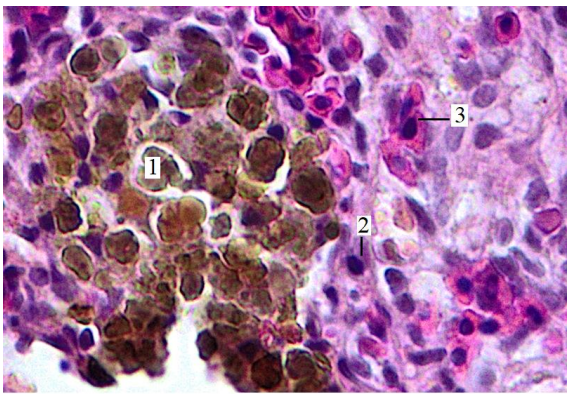


**Figure 2.** Histological section of Prussian carp liver. Hematoxylin and eosin staining. Magnification ×400: 1 – accumulation of melanomacrophages; 2 – hepatocyte; 3 – erythrocyte**.**

Also in the liver tissue, bile ducts are found, formed by the fusion of bile capillaries formed by the cytoplasmic membranes of the bile poles, hepatocytes in contact with each other. The epithelial lining of the bile ducts was represented by a single layer of cubic epithelial cells. In large ducts, it was replaced by a prismatic layer, and their wall contained a developed layer of smooth myocytes and a surrounding layer of connective tissue. Unlike hepatocytes, the epithelial cells of the bile ducts had a single large ovoid nucleus with a light matrix.

In addition to the above structures, the histological sections examined revealed clusters of cells separated from the liver parenchyma by a thin layer of connective tissue. These cells were differentiated by us as endocrine pancreatic cells that form pancreatic islets. They were characterized by a weak basophilic staining of the nuclei and cytoplasm (Figure 3).

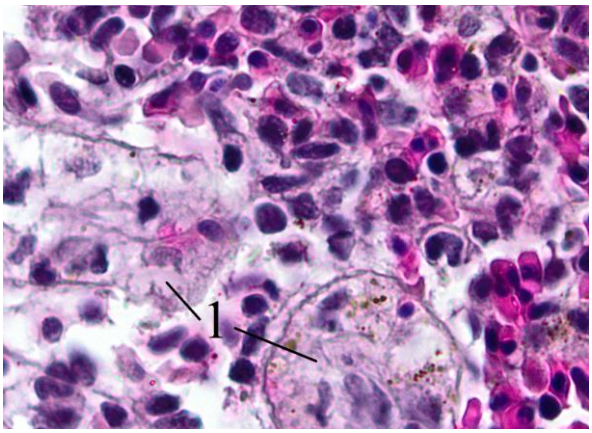


**Figure 3.** Histological section of Prussian carp liver. Hematoxylin and eosin staining. Magnification ×400: 1 – accumulation of endocrine pancryocytes.

Histological preparations of fish liver with induced hepatitis (Figure 4) showed changes characteristic of vacuolar dystrophy. Thus, uneven coloring of the hepatocyte cytoplasm was observed - there was intense coloring near the nucleus, and its peripheral part, forming vacuoles of different sizes filled with cytosol, remained uncolored. Karyolysis was observed in some hepatocytes. It should also be noted that the sinusoids were significantly expanded and heavily filled with blood cells, indicating its stagnation.

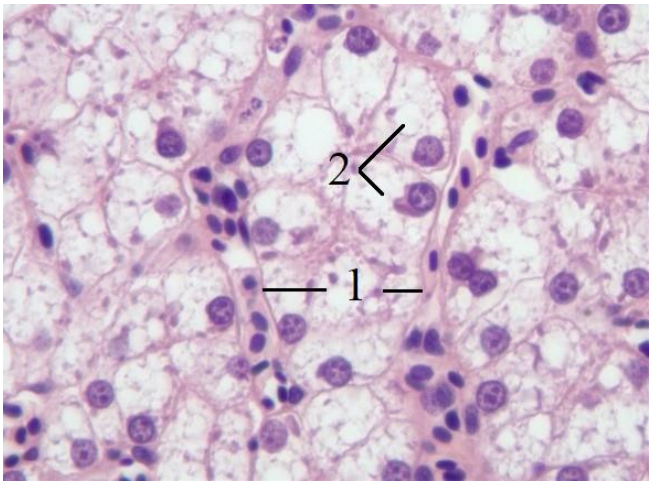


**Figure 4.** Histological section of Prussian carp liver with induced acute hepatitis. Hematoxylin and eosin staining. Magnification ×400: 1 – sinusoids filled with erythrocytes; 2 – vacuolated hepatocytes.

Based on the data in Table 1, in fish that did not have morphological signs of acute hepatitis, the ALT and AST activity was 2.03±0.14 U/L and 2.42±0.11 U/L, and the concentration of bile acids was 5.88±0.36 μmol/L.

**Table 1** The value of biochemical parameters of the blood of the studied fish.

| Parameter | Units | Healthy Prussian carp | Prussian carp with induced acute hepatitis |
|---|---|---|---|
| ALT | U/L | 2,03±0,14 | 41,39±2,55 |
| AST | U/L | 2,42±0,11 | 39,16±3,04 |
| Bile acids | μmol/L | 5,88±0,36 | 26,8±2,77 |

In fish with induced acute hepatitis, ALT and AST activity was 20.39 and 16.18 times higher, compared to fish without morphological signs of acute hepatitis. At the same time, the total level of bile acids in them was 4.56 times higher than in healthy fish.

## 4 Discussion

The conducted study was aimed at a comprehensive characterization of the clinical and morphological manifestations of acute toxic hepatitis induced by phenol in Prussian carp (Carassius gibelio). The results obtained demonstrate a clear correlation between the severity of the pathological process and the severity of disorders at the biochemical, cellular, tissue, and organismal levels.

The most pronounced morphological sign of acute hepatitis in the study was the development of vacuolar (hydropic) dystrophy of hepatocytes. The rarefaction of the cytoplasm with the formation of vacuoles, karyolysis, and uneven staining observed in histological sections are classic signs of cell damage. The phenomenon indicates a profound disruption of metabolism and energy balance in hepatocytes caused by the toxic effects of phenol, which leads to dysfunction of ion pumps and accumulation of fluid in the cells. An

important pathophysiological sign was also a significant expansion of the sinusoids, filled with erythrocytes. This fact indicates the development of venous congestion and impaired microcirculation in the liver, which aggravates hypoxia and contributes to the further progression of dystrophic processes.

The findings of this study align with previously reported hepatotoxic effects of phenolic compounds in aquatic organisms but provide novel quantitative correlations between histological damage and bile acid dysregulation. Phenol, a common industrial pollutant, is known to induce oxidative stress in fish liver by generating reactive oxygen species (ROS) that overwhelm endogenous antioxidant defenses (e.g., glutathione peroxidase, catalase). The observed vacuolar dystrophy likely results from ROS-mediated lipid peroxidation of hepatocyte membranes, leading to impaired $Na^+/K^+$-ATPase activity and subsequent hydropic swelling. This mechanism is supported by the 20-fold increase in ALT, which exceeds typical values reported for subacute phenol exposure in other cyprinids (e.g., 8- to 12-fold increases in common carp), suggesting that Prussian carp may be particularly sensitive to phenol or that the concentration (4 mg/L for 48 h) represents a severe acute challenge.

Importantly, the 4.56-fold elevation of total bile acids (from 5.88 to 26.8 μmol/L) is a critical finding rarely quantified in fish hepatology. In mammals, bile acid accumulation in blood directly correlates with the degree of cholestasis and hepatocellular necrosis. In fish, this marker has been underexplored. The present data indicate that bile acid measurement is a robust, non-lethal (when blood is sampled) indicator of acute liver injury, potentially more specific than transaminases, which can also rise after muscle damage. Future studies should fractionate bile acids into individual species (e.g., cholic acid, chenodeoxycholic acid, and their taurine conjugates) because different profiles may reflect distinct etiologies (toxic, infectious, or nutritional).

Biochemical data are completely consistent with the morphological picture. A 20-fold increase in ALT activity and a 16-fold increase in AST is direct evidence of massive cytolysis (destruction) of hepatocytes, since these enzymes are released into the bloodstream when cell membranes are damaged. Such a significant increase in transaminase activity clearly confirms the acuteness and severity of the pathological process. An equally important diagnostic marker was a 4.5-fold increase in the level of total bile acids (BA) in the blood. This result indicates a serious impairment of the excretory function of the liver. Normally, fatty acids are synthesized in hepatocytes and excreted in bile. When the liver is damaged, this process is disrupted, which leads to cholemia, the accumulation of fatty acids in the blood, which further aggravates the intoxication of the body and can negatively affect other organ systems.

The obtained data on the structural organization of the liver in healthy Prussian carp (absence of a clear lobular structure, spongy appearance of the parenchyma) are consistent with classical concepts of the histology of fish liver. The identified pathological changes (vacuolar dystrophy, increased transaminases, and fatty acids) also correspond to the results of other studies devoted to toxic liver damage in various fish species caused by both chemical pollutants and mycotoxins.

From an ecological monitoring perspective, the proposed biomarker panel (ALT, AST, and total bile acids) offers a cost-effective screening tool for assessing wild fish populations in polluted watersheds. However, baseline values vary among species, seasons, and nutritional states. For Prussian carp, the reference intervals established here (ALT 2.03±0.14 U/L, AST 2.42±0.11 U/L, bile acids 5.88±0.36 μmol/L) should be validated across different age classes, water temperatures, and dietary regimes before broad application. In field settings, confounding factors such as concurrent parasitic infections (e.g., Myxobolus spp.) or nutritional hepatic steatosis may mimic toxic changes. Therefore, histology remains indispensable for confirming etiology.

In aquaculture, the findings underscore the need to monitor feed and water quality for hepatotoxic contaminants. Phenol can leach from certain plastics, paints, or disinfectants used in recirculating aquaculture systems (RAS). Subclinical liver damage may go unnoticed until growth performance or feed conversion ratios decline. The 4.5-fold bile acid elevation observed here likely impairs fat digestion and absorption, leading to malnutrition despite adequate feeding. Aquaculturists should consider periodic blood bile acid measurements as a sentinel indicator of RAS health. Furthermore, hepatoprotective dietary supplements – such as taurine, betaine, and plant-derived flavonoids (e.g., silymarin) – could be tested using the phenol-induced hepatitis model to quantify their efficacy in reducing bile acid elevation and histological lesions.

## 5 Conclusion

The study of liver pathologies in fish is a topical issue of great importance for ecology, aquaculture, veterinary science, biomedical research, and economics. These studies help to improve the state of aquatic ecosystems, increase the efficiency of fish farming, and develop new diagnostic and treatment methods, as well as adapt to global changes and ensure sustainable development of the fishing industry.

While this study provides valuable novel data, several limitations must be acknowledged. First, the sample size (n=10 per group) is relatively small, and no power analysis was reported. Although the observed effect sizes are large (CV% <10% for most parameters), small sample sizes increase the risk of type II errors for subtle differences and limit generalizability. Second, only one time point (48 hours post-induction) was examined; thus, the temporal dynamics of liver injury and recovery remain unknown. A time-course study (e.g., 6, 12, 24, 48, 72 hours, and post-exposure recovery at 1 and 2 weeks) would reveal whether bile acids peak earlier or later than transaminases, which is critical for diagnostic interpretation. Third, the study lacked a sham control group (exposed to solvent or vehicle only if phenol was dissolved in a carrier). Although phenol is water-soluble and no solvent was mentioned, it is good practice to include a group handled identically but without phenol to control for handling stress effects. Fourth, only one sex was not specified – Prussian carp used were two-year-olds but sex was not determined; sex-dependent differences in susceptibility to hepatotoxicants are known in teleosts (often females are more sensitive due to estrogen effects on bile acid metabolism). Future studies should include sex as a biological variable.

The results of the conducted research have significant practical significance for several areas:

1. Environmental monitoring and bioindication. The proposed model of induced toxic hepatitis in fish and a set of histopathological criteria (such as vacuolar dystrophy, hepatocyte necrosis, and liver plate lysis) in combination with a biochemical marker (the level of bile acids) can be used as a sensitive bioindication system. This allows to assess the degree of pollution of aquatic ecosystems by xenobiotics with hepatotoxic effects (pesticides, heavy metals, and industrial waste) at early stages, even in the absence of mass death of aquatic organisms.

2. Aquaculture. The data obtained are important for developing measures for the prevention and treatment of liver diseases in fish in commercial farming conditions. Understanding the relationship between histological damage and bile acid metabolism disorders opens up opportunities for adjusting feed additives that improve liver function and for screening hepatoprotective drugs.

3. Fundamental biology. The work contributes to the understanding of the pathophysiological mechanisms of liver damage in fish under the influence of toxicants. The established correlations between structural changes in the liver and the imbalance of

bile acids, which are key regulators of digestion and metabolism, deepen the knowledge of the consequences of chemical stress for the fish organism as a whole.

Prospects for further research are seen in the following directions:

1. Deepening into molecular mechanisms. It is advisable to study the expression of genes associated with the synthesis, transport, and conjugation of bile acids (e.g., CYP7A1, BSEP, and NTCP genes), as well as markers of apoptosis and inflammation (caspases and cytokines), for a more accurate understanding of the primary molecular targets of the studied toxicant.

2. Evaluation of the recovery potential. An important stage is conducting post-intoxication studies to assess the ability of fish liver to regenerate after exposure to the toxicant has ceased. It is necessary to establish the time frame and the degree of reversibility of the identified histological and biochemical disorders.

3. Search for hepatoprotective agents. Based on the developed model, it is possible to screen natural or synthetic compounds (e.g., selenium, silymarin, pre- and probiotics) to assess their effectiveness in reducing histopathological damage and normalizing the bile acid profile.

4. Study of remote consequences. A promising direction is the study of the effect of sublethal toxic liver damage on other vital functions of fish: reproductive success, growth, immune status, and resistance to pathogens.

5. Expansion of the species composition. The obtained results need to be verified on other fish species of economic importance or acting as key species in aquatic ecosystems to assess the generality of the identified patterns.

Thus, acute hepatitis in fish is morphologically manifested by the development of vacuolar dystrophy of the liver. At the same time, its clinical manifestation is an increase in the activity of ALT and AST, as well as the general level of bile acids.

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