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Original Article

Impact of Phytobacterial Agent on the Toxic Damage to the Liver and Ileum of White Rats

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ABSTRACT

Numerous pharmaceutical agents can induce adverse reactions in the human body, including toxicity to the liver and the inflammation of intestines. Therefore, nowadays one of the most urgent problems in modern medical science is the prevention and restoration of morphological and dysbiosis disorders caused by numerous medications. With this background in mind, we aimed to evaluate the efficacy of phytobacteria on toxic damage to the structure and function of the liver and ileum, as well as the composition of the large intestine microflora in white rats with intestinal dysbacteriosis due to carbon tetrachloride (CCl4) and ampicillin trihydrate. In order to prevent toxic damage to the liver and ileum of experimental animals, a phytobacterial agent was used. This test agent was composed of a mixture of commercial lactobacteria Lactobacillus helveticus with a water-soluble extract of thyme (Thymus Serpyllum L.) on a sterile milk basis. Our results showed that the introduction of phytobacterial agent led to reduced inflammation, accelerated regeneration of the ileum mucous membrane, and a positive effect on the damaged intestine. The phytobacterial agent increased the resistance of the body to potentially pathogenic microorganisms and toxic compounds by restoring the microflora of the large intestine. It was established that the phytobacterial remedy resulted in the normalization of the intestinal microflora of white rats, which had toxic damage to the liver and ileum caused by CCl4 and ampicillin trihydrate administration. Moreover, the usage of phytobacteria was correlated with improvement in the structure and function of the liver and ileum.

Keywords: Dysbiosis, Ileum, Liver, Phytobacterium, Probiotics

Impact de L'agent Phytobactérien sur les Dommages Toxiques au Foie et à L'iléon des Rats Blancs

Résumé: De nombreux agents pharmaceutiques peuvent induire des réactions indésirables dans le corps humain, notamment une toxicité hépatique et une inflammation de l'intestin. Par conséquent, l'un des problèmes les plus urgents de la science médicale moderne est la prévention et la restauration des troubles morphologiques et de la dysbiose provoqués par de nombreux médicaments. Dans cet esprit, nous souhaitions évaluer l'efficacité des phytobactéries sur les dommages toxiques causés à la structure et au fonctionnement du foie et de l'iléon, ainsi que la composition de la microflore du gros intestin chez des rats blancs atteints de dysbactériose intestinale due au tétrachlorure de carbone (CCl4) et trihydrate d'ampicilline. Afin de prévenir les dommages toxiques au foie et à l'iléon d'animaux expérimentaux, un agent phytobactérien a été utilisé. Cet agent d'essai était composé d'un mélange de lactobactéries commerciales *Lactobacillus helveticus* et d'un extrait hydrosoluble de thym (*Thymus Serpyllum L.*) à base de lait stérile. Nos résultats ont montré que l'introduction d'un agent phytobactérien entraînait une réduction de l'inflammation, une régénération accélérée de la membrane muqueuse de l'iléon et un

effet positif sur l'intestin endommagé. L'agent phytobactérien a augmenté la résistance des rats aux micro-organismes potentiellement pathogènes et aux composés toxiques en restaurant la microflore du gros intestin. Il a été établi que le traitement phytobactérien entraînait la normalisation de la microflore intestinale de rats blancs, qui présentait des lésions toxiques du foie et de l'iléon causées par l'administration de CC14 et de trihydrate d'ampicilline. De plus, l'utilisation de phytobactéries était corrélée à une amélioration de la structure et de la fonction du foie et de l'iléon.
Mots-clés: Dysbiose, Iléon, Foie, *Phytobacterium*, *Probiotiques*

INTRODUCTION

In recent years, there has been great concern regarding damage to the liver and intestinal tract associated with the chronic use of pharmaceutical interventions. In Russia, acute drug-induced damage to the liver was detected in patients taking anti-tuberculosis, antibacterial, analgesic, hormonal, cytostatic, hypotensive, and anti-rhythmic medications. Medicinal lesions of the liver are often asymptomatic and difficult to identify. The frequency of these conditions has increased significantly due to a large number of medicines in the market (Aruin et al., 1998; Riordan and Williams, 2006). The liver is one of the major links in the biotransformation of medications in the human body as it holds the main responsibility for drug metabolism. In addition, the gastrointestinal ecosystem is considered as one of the body defense systems. Normal intestinal microflora, mainly the anaerobic representatives, make the mucous membranes of the gastrointestinal tract resistant to the colonization of conditionally pathogenic or pathogenic bacteria (Collins and Gibson, 1999). Various factors can contribute to dysbacteriosis, which is known as a microbial imbalance of the natural human flora leading to pathogenic bacterial growth. This issue is of particular concern when affecting the digestive tract (Vorobyev, 2004; Bondarenko and Gracheva, 2005). Under such conditions, the intestinal microflora cannot perform its normal protective functions. Therefore, a pressing problem in the age of numerous pharmaceutical interventions is to prevent and restore the morphological and dysbiotic disorders of the intestines (Zlatkina, 1999; Aleshkin et al., 2005; Tarmakova et al., 2010). Damage to the liver is associated with the general condition of the body, including protective and adaptive reactions. The procedures in the body cause a variety of mechanisms for restoring the structure and function of the liver that possesses significant compensatory and regenerative capabilities. (Tarmakova, 1999). Any disease is considered as a process that has spread throughout the body. As a result, in the European and Eastern medicine, particular attention is paid to stimulating the whole organism when treating human diseases. Therefore, it is necessary to make extensive use of complex pharmacological therapies with the aim of improving trophism and promoting body defenses (Saulnier et al., 2009; Vandenplas and Benninga, 2009). In recent years, there has been an augmented interest in probiotics involved in almost all physiological processes in the body. Currently, the application of oral probiotic cultures can restore the microflora of the gastrointestinal tract and displace the influence imposed by the consumption of a number of chemical medications (Collins and Gibson, 1999; Folwaczny, 2000; Aleshkin et al., 2005; Saulnier et al., 2009; Tarmakova et al., 2010; Ivashkin and Ivashkin, 2017). The present study aimed to assess the impact of a phytobacterial agent on the structure and function of the liver, ileum, and the microflora of the large intestine in white rats with both liver and intestinal damage due to carbon tetrachloride (CCl_4) and ampicillin trihydrate. The authors of this study adhered to the idea of an

integrated approach for treating various diseases and attempted to modify herbal medicines with biological bacterial agents to prevent toxic damage to the liver, ileum, and intestinal dysbacteriosis.

MATERIAL AND METHODS

Animal models. The present investigation was carried out on 130 white outbred rats of both genders weighing 180-210 g obtained from the nursery of the Institute of Biophysics of the Federal Administration of "Medbioextrem" at the Ministry of Health of Russia in Angarsk. The animals were handled according to the regulations of Good Laboratory Practice (GLP) and the order number of 708H from the Ministry of Health of the Russian Federation on August 23, 2010, titled as "On the Approval of the Rules of Laboratory Practice". The subjects were assigned into two control groups and three experimental groups. Damage to the liver and ileum was simulated in the animals of the control groups using CCl_4 for the first control group and ampicillin trihydrate for the second control group (Tarmakova, 1999). To reproduce toxic liver damage in the animals of the first control group, CCl_4 was diluted in sterile olive oil at a ratio of 1:1 and was injected subcutaneously in a volume of 0.4 ml per 100 g of animal body weight for four days. The second control group of animals was divided into two subgroups. To reproduce toxic ileum damage, ampicillin trihydrate was administered *per os* to the animals of the first and second subgroups at the doses of 50 and 150 mg/kg for three days, respectively. Some pieces of the liver and ileum muscle were fixed in a 10% solution of neutral formalin and Carnoy's solution followed by paraffin embedding. The sections were prepared on a sliding microtome with a thickness of 5-6 μm. The histological sections were stained with hematoxylin-eosin by Van Gieson (Merkulov, 1969). The photomicrography of the study samples was performed using an Axiostar plus microscope (C. Zeiss, Jena, Germany) with an integrated MicroCam 5M video camera (C. Zeiss, Jena, Germany). After simulating the damage to the liver and ileum of white rats, the animals were injected with a phytobacterial agent representing a mixture of *L. helveticus* lactobacilli with a water-soluble thyme extract (*Thymus Serpyllum L.*) on a sterile milk basis. The agent was administered intragastrically at a dose of 1 ml/100 g of animal weight for 21 days.

Microbiological Analysis. For the microbiological evaluation, the freshly excreted feces of separate animals were used in compliance with the rules of sterility (Steinbach and Shetty, 2001). Dilutions were prepared on the thioglycolic buffer (pH=6.8). In order to identify the species and determine the biochemical properties of the isolated bacteria, the bacteria were isolated and incubated on the special nutrient mediums. *Bifidobacterium* on Blaurok's medium modified by Goncharova, *Lactobacillus* on MPC-4 medium, *Clostridium* on Wilson-Blair medium, *Enterococcus* on Kalina medium, *Escherichia coli* (E.coli*)* on media Endo, Levin, Ploskirev, *Staphylococcus* on yolk-salt agar, fungi of the genus *Candida* on the medium Saburo. The staining of the preparations was completed according to the Gram status.

Pathogenic Analysis. The isolation and generic identification of microorganisms was carried out based on the techniques set in the Bergey's Manual of Systemic Bacteriology (Holt, 1997) and Use of the Diagnostic Bacteriology Laboratory: a Practical Review for the Clinician (Steinbach and Shetty, 2001).

Statistical Analysis. In terms of the descriptive statistics, mean ± standard deviation (SD) was utilized to summarize the data. Moreover, Student's t-test was applied to compare the results. $P < 0.05$ was considered statistically significant for comparing with the control groups.

RESULTS

Control Groups Results. Our findings demonstrated that the subcutaneous administration of CCl_4 to the white rats caused a reduction in the number of *Bifidobacterium, Lactobacillus*, and normal *E.coli* during the whole observation periods. On the other

hand, an elevation in the number of lactose-negative *E.coli, Enterococcus, Staphylococcus, Clostridia*, and *Candida* fungi was found (Table 1).

Table 1. Species and the quantitative composition of intestinal microflora of white rats with toxic liver damage due to carbon tetrachloride (lg CFU/g)

Bacteria	Intact group	Control group (CCl_4)
Bifidobacterium sp.	6.97±0.25	4.21±0.17*
Lactobacillus sp.	6.28±0.21	4.03±0.14*
Clostridium sp.	1.66±0.12	3.94±0.17*
E. coli	5.35±0.19	5.81±0.2
Enterococcus sp.	4.27±0.15	6.62±0.23*
Staphylococcus	3.52±0.14	5.43±0.19*
Candida sp.	1.23±0.09	3.85±0.17*

* $P \leq 0.05$

Histopathologic studies revealed dramatic structural changes in the liver of animals after the administration of CCl_4. These alterations manifest in significant hemodynamic disturbances, such as the plethora of interlobular vessels, dilation of the central vein lumen, thickening of vein walls with the migration of blood elements in the parenchyma of the organ, perivascular edema, and expansion of the perivascular space. In addition, marked changes may occur in hepatocytes in the form of ballooning, granular, and fatty degeneration (Figure 1).

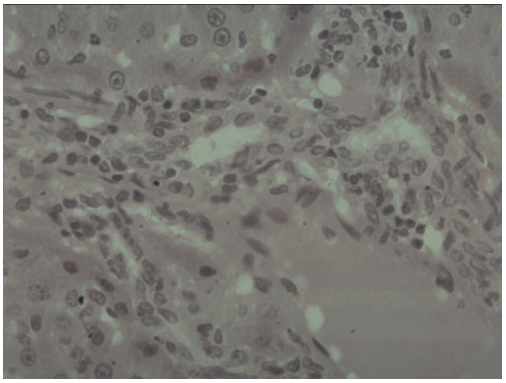

Figure 1. White rat liver with toxic damage caused by CCl4 administration. Hepatocyte necrosis foci with nuclear lysing, cell infiltration, and fatty degeneration. Hematoxylin and eosin staining (magnification 10×20).

We observed in this investigation that the introduction of CCl_4 caused structural changes not only in the liver but also in the ileum of white rats. This was presented as splitting and swelling of the lower layers of the mucosa and infiltration of the mucous layer by cellular elements (Figure 2).

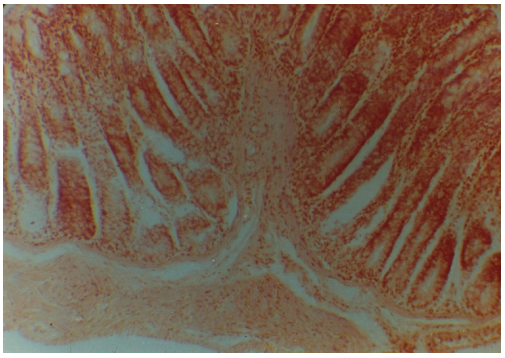

Figure 2. White rat ileum with toxic damage caused by CCl_4 administration. The splitting and edema of the lower layers of the mucous membrane and infiltration of the mucous layer with cellular elements. Hematoxylin and eosin staining (magnification 10×10).

Greater dysbiotic changes were found in the group of white rats treated with ampicillin at the doses of 50 and 150 mg/kg ($P \leq 0.05$). A sharp decrease was reported in the number of *Bifidobacterium, Lactobacillus,* and *E. coli*. Furthermore, the complete disappearance of *Clostridia, Enterococcus, Staphylococcus*, and *Candida* fungi was observed (Table 2). In the animals that received ampicillin at the dose of 150 mg/kg, the number of *Bifidobacterium* and *Lactobacillus* was lower than those treated with 50 mg/kg. The latter finding could be attributed to the application of higher antibiotic dose. *Clostridia, Enterococcus, Staphylococcus*, and fungi of the genus *Candida* were found to be completely disappeared.

Experimental Group Results. Table 3 presents the results of a study on the microbial ecology of the intestine and the morphofunctional state of the liver of experimental animals after using a phytobacterial agent for toxic liver damage by CCl_4 on days 7, 14, and 21. Analysis of the effects of the phytobacterial agent on the liver and ileum damaged by CCl_4 revealed that the phytobacterial agent improved the microbiological indicators of the intestinal flora and the morphological parameters of the liver in the experimental animals. This was reflected in the appearance of hypertrophied hepatocytes indicating the activity of regenerative processes (Figure 4). After 14 days of treatment with the phytobacterial agent in the experimental animals,

Table 2. Microflora of the large intestine of white rats following the introduction of ampicillin (M±m) lg CFU/g.

Bacteria	Intact Group	Control group (ampicillin)			
		50 mg/kg (N=10)		150 mg/kg (N=10)	
		Day 2	Day 7	Day 2	Day 7
Bifidobacterium sp.	7.25±0.32	5.31±0.28*	3.85±0.20*	4.16±0.19*	4.87±0.27*
Lactobacillus sp.	6.41±0.26	5.69±0.30	4.24±0.23*	3.47±0.14*	4.52±0.19*
Clostridium sp.	1.73±0.13	< 1*	< 1*	0*	0*
E. coli	5.28±0.2	3.83±0.14*	4.21±0.13	1.35±0.12*	2.21±0.13*
Enterococcus sp.	4.18±0.16	1.22±0.07*	< 1*	0*	< 1*
Staphylococcus	3.63±0.19	1.24±0.09*	< 1*	0*	< 1*
Candida sp.	1.27±0.09	< 1*	< 1*	0*	< 1*

Table 3. Composition of the intestinal microflora of white rats treated with the phytobacterial agent (M±m, N=10) lg CFU/g.

Bacteria	Intact Group (H_2O)	Control Group (Cl_4)	Experimental groups treated with the phytobacterial agent		
			Day 7	Day 14	Day 21
Bifidobacterium sp.	7.03±0.35	4.25±0.18*	4.68±0.12	7.15±0.45**	7.24±0.11**
Lactobacillus sp.	6.12±0.34	4.05±0.16*	4.81±0.35	6.34±0.31	6.68±0.25**
Clostridium sp.	1.62±0.12	3.72±0.15*	2.93±0.21	2.71±0.12	2.10±0.18**
E.coli	5.30±0.24	5.64±0.19	5.59±0.15	5.64±0.19	5.67±0.11
Enterococcus sp.	4.33±0.19	6.59±0.21*	6.00±0.13	4.96±0.28**	4.23±0.18**
Staphylococcus	3.08±0.21	5.17±0.21*	4.8±0.26	4.15±0.14	4.03±0.18
Candida sp.	1.27±0.09	3.73±0.16*	3.45 ±0.11	3.31±0.25	2.73±0.15**

* Data are reliable compared to the intact group with $P \leq 0.05$
** Data are reliable compared to the control group with $P \leq 0.05$

Table 4. Composition of the intestinal microflora of white rats treated with the phytobacterial agent and ampicillin at the dose of 50 mg/kg (M±m, N=10) lg CFU/g

Bacteria	Intact Group (H_2O)	Control Group (ampicillin)	Experimental groups treated with the phytobacterial agent		
			Day 7	Day 14	Day 21
Bifidobacterium sp.	7.03±0.35	5.31±0.28*	6.21±0.24**	6.63±0.3**	7.14±0.24**
Lactobacillus sp.	6.12±0.34	4.49±0.2*	4.85±0.23	5.04±0.19**	5.83±0.21**
Clostridium sp.	1.62±0.12	< 1*	1.07±0.18	< 1	< 1
E.coli	5.30±0.24	3.83±0.14*	4.16±0.19	4.62±0.2**	5.22±0.21**
Enterococcus sp.	4.33±0.19	1.22±0.07*	2.28±0.15**	2.16±0.19**	2.31±0.35**
Staphylococcus	3.08±0.21	1.24±0.09*	1.22±0.2	1.13±0.25	1.26±0.19
Candida sp.	1.27±0.09	< 1	< 1	< 1	< 1

Table 5. Composition of the intestinal microflora of white rats treated with the phytobacterial agent and ampicillin at the dose of 150 mg/kg (M±m, N=10) lg CFU/g

Bacteria	Intact Group (H_2O)	Control Group	Experimental groups treated with the phytobacterial agent		
			Day 7	Day 14	Day 21
Bifidobacterium sp.	7.03±0.35	4.16±0.19*	5.57±0.21**	6.09±0.33**	6.82±0.25**
Lactobacillus sp.	6.12±0.34	3.47±0.14*	4.34±0.18	5.23±0.18**	5.57±0.23**
Clostridium sp.	1.62±0.12	0*	0	0	< 1
E.coli	5.30±0.24	1.35±0.12*	2.38±0.17**	3.44±0.31**	4.63±0.29**
Enterococcus sp.	4.33±0.19	0*	< 1	2.21±0.14*	2.83±0.17*
Staphylococcus	3.08±0.21	0*	< 1	1.56±0.13*	2.09±0.14*
Candida sp.	1.27±0.09	0*	< 1	< 1	1.29±0.13*

denotes * P≤0,05; ** P≤0,05

restored to the initial composition of the microflora similar to intact animals (Steinbach and Shetty, 2001). Following the usage of the phytobacterial agent in animals receiving ampicillin at the dose of 50 mg/kg, an increase was found in the number of all evaluated microorganisms in all periods of the study with the exception of *Staphylococcus* and *Candida* sp. Moreover, no significant change was found regarding the *Clostridium* sp. An increase in the content of *Bifidobacterium* was noted in all observation periods from 5.31±0.28 to 6.21±0.24, 6.63±0.3, and 7.14±0.24 lg CFU/g, in comparison with the control groups. In addition, elevation in *Lactobacillus* from 4.49±0.2 to 4.85±0.23, 5.04±0.19, and 5.83±0.21 lg CFU/g was revealed, compared to the animals of the control group (Table 4). The results of the present study showed that the phytobacterial agent administration in the ampicillin-treated animals at the dose of 150 mg/kg resulted in a significant augmentation in the number of all assessed groups of bacteria on days 14 and 21. Furthermore, the content of *Bifidobacterium* was noted to rise at all observation periods from 4.16±0.19 to 5.57±0.21, 6.09±0.33, and 6.82±0.25 lg CFU/g. we observed that *Lactobacillus* number increased from 3.47±0.14 to 4.34±0.18, 5.23±0.18, and 5.57±0.23 lg CFU/g, in comparison with the animals of the control groups (Table 5).According to the findings of the current study, the treatment of white rats, which suffered from ampicillin trihydrate toxic damage to the ileum, with a phytobacterial agent led to the restoration of the mucous membrane without the infiltration of cellular elements (Figure 3).

DISCUSSION

The microorganisms that inhabit the intestines could be an indicator of the abnormalities in the functional activity of the body. The subcutaneous introduction of CCl_4 to the animals leads to a shift in the quantitative and qualitative composition of the large intestine microflora along with damage to the liver. The findings of the present investigation showed that the resultant dysbiotic changes were reflected in all the studied groups of microorganisms. These alterations led to a decrease in the number of anaerobic bacteria (*Bifidobacterium* and *Lactobacillus*).

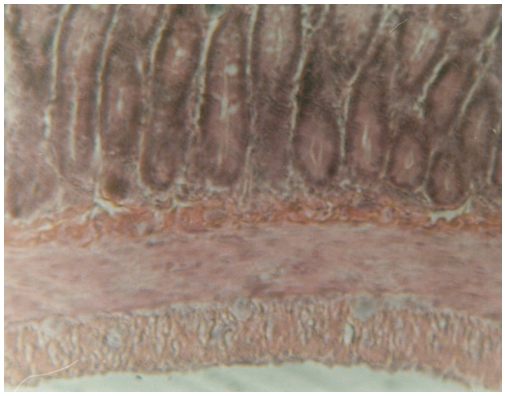

Figure 3. White rat ileum with toxic damage caused by ampicillin trihydrate administration. After 21 days of treatment with the phytobacterial agent: the restoration of the mucous membrane without the infiltration of cellular elements. Hematoxylin and eosin staining (magnification 10×20).

On the other hand, an increase was found in the number of aerobic bacteria (*E. coli, Enterococcus,* and *Staphylococcus*) indicating a diminish in the resistance of the intestines to bacterial colonization. Currently, there is a wealth of information in the literature regarding the normal intestinal microflora of humans and animals (Tarmakova, 1999; Tarmakova et al., 2010). However, questions about the morphological changes of the intestine exposed to the damaging factors of distinct etiologies are insufficiently covered. As a result of the violation of colonization resistance of the intestine, the enterohepatic circulation is disturbed, in which the intestinal microflora takes an active role (Fedos'ina et al., 2009). Toxic damage to the liver of white rats with CCl_4 leads to fatty degeneration with foci of necrosis in hepatocytes and lysis of nuclei. Hepatocytes become enlarged and hemodynamic changes, as well as the fullness of vessels with cell infiltration, are noted (Figure 1) (Tarmakova, 1999). When exposed to CCl_4, acute inflammatory changes were observed in the intestines of white rats, including the mucosal edema, infiltration of the mucous membrane with cellular elements, and destruction of the mucosal epithelium (Tarmakova, 1999). The edema

was also reported around the crypts accompanied by the squeezing of the infiltrated glands (Figure 2). The results of this study indicated that the phytobacterial agent had a positive effect on the structural organization of the ileum of white rats during all the periods of observation. On days 14 and 21 of the introduction of the phytobacterial agent, the laboratory animals with toxic liver damage due to CCl_4 and ampicillin showed a decrease in the cellular infiltration and edema of the mucous membrane of the ileum, compared to the control group (Figure 3). Due to the inflammatory process caused by ampicillin at the dose of 150 mg/kg, the edema of the mucous membrane, edema and splitting of the submucosa, and infiltration of the mucous layer with cellular elements were observed in the ileum. The toxic damage was confirmed by morphological research methods (Figure 3). The massive doses of antibiotic provoke inflammatory bowel reactions that can turn into the chronic forms of the disease. The inflammatory procedure interferes with the regenerative processes in the intestinal mucosa of rats. Consequently, the inflammatory process progresses, hypoxia develops, trophism decreases, crypts shorten, and the lumen expands. The morphological evaluation of the phytobacterial agent efficacy revealed that the introduction of the phytobacterial agent played a role in reducing the inflammatory process and accelerating the regeneration of the mucous membrane of the ileum. In addition, it had a positive impact on the damaged intestine leading to the normalization of the histological structure of the ileum in a short time, in comparison with the control group. The mentioned influence may be related to the possibility of the formation of a physical barrier by beneficent bacteria. As a result, the contact of pathogenic gram-negative microorganisms with the pattern-recognizing receptors of the microorganism is blocked. This effect is likely to be caused by the microbial molecular patterns of beneficial bacteria, which are involved in ligand-receptor interactions. Therefore the occurrence of inflammatory reactions is prevented (Zhou et al., 2015). The positive impact of the phytobacterial agent was found as an increase in the resistance of the body to the effects of potentially pathogenic microorganisms and toxic compounds along with the restoration of the large intestine normal flora. The morphological assessments revealed different signs of regeneration in the liver of animals expressed by a reduction in the hemodynamic and edematous processes and the disappearance of cell infiltrates (Figure 4).

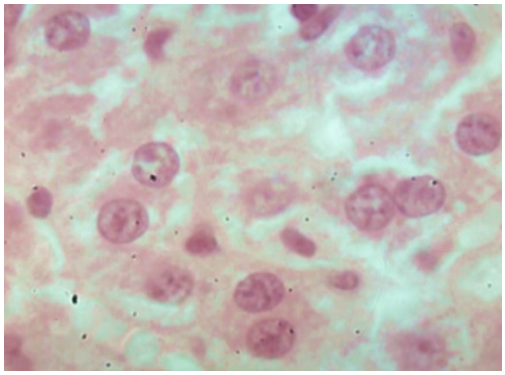

Figure 4. White rat liver after 14 days treatment with the phytobacterial agent. Hypertrophied hepatocytes indicate the activity of regenerative processes. Hematoxylin-eosin staining (magnification 10x90).

The phytobacterial agent application improved the microbiological indicators of intestinal flora and the morphological parameters of the liver in the experiment subjects. Following 14 days of treatment with the phytobacterial agent, the microbial community of the large intestine was restored to the initial microflora composition of intestine similar to intact animals. A significant elevation in the number of all the studied groups of bacteria was found on days 14 and 21. The therapeutic efficacy of this agent could be considered as the ability of a large number of viable bifidobacteria and lactobacilli to settle down in the intestine. Moreover, it shows antimicrobial activity against conditionally pathogenic microflora and the ability to accelerate the regeneration of morphologically modified intestinal mucosa (Tarmakova, 1999; Tarmakova et al., 2010).

In conclusion, when the phytobacterial agent is added to the animal ration, the intestinal microflora composition of white rats with toxic damage to the liver and ileum with CCl_4 and ampicillin trihydrate is normalized. Furthermore, it was observed that this normalization is correlated with an improvement in the structural and functional state of the liver and ileum.

Ethics

The authors of the current study hereby declare that all the ethical standards have been respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest for this investigation.

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