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PHARMACEUTICAL COMPOSITION BASED ON HEPATOPROTECTOR AND PREBIOTIC, THE PRODUCTION AND USE THEREOF

The invention relates to a pharmaceutical composition based on a liver-protective medication and a prebiotic for use according to the preamble of claim 1, and to the production thereof.

The invention is usable in medicine and especially in hepatology and pharmacology. It can be used for the production and use of a pharmaceutical composition based on a liver-protective medication (hepatoprotector) and a prebiotic for the treatment and prevention of liver diseases from the following group of diseases: gallstone conditions, fatty liver and non-alcoholic steatohepatitis, primary biliary cirrhosis, gallbladder cholesterosis, and drug-induced and toxic liver damage.

The topicality of the problem is associated with the increase in prevalence and severity of the liver diseases. The liver is the main organ concerned with the detoxification of exogenous poisons. The causes of the rise in liver disease rates can be traced back to an extremely unfavourable environmental status in the majority of regions of the world.

The exposure to environmental factors is associated, on their part and directly, with a weakening of immunity in the population. This causes a considerable growth of infectious diseases of the liver and especially of viral hepatitis.

The source of infection in the case of a viral hepatitis is a human already affected by the disease. The route of transmission of infection is either faecal/oral or parenteral, depending on the viral species: A, B, C, D, G, E. The sensitivity of the population to this infection is high.

Irrespective of the site of entry of the disease, the virus ultimately reaches the liver. Here, it exerts a direct toxic action on the liver cells. Furthermore, this

poisoning action takes place basically at the same time as the cell membranes take immune-mediated damage. In all forms of viral hepatitis, one of the common and most severe complications is the disturbance in the normal processes of bile formation and secretion. This is a so-called cholestatic syndrome, which is accompanied by jaundice. This syndrome is found most frequently in the case of virus hepatitis A (HAV) – the so-called "enteral" viral hepatitis – and in the case of virus hepatitis E, where the rate of jaundice forms is 100%.

In severe forms of an acute viral hepatitis and in recurrences of a chronic hepatitis, the disturbance in the structure and the functional activity of the biliary ducts is one of the causes of the development of severe complications in biliary cirrhosis.

Apart from viral hepatitis, a large proportion of liver diseases is associated with an exposure to foodstuff poisons (alcohol, other toxic substances and poisons, various medications).

One of the earliest pathological complications in a toxic liver affection is a steatohepatitis. Steatohepatitis is the consequence of the disturbance in the positive balance between the entry of fats into the body and their metabolism.

It should be emphasized that the disturbance in the normal processes of bile formation and the biliary passage thereof is only one of the many widespread effects of a large dose of many medications (antibiotics, p-aminobenzenesulphonamides, chlorpromazine, blockers of histamine receptors and of oestrogen hormones and also cytostatics).

In the last ten years, so-called autoimmune hepatitis has been diagnosed with increasing frequency. This hepatitis is the consequence of a far-reaching disturbance in the cell-associated immune system. One of the most severe sequela thereof is a primary biliary cirrhosis.

One of the most striking forms of the disturbance in the processes of bile formation and bile secretion is the gallstone condition (cholelithiasis). What can be observed here is a superfluous bile accumulation (bile stasis) in the gallbladder with subsequent stone formation (choleliths or gallstones).

In all of the above-mentioned liver diseases, one of the important aetiological and pathogenetic factors is the disturbance in the normal process of bile acid metabolism. Bile acid metabolism is one of the most important factors for a proper digestion.

Bile acid is formed in the liver from cholesterol (see Hofmann A.F. Bile acid secretion, bile flow and biliary lipid secretion in humans. Hepatologi. 1990; 12; 17S; Meier P.J. The bile salt secretory polarity of hepatocytes. J. Hepatol. 1989; 9: 124).

The main bile acids detected in human bile include cholic acids (3α,7α,12α-trihydroxy-5β-cholanic acid), chenodeoxycholic acid, deoxycholic acids (3α,12α-dihydroxy-5β-cholanic acid). Furthermore, much smaller amounts of stereoisomers of cholic and deoxycholic acids in the form of allocholic acid, ursodeoxycholic acid and lithocholic acid (3α-hydroxy-5β-cholanic acid) can be found in bile.

Cholic acid and chenodeoxycholic acid are part of the so-called primary bile acids. They are formed in the liver during cholesterol oxidation. Deoxycholic acid and lithocholic acid are formed in the intestines from the primary bile acids under the influence of the enzymes of microorganisms of the intestinal flora.

Normally, the proportion of cholic acid, chenodeoxycholic acid and deoxycholic acid in bile is 1:1:0.6.

In the gallbladder, the bile acids are usually present in the form of parts of pairs

(conjugates). The bile acids are absorbed into blood in the intestines and usually in the ileum. Via the blood, the bile acids return to the liver again and are secreted in bile again. This is the so-called portal-biliary bile acid circulation. Therefore, 85 to 90% of the total bile acid amounts present in bile account for the bile acids absorbed in the intestines.

The portal-biliary bile acid circulation contributes to the conjugates of bile acid being readily absorbed into the intestines owing to their solubility in water. In said circulation, 10–15% of the total bile acid amount in the intestines is cleaved under the influence of the enzymes of the microorganisms of the intestinal flora. The products of their degradation are discharged in the faeces.

The bile acids emulsify fats and therefore ensure the absorption of insoluble fatty acids and cholesterol and also of vitamins B, K, E and of calcium salts into the small intestine.

Furthermore, the bile acids have a very pronounced choleretic action. They simulate intestinal motor function and have in addition a bacteriostatic and anti-inflammatory action.

Taking into account what has been presented above, the potential component of the method for the treatment and prevention of many pathological liver states is the use of bile acid preparations and especially of ursodeoxycholic acid.

Ursodeoxycholic acid is a tertiary bile acid. It was first discovered in 1902 in the bile of a Chinese bear. Ursodeoxycholic acid has been used in medicine over several centuries. In ancient China, dried-out bear bile was used in the treatment of stomach, intestinal and liver diseases. Under the influence of bacterial enzymes, ursodeoxycholic acid is formed from a 7-ketolithocholic acid, which enters the liver from the small intestine.

The chemical formulae of ursodeoxycholic acid and of the hydrophobic

chenodeoxycholic acid are identical ($C_{24}H_4O_4$).

The use of ursodeoxycholic acid for the treatment of liver diseases in particular requires a dose-dependent change in the above-mentioned bile acid ratio: ursodeoxycholic acid becomes the main constituent of bile, whereas the content of chenodeoxycholic acid and of other bile acids decreases.

The lower accumulation of ursodeoxycholic acid in bile can be observed in the patients affected by liver diseases. This may be associated with a reduction in absorption as a consequence of the decrease in formation of endogenous micelles from the bile acids in the duodenal bile or with a reduction in the secretion of the actual bile acids.

As already mentioned, ursodeoxycholic acid and lithocholic acid are found in only very small amounts (0.1% – 5%) in human bile.

Despite the good absorption ability of ursodeoxycholic acid in the intestines, its level in blood plasma remains comparatively low owing to liver clearance, because an effective conjugation of ursodeoxycholic acid with glycine, taurine, glucuronic acid and sulphate comes about in the liver.

In bile, ursodeoxycholic acid exerts a comprehensive action on cholesterol.

Cholesterol absorption in the intestines, its synthesis in the liver and secretion into bile decreases. However, the cholesterol level in blood is not substantially reduced under the influence of ursodeoxycholic acid.

Ursodeoxycholic acid, and its conjugates which are not absorbed in the small intestine, in the distal segments of the small intestine and in the large intestine, are metabolized by the indigenous bacteria.

Ursodeoxycholic acid is cleaved in the intestines and dehydrogenated to

lithocholic acid. The amount of lithocholic acid is very low in human bile. It is formed in the small intestine under the influence of the microflora during the recovery of many fats. From the small intestine, lithocholic acid enters the large intestine and the rectum. Here, it is partly absorbed and reaches the liver.

In the liver, lithocholic acid is bonded with sulphate anions and then with glycine and taurine and is secreted this way into bile. Its derivatives are taken up only slightly in the intestines and discharged in the stool.

This process is an effective mechanism for the elimination of toxic lithocholic acid from the body.

Chenodeoxycholic acid causes a reduction in the activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. This is an enzyme which takes part in cholesterol synthesis and contributes to reducing the absorption of cholesterol in the intestines. This brings about a change in the ratio of bile acids and cholesterol, and so chenodeoxycholic acid dominates amongst all the bile acids.

The stated mechanisms determine the use of chenodeoxycholic acid in the disintegration of gallstones, which predominantly consist of cholesterol.

Deoxycholic acid is the bile acid which is formed in human intestines under the influence of the enzymes of the intestinal flora. It is drawn into blood and secreted by the liver as a constituent of bile.

It is assumed that the hydrophobic salt of deoxycholic acid may be a link between impaired intestinal motor function and bile lithogenicity. The main bile acids in humans are cholic acid and chenodeoxycholic acid. These are primary bile acids, which are synthesized in the liver from cholesterol.

Secondary deoxycholic acid arises in the distal segments of the small intestine and in the large intestine from cholic acid under the influence of the enzymes of

the intestinal flora, specifically bacterial 7α-dehydroxylase. The deoxycholic acid is partly absorbed from the intestines and absorbed into the recirculation of bile acids after its conjugation with taurine or glycine in the liver.

Prolonged residence time in the intestines intensifies the formation of deoxycholic acid owing to the bacterial metabolism, whereas shortening of said residence time causes the opposite effect.

As a result, the amount of deoxycholic acid varies within a fairly large range of from 10 to 30% of the total pool of bile acids.

Recently, it has been demonstrated that the amount of Gram-positive anaerobic bacteria and their 7α-dehydroxylase-related activity in the appendix is increased in the case of gallstone condition-affected patients with respect to healthy patients.

In this connection, a correlative link was found between the slowed transit across the intestines, the increased proportion of deoxycholic acid, the oversaturation of bile with cholesterol, and the formation of stones. It is assumed that deoxycholic acid contributes to bile lithogenicity and stone formation by the residence time being extended across the intestines. This increases, on its part, the absorption of cholesterol and, in accordance with the mechanism of positive feedback, the formation of deoxycholic acid itself is promoted.

Furthermore, deoxycholic acid can intensify the secretion of cholesterol into bile by stressing the liver cell membrane, where cholesterol is found in sphingomyelin domains. It can also intensify a crystallization of cholesterol in bile by destabilizing the vesicles containing cholesterol.

In the gallbladder, the bile acids predominantly occur in the form of pairs (conjugates). The conjugation of the bile acids with amino acid glycine results in the formation of glycocholic acid or glycochenodeoxycholic acid. In the case of

the conjugation of the bile acids with taurine ($C_2H_7O_3N_5$, degradation product of the amino acid cysteine), taurocholic acid or taurodeoxycholic acid is formed.

The conjugation of the bile acids comprises the stages of the formation of CoA – of the bile acid esters – and of the linking of the bile acid molecule with glycine or taurine by means of the amide bond involving the acyltransferase lysosome enzyme. The ratio of glycine conjugate and taurine conjugate of the bile acids in bile is, on average, 3:1 and can vary depending on the dietary composition and the hormonal state of the body.

Therefore, the disturbance of bile acid metabolism is one of the most important pathogenetic factors in the development of an entire range of liver diseases.

The patent RU 2002123352 (27.03.2004) discloses a method for the treatment of the above-mentioned liver diseases, which method envisages the use of ursodeoxycholic acid preparations in the form of monotherapy or complex therapy.

A method for the treatment of liver diseases by means of the use of a complex therapy with chenodeoxycholic acid preparations is also known (see List of medicinal products of Russia. Pharmacopoeia. Edited by G.L. Vyshkovsky. M.: “RLS-2006”, 2005, pages 895–896).

However, the therapeutic effect in accordance with the cited treatment methods can only be felt after a fairly long time (from a few months up to 6–12 months) of using the medicine. A life-long intake of said medicine is basically required in many cases.

This is substantially related to the fact that the use of bile acid preparations as monotherapy cannot completely eliminate such an important pathogenetic factor such as intestinal dysbiosis and the comprehensive metabolic system disturbances caused thereby.

Substances having a different structure and having different mechanisms of actions have been used for a long time as liver-protective medication (hepatoprotectors). However, many experts dispute that said substances belong to actual liver-protective medication.

In many cases, this includes, for example, the class of the so-called essential phospholipids.

Phospholipids, or phosphoglycerides, are highly specific lipids and are important fundamental components of cell membranes and of the membranes of the structural units of cells, for example mitochondria. They can be referred to as “essential” (indispensable) elements for growth, development, and proper functioning of all somatic cells. In addition to their role in the structure of cell membranes, it can be stated that phospholipids are important constituents of lipoproteins, of “lung surfactants” and of bile. They participate in the functioning of the nervous system, in the enzyme reactions of membranes, and play an important role in metabolism and in oxidation processes. Phospholipids are a component of lipoproteins and influence as such the cholesterol levels in blood.

The phospholipids embedded in thrombocytes participate in the processes of blood coagulation. This ultimately shows their influence on the protective functions of blood and shows the haemodynamics in the body of mammals and of humans. The chemical structure of phospholipids, their biphilic nature, and the presence of charged groups defines the uniqueness of their physiological properties.

The main function of phospholipids is to form the lipid bilayer in cell membranes. The structure and the function of the cell membrane are of very great significance to human health. The general feeling of being unwell, functional disturbance and various diseases can be explained in many cases by membrane damage or by membrane instability. The introduction of

phospholipids can influence membrane functions, which are linked to membrane proteins. In some cases, said functions can even be improved up to a certain extent. In some cases, the disturbed functions can be completely corrected.

Essential phospholipids usually penetrate into liver cells. They become embedded in the membranes of hepatocytes, normalize liver functions, lipid metabolism and protein metabolism, contribute to activating and protecting phospholipid-dependent enzyme systems, and improve the detoxification function of the liver. They regenerate the cell structure of the liver, improve the regeneration processes, and slow the formation of connective tissue in the liver.

The medication reduces the energy expenditure in the liver, converts neutral fats and cholesterol to easily metabolizable forms, and stabilizes the physical and the chemical properties of the liver.

Essential phospholipids indirectly normalize the digestion of both fats and solid foodstuffs in the intestines, and this is specifically due to the restoration of liver cell structure. This causes a normalization of bile formation and bile secretion (K.G. Gurevich. Essentielle Phospholipide bei der Behandlung der Leberkrankheiten [Essential phospholipids in the treatment of liver diseases]. Klinische Qualitätspraxis [Clinical quality practice]. 2002, issue 4, pages 108–111).

Another representative of the group of medicines having liver-protective properties is a plant extract from milk thistles as main active ingredient and the silymarin constituent. This medicine has a liver-protective, regenerative and detoxification action.

This medication neutralizes the free radicals in the liver, prevents the destruction of cell structures, stimulates RNA polymerase in a specific manner, and activates the synthesis of structural and functional proteins and phospholipids in the damaged hepatocytes. It stabilizes cell membranes, prevents the loss of

intracellular components (transaminases), and quickens the regeneration of liver cells. It slows the penetration of some poisonous and liver-threatening substances into the cell (e.g. the poison from the death cap).

Clinical pharmacology: Improvement in the general state of patients affected by liver diseases, reduction in subjective complaints (weakness, heaviness in the right lower rib region, loss of appetite, emesis, itching), normalization of laboratory values: activity of transaminases, of gamma-glutamine transferase, of alkaline phosphatase, of bilirubin values. Long-term intake extends the life of patients with cirrhosis.

Some amino acids or their derivatives are frequently also considered to be liver-protective medication.

The best known of these is ademetionine (List of medicinal products of Russia. Pharmacopoeia. Edited by G.L. Vyshkovsky. M.: “RLS-2006”, 2005, page 51).

This preparation supplements ademetionine deficiency and stimulates generation of ademetionine in the body, especially in the liver and in the brain. The molecule of S-adenosyl-L-methionine (ademetionine) releases the methyl group in methylation reactions of phospholipids of cell membranes of proteins, hormones, neurotransmitters, etc. (transmethylation). It is the precursor of a physiological thiol compound: cysteine, taurine, glutathione (to ensure the redox mechanisms of cell detoxification), CoA, etc. in transsulfphuration reactions. After decarboxylation, said preparation takes part in the processes of aminopropylation as a precursor of polyamines: putrescine (stimulator of cell regeneration and of proliferation of hepatocytes), spermidine and musculamine, which belong to the structure of the ribosomes. The preparation has an anti-cholestatic action. It is useful in the intralobular form of cholestasis (i.e. in the disturbance of synthesis and flow of bile). The anti-cholestatic action is caused by the motility and the polarization of hepatocyte membranes as a result of the simulation of the synthesis of phosphatidylcholine in said membranes. This

improves the function of hepatocyte membrane-associated systems relating to bile acid transport and contributes to the passage of bile acids into the efferent bile system. The preparation stimulates the detoxification of bile acids, increases the content of conjugated and sulphated bile acids in hepatocytes. Conjugation with taurine increases the solubility of bile acids and their discharge from the hepatocyte. Sulphation allows an elimination through the kidneys, facilitates passage through the liver cell membrane and discharge with bile. In addition, the sulphated bile acids protect liver cell membranes from the toxic action of non-sulphated bile acids (these are present at high concentrations in hepatocytes in the case of intrahepatic cholestasis).

In the case of patients with diffuse liver diseases (cirrhosis, hepatitis) with the intrahepatic cholestasis syndrome, this medication reduces itching and the extent of changes in biochemical values, including direct bilirubin, the activity of alkaline phosphatase (AP), of aminotransferases, etc.

During treatment, the asthenic syndrome disappeared for 54% of patients; for 46% of patients, its intensity decreased. The anti-asthenic, anti-cholestatic and liver-protective action was maintained over a period of 3 months after the end of treatment. Effectiveness in the hepatopathies caused by the intake of liver-poisoning medications (paracetamol and others) has been demonstrated. In the case of the treatment of patients following opium abuse with accompanying liver damage, a regression of the clinical symptoms of abstinence, improvement in the functional state of the liver and in microsome oxidation processes, and a mood-lifting action were observed.

Use: Intrahepatic cholestasis; liver affections, including toxic, alcohol-induced, virus-induced, drug-induced (antibiotics, anti-tumour agents, tuberculostatics, virus-killing preparations, tricyclic antidepressants, oral contraceptives); cirrhotic and pre-cirrhotic states; encephalopathy, including encephalopathy with liver failure (alcohol-induced und others); depressive syndrome and withdrawal symptoms.

Besides the immediate influence on liver tissue, ademetionine has some additional pharmacological effects, for example antidepressant action (it develops within the 1st week of treatment and is stabilized in the 2nd week of treatment). Furthermore, this preparation is used in the case of osteoarthritis according to experience. This is accompanied by a reduction in the pain syndrome and a stimulation of the synthesis of proteoglycans and a partial regeneration of cartilage tissue.

However, the use of essential phospholipids in the treatment of liver diseases, like the use of other liver-protective medications, normally does not result in complete recovery. After removal, recurrences or acute symptoms of liver diseases are frequently observed, and one of the reasons for this is that the disturbance in the intestinal biocoenosis persists. The intestinal biocoenosis is usually very persistent in many diseases of organs of the gastrointestinal tract and of the liver.

Currently, a concept of unity (of togetherness) of all the processes which take place in the pathologies of the gastrointestinal tract is gaining increasing recognition in gastroenterology. As part of this concept, one of the most important constituents of this normal state is the normalization of the microflora of the large intestine.

The microflora of the gastrointestinal tract (GIT) and the liver work together continuously in the processes of detoxification of the organism. The microbiota as constituent of the biofilm comes first in contact and in the subsequent metabolic reactions with all substances which reach the body via foodstuffs, water or fresh air. The microbiota converts the chemical substances into non-poisonous end products or into intermediate compounds which are easily broken down in the liver and can be subsequently removed from the body.

The organism has two main organs for detoxification: the liver ensures

protection of the organism by means of oxidation reactions. In return, the microflora of the digestive tract uses hydrolytic reduction processes. The disturbance in the interaction of these systems causes mutual functional and structural changes both in said organs and in the entire body.

Therefore, the enterohepatic circulation of different organic and inorganic compounds can, without exaggeration, be referred to as one of the fundamental homeostatic mechanisms. The reduction in the detoxification function of the GIT microflora in the dysbiosis caused by various pathogens (medicaments, foodstuffs, stress, etc.) increases the stress on the enzymatic systems of the liver and contributes, under certain conditions, to metabolic and structural changes.

In the event of an imbalance in the microenvironment conditions of the digestive tract, the increase in the proportion of potentially pathogenic Gram-negative bacteria leads to a considerable accumulation of endotoxins in the intestinal lumen.

The latter penetrate through the mucous membrane of the intestines into the local circulatory system, and subsequently through the portal vein into the liver and cause injuries to hepatocytes or allow other poisons to act unfavourably. 90% of all endotoxins are optionally released by the anaerobic Gram-negative bacteria. The endotoxins damage cell membranes, disturb ion transport, cause the fragmentation of nucleic acids, induce the formation of products of free-radical oxidation, stimulate apoptosis, etc. (N.M. Gracheva et al. Hylak forte in the complex treatment of patients with acute intestinal infections and chronic diseases of the gastrointestinal tract with dysbacteriosis symptoms. Consilium medicum. 2004. Issue 1. Pages 31–34).

Consequently, one of the possible means of restoration after the disturbances in the entirety of the interactions of microbiota and liver is the control of intestinal dysbiosis.

The paper by V.F. Demin et al. (Erfahrung bei der Anwendung von der biophytogenen Korrektur bei Kindern mit Dysbiose [Experience in the use of biophytogenic correction in children with dysbiosis]. Probleme der modernen Kinderheilkunde [Problems in modern paediatrics], 2003, issue 3, volume 2, pages 33–36) discloses a method for normalizing the intestinal microflora due to the administration (per os) of probiotics. This concerns living bacteria of those species and genera which normally colonize the large intestine of humans and other mammals. However, the use of probiotics in the form of monotherapy does not yield any lasting results owing to the "foreignness" of the bacterial strains introduced from outside and owing to their fairly rapid (3–5 days) removal after intake of the medicine has been terminated.

This deficiency does not exist for other preparations used to correct disturbances in the intestinal microbiota (the so-called prebiotics).

Prebiotics include in particular many oligosaccharides. They are not recovered by the human body because there are no endogenous enzymes in the intestines which can cleave such sugars. Digestible oligosaccharides include, inter alia: fructooligosaccharides, maltooligosaccharides, galactooligosaccharides, inulin, lactulose and some other oligosaccharides which can be used as prebiotics (S.A. Sheveleva. Probiotika, Präbiotika und probiotische Produkte [Probiotics, prebiotics and probiotic products]. Stand von Heute [Position of today]. Ernährungsangelegenheiten [Nutrition affairs], 1999, issue 2, pages 33–39; Shoaf L. et al. Prebiotic galactooligosaccharides reduce adherence of Enteropathogenic Escherichia coli to tissue culture cells. Infect Immun. 2006 September 18. Abstract.).

According to the chemical structure, fructooligosaccharides are oligofructosaccharides in which the residues of β-D-fructofuranose are linked to one another by β-2,1-glycosidic bonds and have at one end of the chain an α-glucose remainder which is linked to the fructose by an α-1,2 bond. They can be considered to be sucrose derivatives, on the fructose part of which 1 to 3

fructofuranose residues are attached by β-2,1 bonds. The main components of fructooligosaccharides are 1-kestose (GF_2), nystose (GF_3) and 1F-fructofuranosyl nystose (GF_4).

Fructooligosaccharides have a pronounced prebiotic action. They are not utilized in the upper segments of the GIT, inhibit the growth of sluggish microflora, promote the normalization of blood pressure and the lipid level in blood, improve the uptake of calcium and magnesium, increase immunity, have a good action in constipations and suppuration processes, and prevent cancer of the large intestine.

Like all prebiotics, fructooligosaccharides are not exposed to hydrolysis by the GIT enzymes. They are not absorbed into the small intestine and, after they have reached the large intestine unmodified, they serve as a selective substrate for a growth of normal microflora.

Lactulose is a disaccharide consisting of galactose and fructose (4-O-D-galactopyranosyl-D-fructose). Under natural conditions, lactulose can be formed in small amounts upon a heating of milk to over 100°C. Lactulose is readily soluble in water and is about 1.5 to 2 times sweeter than lactose.

The prebiotic action of lactulose is shown by an enlargement of the extent of the large intestine, a reduction of pH, and also by a reduction in the ammonia content in the large intestine and an increase in the content of short-chain fatty acids, more particularly propionic acid (ZDUNCZYK Z et al. Physiological effects of lactulose and inulin in the caecum of rats. Arch Anim Nutr. – 2004. – Vol. 58(1). – Pages 89–98).

The influence of lactulose on the intestinal microflora through an increase in the amount of bifidobacteria with increase in activity of microbial β-galactosidases is also known (BOUCHNIC Y. et al. Prospective, randomized parallel-group trial to evaluate the effects of lactulose and polyethylene glycol-4000 on colonic flora

in chronic idiopathic constipation. Aliment. Pharmacol. Ther. – 2004. – Vol. 19(8). – Pages 889–899).

While lactulose is a prebiotic, it has so far been usually and even exclusively used in therapy as a soft and effective laxative. The laxative properties of lactulose are directly linked to the prebiotic effect and are caused by an enlargement of the extent of the large intestine capacity (by about 30%) because the bacterial population is increased.

Thus, there is, for example, disclosure of a method for normalizing the disturbed state of the intestinal microflora, which method includes the use of prebiotics and, in particular, of digestible oligosaccharide (lactulose, fructooligosaccharide and others) (JP 2003-155242, 27.05.2003).

In the technical literature, there is fairly little information about experiences with the use of prebiotics for treating liver diseases.

According to published information (I.G. Nikitin et al. Duphalac (Laktulose) bei der Behandlung der Darmdysbiose bei nicht alkoholischer Steatohepatitis [Duphalac (lactulose) in the treatment of intestinal dysbiosis in non-alcoholic steatohepatitis]. Klinische Aussichten der Gastroenterologie, Hepatologie, Koloproktologie [Clinical views in gastroenterology, hepatology, coloproctology], 2002, issue 1, pages 24–29; V.S. Saveliev. Lipid distress syndrome in surgery. Information sheet from the Russian military medicine academy. 1999, issue 1, pages 36–39), their use by themselves as monopreparations in the experiments for treating liver diseases is found to be only a little effective because cell damage and consequently the functions of the affected liver tissues are not completely eliminated in this case.

The therapeutic effect of the use of only prebiotics appears only after a fairly long time. In this case, there is a failure to achieve a complete restoration of the disturbed lipid and, in particular, cholesterol metabolism. Furthermore, lactulose

was used in large amounts (of about 30 ml of the concentrated syrup per person) in the described research. However, this is not known as prebiotic action, but as a laxative action.

In hepatology, such large doses of lactulose are normally predominantly used for attenuating the symptoms of a hepatic encephalopathy, i.e. for palliative symptomatic care in the case of patients who are already virtually incurable (cirrhosis in the last stage). In such cases, the normalization of the intestinal microflora is not capable of ensuring a more or less long-term effect.

Therefore, in the therapies of various diseases, there is an increasing preference for a comprehensive use of medications of different groups, for example with immunomodulatory action and antibiotics, choleretic agents, etc.

In this connection, there is, for example, disclosure of a method for treating chronic non-calculous cholecystitis with dysbiosis symptoms due to the use of the combination of liver-protective medication (glutargin) and eubiotic (Bifiform) (UA 70018, 15.09.2004).

However, the therapeutic effect of this combination is short because of the above-mentioned limitations associated with the use of probiotics.

A method for treating liver diseases with cholestatic syndrome, by using the combination of liver-protective medication of plant origin (Silybum marianum – extract) with the probiotic strain of Lactobacillus bulgarinii and some other substances, is also known (BG 108250U, 30.04.2005).

However, in this case too, the positive therapeutic effect achieved is relatively short.

The known method for correcting the Saveliev lipid distress syndrome using a complex therapy comprises a liver-protective medication of plant origin –

Hepabene – and the probiotic metabolite Hylak forte (V.A. Petukhov. Funktionsstörung der Leber und Dysbiose beim Lipiden-Distress-Syndrom [Functional disturbance of the liver and dysbiosis in lipid distress syndrome]. RMZh, 2002, issue 10, volume 4, pages 158–160).

However, in this comparison, neither the treatment scheme with the stated medicaments nor their doses or ratios is specified. The interaction of the medicines used is not described either.

EA 5166 (30.12.2004) also discloses the use of a combined preparation for treating liver diseases, comprising, as main active component, an alkaline sphingomyelinase and, as supplement, various substances, including probiotics (Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus catenaforme), prebiotics and ursodeoxycholic acid.

In the above-mentioned state of the art, the main role is assigned to sphingomyelinase (lysosome enzyme). Sphingomyelinase is used for preventing or for treating various diseases belonging to the following group: disturbance in small intestine activity, malignant tumours, disturbances in the immune system, inflammations and desquamation of the mucous membrane of the small intestine, states associated with disturbances in cholesterol synthesis, disturbances in the absorption capability of the small intestine, and allergic small intestine diseases.

The auxiliary substances in the aforementioned pharmaceutical composition include: probiotics (Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus casei, and much more), derivatives of bile acids – ursodeoxycholic acid – and the prebiotic lactulose.

However, the patent document does not contain any information about the role of said auxiliary substances in the treatment of liver diseases. It does not disclose any scientific rationale for including them in said composition.

The same main deficiency is immanent in this use of the pharmaceutical composition for treating the stated diseases – an excessively short action time caused by the exogenous probiotic strain. In this connection, the prebiotic components present in the composition are utilized to a large extent by the above-introduced exogenous strain.

The large amount of components having different actions does not make it possible to properly assess the role of said components and their therapeutic effect. It also does not rule out a mutual antagonism of said effects. This increases the probability of individual variations in the response to the simultaneous introduction of so many preparations.

The prior art closest to the invention is an agent which improves the functions of the liver and acts as methyl group donor. It contains a poorly digestible oligosaccharide which, on its part, contains galactose and is used as product of a functional diet (JP 2003-155242, 27.05.2003).

In this connection, the methyl group donor selected is a group of amino acids which comprises S-adenosylmethionine. The galactose-containing oligosaccharide is selected from a group which includes, inter alia, lactulose or galactooligosaccharide.

However, in the above-cited patent document, the inventors do not consider any prebiotic effects at all of the composition. The role of the oligosaccharides in the proposed agent is (presumably) ascribed by the inventors only to the elimination of the hepatoencephalopathic intoxication induced by ammonia.

Therefore, according to the cited patent document, the inventors do not see any link between the lipid metabolism-normalizing action of the compounds which act as methyl group donors, and the normalization of the state of the intestinal microbiocoenosis. This state is not mentioned at all in the cited examples in the

cited patent document.

The lack of understanding of a steady link between the state of the intestinal microbiocoenosis and the reactions of lipid metabolism, of the involvement of the representatives of normal microflora in breaking the vicious circle of an enteropathogenic recirculation of bile acids, does not make it possible to select the correct composition of components depending on the degree of disturbance of these most important constituents of the metabolic process and to monitor the adequacy and the effectiveness of the performed therapy of liver diseases to the required extent.

Since the role of the first component of the composition is only limited to the function of methyl group donor, the inventors leave other mechanisms of disturbance of lipid metabolism unnoticed for no reason. Cholesterol metabolism is concerned here in particular. Such mechanisms, meanwhile, play a very important role in the development of many liver diseases.

The difference between the invention and the closest prior art is as follows:

- the liver diseases are precisely defined and differ with respect to the closest prior art. The pharmaceutical composition is just developed for treating defined liver diseases,
- the developed pharmaceutical composition comprises a liver-protective medication and a prebiotic selected from a strictly limited number of representatives of this group of medicines,
- the usefulness of a simultaneous introduction of the liver-protective medication and a prebiotic in a pharmaceutical composition taking into account their synergistic influence on one another is demonstrated,
- the ratio of the liver-protective medication and the prebiotic in the pharmaceutical composition is defined.

It is an object of the invention to achieve a substantial positive effect such as a

quickening of the normalization of the state of health and the reduction in the extent of the symptoms of the disease in a complex treatment of individual liver diseases.

The stated object is achieved by the feature of claim 1.

This goal is achieved through the use of a combined (complex) medicine comprising a mixture of liver-protective medication and prebiotic substance, viz. oligosaccharides not digestible in the intestines, in the treatment of liver diseases.

The technical result achieved by this invention is a very rapid restoration of the functions of the liver and the prevention of recurrences of the diseases through a rebuilding of cholesterol metabolism and of the intestinal biocoenosis, which restoration is caused by a synergistic interaction between the liver-protective medication (hepatoprotector) and the prebiotic. This also causes a prevention of adverse effects of the liver-protective medication.

Bile acids / bile acid salts selected from the following group are used as liver-protective medication: glycocholic acid, glycochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid and essential phospholipids.

The presence of the liver-protective medication and the prebiotic in the pharmaceutical composition ensures a pronounced and persisting therapeutic action due to a synergistic action of the liver-protective medication and the prebiotic.

The synergistic action of the liver-protective medication, for example ursodeoxycholic acid, and the prebiotic is caused by ursodeoxycholic acid normalizing the digestion of not only fats, but also solid food, in the intestines owing to the increase in synthesis of bile acids and in actual ursodeoxycholic

acid in the liver. This contributes to normalizing the intestinal microflora. The prebiotic itself promotes the normalization of the microflora through a stimulation of the growth of the resident strains. This involves the appearance of immunostimulatory effects and other effects of the normal flora. Consequently, the digestive processes and the detoxification of exogenous poisons in the microflora are improved. This also reduces a metabolic stress on the liver and contributes to normalizing fatty acid and cholesterol metabolism. This also exerts a stabilizing action on all body cells including hepatocytes.

Therefore, the combination of bile acids or bile acid salts with oligosaccharides which are digestible in the small intestine and from the group of lactulose or fructooligosaccharides, maltooligosaccharides, galactooligosaccharides and inulin in a pharmaceutical composition makes it possible to restore the function of hepatocytes and of the entire liver through normalization of the intestinal biocoenosis. This ensures, on its part, a stabilization of the achieved therapeutic result for a long time.

In the case of liver diseases and gallstone conditions involving predominantly cholesterol calculi, it is known that an increased content of toxic products can be observed in the blood of the patient, which products enter the blood from the large intestine. This concerns ammonia in particular. This is a nitrogen compound which is formed in the large intestine by proteolytic microflora during the bacterial decomposition of proteins. This increases the toxic stress on the liver.

Therefore, the restoration of the microflora in the intestines indirectly contributes to reducing the toxic stress on the liver and to increasing ursodeoxycholic acid in the liver. This promotes, on its part, the rebuilding of the bile composition and, particularly, the increase in bile acids owing to the reduced cholesterol synthesis by the liver. This avoids the recurrences of the disease and, more particularly, the gallstone conditions involving predominantly cholesterol calculi.

Taking into account what has been presented above, the active components of the pharmaceutical composition according to the invention mutually intensify the existing curative properties of each of the components.

The pharmaceutical composition according to the invention is defined by claim 1.

The pharmaceutical composition according to the invention is produced in the form of tablets (pills) (with or without a shell), granules, pellets, powders, capsules, suspensions, emulsions or gels.

In this connection, the pharmaceutical composition according to the invention additionally contains the auxiliary substances generally used in pharmaceutical production, such as microcrystalline cellulose, lactose, cornstarch, stiffening, hydroxypropylmethylcellulose, carboxymethylcellulose, oxypropylmethylcellulose, oxypropylcellulose, their pharmaceutically permissible salts, Ludipress, calcium stearate, magnesium stearate, polysorbate, polyvinylpyrrolidone, polyethylene glycol, talcum, titanium dioxide or silicon dioxide.

The composition according to the invention is produced by mixing the related ingredients. These include both active components (liver-protective medication and prebiotic according to claim 1) and auxiliary substances selected from the following group: microcrystalline cellulose, lactose, cornstarch, stiffening, hydroxypropylmethylcellulose, carboxymethylcellulose, oxypropylmethylcellulose, oxypropylcellulose, their pharmaceutically permissible salts, Ludipress, calcium stearate, magnesium stearate, polysorbate, polyvinylpyrrolidone, polyethylene glycol, talcum, titanium dioxide or silicon dioxide.

The pharmaceutical composition according to the invention is taken orally with

much water over a period of 1.5 to 3 months.

The pharmaceutical composition according to the invention is usable for treating patients with liver diseases from the following group: gallstone conditions involving predominantly cholesterol calculi, alcoholic and non-alcoholic steatohepatitis, primary biliary cirrhosis, gallbladder cholesterosis, and drug-induced and toxic liver damage. Said composition makes it possible to achieve a lasting remission of the disease within a relatively short time (from 6 to 12 weeks).

In this connection, the therapeutic effectiveness is from 89% to 95%.

The pharmaceutical composition according to the invention does not have any contraindications. It is usable for treating patients with the above-mentioned liver diseases, including severe comorbidities in the background (with the exception of the late stages of cirrhosis, of malignant neoplasms of the gastrointestinal tract or other organs), irrespective of the age of the patient.

The composition according to the invention does not have any substantial adverse effects, since its active constituents are used in small and midsized individual curative doses and within a short period.

The inventive embodiments of the composition are notable for low original costs and are accordingly accessible to all patient social groups.

The treatment of patients takes place in the form of outpatient care. The patient does not require any strict or semi-strict bed rest. Therefore, the patients can carry on with their normal lifestyle.

The pharmaceutical composition according to the invention is usable with success for not only treating, but also preventing recurrences of liver diseases from the following group: gallstone conditions predominantly involving

cholesterol calculi, alcoholic and non-alcoholic steatohepatitis, primary biliary cirrhosis, gallbladder cholesterosis, and drug-induced and toxic liver damage. This is possible owing to the rebuilding of the structure and the function of both hepatocytes and the entire liver, and this is caused by the normalization of the intestinal microflora.

The influence of the pharmaceutical composition according to the invention in a particular dose provides a gradual and rising intensification in curative action and the entry of new regulatory levels of homeostasis: subcellular, intracellular, and at the level of tissue, organ, system and organism; this is specifically due to the rebuilding of lipid metabolism, more particularly cholesterol metabolism, which is caused by the normalization of the intestinal microbiocoenosis.

During outpatient treatment, the patient is advised to observe certain daily regimens, at least three meals per day during the entire treatment period; recommendations are given against the consumption of alcohol, fatty foods and spicy foods and the intake of other medicines. During the treatment, a starvation diet and heavy physical work is forbidden.

The final diagnosis is made on the basis of additional tests (ultrasound scan of the liver or radiography of the efferent bile pathways) and laboratory blood tests (biochemistry: cholesterol and its fractions, bilirubin and its fractions, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood cell sedimentation rate, and others).

The inventors tested 60 patients. In the tests, clinical symptoms of liver affections with varying severity are detected in all the patients: yellowing of the skin and the sclera, itching, uncomfortable sensations or heaviness in the right lower rib region, dyspepsia symptoms: nausea, loss of appetite, emesis, weakness, lethargy, change in urine colour (darker colour) and in stool (laxation or diarrhoea).

All the patients receive both outpatient and inpatient treatment. In this treatment, various medicines were used.

Most of the patients (37 out of 60) had comorbidities: chronic gastroduodenitis; cardiovascular system diseases such as hypertension, ischaemic heart disease; pulmonary diseases: pneumosclerosis, bronchial asthma, and others. In addition, accompanying disturbances in the state of the microbiocoenosis of the large intestine were detected in 85% of the patients. All the patients from the test groups (50) are treated with the pharmaceutical composition according to the worked-out regimen of the introduction: 3 times daily during meals over a period of 1.5 – 3.0 months.

Towards the end of the 2nd week, detoxification and synthesis functions of the liver were established in the background of the intestinal biocoenosis for all 50 patients despite the liver affection character.

All the patients noticed a reduction in the uncomfortable feelings in the right lower rib region and an improvement in the general condition as early as the 5th day. At the start of the 2nd week, it is possible to subjectively notice in all the patients a disappearance of the dyspepsia disturbances, a restoration of appetite, a normalization of urine and stool, a disappearance of itching and an original skin colour in the background of a considerable improvement in the general state and general mood. In parallel to this, a reduction or a disappearance of the symptoms of an accompanying pathology is detected. At the end of the 3rd week, the biochemical analysis of the blood revealed the normalization of all biochemical values which characterize liver function and lipid metabolism.

Examples of carrying out the method:

1. Patient I., 46 years old.

Upon admission to hospital: Complaints concerning pulling pains in the right

lower rib region which spread to the right shoulder. Said pains arise 3–4 hours after the consumption of fatty foods or after having a large meal, or after physical strains; general weakness, loss of appetite, nausea, periodic emesis, bitter taste in the mouth, watery stool or sometimes diarrhoea, itching, change in urine colour (darker colour) and in stool colour (brighter).

Medical history: Gallstone condition over a period of 10 years. No operations. Outpatient treatment without any particular effect. Deterioration of state after physical stress.

Objective picture: Overeating, weight 75 kg, height 167 cm, pale skin coverings, scratch mark on the back and on the abdomen. Yellowing of the sclera of the eyes.

Soft abdomen, painful in Kerr's point. Positive Kerr symptoms, phrenicus symptom and Murphy's symptom. Liver at the edge of the costal arch. Vesicular breathing in the lungs. Frequency of breathing movements: 18 per minute.

Heart boundaries are within the age-related norm. The sounds are moderately muffled. Regular rhythm, heart rate 78 per minute, arterial pressure 140/85 mmHg. Negative Pasternacki's sign on both sides.

Test:

General blood sample: Hb 123 g/l; erythrocytes (Er) $4.11*10^{12}$/l; colour index (C.I.) 0.89; leukocytes $4.0*10^{9}$/l; leukocytes with non-segmented nucleus (NSN) 2%; leukocytes with segmented nucleus (SN) 46%; eosinophilic leukocytes (Eph) 5%; lymphocytes (Lph) 45%; monocytes (M) 2%; blood cell sedimentation rate 40 mm/hour.

General urine test: Relative density 10^{16}; no detection of protein and glucose; leukocytes 0-1-3 in field of view; erythrocytes 0 in field of view; urine amylase 16.2 mgc/l.

Coprogram: Few non-striated muscle fibres; fatty acids, moderate amount; undigested plant cellular tissue (much), starch; individual cells.

Faecal sample – Dysbacteriosis: Reduction in the amount of bifidobacteria and lactobacteria, in each case: 10^5/g, 10^6/g, through the increase in fungi from the genus Candida.

Blood biochemistry:

- Bilirubin and its fractions: General bilirubin (GB) – 22.8 μmol/l (N 3.4–20.5 μmol/l); direct bilirubin (DB) – 3.8 μmol/l (N – 0.85–3.4 μmol/l); indirect bilirubin (IB) – 11.7 μmol/l (N – 2.56–10.3 μmol/l);
- Thymol sample (TS) – 12.1 units (N – 4 units), aspartate aminotransferase – 79 units (N – 60 units), alanine aminotransferase – 72 units (N – 50 units), thymol sample (TS) 1.7 units (N – 4 units), alkaline phosphatase (AP) – 362 units (N – up to 295 units), cholate-cholesterol factor 15.3; sugar 3.5 mmol/l (N – 4.4–6.6 mmol/l);
- Cholesterol and its fractions: General cholesterol (GCh) – 5.5 mmol/l (N – 3.65–5.2 mmol/l), cholesterol of high-density lipoproteins – 0.8 mmol/l (N – 0.9–1.9 mmol/l), cholesterol of low-density lipoproteins – 3.2 mmol/l (N – 1.91–2.6 mmol/l), cholesterol factor of atherogenicity CFA 3.5 reference units (N – up to 3 reference units), cholate-cholesterol factor 15.3 (N – to 12);
- Protein fractions: Total protein 67 g/l (N 65–85 g/l); albumins 34 g/l (N – 36-50 g/l);
- Anti-nuclear antibodies: Anti-microsomal antibodies in titre 1:10;
- Coagulogram: Prothrombin index 24 s – 79%; thrombin time 35 s; free heparin 12 s; fibrinogen 2.2 g/l; fibrinolytic activity > 240 minutes.

Coprogram: Dysbacteriosis through the reduction in the number of lactobacteria and bifidobacteria: lactobacteria (10^5) (N >= 10^7/g), bifidobacteria (10^7) (N >= 10^9).

Radiography of the liver and the efferent bile pathways – Indirect signs of gallstone condition, no calculi can be contrasted.

Recommendation

Ultrasound scan of liver and gallbladder.
Ultrasound scan of the liver: Chronic cholecystitis, cholesterol calculi: 0.9, 1.2, 1.5, 1.3 mm, smooth edges.

ECG – Sinus rhythm, signs of moderate hypertrophy of the left ventricle. Arterial pressure 150/85 mmHg; heart rate 74 per minute.

Final diagnosis: Lipid metabolism disturbance, hypercholesterolaemia. Chronic gallstone condition (cholesterol calculi) in the recurrence phase.

Treatment: Use of the composition according to the invention, the active ingredients of which (liver-protective medication – ursodeoxycholic acid – and lactulose) in a 1:2 ratio (individual dose of ursodeoxycholic acid is 325 mg), intussusception 3 times daily during meals over a period of 1.5 months and, at the same time, diet No. 5.
Test repeated after 1.5 months:
Ultrasound scan: Individual calculi with dimensions of 1 and 2 mm.

Findings of the laboratory test – No pathology determined.
Recommendation: Continuation of treatment over a period of 3 months.

After 3 months
Ultrasound scan: Signs of chronic cholecystitis, no calculi present.
Opinion: Chronic cholecystitis in the remission phase.

2. Patient B., 45 years old.

Upon admission to hospital: Complaints concerning loss of appetite, weakness, nausea, periodic emesis, pulling pains in the right lower rib region after having a large meal or after fatty meals.

Medical history: Chronic alcoholism. Primary biliary cirrhosis. Irregular

treatment.

Objective picture: Undernourishment. Dry hot skin. Light yellow skin coverings, yellowing of the sclera of the eyes.

Pulmonary sound across the lungs. Weak breathing, scattered dry rattling noises over the entire lung surface. Frequency of breathing movements: 20 per minute.

Heart boundaries are expanded by 1.0 cm to the left. Muffled sounds. Regular rhythm, stress of the 2nd sound over the aorta.

Soft abdomen, right edge of liver protrudes by 2.0 cm beyond the edge of the costal arch, tight edge. The spleen is not enlarged.

Questionable Pasternacki's sign.

Preliminary diagnosis: Primary biliary cirrhosis?

Test:

General blood sample: Hb 117 g/l; erythrocytes (Er) $3.5*10^{12}$/l; colour index (C.I.) 0.9; leukocytes $4.0*10^{9}$/l; leukocytes with non-segmented nucleus (NSN) – 17%; leukocytes 6%; leukocytes with segmented nucleus (SN) 36%; eosinophilic leukocytes (Eph) 5%; lymphocytes 35%; monocytes (M) 1%; blood cell sedimentation rate 40 mm/hour.

General urine test: Relative density 10^{12}; no detection of protein and glucose; leukocytes 0-2-3 in field of view; erythrocytes 0-2 in field of view; urine amylase 14.7 mgc/l.

Coprogram: Non-striated muscle fibres – not many; fatty acids – moderate amount; plant cellular tissue.

Blood biochemistry:

- Bilirubin and its fractions: General bilirubin (GB) – 28.4 μmol/l (N 3.4–20.5 μmol/l); direct bilirubin (DB) – 4.8 μmol/l (N – 0.85–3.4 μmol/l); indirect bilirubin (IB) – 15.0 μmol/l (N – 2.56–10.3 μmol/l);
- Thymol sample (TS) – 16.1 units (N – 4 units), aspartate aminotransferase – 90 units (N – 60 units), alanine aminotransferase – 74 units (N – 50 units), alkaline phosphatase (AP) – 700 units (N – up to 295 units), sugar 6.6 mmol/l (N – 4.4–6.6 mmol/l);

– Cholesterol and its fractions: General cholesterol (GCh) – 5.9 mmol/l (N – 3.65–5.2 mmol/l), cholesterol of high-density lipoproteins – 10.8 mmol/l (N – 0.9–1.9 mmol/l), cholesterol of low-density lipoproteins – 3.6 mmol/l (N – 1.91–2.6 mmol/l), cholesterol factor of atherogenicity CFA 3.9 reference units (N – up to 3 reference units), cholate-cholesterol factor 16.3 (N – to 12); (norm: up to 50);

– Protein fractions: Total protein 63 g/l (N 65–85 g/l); albumins 34 g/l (N – 36-50 g/l);

– Antibodies: Anti-microsomal antibodies in titre 1:45;

– Coagulogram: Prothrombin index 24 s – 79%; thrombin time 31 s; free heparin 11 s; fibrinogen 2.0 g/l; fibrinolytic activity > 221 minutes.

Faecal sample – Dysbacteriosis: Reduction in the amount of lactobacteria and bifidobacteria: lactobacteria (10^4), bifidobacteria (10^6).

Radiography of the liver and the efferent bile pathways: Enlargement of the liver in the right liver lobe by 2.5 cm, distinct edges, indirect signs of biliary cirrhosis.

Recommendation: Ultrasound scan of liver and gallbladder.

Ultrasound scan of the liver: Signs of slight fat infiltration into the liver and of gallbladder cholesterolosis. The pancreas is not enlarged. The intrahepatic and the extrahepatic bile pathways are not expanded. No signs of portal hypertension were found.

No portal hypertension found.

Liver biopsy: Expanded portal pathways are infiltrated by lymphocytes, plasma cells, macrophages and eosinophilic leukocytes. Formed lymphoid follicles occur among the infiltrate cells of the portal pathways. The infiltrates are detected in the walls of some lymphoid follicle-intralobular bile ducts. The entirety of the basal membrane of the bile ducts is partly damaged. Besides the affected bile ducts, there are granulomas constructed from the epithelioid cells and the polynuclear giant cells.

Opinion: Biliary cirrhosis

ECG: Sinus rhythm, signs of moderate hypertrophy of the left ventricle. Arterial pressure 150/85 mmHg; heart rate 76 per minute.

Final diagnosis: Lipid metabolism disturbance, hypercholesterolaemia. Primary biliary cirrhosis.

Treatment: Use of the composition according to the invention, the active ingredients of which (liver-protective medication – chenodeoxycholic acid – and fructooligosaccharide) in a 1:250 ratio (individual dose of chenodeoxycholic acid is 250 mg), intussusception 3 times daily during meals over a period of 1.5 months in the background of diet No. 5.

Test repeated after 1.5 months:

Ultrasound scan: Positive dynamics: reduction in fat infiltration into the liver.

Blood sample: Decrease in hypercholesterolaemia – 4.6 mmol/l, cholesterol of high-density lipoproteins – 1.2 mmol/l, cholate-cholesterol factor 13.2; general bilirubin – 21.5 μmol/l, anti-microsomal antibodies 1:30; total protein – 72 g/l, albumins – 34%, blood sugar 5.3 mmol/l, alkaline phosphatase – 301 units, aspartate aminotransferase – 70 units, alanine aminotransferase – 62 units, TS – 8.1 units.

Faecal sample for dysbacteriosis: Lactobacteria 10^6, bifidobacteria – 10^7.

Recommendation: Continuation of treatment over a period of 3 months.

After 3 months – Anti-microsomal antibodies 1:15, bifidobacteria 10^9/g, lactobacteria 10^7/g.

Opinion: Primary biliary cirrhosis of stage 1 (considerable positive dynamics)

3. Patient F., 50 years old

Upon admission to hospital: Complaints concerning pronounced general weakness, heaviness and pulling pains in the right upper part of the abdomen, the pains come without any visible reasons, loss of appetite, nausea, periodic emesis, bitter taste in the mouth, watery stool, sometimes diarrhoea, itching, change in

urine colour (darker colour) and stool colour (brighter)

Medical history: Type II diabetes over a period of 15 years. The patient takes the hypoglycaemic preparation bucarban. The patient is monitored in the hospital by the endocrinologist and the internal medicine specialist. The patient receives regular inpatient treatment in the endocrinology department, but without any particular results. The last case of deterioration is due to viral infection (delayed progression; complications: acute bronchitis, intake of the antibacterial preparations cephalosporins).

Objective picture: Overeating: stage 3 obesity, body mass index (BMI) 34, pale skin coverings, scratch marks on the abdomen and on the inner surface of the hips. Yellowing of the sclera of the eyes.

The dimensions of the abdomen are substantially enlarged. The abdomen is soft, painful in Kerr's point. The liver protrudes from the edge of the costal arch by 2.5 cm. Tight edge. The spleen is not enlarged.

Attenuated vesicular breathing (because of adipose cell material) in the lungs. Frequency of breathing movements 22 per minute.

The heart boundaries are expanded: to the right by 1.0 cm, to the left 1.5 cm. Muffled sounds, regular rhythm, soft systolic heart murmur over the cardiac apex. Heart rate 76 per minute, arterial pressure 160/85 mmHg.

Questionable Pasternacki's sign on both sides.

Preliminary diagnosis: Fatty hepatosis (?), type II diabetes, stage 3 obesity.

Test:

General blood sample: Hb 121 g/l; erythrocytes (Er) – $4.15*10^{12}$/l; colour index (C.I.) – 0.89; leukocytes – $3.8*10^{9}$; leukocytes with non-segmented nucleus (NSN) – 7%; leukocytes with segmented nucleus (SN) – 40%; eosinophilic leukocytes (Eph) – 5%; lymphocytes 45%; monocytes (M) 3%; blood cell sedimentation rate 39 mm/hour; blood glucose 6.8 mmol/l.

General urine test: Relative density 10^{16}; traces of protein; leukocytes 3-5 in

field of view; erythrocytes 0 in field of view; urine amylase 16.2 mgc/l.

Coprogram: Non-striated muscle fibres – not many; fatty acids – moderate amount; undigested plant cellular tissue – much; starch; individual cells.

Faecal sample for dysbacteriosis: Reduction in the amount of bifidobacteria and lactobacteria, 10^5/g, 10^6/g (in each case), through the increase in fungi from the genus Candida.

Blood biochemistry:

- Bilirubin and its fractions: General bilirubin (GB) – 27.0 μmol/l (N 3.4–20.5 μmol/l); direct bilirubin (DB) – 3.6 μmol/l (N – 0.85–3.4 μmol/l); indirect bilirubin (IB) – 11.2 μmol/l (N – 2.56–10.3 μmol/l);
- Thymol sample (TS) – 8.0 units (N – 4 units), aspartate aminotransferase – 69 units (N – 60 units), alanine aminotransferase – 76 units (N – 50 units), thymol sample (TS) 1.7 units (N – 4 units), alkaline phosphatase (AP) – 346 units (N – up to 295 units), cholate-cholesterol factor 15.3, sugar 6.9 mmol/l (N – 4.4–6.6 mmol/l);
- Cholesterol and its fractions: General cholesterol (GCh) – 5.9 mmol/l (N – 3.65–5.2 mmol/l), cholesterol of high-density lipoproteins – 0.8 mmol/l (N – 0.9–1.9 mmol/l), cholesterol of low-density lipoproteins – 3.6 mmol/l (N – 1.91–2.6 mmol/l), cholesterol factor of atherogenicity CFA 3.8 reference units (N – up to 3 reference units), cholate-cholesterol factor 16.3 (N – to 12);
- Triglycerides: 1.94 mmol/l (N – 0.45–1.82 mmol/l);
- Protein fractions: Total protein 67 g/l (N 65–85 g/l); albumins 38 g/l (N – 36-50 g/l);
- Coagulogram: Prothrombin index 24 s – 79%; thrombin time 35 s; free heparin 12 s; fibrinogen 2.2 g/l; fibrinolytic activity > 240 minutes.

Coprogram: Dysbacteriosis as a result of the reduction in the number of lactobacteria and bifidobacteria: lactobacteria (10^4) (N >= 10^7/g), bifidobacteria (10^6) (N >= 10^9/g).

Radiography of the liver and the efferent bile pathways: Even liver enlargement. No calculi present.

Recommendation: Ultrasound scan of the liver and the bile ducts.

Ultrasound scan of the liver: Stage 2 hepatomegaly. The pancreas is not enlarged. The intrahepatic and the extrahepatic bile pathways are not expanded. Signs of portal hypertension.

Oesophagogastroduodenoscopy: Expansion of oesophageal veins b/3.

ECG – Sinus rhythm, signs of moderate hypertrophy of the left ventricle, partial bundle branch block (right). Arterial pressure 160/85 mmHg; heart rate 74 per minute.

Final diagnosis: Lipid metabolism disturbance, triglyceridaemia. Fatty hepatosis. Type II diabetes. Stage 3 obesity.

Treatment: Use of the composition according to the invention, the active ingredients of which (liver-protective medication – essential phospholipids – and fructooligosaccharides) are in a 1:50 ratio (individual dose of essential phospholipids is 50 mg). Intussusception 3 times daily during meals over a period of 1.5 months in the background of diet No. 5.

Test repeated after 1.5 months:

The patient noticed the reduction in itching (virtually no itches). Increased activity. Weight loss of 5 kg.

Ultrasound scan: Smaller liver dimensions.

Opinion: Fatty hepatosis, stage 1 hepatomegaly.

Laboratory test data: Reduction in triglyceride content – 1.82 mmol/l and in general cholesterol content – 5.0 mmol/l. Normalized blood sugar – 4.9 mmol/l.

Faecal sample: Considerable increase in lactobacteria and bifidobacteria – 10^7/g and 10^9/g (in each case). Candida fungi were not detected.

Recommendation: Continuation of treatment over a period of 3 months.

After 3 months:

Ultrasound scan: Signs of moderate hepatomegaly. No peculiarities in the blood sample. The intestinal microflora was rebuilt: lactobacteria 10^7/g, bifidobacteria 10^9/g.

Opinion: Fatty hepatosis, signs of moderate hepatomegaly. Type II diabetes, compensation. Stage 3 obesity.

Example 4.

Testing of composition for acute toxicity

The composition of ursodeoxycholic acid and lactulose in a 1:2 ratio (group 1), ursodeoxycholic and fructooligosaccharides in a 1:50 ratio (group 2), essential phospholipids (lecithin) and galactooligosaccharides in a 1:30 ratio (group 3), ademetionine and lactulose in a 1:50 ratio (group 4) was orally administered to white mice (of no strain) having a body weight of 15–20 g.

The control group (group 6) received the same amount of starch suspension. The animals were observed for 96 hours. Their general state (appearance, activity, regularity of food and water uptake, type and nature of excrements) was recorded regularly.

Mixture (group)	Max. amount of administered preparation, g	Survivability (alive/dead)	Appearance	Nourishment	Mobility	Type and nature of excrements
1	2	6/0	N	N	N	N
2	2	6/0	N	N	N	N
3	2	6/0	N	N	N	N
4	2	6/0	N	N	N	N
5	2	6/0	N	N	N	N
6	2	6/0	N	N	N	N

The test results reveal that the acute toxicity of each composition cannot be detected under experimental conditions. All the mixtures belong to the class of substances which are low in harmful substances (the LD-50 values exceed 100 g/kg of body weight).

Therefore, the pharmaceutical composition according to the invention, containing liver-protective medication and prebiotic composed of oligosaccharides indigestible in the intestines as active ingredients, can be recommended for use under clinical conditions for treating and preventing liver diseases from the following group: gallstone conditions, fatty hepatosis and non-alcoholic steatohepatitis, primary biliary cirrhosis, gallbladder cholesterosis, and drug-induced and toxic liver damage.

Patentkrav

1. En farmasøytisk sammensetning for anvendelse ved behandling og forebyggelse av tilbakefall av leversykdommer som er forårsaket av en forstyrrelse i lipid-kolesterol-metabolismen, der sykdommene er valgt fra gruppen bestående av gallestein-tilstander som involverer hovedsakelig kolesterolstein, alkoholisk og ikke-alkoholisk steatohepatitt, primær biliær cirrhose, galleblære-kolesterose og medikament-indusert og toksisk leverskade, karakterisert ved at den farmasøytiske sammensetningen blir brukt innvendig og har som sin aktiv ingrediens et leverbeskyttende medikament valgt fra gruppen bestående av kolsyrer, kenodeoksykolsyrer, deoksykolsyrer, ursodeoksykolsyrer, lithokolsyrer, tauroursodeoksykolsyrer, glycodeoksykolsyrer, glykokolsyre, taurokolsyrer og laktulose som et prebiotikum, som velges i effektive doser.

2. Farmasøytisk sammensetning for anvendelse i henhold til krav 1,
karakterisert ved at
de blir tatt internt som ernæringstilskudd.

3. Farmasøytisk sammensetning for anvendelse i henhold til krav 1 eller 2,
karakterisert ved at
den som leverbeskyttende medikament, har gallesyre eller gallesalt og prebiotika i et forhold på 1:2 til 1:250 etter vekt til den rene substansen.

4. Farmasøytisk sammensetning for anvendelse i henhold til krav 1,
karakterisert ved at
den som leverbeskyttende medikament, har essensielle fosfolipider valgt fra gruppen bestående av fosfatidylkolin, cefalin, og fosfatidyl-inositol.

5. Farmasøytisk sammensetning for anvendelse i henhold til krav 1 eller 4,
karakterisert ved at
den som leverbeskyttende medikament, har essensielle fosfolipider og prebiotika i et forhold på 1:0,1 til 1:100 etter vekt av den rene substansen.

6. Farmasøytisk sammensetning for anvendelse i henhold til krav 1,
karakterisert ved at
den i tillegg har mikrokrystallinsk cellulose, laktose, maisstivelse, stivelsesmidler, hydroksypropylmetylcellulose, karboksymetylcellulose, oksypropylmetylcellulose,

oksypropylcellulose, deres farmasøytisk tillatte salter, Ludipress, kalsiumstearat, magnesiumstearat, polysorbat, polyvinylpyrrolidon, polyetylenglykol, talkum, titandioksid eller silisiumdioksid.

7. Farmasøytisk sammensetning for anvendelse i henhold til krav 1, **karakterisert ved at** den er utformet i form av tabletter, granuler, pellets, pulver, kapsler, suspensjoner, pastaer, siruper, emulsjoner eller geler, som er beregnet for innvendig bruk i doser svarende til de kjente doser av leverbeskyttende medisiner og prebiotika, 2 til 3 ganger per dag.

8. Fremstilling av en farmasøytisk sammensetning i henhold til krav 1, for behandling av leversykdommer valgt fra gruppen bestående av gallestein-tilstander som involverer hovedsakelig kolesterolstein, alkoholisk og ikke-alkoholisk steatohepatitt, primær biliær cirrhose, galleblærekolesterose, og medikament-indusert og toksisk leverskade, karakterisert ved at de aktive komponentene – leverbeskyttende medikamenter og prebiotika – tatt i effektive doser, blandes med mikrokrystallinsk cellulose, laktose, maisstivelse, potetstivelse, hydroksypropylmetylcellulose, karboksymetylcellulose, oksypropylmetylcellulose, oksypropylcellulose, sammen med deres farmasøytisk tillatte salter, Ludipress, kalsiumstearat, magnesiumstearat, polysorbat, polyvinylpyrrolidon, polyetylenglykol, talkum, titandioksid eller silisiumdioksid.