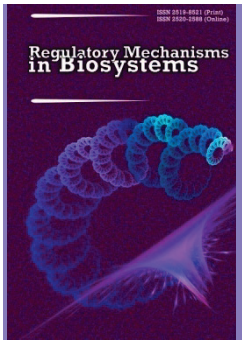

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Pathomorphological changes in laboratory animals exposed to lethal doses of disinfectants

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The use of disinfectants is a crucial aspect of preventive and health improvement measures for infectious diseases in farm and domestic animals. Regulatory documents require the determination of toxicity to macroorganisms, including the establishment of lethal doses and toxicity groups, during the development and registration of antimicrobial agents. This study aimed to investigate the histological changes in the internal organs of laboratory animals when determining lethal doses of innovative disinfectants. The experiments used domestic disinfectants containing glutaraldehyde as the active ingredient. Histological studies were conducted on the internal organs (kidney, liver, stomach, intestines, and spleen) of 75 laboratory animals using an Axioskop 40/40FL microscope (Carl Zeiss, Germany) with video microscopic photography. The methods used were under current standards. Hemodynamic disorders were observed in the renal tissue under the influence of lethal doses of aldehyde disinfectants. These disorders were characterized by capillary dilation and blood filling. Glomerular capillary dilation and overflow with blood cells were also detected. Additionally, stasis was observed in the lumen of the microcirculatory vessels throughout the entire length. The examination of histological sections from animal liver samples revealed a significant expansion of Dissé spaces, variations in the size of hepatocyte nuclei, beam decomposition and fragmentation, small acute perivascular hemorrhages, leukostasis in sinusoids, and hemodynamic disorders. The structure of the organ's beams was also disturbed, and a significant number of venous vessels were dilated and excessively filled with blood cells. Minor changes were detected in the stomach, including desquamation of the epithelial cells of the glands and their exfoliation into the gastric lumen, as well as circulatory disorders. Epithelial desquamation, blood vessel dilation, and signs of connective tissue edema were observed in the intestine. The kidneys exhibited signs of acute venous hemorrhage and stasis in vessels of various calibers, with the development of small acute parenchymal hemorrhages and localized lymphoid cell death in the white pulp. The prospect of further research is to investigate the histomorphological changes in the internal organs of laboratory animals when exposed to modern complex disinfectants with different active ingredients.

Keywords: disinfectant; LD_{50}; toxicity; laboratory animals; pathological changes; histology; internal organs.

Introduction

Prevention and control of infectious animal diseases is a crucial task in modern veterinary science. Currently, the epizootic situation of most economically important and emerging animal diseases in Ukraine is under control. However, anthropogenic challenges pose unpredictable risks of infection outbreaks among productive livestock and domestic animals. There is an urgent need to provide veterinary specialists with modern means of diagnosis, treatment, and prevention of pathological states in animals (Francis, 2020; Aida et al., 2021). Strict implementation of zootechnical, technological, and sanitary measures on livestock farms and complexes can prevent most diseases (Wang & Hu, 2023). Vaccines and treatments have been developed and introduced for many infectious diseases. However, disinfection remains the only effective measure to prevent certain diseases (Paliy, 2018; Buzun et al., 2023; Frost et al., 2023).

Various detergents and disinfectants with different compositions are used for veterinary and sanitary purposes (Rodionova et al., 2021; Frost et al., 2023; Rhee et al., 2023). The choice of disinfectant depends on several factors, including the spectrum of bactericidal action, cost, and environmental impact (Montagna et al., 2019; Alajlan et al., 2022). The use of disinfectants has become widespread, resulting in the development of a resistant microbiota. This, in turn, reduces the effectiveness of some disinfectants (Rozman et al., 2021; Tong et al., 2021). Studies have shown that resistance to certain chemicals can lead to cross-resistance to other biocides (Burgess et al., 2017). Glutaraldehyde, either alone or in combination with other antimicrobial compounds, is widely used as a high-level disinfectant worldwide (Burgess et al., 2017). It has a strong bactericidal effect in solution and is also used to create new antibacterial polymers (Sehmi et al., 2016) and combined disinfectants with high stability and antimicrobial action (Lin et al., 2018). When developing and registering new antimicrobial compounds, determining their toxicity is a key step. This criterion assesses the practical feasibility of using disinfectants in the presence or absence of staff and animals.

Various methods and biological objects are used to assess the toxicity of drugs (Laingam et al., 2012; Ren et al., 2022; Seo et al., 2022). However, the most informative and closest to the practical conditions of using chemical compounds is the use of laboratory animals (Marxfeld et al., 2019; Jantawong et al., 2021; Lieshchova & Brygadyrenko, 2022). Mice are reported to be more sensitive than rats to acute oral toxicity (Ballantyne & Myers, 2001). The objective of this study is to assess the morphological changes in the internal organs of laboratory animals following the oral administration of lethal doses of aldehyde-containing disinfectants.

Materials and methods

Experimental studies were conducted at the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv). The experiments used the latest disinfectants developed at the NSC "IECVM": "DZPT-2" (composition: 25% glutaraldehyde; surfacetants; fragrance); "FAG" (composition: 25% glutaraldehyde; 37% formaldehyde). The study was conducted on white mice (n = 75). In the preliminary stage, experiments were conducted to determine the acute toxicity (LD_{50}) of disinfectants. The acute toxicity (LD_{50}) of the drugs was determined by processing experimental data using the method of Kerber (1931) (Karpenko et al., 2022). For histomorphologic studies, organ samples were fixed in a 10% buffered formalin solution. To perform paraffin embedding of the organ samples, the material was washed with running tap water until the fixative was removed. For dehydration and compaction of the material, it was placed in solutions of ethyl alcohol, alcohol-chloroform, chloroform of increasing concentration (70%, 80%, 90%, 96%, 100%). Histological sections of 5–7 μm thickness were made on a rotary microtome MPS-2 according to the methods used in histological studies (Alturkistani et al., 2015; Szunyogova & Parson, 2016), followed by hematoxylin-eosin (H+E) staining. The magnification (bars) of the presented objects is 50, 100, 200 μm. Histological preparations were studied using an Axioskop 40/40FL microscope (CarlZeiss, Germany) with subsequent video microscopic photography.

Selection of laboratory animals, formation of research groups and manipulations with them were carried out in accordance with generally accepted methods and requirements in compliance with bioethics. The experiments conducted on the animals did not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On the protection of animals from cruelty) and the "General ethical principles for experiments on animals" adopted by the First National Congress on Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals used for Experimental and other Purposes, Strasbourg, 1985) (Simmonds, 2017). The research program was reviewed and approved by the Bioethics Committee of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" under the current procedure.

Results

Pathological changes were observed in the tissues and organs of laboratory animals following oral administration of LD_{50} doses of "DZPT-2" (251.2 ± 0.5 mg/kg) and "FAG" (283.1 ± 1.0 mg/kg). The respiratory tract was characterized by the presence of a significant amount of foamy fluid. The mucous membrane appeared pale, while the lungs were light red and contained fluid. The heart was blood-filled and the myocardium was red. The liver was enlarged, flabby, and dark in color with rounded edges. The spleen appeared dark red, while the kidneys were flabby. The mesenteric lymph nodes were distinguishable, as shown in Figure 1.

Also, the effect of lethal doses of new disinfectants on the body of white mice after their oral administration was studied by examining histological disorders in organs and tissues. The results of histomorphological studies of renal tissue in normal conditions and after exposure to disinfectants are shown in Figure 2.

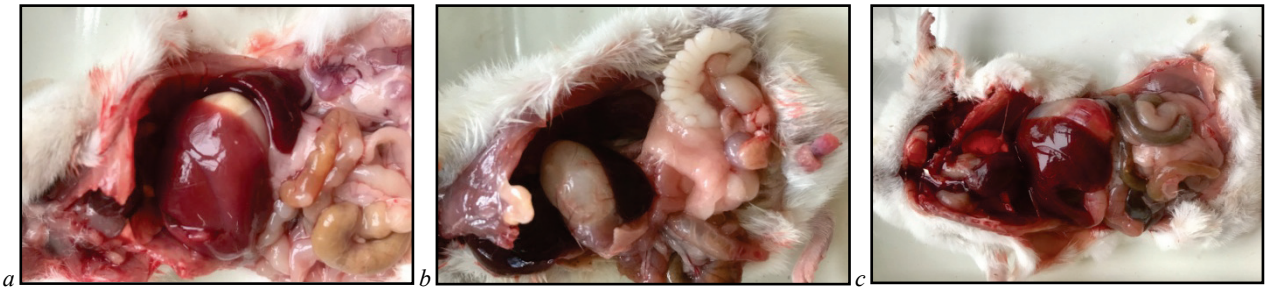

Fig. 1. Internal organs of a white mouse: *a* – normal; *b* – under the influence of "DZPT-2"; *c* – under the influence of "FAG"; n = 15

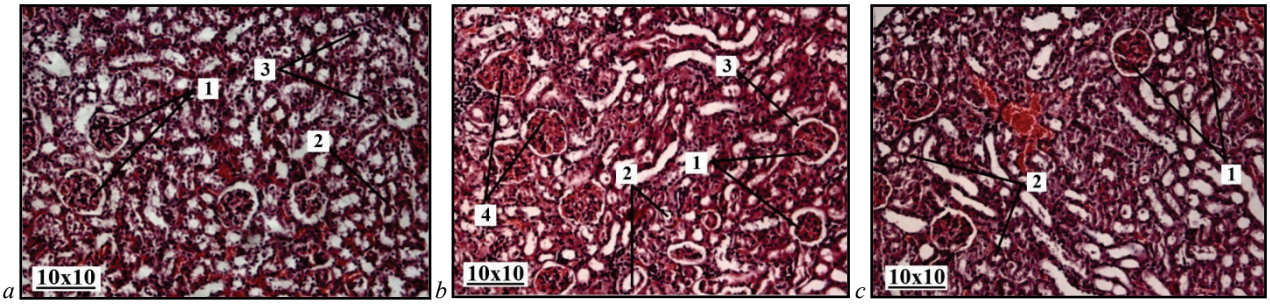

Fig. 2. Renal tissue of a white mouse (×100): *a* – normal (*1* – renal cells; *2* – urinary tubules; *3* – venous sinuses); *b* – under the influence of "DZPT-2" (*1* – renal cells; *2* – urinary tubules; *3* – nephron capsule; *4* – capillaries); *c* – under the influence of "FAG" (*1* – renal cells; *2* – urinary tubules); n = 15

The histological examination revealed (Fig. 2) that the kidney of the control group was covered with a fibrous capsule. The cortical and cerebral substances were clearly expressed. The nephrons were mainly located in the cortical substance. They are an epithelial urinary tubule closely connected to blood vessels. In the lower part of the cortex, at the border with the cerebral cortex, there are the renal corpuscles of the juxtamedullary nephrons. In the renal corpuscles, capillary glomeruli are densely arranged in a double-walled capsule. The capsule is thin, without stratification, without deposits on the surface. The glomeruli have a "paw-like" appearance, formed of capillaries with a distinct endothelium. There is a clear, narrow gap between the glomerulus and the glomerular capsule, containing neither fluid nor blood. There are no fusions between the capsule and the glomerulus. The glomeruli are surrounded in different planes by sections of the proximal and distal convoluted tubules. Urinary tubules are of the same diameter, the lining of the tubules is represented by a single-layer cuboidal epithelium located on the basement membrane. The stroma is composed of loose connective tissue and vessels of different caliber.

Hemodynamic disorders were found in samples of renal tissue from laboratory animals after the use of disinfectants "DZPT-2" and "FAG". These disorders were characterized by paretic dilation and excessive blood filling of capillaries, particularly those in the subcapsular zone. Glomerular capillaries were slightly dilated and filled with blood cells, resulting in enlarged renal corpuscles. Stasis and sludge were observed in the lumen of microcirculatory vessels, while in venous vessels, the phenomenon of marginal leukocyte standing was observed as a manifestation of acute stagnation in this part of the bloodstream. The vessels showed an endothelial reaction of the 'palisade' type without signs of detachment, as well

as perivascular cell-free spaces, indicating perivascular edema. The histological structure of the urinary tubules was normal, with no cells or cell-free masses in the lumen. However, the lumen of the distal tubules was slightly dilated. Figure 3 displays the results of histomorphologic studies conducted on liver tissue, both in normal conditions and after exposure to disinfectants.

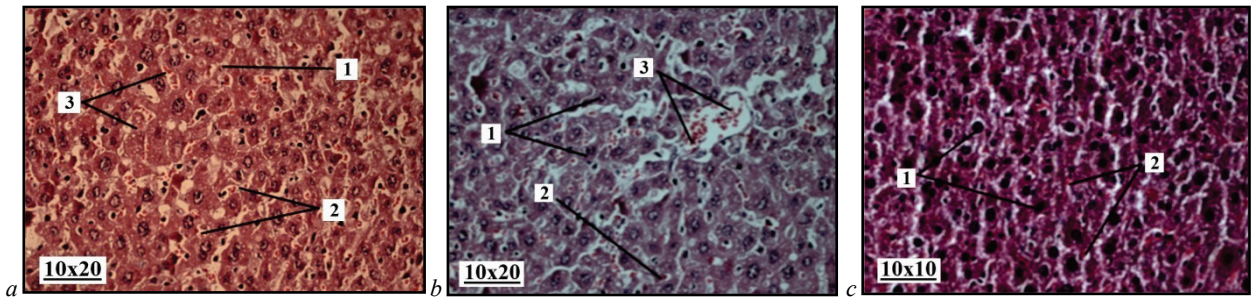

Fig. 3. Liver tissue of a white mouse (×100, ×200): *a* – normal (*1* – hepatic beams; *2* – capillaries; *3* – red blood cells); *b* – under the influence of "DZPT-2" (*1* – hepatocytes; *2* – capillaries; *3* – blood cells); *c* – under the influence of "FAG" (*1* – hepatocytes; *2* – blood cells); n = 15

Histological studies have revealed that the liver was encapsulated by fibrous tissue, which extended into thin partitions that divided the gland into lobules. These lobules were composed of hepatic laminae, consisting of hepatocytes and sinusoids, which are capillaries of a unique 'open' type without a basement membrane or endothelium. The interlobular bile ducts were lined with a single-layer of low epithelium on the basement membrane. As the diameter of the ducts increases, the epithelial cells became taller and adopted a prismatic shape. Loose connective tissue appeared around the ducts. The arteries and veins had a typical histological structure and, together with the bile duct, are part of the "triads". The central vein is located in the center of the liver lobe. The shape of the liver lobe was often irregular, mostly close to triangular and quadrangular. Hepatocytes formed beams, the cells were predominantly uninucleated (up to 30% are binucleated and multinucleated), with a distinct granular eosinophilic cytoplasm and indistinct intercellular boundaries.

The histological sections of animal liver samples were studied after the use of "DZPT-2". The results showed significant expansion of Dissé spaces. The nuclei of hepatocytes varied in size, with 1 to 3 large nuclei clearly defined in hepatocytes against the background of enlightened chromatin. There was a discomplementation of the beams, their fragmentation, the boundaries of hepatocytes were focally indistinct, and the membrane of hepatocytes was focally uneven and had 'torn' edges. Small acute perivascular hemorrhages, mainly around the central veins, and leukostasis were present in the sinusoids. The cytoplasm of hepatocytes displayed uneven granulation with small light vacuoles, indicating hydropic dystrophy. Following treatment with the "FAG" disinfectant, we observed dystrophic changes in hepatocytes, including chromatin condensation and cytoplasmic coagulation (a severe form of protein parenchymal dystrophy), as well as hemodynamic disorders such as stasis in blood vessels, sludge, and red blood cell adhesion. The beam structure of the organ was also disturbed, and hepatocytes exhibited nuclei of varying sizes. Additionally, we observed many cells with dark nuclei lacking nucleoli, as well as phenomena of karyorexia and perinuclear halos. Some hepatocytes exhibited signs of granular hepatocyte dystrophy, characterized by fine eosinophilic granularity in the cytoplasm. Additionally, a significant number of venous vessels were dilated and filled with blood cells.

The results of histomorphologic studies of the stomach in normal conditions and after exposure to disinfectants are shown in Figure 4. The histological examination of stomach samples from the control group (Fig. 4) revealed that the cardiac part of the stomach was covered with a single-layer cylindrical glandular epithelium. The cardiac glands were located in the mucosa's own plate, extending from the muscular plate of the mucosa. The submucosal base and glandular ducts were separated by an intrinsic mucosal plate that was heavily infiltrated by diffuse lymphoid tissue. This tissue formed dense conglomerates of lymphoids, plasma cells, and macrophages. The secretion of mucous cells in the gastric glands was moderate. The muscular membrane was composed of smooth muscle tissue. The outer membrane was serous, consisting of connective tissue covered with mesothelium.

Minor changes were observed after the use of disinfectants "DZPT-2" and "FAG". These changes were manifested by the desquamation of glandular epithelial cells with their exfoliation into the lumen of the stomach or glands and dilation of the lumens of the glands and crypts. Circulatory disorders were also verified, including interstitial edema and full blood vessels of the microcirculatory channel.

Figure 5 displays the results of histomorphologic studies conducted on the small intestine, both in its normal state and after exposure to disinfectants. Figure 5 illustrates the finger-like outgrowths of the intestinal villi, which are composed of loose fibrous connective tissue. The villi are covered by a single layer of cylindrical epithelium. The connective tissue base of the villi is composed of microcirculatory vessels, loose connective tissue, lymphoids, and other cellular elements. The basement membrane is moderately infiltrated with lymphoid cells, forming mucosa-associated lymphoid tissue. The lamina propria contains blood vessels. The muscle membrane is composed of two layers of myocytes: the inner circular layer and the outer punctate layer. The connective tissue between these layers contains the autonomic nerve plexus.

Based on the materials presented in Figures 5b and 5c, it was observed that the use of "DZPT-2" and "FAG" in the intestine resulted in the fragmentation of villus tops, desquamation of the epithelium, and exfoliation of individual cells into the intestinal lumen. Additionally, the vessels of the lamina propria were dilated and filled with blood without signs of erythrocyte lysis. Connective tissue with unevenly expressed signs of edema was also observed.

Figure 6 displays the results of histomorphologic studies conducted on the spleen under normal conditions and after exposure to disinfectants.

The histological examination showed that the spleen is covered by a capsule of dense fibrous tissue (Fig. 6). Trabeculae, which contain blood vessels, smooth muscle fibers, and nerves, extend from the capsule. In all study groups, the white pulp occupies a large area and is organized into cylindrical clutches of lymphocytes that surround the central arteries forming periarterial lymphoid sheaths. The red pulp consists of sinusoidal vessels and pulp cords located between them.

The study showed that treatment with disinfectants "DZPT-2" and "FAG" caused histological changes in animals. These changes were characterized by the local death of lymphoid cells in the white pulp, as well as signs of acute venous hemorrhage and stasis in vessels of various calibers, with the development of small acute parenchymal hemorrhages. The white pulp appeared to be reduced due to pronounced blood filling, with the appearance of light cell-free zones in the centers of some follicles (edema zone).

Summarizing the obtained results, it is evident that lethal doses of disinfectants "DZPT-2" and "FAG" caused hemodynamic disorders in the liver and kidneys, alternative lesions of hepatocytes and enterocytes, and lympholytic changes in the spleen in experimental animals.

Discussion

When selecting a disinfectant, it is important to consider both its effectiveness and its impact on the environment and macroorganisms (Park, 2016; Chhetri et al., 2019; Zhang et al., 2023). According to the literature, disinfectant chemicals can be found in the environment and a significant

portion of them are toxic to fish, algae, and daphnia (Musee et al., 2023). Determining the toxicity of new chemical compounds is a crucial step in registering a disinfectant. Although glutaraldehyde has received negative criticism in the past (Beauchamp et al., 1992), it is currently widely used as the main active ingredient in many modern disinfectants (Burgess et al., 2017; AlZain, 2019; Brill et al., 2020). It is reported that products containing glutaraldehyde are mainly used as farm disinfectants (99.2%) with an average concentration of 15%. Most products (76.2%) contain excipients such as cationic detergents and formaldehyde (Thumtecho et al., 2021). There is also no clear evidence of genetic toxicity of glutaraldehyde to humans or animals (Takigawa & Endo, 2006). In the experiments, disinfectants with the active ingredient glutaraldehyde were used, in one case with the excipient benzalkonium chloride (DZPT-2), and in the other with formaldehyde (FAG).

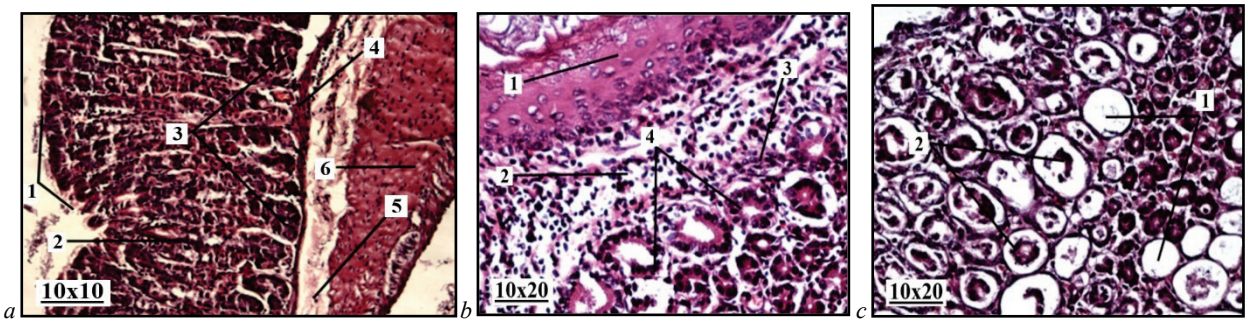

Fig. 4. Stomach of a white mouse (×100, ×200): *a* – normal (*1* – glandular epithelium; *2* – mucosa; *3* – gastric glands; *4* – muscular plate; *5* – submucosa; *6* – muscle layer); *b* – under the influence of "DZPT-2" (*1* – multilayer squamous epithelium of the esophagus; *2* – submucosa; *3* – muscle fibers; *4* – glands); *c* – under the influence of "FAG" (*1* – glands; *2* – desquamated epithelium); n = 15

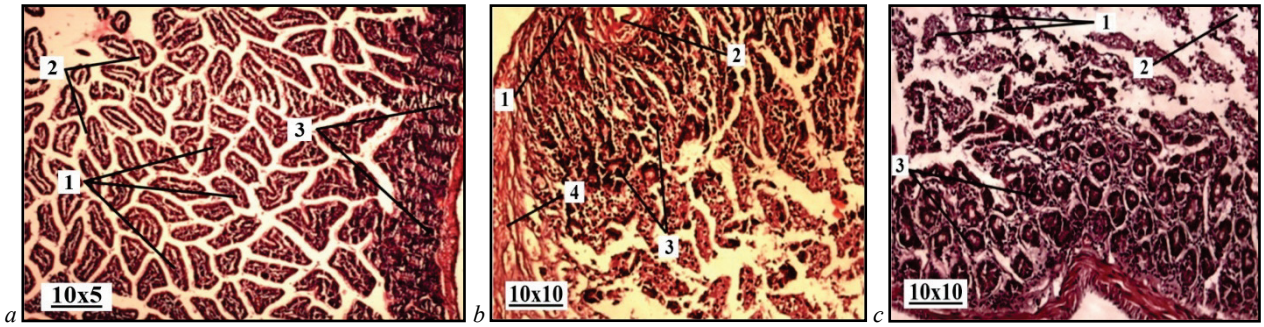

Fig. 5. Small intestine of a white mouse (×50, ×100): *a* – normal (*1* – villi; *2* – unilayer epithelium of villi; *3* – lymphoid cells); *b* – under the influence of "DZPT-2" (*1* – crypts; *2* – epithelium of villi; *3* – glands; *4* – muscle plate); *c* – under the influence of "FAG" (*1* – villi; *2* – crypts; *3* – glands); n = 15

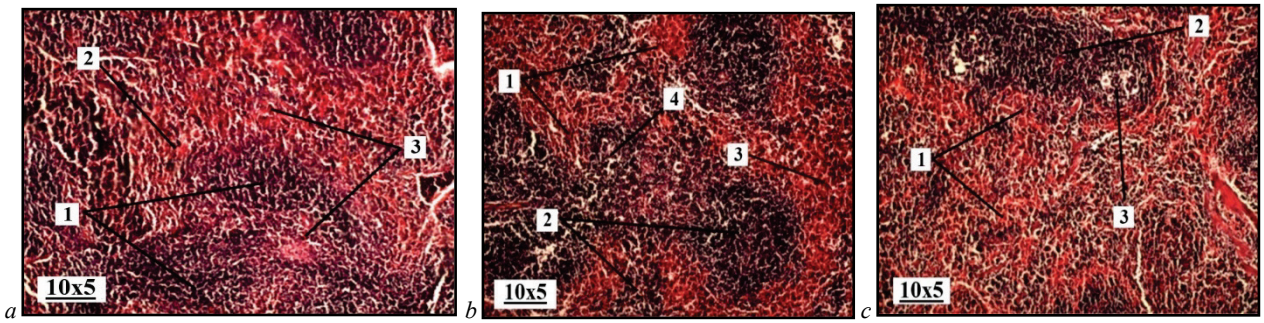

Fig. 6. Spleen of white mouse (×50): *a* – normal (*1* – white pulp; *2* – red pulp; *3* – sinusoidal capillaries); *b* – under the influence of "DZPT-2" (*1* – red pulp; *2* – white pulp; *3* – capillaries; *4* – lymphoid cells); *c* – under the influence of "FAG" (*1* – red pulp; *2* – white pulp; *3* – lymphoid cells); n = 15

Assessing the toxicity of antimicrobial agents is crucial for their widespread testing and should follow modern requirements. Various methodological approaches and regulations have been proposed for this purpose, which can differ significantly from one another. The standard method of cytotoxicity assessment based on direct microscopic examination of cell cultures has been shown to be insensitive and may lead to an underestimation of the risk posed by disinfectants (Sagripanti & Bonifacino, 2000). Therefore, laboratory animals (outbred white mice) were chosen as the subject of study, allowing for a more informative picture of pathological changes under the toxic effects of the studied chemical compounds.

During our research, we considered reports on the morphological assessment of the aerogenic effects of citric acid and sodium hypochlorite (Kim et al., 2023), as well as the effects of aldehyde agents on laboratory animals and humans (Ballantyne & Myers, 2001; Wang et al., 2022). The experimental animals underwent histopathological analysis, which revealed inflammatory lesions and discoloration of the lungs. Exposure to glutaraldehyde aerosols resulted in oxidative stress, inhibition of cilia beating frequency, abnormal mucin production, impaired secretion of cytokines and matrix metalloproteinase, as well as morphological transformation (Wang, et al., 2022).

In the study of acute toxicity of benzalkonium chloride in laboratory animals, histopathology showed inflammatory reactions in the lungs after intratracheal instillation (diffuse congestion and hemorrhage, necrotized alveolar wall, and thrombus formation in small vessels and terminal bronchioles) (Lee & Park, 2019). Therefore, our attention was focused on the study of the toxicity of aldehyde agents when administered orally to laboratory animals. It is also known that functional and morphological changes in the stomach wall, particularly in its low curvature, can cause visceral reflexes that affect vascular tone. This plays a crucial role in cases involving the ingestion of toxic or irritating substances.

Histological changes were observed in the kidney, liver, stomach, and small intestine of white mice when exposed to lethal doses of aldehyde disinfectants. Similarly, acute liver damage was observed in mice exposed to chlorine disinfection by-products. The severity of liver damage was found to increase with the concentration of the agents, possibly due to an increase in hepatic macrophages (Dong et al., 2019). Therefore, it can be inferred that the liver is the primary target organ in cases of oral disinfectant poisoning, regardless of the chemical composition of the disinfectant.

It is noted that exposure to biocides can activate complex damage and repair pathways, including DNA, oxidative, protein, general, and membrane stress. The extent of damage increases with concentration (Yang et al., 2023). Based on the presented data analysis, it can be inferred that the death of animals in our experiments was caused by the toxic effects of aldehydes. An innovative approach in modern toxicology involves the use of biocathode sensors due to their unique function of electroauto-trophic respiration (Liao et al., 2023). Regardless of the properties of any antimicrobial compounds, it is important to exercise caution when using antimicrobial compounds, taking into account their efficacy and safety based on evidence (Ambrosino et al., 2022).

Conclusion

When laboratory animals were exposed to aldehyde disinfectants at a dose of LD_{50}, they experienced acute circulatory failure leading to death. This failure was manifested in the renal tissue by dilation and excessive blood filling of capillaries in the subcapsular zone. The liver of animals showed dystrophic changes in hepatocytes and hemodynamic disorders. The stomach and intestine exhibited desquamation of glandular epithelial cells and dilation of the lumens of the ducts and vessels of the small intestinal lamina propria. Histological changes in the spleen were manifested by localized death of lymphoid cells in the white pulp and hemorrhage.

The authors declare no conflicts of interest regarding the authorship and publication of this article.

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