Prospects of using biotechnology to improve morphofunctional state of liver

M D Kashaeva, A V Proshin, L G Proshina and K V Gavrilova
Yaroslav-the-Wise Novgorod State University, 41, ul. B. St. Petersburgskaya, Veliky Novgorod, Russian Federation
E-mail: kashaevamrd@mail.ru

Abstract. The study examines the effect of hepatoprotectors of plant origin, obtained using biotechnology, on the liver morphofunctional state in 465 patients with chronic diffuse diseases. The patients undergo a complex conservative therapy, including hepatoprotectors of plant origin, which helps to correct homeostasis disorders, microcirculatory disorders and blood rheology, increase blood antioxidant activity, and improve the cell membranes structure and function. It is revealed that with intrahepatic cholestasis, regardless of the nosological form, against the background of dysproteinemia, bilirubinemia, azotemia, moderate transaminasemia, we observe a 2.2 – 4.9 times increase in the of alkaline phosphatase level, moderate cholesterolemia, pronounced phospholipidemia and β-lipoproteinemia. The conducted complex conservative therapy significantly improves the condition of patients and normalizes hemostasis and homeostasis. The inclusion of hepatoprotectors of plant origin in complex conservative therapy is a promising direction in the development of biotechnologies in hepatology.

1. Introduction
Recently, there has been a significant increase in the number of diffuse affects of the liver, leading to cirrhotic transformation of its parenchyma. Such outcomes are due to both the steady increase in the viral hepatitis incidence, and the uncontrolled use of drugs, chemicalization of agriculture, poor nutrition, alcohol abuse, toxic substances, drugs, as well as the fairly common use in medical practice of donated blood and its preparations in violation of precautionary measures [1]. As a result, in 5.4% of patients, the development of an early chronic pathological process in the liver is detected even in the hospital: 2.9% have a transition to the persistent, and 2.7% to the chronic active hepatitis [2]. Within 3-5 years, cirrhosis develops in 41.2% of patients who have undergone viral hepatitis. The most difficult tasks in hepatology are the development of an optimal algorithm for the treatment of patients with chronic diffuse liver diseases (CDLD) and their complications, and the prevention of transition to liver cirrhosis, the frequency of which is constantly growing [3].

Numerous and interrelated factors of liver damage are known, leading to malnutrition of tissues and organs and to the liver cells destruction. The causes of tissue imbalance are structural changes in the cytolemma and cytoplasmic skeleton of hepatocytes, a malfunction of organic compounds oxidation, mitochondrial thermogenesis and a violation of maintaining the dynamic balance of ions inside the cell, increased activity of proteinases, nucleases, phospholipases, a disproportion of lipid peroxidation enzymes. Important components of the intrahepatic biliary stasis development mechanism at the molecular level are violations of the architectonics and characteristic features of cell membranes associated with a change in the composition of lipids, cholesterol and fatty acids of plasmolemma [4].
Changes in the lipid metabolism lead to a violation of the cholesterol removal from the cell, which is accompanied by a breakdown of mitochondria, lysosomes, a change in the structure and functions of the membranes in all cells, including red blood cells, with a decrease in their elasticity and deformability, and hence, of the blood flow. The described changes contribute to the disturbance of microcirculation, impaired oxygen transport, impaired liver and kidney function, and an increase in endogenous intoxication. The main pathogenetic mechanisms for the development of endogenous intoxication are: cytolysis, mesenchymal inflammatory syndrome, stagnation of bile and jaundice. Of great importance is also a violation of cholesterol synthesis and transport, of bile acids metabolism, of bile synthesis and excretion. Thus, a significant metabolic processes violation occurs in the cells. The consequence of the main pathogenetic mechanisms is the development of structural and functional changes in hepatocytes [5]. The development of intrahepatic cholestasis is associated with changes in the morphofunctional state of blocking intercellular contacts, cytolemma of the sinusoid region and bile ducts of the hepatic lobule. Failures in the hepatic-biliary movement of substances, genetic changes in transport proteins, and resulting breakdowns of molecular transfer systems leading to a cholangiogenic secretion disorder can cause the membrane dysfunction; a change in the content and density of the hepatocytes plasma membranes affects the enzymes and receptors activity. The viscosity and ductility of cell membranes is determined by the ratio of phospholipids to cholesterol. A decrease in membrane elasticity is usually associated with high cholesterol, which occurs with drug cholestasis. A change in the cytoplasmic skeleton of hepatocytes leads to the disappearance of microvilli on the apical surface of the cells, a decrease in contractility of the prolapse cytolemma, and can cause increased permeability of the locking intercellular contacts, which leads to the reverse flow of bile into sinusoids [5, 6]. Having an increased surface activity, monocarboxylic hydroxy acids contribute to cell membrane damage, the accumulation of cytosolic calcium, activation of intracellular hydrolases and hepatocyte necrosis. Monocarboxylic hydroxy acids of steroid class inhibit the liver cells restoration, stimulate the connective tissue development, and disrupt the immune response. The free radicals accumulation process triggers the caspases activation, which ultimately leads to of biliary epithelial cells apoptosis. The vast majority of the above agents accompany a decrease in the intensity of the enzyme system that provides the formation of ademetionine. The synthesis of important intracellular compounds, the constituent elements of phospholipids, thiols, sulfates and many other components vital for the hepatocyte metabolism is impaired. The lipid composition of the plasma membrane of the liver cells is pathologically modified, which entails a change in their fluidity. Altered biochemical reactions and substrates lead to a sharp decrease in antioxidant and detoxification abilities of liver cells. [7]. The described phenomena of metabolic disorders lead to morphological changes, destruction of the hepatic cells at their death with a simultaneously forming pathology of bile formation and excretion [8].

Given the occurring violations of the structure and function of hepatocytes that affect all organs and systems, it is necessary to timely correct the revealed changes and carry out complex multicomponent therapy taking into account the severity of the patient's condition. The earlier the optimal treatment is selected and carried out, the faster the removal or termination of the impact of negative mechanisms will occur, which will help slow down the progression of CDLD, improve the quality and life expectancy [9]. To treat cholestasis, many drugs of different action have been proposed that affect individual pathogenesis links. The most promising of them are hepatoprotectors - pharmaceuticals that stimulate the hepatocytes restoration and increase liver resistance to toxic effects. Treatment with hepatoprotective drugs should be reasonably balanced and saving, focused on the suppression of the main clinical and pathogenetic syndromes. The therapy duration is dictated by the pathological process activity and the disease condition. The use of a specially selected hepatoprotector should be aimed at improving the membranes structure and function, enhancing the antioxidant activity of enzymes, regulating the secretion and peristalsis of the hepatic duct system, optimizing metabolic processes, blocking inflammatory reactions and normalizing the functioning of the immune system [10]. Ideal drugs for the treatment of chronic diffuse liver diseases that fully comply with these requirements have not been developed. Modern hepatoprotectors are mainly represented by synthetic medicines, herbal substances, homeopathic remedies. It should be noted that drugs derived from plant materials form a key component
of pharmacological agents. This fact is determined by the lower toxicity and bioavailability of plant material. Biologically active substances of plants have anti-inflammatory, antioxidant, antimicrobial, choleretic effect, activate the immune system [11, 12]. The primary role in achieving the therapeutic effects of herbal remedies is their antioxidant activity. The antioxidant properties of medicinal plant materials are of great importance for the implementation of the key mechanism of action in case of liver damage of viral and toxic genesis. Herbal preparations contain antioxidants that have pronounced anti-inflammatory, immunomodulating and anti-allergic effects, which are formed due to the complex combination of free radicals, slowing down the reaction of excessive lipid peroxidation, suppressing the lipoxygenase enzyme, reducing malon dialdehyde and reducing glutathione consumption [13].

2. Aim of research
In connection with the relevance of the issue of the prospective development and use of biotechnologies of plant origin to restore the morphofunctional state of the liver, this study was conducted. The practical aim was to improve the results of intrahepatic cholestasis treatment by optimizing conservative therapy using hepatoprotectors of plant origin and expanding indications for surgical interventions.

3. Materials and methods
A retrospective and prospective evaluation of the treatment results of 465 patients with chronic diffuse liver diseases was performed. The liver chronic process duration ranged from 2 to 5 years, there were 69.5% of women with the indicated pathology, and 30.5% of men, the age ranged from 16 to 75 years. Patients aged 25–60 years with good working and reproductive abilities accounted for 77.4%. In 67.9% of patients was a history of acute viral hepatitis, 23.1% worked with hepatotoxic substances, in 4.5% of patients - long-term use of hepatotoxic drugs, in 4.5%, the etiological factor could not be established. Patients with intrahepatic cholestasis had complaints of apathy, drowsiness, fatigue, memory impairment, loss of appetite, and unstable stool. Patients with chronic cholestatic hepatitis were most often bothered by debilitating itchy skin (68.1%), which was often the main complaint. All clinical cases of the disease analyzed by severity were divided into two groups:

Control group 1 – patients with inactive cirrhosis, who had no history of liver pathological processes activation – 242 patients;

Main group 2 – patients with chronic active hepatitis with prevailing cholestasis syndrome – 223 patients.

Patients underwent combined studies, the computed tomography and ultrasound were supplemented with endoscopic retrograde pancreatocholangiography or laparoscopy, which made it possible to establish an accurate diagnosis. In addition to the instrumental methods, clinical and laboratory studies were used for cholestasis diagnosis. The full scope of clinical and laboratory studies was performed for all patients who entered the clinic; in both groups, a complete blood count was supplemented by Fonio count of platelets. A detailed biochemical blood test was carried out, the concentration of bilirubin according to Iendrashek, the level of transaminases according to Reitman-Frenkel, alkaline phosphatase (test Bio-La-Test Zachtma, Brno) were determined, the total protein was determined by photocolorimetry, protein fractions – by electrophoresis on paper. The residual nitrogen and lipids (lecithin, β-lipoproteins, total Ilk cholesterol) were detected. These tests allowed us to assess the structural and functional state of the liver. Diagnostics of hemostasis changes was carried out by the Kotovschikova prothrombin index, Sigg heparin identity, Rutberg fibrinogen density, fibrinase and fibrinolytic activity. Clinical, laboratory, biochemical and hemostasiological studies were performed for all patients. Blood rheology indices were determined using an automatic rotational axial-cylindrical viscosimeter using the principle of a free-floating inner cylinder. As a result of blood rheology study, the following indicators were obtained: apparent dynamic viscosity (ADV) (η), yield value (YV) (τ), erythrocyte aggregation coefficient (EAC), hematocrit (H) and erythrocyte deformability (CED). Endogenous intoxication (EI) was determined by the method developed by A.A. Togaybaev and A.V. Kurguzkin for determining the erythrocyte sorption capacity (ESC). At the same time we established: erythrocyte osmotic resistance (EOR), hematocrit, erythrocyte number, hemoglobin content. The
morphological study was carried out on biopsy samples fixed in a 10% formalin solution and subjected to standard histological examination. Paraffin sections of the tissue were made 5 µm thick. Sections were stained with hematoxylin and eosin, as well as picrofuchsin according to Van Gieson. The preparations were analyzed using an Axio Scope A1 microscope (Carl Zeiss, Germany). The results obtained during the study were subjected to statistical processing according to Fisher-Student, Friedman criterion, Kendall concordance and matrix correlation analysis. The calculated indicators were reliable if the significance level corresponded to the condition $p < 0.05$.

The basis of treatment in this category of patients was a comprehensive conservative multicomponent therapy focused on regulating homeostasis and microcirculation disorders, improving blood rheology, increasing its antioxidant activity, normalizing the morphology and functional characteristics of plasmolmma. As a result, membrane-stabilizing therapy was included in the therapeutic measures complex. For patients with inactive cirrhosis, to correct moderate lipid metabolism and rheological disorders it was sufficient to have a 7–10 day course of complex conservative therapy; patients with cholestasis needed a longer treatment – up to 3–5 weeks.

Conservative multicomponent complex therapy consisted of the following fundamental elements: medicinal substances that improve and stabilize the structure and properties of plasmolmma; drugs improving the blood rheological characteristics; allogeneic blood components; agents accelerating the toxins elimination; preparations of protein solutions supplementing the intravascular space proteins insufficiency; blood plasma ionizing polymers; drugs improving metabolism in affected cells due to polarization - glucose-insulin-potassium mixtures; medicinal substances contributing to the liver cells restoration; substances normalizing the immune system; vitamins.

To improve the cytolemma properties was composed of α-tocopherol acetate (200-300 mg intramuscularly), Essentiale (10.0 ml per 200.0 ml of a 0.5% glucose solution intravenously), and freshly frozen plasma – 200–300 ml in a day or two. Also, to stabilize the vascular wall, ascorbic acid was prescribed (10.0 ml of a 5% solution 2–3 times intravenously).

To improve the structural and functional state of the liver and enhance the hepatocytes regeneration, the medicine Hepabene was used (3 pills/day). The Hepabene drug contains the optimal combination of natural active plant components: dry extract of the herb medical haze – 275.1 mg (corresponds to the sum of fumar alkaloids in terms of protopine – 4.13 mg), dry extract of the milk thistle fruits 83.1 mg (corresponds to the content of silymarin – 50 mg, including silibinin no less than 22 mg). Of particular note are the protective, membrane-stabilizing, antioxidant, antifibrotic and anti-inflammatory effects of milk thistle extract. Due to this, the Hepabene drug is justifiably used for HDLD of various etiologies. The beneficial effect of silymarin on the morphofunctional state of the liver is associated with the possibility of protein transporters to contact the hepatocyte membrane and blocking the toxic substances into the cell. The ability of silymarin to invade the cell nucleus causes a positive effect on the synthetic apparatus of the cell. The biological effect of silymarin is manifested in the activation of protein synthesis, stimulation of hepatocyte regeneration, preventing the cell membranes destruction. Thus, the drug has a positive effect on the functional state of the liver, increases its ability to pathological effects, enhances the detoxification ability, increasing the activity of enzyme systems in cases of HDLD or functional disorders of the biliary tract. The antioxidant effect is associated with the properties of silymarin to bind to free radicals, followed by a decrease in lipid peroxidation. Silymarin, in contact with cytolemma elements, stabilizes the hepatocyte membrane, restores metabolic processes associated with lipid metabolism. It also normalizes the glutathione concentration, thereby showing its antitoxic effect, and contributes to the rapid elimination of toxins through the kidneys. It is known that under the influence of intestinal microorganisms up to 40% of silymarin undergoes reverse absorption. Silymarin inhibits the collagen fibers formation, that is, the progression of the fibrous process and even contributes to its reverse development. Hepabene demonstrates pronounced anti-inflammatory properties through the leukotrienes formation inhibition. An important plant ingredient in Hepabene is fumarin, which contains alkaloids and is distinguished by its ability to eliminate biliary tract spasm, normalize peristalsis and prevent the formation of calculi. The main active drug fumitory ingredient is protopin, which increases the cholecystokinin and secretin production. Under physiological conditions, these compounds
also control the biliary tract function. The use of drug fumitory leads to the normalization of both reduced and enhanced process of bile formation in liver cells, stabilization of the volume of secreted bile. This herbal medicine helps to relieve spasm of smooth muscle fibers of the bile ducts, regulates the functioning of the sphincter apparatus, prevents the calculi development in the biliary tract, regulates the bile secretion processes, accelerates the flow of bile into the intestines. Due to the reflex feedback principle, fumarin can reduce the cholesterol absorption in the intestines, its secretion in bile and synthesis in the liver, which affects the ability to form bile stones and normalizes its biochemical composition. An indirect positive effect of fumarin on exocrine pancreatic function, digestion processes and intestinal microorganisms restoration was revealed. The results of the many positive effects of Hepabene were the rationale for its use.

To prevent the endogenous intoxication, the complex of therapeutic measures includes the use of blood plasma, albumin, hemodesis (400 ml/day) and saline solutions, if necessary, forced diuresis. The following drugs were used as blood coagulation inhibitors and to prevent blood clots: Trental (0.5 g / day), Curantyl (0.15 g / day), intravenous infusions of reopoliglukin (400 ml / day) in courses.

Identified disorders of the immune system were regulated by the use of leukocyte suspension, gamma globulin, intravenous infusions of hyperimmune plasma. Levamisole (15 mg / day) was also prescribed in short courses. If a deficiency of vitamins and microelements was detected, they were replenished with the use of vitamin-mineral complexes, with hemoprothrombinemia, vicasol was administered intramuscularly.

4. Results

Morphofunctional changes in the liver, metabolic disorders and endogenous intoxication lead to a change in the properties and structure of the plasma membrane, including erythrocyte membranes. The erythrocyte plasmalemma in patients with CDLD is fragile, the cells lose their elasticity, which becomes crucial in the pathogenesis of the disease, as it affects oxygen transfer, blood rheological properties, microcirculation, regulation of redox processes, the nature of cellular immunity, the hemostasis system functioning and maintaining the blood aggregation state [13].

Patients with inactive liver cirrhosis (table 1) are characterized by moderate dysproteinemia and a 2.2-fold increase in the β-lipoproteins content (16.0 ± 3.2 g/l, against 7.3 g/l normal). The prevalence of low density lipoproteins, stimulating the cholesterol accumulation in the cell membrane, contributes to the disruption of the structure of the lipid-protein layer of the membranes, which leads to a violation of their properties and functions of cells, including blood cells. In these patients, on average, the number of platelets decreases by 23.1% compared with the lower limit of normal, blood viscosity increases by 26%. The erythrocyte aggregation ability is moderately increased (2.4 times, or 51.7%) and their deformability decreases (1.5 times, or 33.1%), with almost normal erythrocyte sorption ability and their osmotic resistance. Coagulogram indicators are at the level of lower limit of normal (tables 1, 2).

All types of hepatic cholestasis are characterized by more pronounced dysproteinemia, hypergammaglobulinemia (p <0.01), azotemia (p <0.01), bilirubinemia (p <0.001), a moderate increase of less than 3 norms (1.9; 2.7; 1.6 times, respectively) of alanine aminotransferase and a sharp increase in alkaline phosphatase, and a significant violation of lipid metabolism. Thus, patients with intrahepatic cholestasis, also have dysproteinemia, azotemia; bile stagnation processes prevail over cytolytic ones. Naturally, the detected protein and lipid metabolism disorders, an increase in the excretory enzymes concentration and azotemia contributed to the endogenous intoxication growth and significant disturbances in the blood aggregation state.

Patients with intrahepatic cholestasis suffered from anemia and moderate thrombocytopenia. During the study, patients with chronic active hepatitis revealed normal or subnormal hematocrit and viscosity indices (table 1). The osmotic resistance of erythrocytes was also moderately reduced (maximum on average to 0.25). The above factors indicate a violation of the morphology and functional characteristics of cell membranes and cells themselves under the endogenous intoxication. The endogenous intoxication effect is confirmed by the high erythrocyte sorption ability, reflecting its degree.
Coagulogram indicators show a decrease in coagulation and activation of anticoagulant and fibrinolytic factors (tables 1, 2).

**Table 1. Indices of homeostasis before and after 7-10 days of conservative therapy.**

| Indices | Nosological forms | Norm |
|---------|------------------|------|
|         | Inactive cirrhosis n=242 | Chr. cholest. hepatitis n=223 |
|         | before | after | before | after |
| Plasma proteins | |
| Total (g/l) | 75.7 | 72.6 | 66.4 | 72.7 | 60-80 |
| A (%) | 52.0 | 52.8 | 46.7 | 49.9 | 55-65 |
| γ-Gl. (%) | 20.8 | 18.4 | 26.2 | 19.9 | 15.1 |
| C A/G | 1.08 | 1.12 | 0.88 | 0.92 | 1.2 |
| Residual nitrogen mm/l | 17.2 | 17.8 | 24.5 | 18.7 | 14.3 |
| Bilirubin mkm/l | 15.5 | 13.0 | 127.4 | 76.3 | 8.55 |
| Direct | - | - | 101.6 | 55.3 | 25% |
| Enzymes | |
| ALT mkm/l | 0.39 | 0.33 | 1.31 | 0.82 | 0.10 |
| AST mkm/l | 0.32 | 0.31 | 0.74 | 0.71 | 0.10 |
| Coef. De | 0.82 | 0.94 | 0.64 | 0.86 | 1.0 |
| Rittis (unit/l) | 68.6 | 58.5 | 292.2 | 137.7 | 10.0 |
| AP | 4.8 | 3.2 | 7.7 | 11.5 | 60.0 |
| Cholestasis (mm/l) | 3.62 | 4.22 | 5.09 | 4.01 | 3.1 |
| Lecithin (mm/l) | 1.52 | 1.10 | 3.71 | 2.23 | 1.0 |
| β-lipoproteins (g/l) | 16.0 | 12.0 | 40.8 | 23.7 | 1.3 |
| As a result of the therapy, the blood protein composition improved in patients of the control group, although mild dysproteinemia remained (albumin-globulin coefficient – 1.12, with a norm of 1.2), the content of β-lipoproteins decreased, lecithin and alkaline phosphatase normalized (tables 1, 2).**

**Table 2. Indices of blood aggregation state before and after 7-10 days of conservative therapy.**

| Indices | Nosological forms |
|---------|------------------|
|         | Inactive liver cirrhosis | Chr. active hepatitis |
|         | before | after | before | after |
| Coagulogram indicators show a decrease in coagulation and activation of anticoagulant and fibrinolytic factors (tables 1, 2).
|                  | before | after | before | after |
|------------------|--------|-------|--------|-------|
| **Blood cells**  |        |       |        |       |
| Erythrocytes (10^{12}/l) | 4.1    | 4.06  | 3.65   | 3.99  |
|                  | 0.20   | 0.10  | 0.11   | 0.82  |
| Hemoglobin (g/l) | 124.4  | 124.6 | 114.9  | 120.7 |
|                  | 3.4    | 4.1   | 4.4    | 3.8   |
| Platelets (10^{9}/l) | 184.6  | 190.2 | 204.9  | 270.4 |
|                  | 5.0    | 4.2   | 16.7   | 13.0  |
| **Blood rheology** |       |       |        |       |
| Viscosity (mPa·s) | 7.51   | 5.72  | 7.29   | 5.28  |
|                  | 0.30   | 0.22  | 0.50   | 0.42  |
|                  |        |       | 0.20   |       |
|                  |        |       |        | 473-5.90|
| YV (n/m²·10^{-3}) | 0.62   | 0.52  | 1.46   | 0.52  |
|                  | 0.10   | 0.05  | 0.62   | 0.12  |
|                  |        |       | 0.08   |       |
|                  |        |       | 0.11-0.89|
| EAC (n/m²·10^{-5}) | 3.24   | 2.00  | 5.18   | 2.13  |
|                  | 0.22   | 0.28  | 0.43   | 0.29  |
|                  |        |       | 0.25   |       |
|                  |        |       | 0.50-2.29|
| CED (unit) | 0.240  | 0.248 | 0.211  | 0.215 |
|                  | 0.003  | 0.004 | 0.002  | 0.06  |
| Hematocrit (%)   | 38.9   | 39.0  | 35.2   | 36.3  |
|                  | 1.7    | 1.6   | 1.3    | 0.9   |
|                  |        |       | 5.3    |       |
|                  |        |       | 36-50  |       |
| **EOR (%)**      |        |       |        |       |
| Min              | 0.42   | 0.43  | 0.40   | 0.42  |
|                  | 0.02   | 0.01  | 0.01   | 0.01  |
|                  |        |       | 0.40-0.45|
| Max              | 0.27   | 0.26  | 0.25   | 0.27  |
|                  | 0.04   | 0.01  | 0.01   | 0.01  |
|                  |        |       | 0.27-0.35|
| ESC (%)          | 39.7   | 40.0  | 45.5   | 42.9  |
|                  | 1.4    | 2.0   | 0.9    | 2.8   |
|                  |        |       | 0.57   |       |
|                  |        |       | 30.0-40.0|
| **Coagulogram**  |        |       |        |       |
| PTI (%)          | 81.5   | 88.1  | 80.1   | 87.9  |
|                  | 1.3    | 6.2   | 2.2    | 4.8   |
| PTH (min)        | 12.4   | 7.8   | 12.5   | 9.9   |
|                  | 0.4    | 0.6   | 0.7    | 0.3   |
| Fibrinogen (g/l) | 2.37   | 2.47  | 2.41   | 2.81  |
|                  | 0.08   | 0.12  | 0.31   | 0.56  |
| Fibrinase (sec)  | 74.1   | 56.3  | 79.7   | 61.0  |
|                  | 1.7    | 6.0   | 3.4    | 2.8   |
| FLA (min)        | 188.6  | 234.5 | 162.3  | 209.8 |
|                  | 2.8    | 10.0  | 8.0    | 14.6  |
Figure 1. Indicators span chart on the blood aggregate state in patients with inactive liver citthosis.

Figure 2. Indicators span chart on the blood aggregate state in patients with chronic active hepatits.

Under the influence of the treatment of cholestatic active hepatitis, the patients' condition significantly improved: dysproteinemia (p <0.05) decreased, the gamma globulin content (p <0.01) and residual nitrogen (p <0.01) normalized, bilirubinemia decreased 1.7 times (De Rittis coefficient increased 1.3 times), and alkaline phosphatase content - more than 2 times (p <0.001).

The lipid metabolism was improved, β-lipoproteins content decreased by 1.7 times (p <0.05). The blood aggregate state improved but not completely. The viscosity returned to normal, however EAC remained increased and CED – decreased (table 2), the endogenous intoxication remained (ESC - 42.9 ± 2.8%, p> 0.05). Coagulogram returned to normal (table 2).
After a week of therapy, YV values decreased to normal, however, with a significant scattering of indicators; the viscosity decreased. EAC and CED, although improved, remained at high values. Normalization of EAC began at 3–5 weeks of preoperative preparation, although CED increased but did not reach normal values, which is combined with the dynamics of beta-lipoproteins (tables 1, 2, figures 1, 2). In the group of patients with chronic active hepatitis, where cholestyramine and sorbitol were used, an improvement was noted, and skin itching decreased.

With cholestatic hepatitis, bilirubinemia, transaminasemia, alkaline phosphatase content decreased, lipid indices improved. The blood cell composition improved (red blood cells, platelets – p < 0.05). The blood aggregate state was almost normal. All indicators of blood rheology and coagulograms improved in comparison with 7-10 days of treatment. Endogenous intoxication also decreased. (tables 1, 2).

If from the tables it is possible to judge the scattering of indicators only relatively, then the charts show that with CAH, the indicators of the blood cell composition have a significant scattering. It especially concerns platelets which have a tendency to decrease, with the prevalence of thrombocytopathy. The scattering of the indicators decreased, which indicates a positive trend.

A decrease in the indicators scattering led to their increase to normal values by 3 to 5 weeks of treatment. The scattering in hemoglobin and platelets decreased (figures 1, 2). The viscosity, aggregation capacity (p < 0.05) and erythrocytes osmotic resistance normalized, erythrocyte deformability improved slightly, coagulogram indices normalized (tables 1, 2, figures 1, 2). The blood aggregate state significantly improved, which was a criterion of conservative therapy efficiency.

5. Conclusion

Thus, with intrahepatic cholestasis, regardless of the nosological form, against the background of dysproteinemia, bilirubinemia, azotemia, moderate transaminasemia, an increase in alkaline phosphatase is 2-4.9 times, cholesterolemia is moderate, phospholipidemia and β-lipoproteinemia are pronounced. With inactive liver cirrhosis, unlike hepatic cholestasis, liver functions are practically not impaired, endogenous intoxication is absent, but β-lipoproteinemia is moderate, an increase in viscosity and erythrocytes aggregation ability, as well as a change in their deformability, are observed when the hemostasis system is within the lower normal range.

The algorithm of therapeutic measures in patients with intrahepatic cholestasis should begin with a complex multicomponent conservative therapy which should be membrane-stabilizing, hepatotropic (intravenous Essentiale, α-tocopherol, Hepatobene, ascorbic acid), disaggregant (Rheopolyglucin, Trental, Curantyl), in combination with detoxification and symptomatic therapy. The multicomponent treatment should be aimed primarily at correcting rheological disorders, reducing endogenous intoxication, preventing the occurrence of disseminated intravascular coagulation syndrome and liver failure. The duration of therapy is at least 3–5 weeks.

The conducted complex conservative therapy significantly improves the condition of patients and normalizes hemostasis and homeostasis. The inclusion of hepatoprotectors of plant origin in the complex conservative therapy is a promising direction in the development of biotechnologies in hepatology.

References

[1] Devyatych E A 2001 Rheological properties of blood with lipid distress syndrome Bulletin of Russian State Medical University 2 (17) 42–43
[2] Sokolov S Ya 2000 Herbal medicine and phytopharmacology. Guide for doctors M.:MIA p 976
[3] Gebhardt R 2002 Oxidative stress, plant-derived antioxidants and liver fibrosis Planta Med 68 289–296
[4] Savelyev V S, Petukhov V A, Karapkin A V and Fomin D K 2002 Extrahepatic biliary dysfunctions in lipid distress syndrome: etiopathogenesis, diagnosis and treatment principles Digestive system diseases 2 62–69
[5] Ikchenko L Yu 2007 Use of Hepatene in the treatment of patients with hepatobiliary pathology Difficult patient 12–13 (5) 43–46
[6] Gromovaya V F, Shapoval G S and Mironyuk K E 2008 Antioxidant properties of medicinal plants Chemical Pharmaceutical Magazine 42 (1) 26–29
[7] Bartos G 2003 Total antioxidant capacity Adv Clin Chem 37 219–292
[8] Nadinskaya M Yu 2001 Liver diseases occurring with intrahepatic cholestasis syndrome. Consilium medicum 4 (6) 286–292
[9] Florkmeier V 2002 Cholestatic Liver Disease Dr. Falk Pharma GmbH
[10] Eisenburg J 2003 Cholestasis Guiding Symptom in Liver Disease: Pathogenesis and Clinical Pictures Dr. Falk Pharma GmbH
[11] Jorge A D and Leuschner U 2001 Primary biliary cirrhosis Dr. Falk Pharma GmbH
[12] Lindor K D and Gershwin M E 2009 Poupon R et al. Primary Biliary Cirrhosis Hepatology 50 (1) 291–308
[13] Podymova S D 2004 Intrahepatic cholestasis: pathogenesis and treatment from a modern perspective Consilium Medicum 6 (2) 3–6