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Study the Effect of the Combination of Flavonoids of the Crocus Sativus Plant on Opportunistic Intestinal Microorganisms Against the Background of Acute Toxic Hepatitis Caused in Mice Under Study Conditions

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Abstarct: Not only infectious factors, but also the expanding scale of industrial production today increase the occurrence of liver diseases against the background of the adverse effects of the production process or manufactured artificial synthetic substances, medicines on many human organs and systems. On the other hand, emerging liver diseases themselves cause serious medical, social and even economic problems due to the development of complications that cause disability and death, such as hepatocellular carcinoma or fibrosis and cirrhosis of the liver. The appearance of antibiotic-resistant strains used to prevent secondary infection during the improvement of antiviral or hepatoprotective agents for the treatment of liver diseases causes a decrease in the effectiveness of treatment. In this regard, the priority task is to determine the effectiveness of drugs with high hepatoprotective activity obtained on a plant basis, namely when using antibiotics. To this end, simultaneous activity against hepatoprotective and intestinal pathogenic strains against the background of acute toxic hepatitis was detected in laboratory white mice of Crocus savitus plant extract. Acute toxic hepatitis was caused in white mice by oral administration of a paracetamol solution (JSC Pharmstandard-Lexredstva Russia) at a dose of 1500 mg/kg once 2 times in a row. The solution of the studied plant extract was selected as a comparative drug together with ciprofloxacin 50 mg/kg in dose of 50 mg/kg using a special atraumatic probe from 3 days of the study, the drug Karsil was administered orally to white mice at a dose of 50 mg/kg together with ciprofloxacin 50 mg/kg for 14 days. And the control group was given distilled water corresponding to the volume of the test substance. In the experiments conducted, it was found that the studied plant extract not only exhibits high hepatoprotective activity, but also eliminates cases of dysbiosis, which can develop under the action of ciprofloxacin.

Keywords: Bacterial infections, acute paracetamol hepatitis, antibiotic resistance, Crocus sativus, Karsil, ciprofloxacin.

Introduction. In recent years, severe complications such as cirrhosis of the liver, hepatocellular carcinoma (HCC) and liver failure, arising as a complication of chronic liver diseases that develop as a result of bad habits, infectious and non-infectious factors among different ages and genders in different regions and demographic groups, have also been noted among the causes of a global increase in mortality and have led to to increase the burden on the healthcare system. rotating [1, 2, 3, 4, 5, 6,]. All over the world, a fairly large part of the population suffers from liver diseases of various origins, and almost half of patients with severe complications caused by chronic liver damage and inflammation of various etiologies die every year, that is, about 2 million people. A significant part of the deaths observed due to complications of these liver diseases are due to cirrhosis of the liver, the final stage of liver fibrosis, which develops as a result of a progressive violation of the architecture of the liver [7, 4, 8,9, 2, 10]. On the other hand, HCC deaths in recent years account for more than 30% of all deaths caused by oncological diseases worldwide, and not only by tumors originating in the gastrointestinal tract [3, 11, 12,]. By country or region, the mortality rate from liver cirrhosis in China, the United States and Western European countries is significantly lower than in Central Asian countries.

Differences in mortality rates from cirrhosis of the liver by country or region reflect differences in the prevalence of risk factors, which necessitates the strengthening of preventive measures to control and reduce risk factors for cirrhosis of the liver in regions with high or rapidly increasing mortality rates. [13]. It should be noted that approximately 30% of the world's population has fibrosis or cirrhosis of the liver as a result of the death of hepatocyte cells due to prolonged exposure, there is also at least one risk factor leading to hepatocellular carcinoma [14, 13,].

It is worth noting here that not only the factors provoking them, but also bacterial infections are recognized as provoking factors in the development or complication of liver diseases. It was in conditions of chronic liver disease that patients were found to have low levels of strains such as *Bacteroidetes* and high levels of strains such as *Proteobacteria*, *Enterococcus, Veillonella*. It is known that changes in the composition of the intestinal microbiota are associated with changes in lipid metabolism and activation of inflammatory pathways, which, in turn, can lead to an aggravation of the course of the disease in patients with liver diseases. For this reason, even current regulations recommend the preventive use of antibiotics in certain situations, and enteric antibiotics are prescribed in the acute phase of the disease, and in the parenteral remission phase. The use of antibiotics for liver diseases reduces the risk of aortic development, preventing the addition of additional infections and contributes to improving the quality of life of patients.

Although the preventive use of promising antibiotics with high activity has proven to be effective, when achieving high efficacy, it is necessary to carefully consider the individual characteristics of the patient. This not only highlights the need to assess factors such as antibiotic resistance, kidney function and the presence of concomitant diseases when choosing an appropriate prevention regimen, but also the importance of antibiotics in the context of liver diseases, which, unlike their harmful effects, help to expand the possibilities of their clinical use [15, 16, 17, 18, 19,20]. In this regard, the study examined not only the hepatoprotective activity of biologically active substances derived from plants with high hepatoprotective activity when administered orally, but also their effect on the pathogenic intestinal microflora. The prospect of these studies lies in the fact that, along with the simultaneous suppression of hepatoprotective activity, there are possibilities to increase the effectiveness of some antibiotics used.

The main purpose of the presented research work is to evaluate the effect of an extract containing a set of flavonoids obtained on the basis of the *Crocus savitus* plant against the background of acute toxic hepatitis in laboratory white mice under study conditions on the simultaneous hepatoprotective and pathogenic intestinal microflora.

Research materials and methods. The extract of the *Crocus savitus* plant, colloquially called saffron or saffron, widely distributed in our country, was chosen as the object of research, and its acute toxicity and biological activity were studied. All studies were conducted on infertile white male laboratory mice with a body weight of 20 to 24 grams. both before the experiment and throughout the experiment, all the studied animals were kept in the same conditions for care and feeding [21]. Acute toxic hepatitis was caused in white mice by oral administration of a paracetamol solution (JSC Pharmstandard-Lexredstva In Russia) at a dose of 1500 mg/kg once for 2 consecutive periods [22]. The solution of the studied plant extract was selected as a comparative drug together with ciprofloxacin 50 mg/kg at a dose of 50 mg/kg using a special atraumatic probe from 3 days of the study, Karsil was administered orally to white mice at a dose of 50 mg/kg together with ciprofloxacin 50 mg/kg for 14 days. And the control group was given distilled water corresponding to the volume of the test substance. By fainting the experimental animals under the influence of mild anesthesia, the necessary samples were taken from them for blood analysis and bacterial analysis during the 15 days of the study. The screening assessment of the effect of the studied plant extract on hepatoprotective activity and pathogenic intestinal microflora was carried out in comparison with the control group into which acute toxic hepatitis was administered and the group into which carcinogen icrofloxacin was administered. The statistical processing of the obtained results was developed using the analytical applications Excell and OriginPro7.5 (OriginLab Corporation, SShA), as well as the statistical method

of variation series. The reliability between the indicators of the control and experimental groups was developed by the Student coefficient (T) method.

The results of the study. The acute toxicity of the studied plant extract was dissolved to a state of 10-50% aqueous emulsion and given orally to male white mice in doses from 500 mg to 3000 mg per kg of body weight. At first, they were observed for 1-2 hours, then for a day, and also for 14 days in a vivarium. At doses of 4000 and 5000 mg/kg, a decrease in relative motor activity was observed in the first minutes, followed by a return to normal after 4-5 hours. Based on the experiments conducted, it was found that the acute toxicity of the studied samples of the extract based on the *Crocus savitas* plant with oral single administration in rats belongs to the LD50 -5000 group and higher, i.e. it is practically harmless to class IV according to GOST.

Toxic damage to the liver in rats as a result of the administration of paracetamol led to a violation of the functional state of liver cells. In rats with toxic hepatitis, the activity of serum transaminases (AST and ALT) was 338.6% and 381%, respectively, compared with the levels of these indicators in the control group. The results are shown in the table.

Table 1. Comparison of doses of plant extracts and Carsil preparations by the activity of the functional state of liver marker enzymes in acute paracetamol hepatitis

Experimental groups	Intackt group	Control group	The amount of flavonoids	Karsil
Doses in mg/kg	Dist. wat	Paracetamole 1500 mg/kg	50 mg/kg	50 mg/kg
Total protein (g/l)	85,6	58,7	60,5	63,8
Albumine g/l	48,5	33,3	39,4	36,2
Total Bilirubine mkmol/l	2,15	5,12	3,12	4,27
ALT yed/l	95,8	460,8	256,6	185,8
AST yed/l	156,6	530,2	358,4	246,4
Koeffitsient of de Ritsa(AST/ALT)	1,63	1,15	1,4	1,33
APh, Yed/l	394	434,4	427,5	417,6
LDG, Yed/l	625,5	1532,5	1459,3	1291,2
GGD Yed/l	1,15	1,46	1,41	1,38
Amilaze (Ed/l)	495,7	442,6	448,5	462,6

The administration of *Crocus savitas* plant extract to the studied animals did not reduce the activity of these enzymes compared to the rats of the control group who did not receive this substance, but demonstrated lower rates compared to the mouses of the Intact group (Table 1). However, the activity of alkaline phosphatase remained within the normal range. The above data on the dynamics of activity of endocrine and excretory enzymes in the blood serum of rats treated with these substances under experimental conditions indicate that the administration of a combination of flavonoids did not have a normalizing effect on liver function.

Thus, in the model of acute toxic hepatitis, the amount of flavonoids obtained on the basis of the *Crocus savitas* plant showed hepatoprotective activity, expressed in comparison with the control group in terms of hepatoprotective properties and insignificant in comparison with the drug Karsil.

In studies of the effect of the studied substances on the normal intestinal microflora against the background of an antibacterial drug, there was a tendency to have a positive effect on the number of pathogenic strains under the influence of the amount of flavonoids (Table 2).

Table 2.The effect of the amount of flavonoids on the microflora of the large intestine of mice.

Groups	Doses in mg/kg	The number of microorganisms	
		Enterobacteria	Staphylococcus
Control group	Dis.wat.	$3,3*10^6$	$3,5*10^3$
The amount of flavonoids + ciprofloxacin	50+50	$2,8*10^6$	0
Karsil + ciprofloxacin	50+50	$3,2*10^6$	$3,6*10^3$

Based on these results, the following conclusion can be drawn that the studied compound eliminates the negative effect of the antibacterial drug on the normal intestinal microflora and has a positive effect on the composition of the existing microbiome.

Discussion. Bacterial infections are one of the main causes of hospitalization, morbidity and mortality in patients with cirrhosis. Urinary infections, pneumonia and spontaneous bacterial peritonitis are the most common infections, with an increased incidence of severe complications. Patients with cirrhosis of the liver have an increasing number of multidrug-resistant bacterial infections due to the sweat of using broad-spectrum antibiotics; In particular, patients receiving prophylactic norfloxacin for the treatment of chronic liver diseases have an increased risk of infection with intestinal microflora. Community-acquired infections are more common with Gram-negative bacteria than with Gram-positive bacteria. Antibiotics can also cause other infections, such as invasive fungal infections. Fungal infections are much less common; they are usually nosocomial and have high short-term mortality. Invasive fungal infections occur in 3-7% of infected people with a positive culture result in patients with cirrhosis, and they are more common as secondary or nosocomial infections during acute chronic liver failure. Among them, invasive candidiasis, or candidemia, accounts for 70% to 90% of cases. It is followed by invasive aspergillosis [23,24,25,26].

In this regard, the prospects of drugs obtained from a low-toxic vegetable sauce, which has both hepatoprotective and probiotic activity, are highly appreciated today. The hepatoprotective and probiotic activity of a plant extract containing flavonoids against the background of acute toxic hepatitis has been studied. It is noteworthy that the plant extract showed a higher improvement in functional disorders in the liver caused by pracetamol, compared with the comparable drug karsil. It was also found that the antibacterial drug grofloxin, which was administered with it, also eliminated the negative effect on the normal gut microbiome.

Conclusion. Thus, the studied crocus extract from avitus has hepatoprotective activity, in this activity it surpasses the comparable drug Karsil and is relatively low-toxic.

However, it also exhibits a probiotic effect, and there is a prospect that this activity will help increase the chances of its use.

The ongoing research is screening experiments, which, in turn, requires extensive in-depth scientific research in this area.

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