



Review

Activity of essential phospholipids (EPL) from soybean in liver diseases

Karl-Josef Gundermann¹, Ann Kuenker², Erwin Kuntz³, Marek Drożdżik¹

¹Institute of Pharmacology, Pomeranian Medical Academy, Powstańców Wielkopolskich 72, PL 70-111 Szczecin, Poland

²Elk Rapids Medical Clinic, 516 Bridge Street, Elk Rapids, MI 49629, USA

³Scientific Institute of Hepatology, Auf dem Kronberg 6, 35582 Wetzlar, Germany

Correspondence: Karl-Josef Gundermann, e-mail: gmc-gundermann@netcologne.de

Abstract:

Essential phospholipids (EPL) contain a highly purified extract of polyenylphosphatidylcholine (PPC) molecules from soybean. The main active ingredient is 1,2-dilinoleoylphosphatidylcholine (DLPC), which differentiates it from other phospholipids, lecithins, or extracts from other sources. Although EPL is widely used in liver diseases of various origins, its mode of action and pharmacological and clinical evidence of its efficacy have not yet been concisely reviewed. This paper critically summarizes experimental and clinical results.

With regard to *in-vitro* and animal tests, EPL influenced membrane-dependent cellular functions and showed anti-oxidant, anti-inflammatory, anti-fibrotic, apoptosis-modulating, regenerative, membrane-repairing and -protective, cell-signaling and receptor-influencing, as well as lipid-regulating effects in intoxication models with chemicals or drugs. Clinical studies, primarily from European and Asian countries, have shown improvement in subjective symptoms; clinical, biochemical and imaging findings; and histology in liver indications such as fatty liver of different origin, drug hepatotoxicity, and adjuvant in chronic viral hepatitis and hepatic coma. The available studies characterize EPL as evidence-based medicine, although further long-term controlled clinical trials are required to precisely determine its benefit for alleviating symptoms, improving well-being, inducing histological changes and slowing the progression of liver disease. EPL-related relevant side effects were not observed.

Key words:

essential phospholipids, membrane therapy, fatty liver, chronic hepatitis, intoxication, radicals, fibrosis

Introduction

Acute and chronic liver diseases are important causes of morbidity and mortality worldwide. Conventional medical therapies for many of these disorders and diseases have limited efficacy. Rather than administering these drugs, which have a characteristic action profile and distinctive side effects, complementary and alter-

native medicines are being used by an increasing population. These medicines are often regarded as more natural and safe than conventional medicine. Their use is a challenge for physicians because the composition of the active ingredients is unknown or incompletely understood in many cases. They are often neither standardized nor developed and investigated according to good clinical practice (GCP).

To evaluate the biological activity of essential phospholipids (EPL) for the treatment of liver diseases and to compare it to other complementary and alternative therapies [146], we conducted database research on Medline, Embase, Cochrane Library and country-specific journals with relevant hepatogastrointestinal articles. This screening included alternative names for EPL or polyenylphosphatidylcholine, such as polyenephosphatidylcholine, polyunsaturated phosphatidylcholine, phosphatidylcholine from soybean, and trade names of EPL products (Essentiale[®], Lipostabil[®], Phoschol[®]). Our database research was hampered by the fact that the wording essential phospholipids or EPL is also used for products with purification grades lower than the minimum of 72% (3-sn-phosphatidyl)choline. However, we defined the minimum amount of 72% (3-sn-phosphatidyl)choline as being necessary to treat the patient when incorporating a considerable amount of 1,2-dilinoleoylphosphatidylcholine (DLPC) as a key component in EPL (for details, see below). Therefore, we reviewed only those papers that indicated this minimum purification grade of EPL or that were based on the aforementioned trade names.

Phospholipids

Phospholipids are essential components of all cellular and sub-cellular membranes, with phosphatidylcholine and phosphatidylethanolamine being the most abundant [150], and they can form lipid bilayers. In addition to phospholipids, cholesterol, glycolipids, and peripheral and integral proteins are also incorporated in the membranes. The basic structure of biological membranes is thus a series of recurrent units of lipid-protein complexes [48]. The integrity and function of the external (cellular) and internal (sub-cellular) membrane systems depend on their composition and on the integrity of their phospholipid structure [29, 48]. Membranes are selectively permeable structures, which are essential for effective separation of a cell or organelle from its surroundings. No mammalian membranes or cells are formed without phospholipids. In addition to cell membranes, phospholipids are structural and functional constituents of the surface monolayers of lipoproteins [33], pleura and alveoli of the lung, pericardium, joints, peritoneal and gastrointestinal surfactants [28, 50, 143], and to-

gether with cholesterol and bile acids, they form mixed micelles in the gallbladder [32]. In addition to chylomicrons, lipoproteins are essentially synthesized and/or metabolized in the liver, and their main task is the transport of lipophilic triglycerides and cholesterol through the hydrophilic blood.

Phosphatidylcholines are indispensable for cellular differentiation, proliferation, and regeneration, as well as for the transport of molecules through membranes. They control membrane-dependent metabolic processes between the intracellular and intercellular space, maintain and promote the activity and activation of membrane-bound proteins such as enzymes (e.g., Na⁺-K⁺-ATPase, lipoprotein lipase, lecithin-cholesterol acyltransferase (LCAT) and cytochrome oxidase) and receptors (e.g., of insulin), and contain bound polyunsaturated fatty acids to be released on demand as precursors of cytoprotective prostaglandins and other eicosanoids. They are a source of second messengers in cell signaling (e.g., of diacylglycerol), contain phosphate for cellular processes including ATP formation, participate in fat emulsification in the gastrointestinal tract and bile, are a determinant of erythrocyte and platelet aggregation, and influence immunological processes at the cellular level. For the cellular biosynthesis of phosphatidylcholine molecules, 5,600 cal/Mol or 8 moles of ATP are needed [48, 76].

Membrane fluidity plays a significant role in the function of biological membranes, and fluidity is decisively influenced by the composition of phospholipids. In addition to the content of cholesterol and proteins, and the nature and charge of the polar head-groups of the phospholipids, membrane fluidity depends on the length of the fatty acid chains of the phospholipids as well as the number and type of their double bonds. In mammals, polyunsaturated fatty acids such as linoleic acid are basic constituents of phospholipids, and they influence cellular membrane fluidity and modulate the activities of membrane-bound enzymes, carriers and receptors [48, 66, 101, 154].

EPL/polyenylphosphatidylcholine (PPC)

The EPL with their PPC molecules indicate a well-defined, highly purified extract of the semen of soybeans (*glycine max.*), with standardized contents of

73–79% to 92–96% (3-sn-phosphatidyl)choline. EPL supplies the organism with non-toxic (3-sn-phosphatidyl)choline molecules that have a high content of bound polyunsaturated fatty acids, particularly linoleic acid. The quantitatively and qualitatively dominating molecule is DLPC, representing up to 52% of the administered phosphatidylcholine molecules (Fig. 1) [90]. This high level of DLPC is the primary difference between EPL/PPC and typical phospholipids (e.g., triple lecithin, raw lecithin, egg lecithin), as well as the phosphatidylcholines consumed through the diet and synthesized within the body.

Physiologically, only about 1.3% of the phosphatidylcholine content in serum is DLPC [110]. EPL administration significantly augments its percentage in the membranes of hepatocytes, blood corpuscles and pancreatic tissue, among other tissues [1, 5, 6, 110]. Increasing the amount of DLPC in membranes results in increased membrane fluidity and influences membrane-dependent functions [2, 7, 48, 66, 75]. It is currently accepted that DLPC is the key active ingredient in EPL [8, 17, 21–26, 95, 107, 114, 123, 153].

Pharmacokinetics of exogenous EPL/DLPC

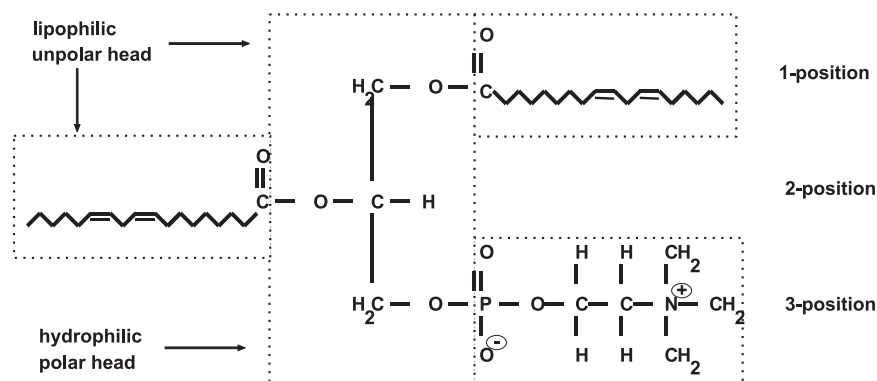
In pharmacokinetic investigations of EPL, the key component DLPC was radioactively or deuterium labeled. Different isotopes and, sometimes, multiple labels with various molecular components were applied [48].

The absorption rate following oral administration of the labeled EPL within 24 h was higher than 90% in animals and humans [80, 157]. In rats, almost 100% of the administered dose of EPL was hydrolyzed to 1-acyl-phosphatidylcholine during absorption, of which about 50% was reacylated to the original molecule. Studies in rats and mice in which EPL was given intravenously showed that approximately 80% of the injected dose was eliminated from plasma after 15 min, about 92% after 75 min and nearly 100% after 10 h [48]. In man, the maximum concentration in blood six hours after oral administration of the labeled substance was about 20% of the administered dose and, thus, approximately four times that measured in rats and monkeys [157]. In blood, phospholipid fractions of the lipoproteins exchange with EPL, with EPL being taken up preferentially by HDL particles [156–158].

EPL is primarily incorporated into the liver, with minor incorporation into other organs such as the gastrointestinal tract, spleen, lung, muscles, kidneys and brain [48, 80, 157]. Analytic examinations of the incorporated $^3\text{H}/^{14}\text{C}$ -EPL showed that radioactivity was primarily localized in the membrane-containing fractions, with the distribution being ubiquitous in all liver cell fractions [48, 80, 156–158].

Renal excretion after a single dose in the first eight days was 17.4% of the administered dose in rats and 17.7% in rhesus monkeys; 15% was expired by breathing [48]. As the excretion in the feces was low, with 3–8% of the dose excreted in the first 5–7 days in rats [80], a considerable part of the EPL must have accumulated within hepatocytes and other cells, blood corpuscles and lipoproteins.

Fig. 1. 1,2-Dilinoleoylphosphatidylcholine (DLPC), the main active ingredient in EPL



Mode of action and pharmacological effects of EPL in the liver

The normal human liver consists of about 300 billion hepatocytes, and the total surface of all cellular and sub-cellular membranes amounts to approximately 33,000 square meters [75]. Many biological reactions take place at and in this membrane surface. Serious disorders may occur due to toxic, inflammatory, allergic, metabolic or immunological effects caused by viruses, organic solvents, alcohol, medication, drugs and fatty food, leading to morphological cell damage. Regardless of the origin, damage to liver cell membranes and the organelles is generally present in liver diseases that are associated with reduced phospholipid levels, altered phospholipid composition and/or decreased membrane fluidity.

Liver function is inhibited by reduced membrane fluidity that leads to impaired membrane-associated enzyme, receptor and carrier activities. Under chronic conditions, these can lead to altered collagen metabolism, promoting the formation of fibrosis and cirrhosis. Furthermore, the liver is the key organ in lipid metabolism. In dyslipidemia, various pathomechanisms (lipid

peroxidation, decreased lipid-metabolizing enzyme activity, modification of lipoprotein structure and function) interact and induce an increase in serum cholesterol and triglyceride levels. Liver disease-induced, lowered high-density lipoprotein (HDL) secretion into blood leads to reduced take up and transport of cholesterol from the periphery back to the liver. In addition to the liver, an elevated cholesterol/phospholipid ratio in the membranes of platelets and red blood cells, with concomitant changes in membrane function, leads to hemorheological disturbances with an increased tendency of platelets and red blood cells to aggregate. Potentially reducing blood flow properties and microcirculation occur within and outside the liver.

Such disorders may be positively influenced or eliminated by the administration of EPL (Tab. 1). These effects of EPL became more pronounced as the drug was administered earlier [75].

The cytoprotective effect of EPL has been corroborated in 25 *in vitro* and 145 *in vivo* experiments with 43 different types of models and 8 different animal species. EPL has primarily been administered to avoid or treat hepatic intoxications by chemicals or drugs: chemical substances such as carbon tetrachloride [7, 52] and galactosamine [12, 131], medication such as

Tab. 1. Mode of action of EPL in liver disease

Mode of action	Reference number
Membrane structure restored and membrane fluidity increased	48, 59, 66, 128, 129
Membrane-associated metabolic functions enhanced	12, 13, 14, 48, 63, 64, 66, 79, 80, 83, 95, 100, 105–107, 119, 128, 130, 141
Peroxidative reactions reduced or normalized	1–3, 5–9, 11, 15, 16, 25, 26, 31, 35–38, 41, 42, 48, 63, 66, 75, 77, 88, 96, 104, 130, 133, 134, 138, 144, 159
Cytolysis decreased; excretory, detoxifying/clearing and synthesizing capacity of the liver improved	38, 42, 46, 52, 63, 67, 83, 115, 138, 144
Immune properties improved	48, 69, 78, 107, 113, 142, 145, 151
Regeneration stimulated	42, 52, 72, 73
Fibrogenic processes inhibited or corrected	7, 17, 34, 40, 56, 61, 63, 82, 87–90, 85, 93, 94, 102, 113, 144
Apoptosis influenced	64, 95, 100, 113, 126, 127, 136, 153
Bile composition stabilized	3, 45, 48, 98, 122, 129
Lipid metabolism modulated; fatty infiltration and hepatocyte necrosis diminished or abolished	11, 19, 27, 30, 39, 41, 48, 83, 99, 100, 105, 106, 117, 121, 122, 128, 144, 156, 158
Experimental hepatocarcinogenesis decreased	126, 127

Tab. 2. Cytoprotective action of EPL *in vivo*

144 experiments			43 different types of models			8 different species of animals		
<i>Intoxication with chemicals</i>			<i>Intoxication with drugs</i>					
CCl ₄	acute/subacute	17	Paracetamol	acute	1			
	chronic	10	Tetracycline	subacute	2			
CCl ₄ + Ethanol	chronic	1	Rifampicin	subacute	1			
Ethanol	acute/subacute	20	Cholic acid	chronic	1			
	chronic	19	Indomethacin	acute	3			
+ Triton, INH/Rifampicin, Cyanate	acute/chronic	4	Diclofenac	subacute	1			
Galactosamine	acute/subacute	10	Choline deficiency	subacute	1			
Allyl alcohol	acute	5	Anesthetics	subacute	3			
Ethionine	subacute	1	INH	subacute	1			
Organic solvents	chronic	2	Platidium ± CCl ₄	acute	1			
Carbon disulfide	chronic	1	Reye syndrome	acute	1			
Thioacetamide	chronic	1	Cyclosporine A	subacute	4			
Na-glutamate	chronic	1	Prevention of hepatic cancer		2			
Hexachlorocyclohexane		1	Cholestasis intoxication		5			
Ammonium fluoride	chronic	1	Antigen-antibody-reaction		1			
HgCl ₂	subacute	1	Radiation-induced intoxication		8			
Diethylnitrosamine (+ Phenobarbital)	acute (chronic)	1	Lipid peroxidation by FeSO ₄		2			
Alloxan	subacute	1	Endogenous oxidative stress		2			
High-fat diet	subacute	7	Ischemia/Reperfusion		1			

cyclosporine A [13, 14] and non-steroidal anti-inflammatory drugs [45, 138], alcohol [9, 90, 113], cholestasis [63, 159], immunological phenomena [12, 108], and exposure to radiation [72, 73] (Tab. 2).

Since the beginning of the 1990's, an increasing number of experimental investigations have been published, especially by C.S. Lieber's group in New York (Tab. 3). This research group has demonstrated anti-inflammatory and anti-peroxidative properties, as well as anti-apoptotic and anti-fibrogenic properties, of EPL.

Many of the previous results were confirmed, and additional experiments are described, in a paper by Okiyama et al. [113]. PPC prevented alcoholic liver disease in PPAR α -null mice through the attenuation of increased oxidative stress, as shown by (1) significant improvement in serum transaminases and the histology of hepatitis; (2) normalization of expression and activity of CYP2E1; (3) cessation of the increases in the lipid peroxides malondialdehyde (MDA) and

4-hydroxynonenal (4-HNE); (4) induction of reactive oxygen species generating enzymes acyl-coenzyme A oxidase (AOX), NADPH oxidases NOX-2 and NOX-4 diminished; (5) suppression of the activation of stress kinases apoptosis signal-regulating kinase (ASK1), p38 mitogen-activated protein kinase (p38 MAPK), protein kinase C (PKC) and phosphatidylinositol-3 kinase (PI3K); (6) normalization of nuclear-factor- κ B (NF- κ B) subunits p65 and p50 in hepatocyte nuclei by increasing I κ B, the inhibitor of NF- κ B, and decreasing the phosphorylated form of I κ B- α ; (7) decreases in TNF- α , interleukin-1 β (IL-1 β), cyclooxygenase 2 (COX-2), inducible nitric oxide synthetase (iNOS), intercellular adhesion molecule 1 (ICAM-1), and monocytes chemotactic protein (MCP-1); (8) suppression or blocking of toll-like receptor 4 (TLR4), cluster of differentiation 14 (CD14), the number of TUNEL-positive hepatocytes, caspase 3 activity, the previously mentioned protein Bax and

Tab. 3. EPL/PPC (DLPC): prevention and treatment of liver disease

Results found by the study group of C.S. Lieber/New York	
1.	Corrected hepatic phospholipid and phosphatidylcholine depletion, restored activity of PEMT [85, 87, 89, 90]
2.	Increased hepatic DLPC (direct influence on membrane fluidity) [2, 6, 90]
3.	Normalized cytochrome oxidase activity and improved hepatic mitochondrial respiration and potential, and decreased hepatic fat accumulation [11, 85, 100, 105–107, 153]
4.	Reduced oxidative stress [2, 6–9, 25, 26, 85, 88] by the inhibition of free radical generation <i>via</i> CYP2E1 [3, 4, 153] and products of peroxynitrite hepatotoxicity [11] decreased LDL-peroxidation [85, 104]
5.	Re-increased S-adenosylmethionine level [9] and increased/restored glutathione level [2, 9, 26, 88, 153]
6.	Decreased activation of Kupffer cells by endotoxin [85, 114] and by acetaldehyde [21, 26]
7.	Attenuated apoptosis [95, 100, 153]
8.	Reduced stellate cell activation, fewer myofibroblast-like cells [85, 87, 90, 123]
9.	Prevented collagen accumulation and improved collagen degradation by increased activity of collagenase [82, 85, 90] and down-regulation of TNF- α [21, 22], TGF- β 1 [23, 26], TIMP-1 [23, 24] & α 1(I) collagen mRNA up to collagen I [23, 25, 26]
10.	No septal fibrosis and cirrhosis in baboons [85, 87–89]
11.	Protection against fibrosis and cirrhosis by CCl ₄ or heterologous albumin in rats [7, 85, 93]
12.	Reversal of pre-existing fibrosis induced by CCl ₄ in rats [85, 93]
13.	Improved fibrosis score in patients with chronic hepatitis C [86]

the truncated Bid, and activation of the mitochondrial permeability transition (MPT); (9) inhibition of collagen type I α 1 (COL1A1) mRNA levels, TGF- β 1, TGF- α and α -smooth muscle actin (α -SMA).

The latest data with DLPC confirm the anti-inflammatory effects of EPL [139]. DLPC-induced hemoxygenase-1 (HO-1) expression through NF-erythroid 2-related factor 2 (Nrf2)-activation in RAW264.7 macrophages. The transcription factor Nrf2 regulated the induction of the antioxidant defense enzymes by binding to antioxidant response elements in the promoter region of their genes. HO-1, as one of these enzymes, is a major protective factor because of its antioxidant and anti-inflammatory properties. Therefore, the DLPC-induced HO-1 expression also suppressed iNOS expression and TNF- α production.

Clinical results with EPL

According to our database research, 248 clinical studies, out of these 46 single-blind and 21 double-blind trials have been conducted with EPL. One hundred ninety-three studies combined subjective criteria, clinical findings and biochemical data, 44 studies in-

cluded histology and 5 studies additionally included electron microscopy. Eighteen studies were performed on newborns and children. The dosage of EPL ranged from 525 to 6,000 mg/day when administered orally (in most studies between 1,800 mg/day and 2,700 mg/day) and from 250 to 3,000 mg/day with intravenous application. The duration of treatment lasted from a few weeks up to 5 years. The main liver indications were fatty infiltration of the liver, followed by chronic hepatitis, toxic liver damage, fibrosis/cirrhosis of the liver, and acute (viral) hepatitis.

Acute viral hepatitis (AVH)

In most patients with AVH, remission sets in after a certain length of time. However, the time until restoration of well-being and physical health can vary widely depending on the severity of the infection, general constitution of the patient, integrity of the patient's immune system and complications or intercurrent diseases. Therefore, the objective of most EPL investigators was to achieve rapid restoration of hepatocyte membrane functions.

Although two double-blind studies were performed with EPL [43, 147], only one focused on a specific type of AVH [147]. The investigator followed up 60 patients for at least 6 months after the onset of AVH

B. They were assigned at random to 2 groups, taking 1.8 g EPL or placebo per day for 60 days. After 4 weeks of treatment, 15 of the 30 test-group patients were negative for HBsAg in serum compared to seven of the controls. At the end of the 6-month period of observation, all but one patient of the control group were HBsAg-negative. At every control date, the test group presented a higher number of cases with normalized ALT values *vs.* control. Apparently, EPL increased the rate of seroconversion of HBsAg in this study.

According to our database research, there are currently no data available from single- or double-blind studies on the use of EPL for acute hepatitis A, C, E or other infections, such as by cytomegalovirus. Only single case reports are published.

Although the EPL study [147] confirmed earlier recovery and biochemical improvements from open controlled studies in patients with AVH [48, 71, 92, 116], further randomized-controlled studies with higher numbers of patients are recommended.

Chronic hepatitis (CH)

Nine out of 91 clinical studies with CH of different origins were performed as randomized double-blind studies *vs.* placebo or compared to standard therapy alone [51, 56, 57, 60, 70, 86, 109, 149, 155]. The efficacy of EPL was assessed in a total of 3,860 patients. In addition to subjective symptoms, clinical findings and laboratory variables, 6 of the 9 double-blind studies included histology as a basis for assessment [51, 56, 57, 60, 70, 86].

In the first double-blind study performed, it was only in the EPL-treated group that the histological findings showed statistically significant improvements with respect to the appearance of the hepatocyte membranes, focal necrosis, Kupffer cell mobilization, and a trend toward reduced ballooning of the liver cells [51]. Improved histological findings after additional EPL therapy became evident in a study with patients with HBsAg-negative chronic active hepatitis [60]. In this British double-blind trial, all patients were treated with immunosuppressive drugs and with either EPL or placebo. A pilot study, carried out in a first run over a treatment period of 6 months, showed a reduction in both portal tract infiltration and piecemeal necrosis in four out of six EPL-treated cases; in contrast, histological deterioration was observed in three out of four patients of the control group. In the following prospective double-blind study, 15 patients per study group were subjected to

the same trial conditions but treated over a period of 12 months. The EPL-treated patients showed relative to the control group a statistically significant reduction in the histological index of disease activity, again, particularly with respect to portal tract infiltration and piecemeal necrosis. In none of the control group patients, but in three of the 15 EPL-treated patients, the disease had passed to the inactive phase at the end of the study. Although the number of cases treated in these two studies was low, the authors came to the conclusion that the additional administration of EPL was of value for the patients with HBsAg-negative chronic active hepatitis in whom conventional therapy failed to control the condition. Histology also improved during the 12-month EPL treatment period of patients with HBsAg⁺ chronic active hepatitis in a double-blind study by Ilić and Begić-Janev [56]. With EPL treatment, intralobular necrosis and portal tract inflammation decreased significantly, and the total liver score improved in 23 of 25 patients *vs.* ten of 25 placebo-group patients. Furthermore, the values of alanine aminotransferase (ALT) and procollagen-III-peptide fell to a greater extent than in the control group, and cholinesterase (CHE) only returned to normal in the EPL group, whereas alkaline phosphatase (AP) fell within the normal range, and aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and γ -glutamyl transferase (γ -GT) were significantly reduced compared to the initial levels. Similar results were observed in two subsequent double-blind studies with smaller numbers of patients [8/7 in ref. 57 and 10/10 in ref. 70]. Two additional double-blind trials with CH B investigated subjective symptoms and biochemical variables alone [149, 155]. Although the study of Wang et al. [149] showed advantages for transaminase levels as well as total and direct bilirubin under EPL, there were no significant differences *vs.* the placebo group; however, this was likely a reflection of the short 3-month duration of treatment. The six-month treatment study of Zhang et al. [155] led to the expected significant differences of AST and ALT in favor of the EPL group. More interesting is a European multicenter, randomized, placebo-controlled double-blind study with chronic viral hepatitis [109]. In this international trial, 24-week treatment with α -interferon was applied three times weekly to patients suffering from CH B (5 mio IU/ day) or CH C (3 mio IU/day). During this period, the patients were given either an additional 1.8 g of EPL or placebo. After this 24-week period, α -interferon treatment was stopped,

but EPL or placebo continued for a further 24 weeks. After the first 24 weeks, 50 of the 70 patients of the hepatitis C group responded to the α -interferon + EPL treatment (71%), as did 30 of the 59 cases of the control group (51%). The difference between the groups was significant. After 48 weeks, a sustained response was observed in 24 of 57 cases (42%) compared to 13 of 41 patients on placebo (32%), suggesting that patients receiving the extended treatment with EPL had a prolonged response to interferon treatment. The improvement in patients on EPL suffering from CH C and demonstrating a sustained reduction of at least 50% or complete normalization of ALT approached statistical significance (41% vs. 15% on placebo). With regard to the patients suffering from CH B, there was no statistical significance between the groups in this study. The positive result on CH C seems to be confirmed by the latest multicenter, double-blind study in CH C [86]. This study combined pegylated interferon and ribavirin with EPL or placebo. The additional administration of EPL produced a significant decrease of transaminases at 12 months that persisted after 18 months of therapy. Furthermore, in 50 patients who had already completed the 3-year treatment, EPL significantly improved the fibrosis score. A drawback of this new trial is the significantly higher dose of EPL administered per day (4.5 g/day) in comparison with the other double-blind studies. Although the analyzed double-blind studies showed positive results with EPL in CH C, recent studies with silymarin reported no such effects [55, 140]. All of the evaluated double-blind studies showed no influence on the viral response.

A systemic review and meta-analysis of six of the randomized double-blind studies confirmed the results of the single studies [55]. Four studies with 451 patients analyzed clinical effectiveness [56, 70, 109, 155], 3 with 100 patients investigated histopathology [56, 60, 70], 2 with 117 patients investigated total bilirubin as a variable for liver functioning [60, 149] and 1 with 87 cases measured ALT decrease [149]. Significant differences of EPL vs. placebo were observed for all of the investigated parameters.

Although the available double-blind and open controlled studies on EPL in CH were partly published in country-specific journals, and although only a few studies fulfilled GCP criteria, the uniformity of the results from an impressive number of papers rationalizes its use as an adjuvant medication for CH treatment. Subjective symptoms such as asthenia and

tiredness, clinical findings such as hepatomegaly, biochemical parameters from cytolysis to hepatic synthesis and the histological picture of the liver seem to be positively influenced by EPL and lead to an accelerated recovery when considering the well-being of the patients. A minimum duration of treatment of 6 months could be necessary, and its long-term administration indicates a stabilization of liver function and a significant reduction in disease activity.

Cirrhosis of the liver (LC) and hepatic coma (HC)

With the exception of one double-blind study on alcoholic hepatitis, in which 58% of the PPC-treated patients and 53% of the placebo group had LC at entry into the study [118], no clinical double-blind studies were found during our database search on LC. Although double-blind studies are questionable in HC, they are necessary in LC. In the double-blind study on alcoholic hepatitis, there was a notable trend toward improved survival in the PPC group compared to placebo (69% vs. 49%, respectively, $p = 0.11$) [118]. According to the pharmacologically observed antifibrotic profile of EPL (DLPC), its administration might be useful, especially in those cases with incomplete or compensated LC. Although papers have been published [48, 58, 103, 120, 129], showing an EPL-induced decrease of serum free phenols, ammonia, and bromphthalein reduction time, unconjugated bilirubin and spur cells [103, 129], as well as a reduction in the uptake and excretion of technetium-labeled parabutyl-IDA [58] and improved serum albumin levels, as favorable prognostic markers [120], these studies are not sufficient without long-term randomized double-blind studies to recommend the continuous administration of EPL for such patients. The lack of qualified clinical trials in this field is not only the case for EPL, but for all complementary and alternative medicines and conventional medical therapies.

Likewise, patients with fulminant hepatitis and HC have not often been the subject of clinical studies. Assessment of the efficacy of a particular treatment is made difficult by the severity of the clinical picture, the heterogeneity of the underlying liver diseases, the small number of cases per clinic and the large number of life-saving measures employed simultaneously. Essentiale[®] is the only registered purified phosphatidylcholine extract from soybean that is suitable for both oral and intravenous administration. There are numerous studies with single case reports, but only

a few randomized, controlled studies. More patients survived when EPL was administered along with normal HC treatment of patients with pre-coma and coma, and the survival period increased in comparison with the control groups [18, 74, 137, 152]. In a randomized, open, controlled study, 28 patients with acute icteric fulminant hepatitis and 22 patients with fulminant hepatitis on chronic active hepatitis or on decompensating LC were divided into two groups [152]. Compared to the group receiving standard therapy, the group additionally administered 500–1,000 mg EPL/day over 14 days increased the cumulative survival from 36.4% to 64.7% in acute inflammatory hepatitis and from 50.5% to 87.5% in chronic active hepatitis/decompensating liver cirrhosis. Brůha and Mareček also treated decompensating LC and observed comparable prolonged survival and improved encephalopathy in comparison with a control group [18]. In an additional single-blind study, oral administration of 1,050 mg EPL per day for 6 to 8 weeks reduced the recovery period from encephalopathy and the mortality rate of patients with fulminant hepatic failure [137]. Kuntz investigated a new galenic EPL application (lyophilizate without deoxycholate as solvent) [74]. The patients with severe liver insufficiency received 3,000 mg of EPL for 8–16 days. Seven of the ten patients showed a clear improvement. After termination of the 4-week period of observation, nine of the ten patients were still alive and had a recompensated and stabilized condition. These data with EPL on HC are promising, and it may be worth adding EPL *iv* to basic treatment and administering the intravenous form for up to 2–4 weeks, followed by oral treatment until the patient's condition is restored.

Toxic liver damage (TLD) and fatty liver of different origin (FL)

Two different kinds of TLD predominate in the literature on EPL, namely due to anti-tuberculous drugs and alcohol.

The reasons for the administration of **EPL in tuberculosis** (TB) were to avoid hepatotoxicity of the anti-TB agents [48, 97], to protect the liver in patients who had concomitant liver damage [16], or to avoid aggravation of concomitant diseases [62]. In a double-blind study, 49 patients with active tuberculosis were treated with 1,800 mg EPL/day plus a combination consisting of isonicotinic acid hydrazide (INH), pyridoxine hydrochloride and ethambutol. These patients were compared

to 52 cases who received anti-tuberculosis treatment plus placebo [97]. Damage to hepatocyte membranes with increases in the transaminases in serum occurred two to three times less frequently in the EPL-treated group.

Randomized, double-blind studies were performed with **EPL in alcoholic liver damage**. An initial study showed significant improvements in favor of EPL in biochemical variables such as transaminases, bilirubin and immunoglobulins in an 8-week trial with patients who did not noticeably change their drinking habits [145]. These positive results were confirmed by a second, small, double-blind study [132]. A third double-blind study followed and showed a trend toward improved survival in a 2-year trial in patients with acute alcoholic hepatitis [118]. This result particularly applied to patients with a Child Pugh B score. A drawback of the study was the daily intake of 12 capsules with, in total, 6,000 mg EPL per day. It is questionable whether the alcohol-abusing patients took 12 capsules every day for 2 years. Finally, a randomized, double-blind, placebo-controlled clinical trial with 789 patients was conducted in the USA. The patients averaged 16 drinks a day (1 drink = 14 g of alcohol) for 19 years [84, 91]. A baseline biopsy confirmed the presence of perivenular or septal fibrosis or incomplete cirrhosis. Three doses of 1,500 mg EPL were administered every day for a minimum of two years. During the study, alcohol intake was reduced in both groups to approximately two and a half drinks a day as a result of a new “brief intervention” approach. Accordingly, there was no further progression of fibrosis and, therefore, no way to test whether EPL could oppose such a progression except in a subgroup who were still consuming six or more drinks a day and showed beneficial effects regarding fibrosis. Ascites, an important secondary clinical condition in liver disease, was less frequently observed during the follow-up of the EPL-treated patients.

The efficacy of EPL does not seem to be limited to TLD due to alcohol abuse or anti-TB agents. In a double-blind study by Gurevich and co-workers, the combined therapy of EPL and a low dose of lovastatin resulted in an effective decrease of LDL-cholesterol in the 8-week treatment course when compared to lovastatin plus placebo [49]. EPL prolonged the achieved hypolipidemic effect of the combined therapy and reduced the sharp increase of plasma lipid levels during interruption of the lovastatin treatment. Furthermore, EPL effectively reduced the ALT activity and spontaneous and induced platelet aggregation, which were

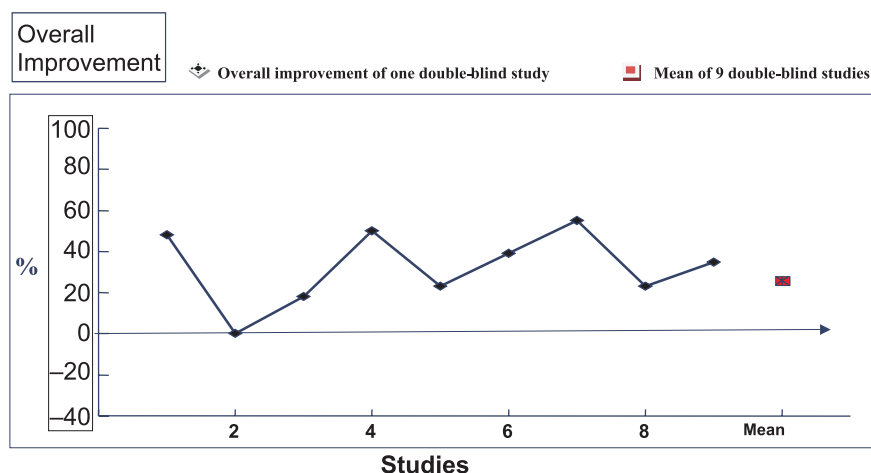


Fig. 2. Differences of responder rates of double-blind trials with EPL in chronic liver diseases – a formal meta-analysis [47]

increased after long-term lovastatin therapy. Another interesting study showed that infusion of EPL in cirrhotic patients decreased the cholesterol-to-phospholipid molar ratio in red blood cell membranes, which correlated well with improved down-regulation abilities of surface insulin receptors [20].

An increasing number of studies have been published regarding the effects of **EPL on non-alcoholic fatty liver disease (NAFLD)** [e.g., ref. 10, 44, 65, 91, 106, 111, 112]. Positive results were found concerning subjective symptoms, hepatomegaly, ultrasonography, liver enzyme levels, liver function and histopathology. However, only three small, double-blind studies were found [10, 44, 106]. A randomized, placebo-controlled double-blind study was performed in patients with FL associated with maturity-onset diabetes mellitus [44]. During the 6-month treatment phase, EPL significantly reduced hepatomegaly. The histological examination of liver bioptic material yielded similar results: in four of the 15 patients under EPL, the hepatic fatty infiltration completely disappeared, and in three additional patients, only focal forms of fatty infiltration remained. Under placebo, one of the 14 patients had no more infiltrations at the end of the treatment period, but transformation to cirrhosis was reported. Focal forms of fatty infiltration were observed in another three patients. Transaminases and bilirubin were within the normal range throughout the study. In the latest double-blind study on 40 patients with ultrasonically proven early stage NAFLD, 20 were given ursodeoxycholic acid (UDCA) and 20 were given EPL [10] for three months. In each group, half of the patients were diabetics and the other half

were obese individuals. In this study, 45% of the patients on EPL expressed significant symptom reduction, as opposed to 30% on UDCA. No noteworthy ultrasonic changes were observed after 4 weeks, but after 12 weeks, 20% of the patients on EPL and 10% of the UDCA group displayed ultrasonographic improvement. ALT and AP significantly improved in the EPL group, whereas there was only an improvement in ALT in the UDCA group. There were no major differences in the response of the diabetic and obese subgroups. Larger double-blind studies are needed to confirm these initial and interesting positive results with EPL.

In 1998, a formal meta-analysis was performed including nine randomized, placebo-controlled double-blind studies [51, 56, 57, 60, 68, 70, 109, 132, 155] on the clinical efficacy of EPL in 409 hospitalized patients who suffered from chronic liver disease (chronic active hepatitis or fatty degeneration of the liver) [47]. The overall effect of these studies showed a mean difference of the responder rates by 26.6% in favor of EPL, supporting the positive findings obtained in the individual trials (Fig. 2). This higher clinical responder rate represents an important dimension of liver disease, as in chronic liver disease, reliable parameters of the assessment of statistically significant alterations of progression, or disease status, are still under discussion. A further systematic review and meta-analysis of six randomized, double-blind studies [44, 68, 83, 91, 118, 132] was published in 2005 [53]. Only one study reported mortality rate, which was 22.6% in the treatment group and 39.2% in the control group and was not significantly different [118]. Overall clinical efficacy (symptoms, findings

and biochemical functions) from four studies [44, 68, 83, 132] and histological improvement from three studies [44, 91, 118] were observed in favor of the EPL group. According to the meta-analysis of this publication, the clinical efficacy rate was significant, with 83.5% in the treatment group vs. 41.7% under control, suggesting that in every 100 patients treated with EPL, 41 show a benefit and respond to the treatment.

Side effects and drug interactions

Because EPL corresponds to membrane-located phospholipids in its chemical configuration, toxic reactions are not expected, and side effects are rare and weak. In all clinical studies, EPL was well tolerated. Serious adverse drug reactions were not observed either in the inpatients or during long-term treatment of the outpatients. The intravenous form of EPL contains deoxycholate as a solvent and was not used for longer than 2–4 weeks in the trials. The good tolerability of EPL distinguishes the preparation from other herbal drugs with adverse reactions, such as glycyrrhizin with fluid retention and/or hypokalemia, in up to 20% of the study population [81], or from other drugs with potential hepatotoxicity [135]. No drug interactions with EPL are known.

Discussion

Treatment of liver diseases has evolved in recent years to include different choices and options that are often still far from being satisfactory. Complementary and alternative medicines belong to this group of treatments, but their effects have frequently been either insufficiently investigated, published in languages other than English, or in journals that are not respected in the medical field. Although the standard of some of the journals does not follow international rules, such as peer-reviewing of the contributions, individual publications may contain interesting methods and results that can contribute to understanding the mode of action and effectiveness of a preparation. It is important to emphasize that the overall therapeutic goal is the restoration of a normal quality of life for

patients with liver disease, and this can perhaps be more effectively achieved if all available scientific journals are carefully searched and evaluated for liver drugs. Therefore, we considered it essential to include lesser-known journals in our evaluation and to publish these results as a review. We selected articles that contained interesting pharmacological models and methods, and from a clinical point of view, publications that followed randomized, controlled study designs or reported important secondary data.

The odds of success in therapy depends on many factors, of which two predominate: the efficacy of the therapeutic agent and the stage of disease when therapy is started. Presently, it is well understood that most liver diseases are too complex to be treated with only a single agent, which makes it difficult to evaluate the efficacy attributable to any one treatment component. However, all liver diseases include membrane-related defects that are at least partly induced by lipid peroxidation as one of the earliest events [75]. Therefore, we considered it relevant to aggregate and summarize the available data on EPL in liver diseases to allow comparison with other drugs or treatment regimens. The result is surprising: essential phospholipids, a highly purified extract from soybean, are quite well investigated from a pharmacological and clinical standpoint, probably better than any other complementary or alternative medicine. The effect of EPL on membranes makes them an interesting drug in liver disease, even though detailed analysis of the quality of the single clinical studies is often disappointing. Published data mostly derive from short-term studies; the number of patients is low; relapses are not included; more children or patients with concomitant diseases should be investigated; and reasons for not responding to EPL treatment are not discussed. If potential non-responders can be predicted, either before beginning treatment or after short-term administration, the therapeutic utility of EPL in liver disease could be optimized.

Histological improvement is a crucial sign regarding the attainment of a therapeutic goal and was surprisingly observed in 44 clinical studies. The pharmacologically documented regression of fibrogenesis and fibrosis is an exciting achievement of EPL, and as a rule, it parallels the observed histological improvement in humans. Even though EPL do not cure chronic liver disease by itself, its membrane-regulating effects slowed the progression of the disease, frequently normalizing the well-being of the patients. However, the recommendation to administer EPL over many years

– up to a lifelong therapy – is hampered by having insufficient long-term, double-blind studies and a lack of available socio-economic data. There is only one clinical-economic study published claiming that a combination of α -interferon plus EPL is more favorable than α -interferon alone [148].

Conclusions and perspectives

According to the analyzed randomized controlled trials, EPL accelerates the improvement or normalization of subjective symptoms (e.g., feeling of well-being), mainly in chronic viral hepatitis, fatty liver disease and hepatic intoxication. These weak characteristics are supported by imaging procedures such as ultrasonography and computed tomography; biochemical markers of hepatic cytolysis, detoxification, excretion, synthesis and clearance; liver histology; and survival as objective end-points. Although increased survival rate and/or duration is observed in hepatic coma after intravenous EPL administration, the outcome is still open in alcoholic liver disease after oral EPL administration. This corresponds with similar data on S-adenosylmethionine [124] and silymarin [125].

Based on compelling experimental evidence, EPL has an interesting profile as an anti-fibrogenic agent. Reduced levels of procollagen-III-peptide [56], albumin-bound hydroxyproline [94] and, especially, improved histology in pharmacological and clinical studies [e.g., ref. 53, 54, 56, 87, 90, 93] support the assumption that EPL is useful in preventing and treating liver fibrosis based on pathogenesis, but further studies are necessary to assess the extent of its efficacy in preventing or treating fibrosis and to optimize the treatment regimen. Although a systematic review with meta-analysis on silymarin came to the conclusion that it does not seem to significantly influence the course of patients with alcoholic and/or hepatitis B or C liver diseases [125], three meta-analyses on EPL showed that it apparently affects liver damage in a positive way. Clinical findings, biochemical values, histological features and responder rates improved vs. the control group. However, because not all the analyzed double-blind studies were well designed, additional adequately conducted, randomized clinical trials on EPL vs. placebo should be performed. It is

an important area for further research and development to combine EPL with other liver active drugs and/or to investigate their respective efficacy, preferably in long-term clinical studies. No severe side effects limit its long-term oral use. Furthermore, detailed cost-effective analysis stratified for age, gender and disease stage should be performed to guide patients and health providers.

Acknowledgment:

Declaration of personal interest: Dr. K.-J. Gundermann has served as a speaker and consultant for the company Sanofi-Aventis. Declaration of funding interests: No funding was received for this study.

References:

1. Aabdallah DM, Eid NI: Possible neuroprotective effects of lecithin and α -tocopherol alone or in combination against ischemia/reperfusion insult in rat brain. *J Biochem Mol Toxicol*, 2004, 18, 273–278.
2. Aleynik AI, Leo MA, Aleynik MK, Lieber CS: Polyenylphosphatidylcholine protects against alcohol but not iron-induced oxidative stress in the liver. *Alcohol Clin Exp Res*, 2000, 24, 196–206.
3. Aleynik MK, Leo MA, Aleynik SI, Lieber CS: Polyenylphosphatidylcholine opposes the increase of cytochrome P-450E1 by ethanol and corrects iron-induced decrease. *Alcohol Clin Exp Res*, 1999, 23, 96–100.
4. Aleynik MK, Lieber CS: Dilinoleoylphosphatidylcholine decreases ethanol-induced cytochrome P450E1. *Biochem Biophys Res Commun*, 2001, 288, 1047–1051.
5. Aleynik SI, Leo MA, Aleynik MK, Lieber CS: Alcohol-induced pancreatic oxidative stress: protection by phospholipid repletion. *Free Radic Biol Med*, 1999, 26, 609–619.
6. Aleynik SI, Leo MA, Lieber CS: Polyenylphosphatidylcholine intake increases dilinoleoylphosphatidylcholine content and antioxidant capacity in human plasma. *Hepatology*, 2000, 25, Suppl. 544A.
7. Aleynik SI, Leo MA, Ma X, Aleynik MK, Lieber CS: Polyenylphosphatidylcholine prevents carbon tetrachloride-induced lipid peroxidation while it attenuates liver cirrhosis. *J Hepatol*, 1997, 27, 554–561.
8. Aleynik SI, Leo M-A, Takeshige U, Aleynik MK, Lieber CS: Dilinoleoylphosphatidylcholine is the active antioxidant of polyenyl-phosphatidylcholine. *J Invest Med*, 1999, 47, 507–512.
9. Aleynik SI, Lieber CS: Polyenylphosphatidylcholine corrects the alcohol-induced hepatic oxidative stress by restoring S-adenosylmethionine. *Alcohol Alcohol*, 2003, 38, 208–212.
10. Arvind N, Savaikar P, Rajkumar JS: Therapy for NAFLD – a comparative study of essential phospholipids vs. ursodeoxycholic acid. *Ind J Clin Pract*, 2006, 16, 21–24.
11. Baraona E, Zeballos GA, Shoichet L, Mak KM, Lieber CS: Ethanol consumption increases nitric oxide produc-

- tion in rats, and its peroxynitrite-mediated toxicity is attenuated by polyenylphosphatidylcholine. *Alcohol Clin Exp Res*, 2002, 26, 883–889.
12. Barbarino F, Neumann E, Toganel E, Stroila C, Gundermann KJ: Effect of “essential” phospholipids in experimental D-galactosamine-induced liver cell damage. *Therapiewoche (Austria)*, 1990, 5, 415–419.
 13. Benkoel L, Chanussot F, Dodero F, de la Maisonneuve C, Lambert R, Brisse J, Chamlian A: Effect of dietary lipids on hepatic Na^+ , K^+ -ATPase in cyclosporine A-treated rats. *Dig Dis Sci*, 1999, 44, 1643–1649.
 14. Benkoel L, Chanussot F, Dodero F, De La Maisonneuve C, Lambert R, Brisse J, Delmas M, Chamlian A: Modification of Ca^{2+} , Mg^{2+} -ATPase and F-actin distribution in hepatocytes of cyclosporine A treated rats. Effect of soyabean lecithin and triacylglycerol. *Cell Mol Biol*, 1998, 44, 1221–1227.
 15. Biagi PL, Bordonì A, Hrelia S, Celadon M, Turchetto E: The effect of dietary polyenylphosphatidylcholine on microsomal delta-6-desaturase activity, fatty acid composition, and microviscosity in the rat liver under oxidative stress. *J Nutr Biochem*, 1993, 4, 690–694.
 16. Blikhar YM, Skakun NP: Essentiale in the therapy of chronic liver disease in patients with pulmonary tuberculosis (Russian). *Probl Tuberk*, 1988, 3, 41–44.
 17. Brady LM, Fox ES, Fimmel CJ: Polyenylphosphatidylcholine inhibits PDGF-induced proliferation in rat hepatic stellate cells. *Biochem Biophys Res Commun*, 1998, 248, 4–179.
 18. Brůha R, Mareček Z: Essentiale phospholipids in the treatment of hepatic encephalopathy (Czech). *Vnitř Lék*, 2000, 46, 199–204.
 19. Buyeverov AO, Yeshanu VS, Mayevskaya MV, Ivashkin VT: Application of essential phospholipids in complex therapy of steatohepatitis of the mixed origin (Russian). *Klin Persp Gastroenterol Hepatol*, 2008, 1, 17–22.
 20. Cantafora A, Masella R, Angelico M, Gandin C, Blount RJ, Peterson SW: Effect of intravenous polyunsaturated phosphatidylcholine infusion on insulin receptor processing and lipid composition in patients with liver cirrhosis. *Eur J Clin Invest*, 1992, 22, 777–782.
 21. Cao Q, Mak KM, Lieber CS: Dilinoleoylphosphatidylcholine decreases acetaldehyde-induced TNF- α generation in Kupffer cells of ethanol-fed rats. *Biochem Biophys Res Commun*, 2002, 299, 459–464.
 22. Cao Q, Mak KM, Lieber CS: Dilinoleoylphosphatidylcholine decreases LPS-induced TNF- α generation in Kupffer cells of ethanol-fed rats, respective roles of MAPKs and NF- κ B. *Biochem Biophys Res Commun*, 2002, 294, 849–853.
 23. Cao Q, Mak KM, Lieber CS: Dilinoleoylphosphatidylcholine prevents transforming growth factor β 1-mediated collagen accumulation in cultured rat hepatic stellate cells. *J Lab Clin Med*, 2002, 139, 202–210.
 24. Cao Q, Mak KM, Lieber CS: DLPC and SAME combined prevent leptin-stimulated TIMP-1 production in LX-2 human hepatic stellate cells by inhibiting HO-mediated signal transduction. *Liver Int*, 2006, 26, 221–231.
 25. Cao Q, Mak KM, Lieber CS: DLPC and SAME prevent α 1(I) collagen mRNA up-regulation in human hepatic stellate cells, whether caused by leptin or menadione. *Biochem Biophys Res Commun*, 2006, 350, 50–55.
 26. Cao Q, Mak KM, Lieber CS: DLPC decreases TGF- β 1-induced collagen mRNA by inhibiting p38 MAPK in hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol*, 2002, 283, G1051–G1061.
 27. Chatterjee C, Young EK, Pussegoda KA, Twomey EE, Pandey NR, Sparks DL: Hepatic high-density lipoprotein secretion regulates the mobilization of cell-surface hepatic lipase. *Biochemistry*, 2009, 48, 5994–6001.
 28. Chen Y, Hills BA, Hills YC: Unsaturated phosphatidylcholine and its application in surgical adhesion. *ANZ J Surg*, 2005, 1111–1114.
 29. Chien S, Sung LP: Molecular basis of red cell membrane rheology. Part 1. *Biorheology*, 1990, 27, 327–344.
 30. Chirkin AA, Konevalova NY, Grebennikov IN: Effect of polyunsaturated phosphatidylcholine on lipid transport system in alcoholic liver injury. *Addiction Biol*, 1998, 3, 65–70.
 31. Chirkin AA, Konevalova NY, Grebennikov IN, Kulikov VA, Filipenko GV: Effect of polyunsaturated phosphatidylcholine on the activity of antioxidative and lipolytic enzymes in irradiated rats with alimentary hypercholesterolemia (Russian). *Vopr Med Khim*, 1994, 40, 24–28.
 32. Cohen DE, Carey MC: Physical chemistry of biliary lipids during bile formation. *Hepatology*, 1990, 12, 143S–147S, discussion 147S–148S.
 33. Cushley RJ, Okon M: NMR studies of lipoprotein structure. *Annu Rev Biophys Biomol Struct*, 2002, 31, 177–206.
 34. Demirbilek S, Aydin G, Yücesan S, Vural H, Bitiren M: Polyunsaturated phosphatidylcholine lowers collagen deposition in a rat model of corrosive esophageal burn. *Eur J Pediatr Surg*, 2002, 12, 8–12.
 35. Demirbilek S, Ersoy MO, Demirbilek S, Karaman A, Akin M, Bayraktar M, Bayraktar N: Effects of polyenylphosphatidylcholine on cytokines, nitrite/nitrate levels, antioxidant activity and lipid peroxidation in rats with sepsis. *Intensive Care Med*, 2004, 30, 1974–1978.
 36. Demirbilek S, Gürses İ, Sezgin N, Karaman A, Gürbüz N: Protective effect of polyunsaturated phosphatidylcholine on stress ulcer formation in rats. *J Pediatr Surg*, 2004, 39, 57–62.
 37. Demirbilek S, Karaman A, Baykarabulut A, Akin M, Gürnlüoğlu K, Türkmen E, Taş E et al.: Polyenylphosphatidylcholine pre-treatment ameliorates ischemic acute renal injury in rats. *Int J Urol*, 2006, 13, 747–753.
 38. Demirbilek S, Karaman A, Gürnlüoğlu K, Taş E, Akin M, Aksoy RT, Türkmen E et al.: Polyenylphosphatidylcholine pre-treatment protects rat liver from ischemia/reperfusion injury. *Hepatol Res*, 2006, 34, 84–91.
 39. Desreumaux C, Dedonder E, Dewailly P, Sézille G, Furchart JC: Effects of unsaturated fatty acids in phospholipids on the in vitro activation of the lipoprotein lipase and the triglyceride lipase. *Drug Res*, 1979, 29, 1581–1583.
 40. Dinakaran N: Safety and efficacy of Essentiale-L on the treatment of non-alcoholic fatty liver disease. *Indian J Clin Practice*, 2003, 14, 51–58.
 41. Feng Zh, Shen Zh, Tan SH, Qi Ch: Prevention and treatment on alcoholic fatty liver by Essentiale (Chinese). *Chin J Gastro Hepa*, 2005, 14, 480–486.

42. Fomina TI, Vetoshkina TV, Dibskaya TY: Pharmacological corrections of platidiam hepatotoxic effect (Russian). *Eksp Klin Farmakol*, 1999, 62, 62–64.
43. Ghose N, Santra A, Pal A, Mazumdar DN: Role of essential phospholipids (EPL) in acute viral hepatitis. *Indian Med Gazette*, 1993, 6, 179–183.
44. Gonciarz Z, Besser P, Lelek E, Gundermann K-J, Johannes K-J: Randomized placebo-controlled double-blind trial on “essential” phospholipids in the treatment of fatty liver associated with diabetes. *Méd Chir Dig*, 1988, 17, 61–65.
45. Gryshchenko VA, Lytvynenko ON: Peculiarities of the bilious acid spectrum of bile and duodenal content in mice at medicamentous hepatitis and use of correction therapy (Ukrainian). *Ukr Biokhim Zh*, 2007, 79, 97–101.
46. Gubsky VI, Radzinsky VO: Effects of the antioxidants alpha-tocopherol and Essentiale on protein biosynthesis in the chemically damaged liver and placenta (Russian). *Farm Zh*, 1998, 4, 72–73.
47. Gundermann K-J, Lehmacher W: The essential phospholipids as liver therapeutic – a meta-analysis of double-blind trials in chronic liver disease (Polish). *Gastroenterol Pol*, 1998, 5, 553–539.
48. Gundermann K-J: The “Essential” Phospholipids as a Membrane Therapeutic. Szczecin, Jota Press, 1993.
49. Gurevich V, Bondarenko B, Gundermann K-J: Polyunsaturated phospholipids increase the hypolipidemic effect of lovastatin. *Eur J Int Med*, 1997, 8, 13–18.
50. Hills BA: Role of surfactant in peritoneal dialysis. *Perit Dial Int* 2000, 20, 503–515.
51. Yano M, Koga M, Shirahama S, Toda T, Ohta Y, Hirayama C: Blind assessment of liver biopsy findings in chronic hepatitis: drug efficacy trial of polyenephosphatidylcholine (Japanese). *Shindan to chiryo*, 1978, 9, 1783–1789.
52. Holeček M, Mráz J, Koldová P: “Essential” phospholipids stimulate liver regeneration after 70% hepatectomy and after carbon tetrachloride induced liver damage. *Prog Hepato-Pharmacol*, 1995, 1, 202–212.
53. Hu G, Liu K, Zhao L: Polyunsaturated phosphatidylcholine (Essentiale) in the treatment of alcoholic liver disease and fatty liver disease, a systematic review (Chinese). *Liver*, 2005, 10, 5–7.
54. Hu G-P, Liu K, Wang S, Tang H, Zhao L-S: Polyunsaturated phosphatidylcholine (Essentiale) for chronic hepatitis, a systematic review (Chinese). *Chin J Evidence-Based Med*, 2005, 5, 543–548.
55. Huber R, Futter I, Lüdtker R: Oral silymarin for chronic hepatitis C – a retrospective analysis comparing three dose regimens. *Eur J Med Res*, 2005, 10, 6870.
56. Ilić V, Begić-Janeva A: Treatment of HBsAg positive chronic active hepatitis. Efficacy of “essential” phospholipids (German). *Med Welt*, 1991, 42, 523–525.
57. Ilić V, Kordač V, Alvarez SZ: Clinical experience with long-term administration of “essential” phospholipids in chronic active hepatitis. Review of 3 double-blind studies (Czech). *Cas Lék Čes*, 1992, 131, 801–804.
58. Indovina I, Licata G, Scaglione R, Parrinello G: Effect of i.v. administered polyunsaturated phosphatidylcholine (EPL) in hepatopathies. Study on morphology and function of ^{99m}Tc parabutyl-IDA (Italian). *Epatologica*, 1981, 27, 261–278.
59. Jayaraman T, Kannappan S, Ravichandran MK, Anuradha CV: Impact of Essentiale L on ethanol-induced changes in rat brain and erythrocytes. *Singapore Med J*, 2008, 49, 320–327.
60. Jenkins PJ, Portmann BP, Eddleston ALWF, Williams R: Use of polyunsaturated phosphatidylcholine in HBsAg negative chronic active hepatitis, results of prospective double-blind controlled trial. *Liver*, 1982, 2, 77–81.
61. Kanmaz T, Karakayali H, Sakallıoglu AE, Ozdemir BH, Haberal M: Polyunsaturated phosphatidylcholine protects against wound contraction in experimental skin burn injury. *J Invest Surg*, 2004, 17, 15–22.
62. Karachunsky MA, Adamovich NV, Abdullayev AA: Specific features of chemotherapy of pulmonary tuberculosis and its effectiveness in diabetes mellitus patients (Russian). *Probl Tuberk*, 1991, 5, 31–33.
63. Karaman A, Demirbilek S, Sezgin N, Gürbüz N, Gürses İ: Protective effect of polyunsaturated phosphatidylcholine on liver damage induced by biliary obstruction in rats. *J Pediatr Surg*, 2003, 38, 1341–1347.
64. Katz GG, Shear NH, Malkiewicz IM, Valentino K, Neumann MG: Signalling for ethanol-induced apoptosis and repair in vitro. *Clin Biochem*, 2001, 34, 219–227.
65. Kharchenko NV, Korulya IA: Efficacy and tolerability of Essentiale forte N preparation in the treatment of patients with non-alcoholic steatohepatitis (Ukrainian). *Such Gastroenterol*, 2004, 19, 46–49.
66. Klinger W, Müller D, Kleeberg U, Kretzschmar M, Splinter FK: The influence of “essential” phospholipids (EPL) on phase-I- and phase-II-reactions and on the glutathione status in the liver of aging rats. *Exp Pathol*, 1991, 41, 151–156.
67. Knauff HG, Georgii A, Matzen K: Influence of choline phospholipids on the spectrum of free plasma amino acids of toxic liver damage (German). *Klin Wochenschr*, 1963, 41, 915–919.
68. Knüchel F: Double-blind study in patients with alcoholic fatty liver (German). *Med Welt*, 1979, 30, 411–416.
69. Konoplia YN, Prokopenko LG: Essentiale as immunomodulator in toxic liver damage (Russian). *Eksp Klin Farmakol*, 1992, 2, 5–8.
70. Kordač V, Brodanová M, Mareček Z, Jirásek A: Essentiale® forte in the treatment of chronic active hepatitis (Czech). *Prakt Lék (Praha)*, 1985, 65, 834–837.
71. Kowala-Piaskowska A, Mozer-Lisewska I, Służewski W, Bujnowska A: Intravenous administration of essential phospholipids for the treatment of acute and chronic viral hepatitis in children (Polish). *Klin Ped*, 1999, 7, 425–429.
72. Kožurková M, Mišurová E, Kropáčová K, Haková H: The effect of Essentiale on histones and nucleic acids in liver and blood-forming tissues of rats irradiated with γ-rays. *Radiats Biol Radioecol*, 1999, 39, 388–393.
73. Kropáčová K, Mišurová E: The influence of essential phospholipids (ESSENTIALE) on liver regeneration in gamma irradiated rats. *Physiol Res*, 1995, 44, 241–247.
74. Kuntz E: Pilot study with polyenylphosphatidylcholine in severe liver insufficiency (German). *Med Welt*, 1989, 40, 1327–1329.

75. Kuntz E: The “essential” phospholipids in hepatology – 50 years of experimental and clinical experiences (German). *Z Gastroenterol*, 1991, 29, Suppl 2, 7–13.
76. Kuntz E: The “essential” phospholipids in hepatology – experimental and clinical experiences. *Prog Hepato-Pharmacol*, 1995, 1, 156–167.
77. Lazareva GA, Brovkina IL: Protective effect of biological oxidation activators in nitrite intoxication of rats (Russian). *Antibiot Khimioter*, 2005, 50, 8–12.
78. Lazareva GA, Brovkina IL: The protective action of Essentiale, Riboxin, and Elkar against hemotoxic anemia (Russian). *Eksp Klin Farmakol*, 2006, 69, 48–51.
79. Lee SH, Han YM, Min BH, Park IS: Cytoprotective effects of polyenylphosphatidylcholine (PPC) on β -cells during diabetic induction by streptozotocin. *J Histochem Cytochem*, 2003, 51, 1005–1015.
80. LeKim D, Graf E: Animal experimental studies on the pharmacokinetics of “essential” phospholipids (German). *Drug Res*, 1976, 26, 1772–1782.
81. Levy C, Seeff LD, Lindor KD: Use of herbal supplements for chronic liver disease. *Clin Gastroenterol Hepatol*, 2004, 2, 947–956.
82. Li J, Kim C-I, Leo MA, Mak KM, Rojkind M, Lieber CS: Polyunsaturated lecithin prevents acetaldehyde-mediated hepatic collagen accumulation by stimulating collagenase activity in cultured lipocytes. *Hepatology*, 1992, 16, 373–381.
83. Li J-H, Chen X-Y, Zhong C-F, Min J: A randomized controlled study of essential phospholipids (Essentiale capsules) in the treatment of fatty liver (Chinese). *Infect Dis Information*, 2000, 13, 180–181.
84. Lieber CS: New concepts of the pathogenesis of alcoholic liver disease lead to novel treatments. *Curr Gastroenterol Rep*, 2004, 6, 60–65.
85. Lieber CS: Prevention and treatment of liver fibrosis based on pathogenesis. *Alcohol Clin Exp Res*, 1999, 23, 944–949.
86. Lieber CS, Anand BS, Bibi EJ: Polyenylphosphatidylcholine (PPC) is beneficial for the treatment of hepatitis C. *Hepatology*, 2005, 42, Suppl 1, 695A.
87. Lieber CS, DeCarli LM, Mak KM, Kim C-I, Leo MA: Attenuation of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. *Hepatology*, 1990, 12, 1390–1398.
88. Lieber CS, Leo MA, Aleynik SI, Aleynik MK, DeCarli LM: Polyenylphosphatidylcholine decreases alcohol-induced oxidative stress in the baboon. *Alcohol Clin Exp Res*, 1997, 21, 375–379.
89. Lieber CS, Robins SJ, Leo MA: Hepatic phosphatidylethanolamine methyltransferase activity is decreased by ethanol and increased by phosphatidylcholine. *Alcohol Clin Exp Res*, 1994, 18, 592–595.
90. Lieber CS, Robins SJ, Li J, DeCarli LM, Mak KM, Fasulo JM, Leo MA: Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. *Gastroenterology*, 1994, 106, 152–159.
91. Lieber CS, Weiss DG, Groszmann R, Paronetto F, Schenker S: II. Veterans affairs cooperative study of polyenylphosphatidylcholine in alcoholic liver disease. *Alcohol Clin Exp Res*, 2003, 27, 1765–1772.
92. Liu E: The curative effect observation of Essentiale on treating severe jaundice type of hepatitis (Chinese). *Mod J Integr Trad Chin West Med*, 2003, 12, 402.
93. Ma X, Zhao J, Lieber CS: Polyenylphosphatidylcholine attenuates non-alcoholic hepatic fibrosis and accelerates its regression. *J Hepatol*, 1996, 24, 604–613.
94. Magomedov S, Shipulin VP: Serum metabolism of the connective tissue in patients with chronic hepatitis C (Russian). *Such Gastroenterol*, 2001, 2, 50–53.
95. Mak KM, Wen K, Ren C, Lieber CS: Dilinoleoylphosphatidylcholine reproduces the antiapoptotic actions of polyenylphosphatidylcholine against ethanol-induced hepatocyte apoptosis. *Alcohol Clin Exp Res*, 2003, 27, 997–1005. Erratum in, *Alcohol Clin Exp Res*, 2003, 27, 1212.
96. Maneesh M, Jayalekshmi H, Dutta S, Chakrabarti A, Vasudevan DM: Effect of exogenous lecithin on ethanol-induced testicular injuries in Wistar rats. *Indian J Physiol Pharmacol*, 2005, 49, 297–304.
97. Marpaung B, Tarigan P, Zein LH, Barus AP, Soekiman I: Tuberculostatic combination treatment with INH/RMP/EMB and administration of Essentiale forte for the prevention of liver damage (German). *Therapie-woche*, 1988, 38, 734–740.
98. Mastellone I, Polichetti E, Grës S: Dietary soybean phosphatidylcholine lowers lipidemia. Mechanisms at the levels of intestine, endothelial cell, and hepatobiliary axis. *J Nutr Biochem*, 2000, 11, 461–466.
99. Mathur SN, Simon I, Lokesh BR, Spector AA: Phospholipid fatty acid modification of rat liver microsomes affects acylcoenzyme A: cholesterol acyltransferase activity. *Biochim Biophys Acta*, 1983, 751, 401–411.
100. Mi L-J, Mak KM, Lieber CS: Attenuation of alcohol-induced apoptosis of hepatocytes in rat livers by polyenylphosphatidylcholine (PPC). *Alcohol Clin Exp Res*, 2000, 24, 207–212.
101. Minto RE, Adhikari PR, Lorigan GA: A 2H solid-state NMR spectroscopic investigation of biomimetic bicelles containing cholesterol and polyunsaturated phosphatidylcholine. *Chem Phys Lipids*, 2004, 132, 55–64.
102. Mourelle M, Guarner F, Malagelada JR: Polyunsaturated phosphatidylcholine prevents stricture formation in a rat model of colitis. *Gastroenterology*, 1996, 110, 1093–1097.
103. Mütting D, Dohn P, Reikowski J: The action of intravenous and oral administration of high doses of “essential” phospholipids on protein and lipid metabolism and on enzyme activity in chronic liver disease (German). *Verh Dtsch Ges Inn Med*, 1972, 78, 1389–1392.
104. Navder KP, Baraona E, Leo MA, Lieber CS: Oxidation of LDL in baboons is increased by alcohol and attenuated by polyenylphosphatidylcholine. *J Lipid Res*, 1999, 40, 983–987.
105. Navder KP, Baraona E, Lieber CS: Polyenylphosphatidylcholine attenuates alcohol-induced fatty liver and hyperlipemia in rats. *J Nutr*, 1997, 127, 1800–1806.
106. Navder KP, Baraona E, Lieber CS: Polyenylphosphatidylcholine decreases alcoholic hyperlipemia without affecting the alcohol-induced rise of HDL cholesterol. *Life Sci*, 1997, 61, 1907–1914.
107. Navder KP, Lieber CS: Dilinoleoylphosphatidylcholine is responsible for the beneficial effects of polyenylphospha-

- tidylcholine on ethanol-induced mitochondrial injury in rats. *Biochem Biophys Res Commun*, 2002, 291, 1109–1112.
108. Neuberger J, Hegarty JE, Eddleston ALWF, Williams R: Effect of polyunsaturated phosphatidylcholine on immune mediated hepatocyte damage. *Gut*, 1983, 24, 751–755.
 109. Niederau C, Strohmeyer G, Heintges T, Peter K, Göpfert E: Polyunsaturated phosphatidyl-choline and interferon alpha for treatment of chronic hepatitis B and C. A multicenter, randomized, double-blind, placebo-controlled trial. *Hepato-Gastroenterol*, 1998, 45, 797–804.
 110. Oette K, Kühn G, Römer A, Niemann R, Gundermann KJ, Schumacher R: The absorption of dilinoleoyl-phosphatidylcholine after oral administration (German). *Drug Res*, 1995, 45, 875–879.
 111. Ohbayashi H, Fujimoto M, Yamase H, Ito M: Improvement of NASH with oral polyenephosphatidylcholine. *J Rural Med*, 2007, 1, 67–73.
 112. Ohbayashi H, Fujimoto M, Yoshida M, Ito M, Yamase H: The therapeutic effect of polyenephosphatidylcholine (EPL) on NASH (Japanese). *Liver, Bile and Pancreas*, 2006, 52, 637–642.
 113. Okiyama W, Tanaka N, Nakajima T, Tanaka E, Kiyosawa K, Gonzalez FJ, Aoyama T: Polyenylphosphatidylcholine prevents alcoholic liver disease in PPAR α -null mice though attenuation of increases in oxidative stress. *J Hepatol*, 2009, 50, 1236–46.
 114. Oneta CM, Mak KM, Lieber CS: Dilinoleoylphosphatidylcholine selectively modulates lipopolysaccharide-induced Kupffer cell activation. *J Lab Clin Med*, 1999, 134, 466–470.
 115. Ortenberg EA, Tazhudinova SJ: Comparative study of the efficacy of certain hepatoprotective agents in liver damage caused by combined ethanol and tuberculostatic drugs (Russian). *Farmakol Toksikol*, 1984, 471, 102–105.
 116. Panda RS, Patel DK: Polyunsaturated phosphatidylcholine (EPL, Essentiale) in viral hepatitis. *Indian Pract*, 1994, 47, 751–756.
 117. Pandey NR, Renwick J, Misquith A, Sokoll K, Sparks DL: Linoleic acid-enriched phospholipids act through peroxisome proliferator-activated receptors α to stimulate hepatic apolipoprotein A-I secretion. *Biochemistry*, 2008, 47, 1579–1587.
 118. Panos MZ, Polson R, Johnson R, Portmann B, Williams R: Polyunsaturated phosphatidyl choline for acute alcoholic hepatitis, a double-blind, randomized, placebo-controlled trial. *Eur J Gastroenterol Hepatol*, 1990, 2, 351–355.
 119. Perederiy VG, Babak OY, Zadorozhna TD: Should patients with chronic hepatitis C and without indication to α -interferon therapy be treated? (Russian). *Such Gastroenterol*, 2001, 1, 60–63.
 120. Petera V, Prokop V: The compensated cirrhosis of the liver (German). *Therapiewoche*, 1986, 36, 540–544.
 121. Polichetti E, Janisson A, Iovanna C: Stimulation of the apo AI-high density lipoprotein system by dietary soyabean lecithin in humans. *J Nutr Biochem*, 1998, 9, 659–664.
 122. Polichetti E, Janisson A, Lechčne de la Porte P, Portugal H, Léonardi J, Luna A, La Droite P et al.: Dietary polyenylphosphatidylcholine decreases cholesterolemia in hypercholesterolemic rats, role of the hepato-biliary axis. *Life Sci*, 2000, 67, 2563–2576.
 123. Poniachik J, Baraona E, Zhao J, Lieber CS: Dilinoleoyl-phosphatidylcholine decreases hepatic stellate cell activation. *J Lab Clin Med*, 1999, 133, 342–348.
 124. Rambaldi A, Gluud C: S-adenosyl-L-methionine for alcoholic liver diseases. *Cochrane Database Syst Rev*, 2006, 2, CD002235.
 125. Rambaldi A, Jacobs BP, Iaquinto G, Gluud C: Milk thistle for alcoholic and/or hepatitis B or C liver diseases – a systematic cochrane hepato-biliary group review with meta-analyses of randomized clinical trials. *Am J Gastroenterol*, 2005, 100, 2583–2591.
 126. Sakakima Y, Hayakawa A, Nagasaka T, Nakao A: Prevention of hepatocarcinogenesis with phosphatidylcholine and menaquinone-4, in vitro and in vivo experiments. *J Hepatol*, 2007, 47, 83–92.
 127. Sakakima Y, Hayakawa A, Nakao A: Phosphatidylcholine induces growth inhibition of hepatic cancer by apoptosis via death ligands. *Hepato-Gastroenterol*, 2009, 56, 481–484.
 128. Salvioli G, Pula B, Pradelli JM: Variation of membrane composition during EPL feeding. *Prog Hepato-Pharmacol*, 1995, 1, 191–201.
 129. Salvioli G: The effect of EPL administration in liver cirrhosis. *Z Gastroenterol*, 1991, 29, Suppl 2, 30–33.
 130. Saratikov AS, Vengerovskii AI, Mozzhelin ME, Sukhodolo IV: The effect of hepatoprotectors containing phospholipids on rats with experimental acute pancreatitis (Russian). *Eksp Klin Farmakol*, 2004, 67, 35–37.
 131. Saratikov AS, Vengerovskii AI, Sedykh IM: Effect of hepatoprotective drugs on the liver structural and metabolic disturbances in D-galactosamine-induced intoxication (Russian). *Farmakol Toksikol*, 1990, 53, 38–40.
 132. Schüller-Peréz A, González-San Martín F: Placebo-controlled study with polyunsaturated phosphatidylcholine in alcoholic steatosis of the liver (German). *Med Welt*, 1985, 36, 517–521.
 133. Seifulla NR, Kovalev IE, Rozhkova EA, Panyushkin VV: Effect of Carnosine and its combination with Essentiale on lipid peroxidation and work capacity of experimental animals (Russian). *Eksp Klin Farmakol*, 2005, 68, 44–46.
 134. Shalina RI, Kusch IB, Oreshkina VP, Azizova OA, Kozlov AV, Panasenkov OM: Antioxidants as a part of combined treatment of patients with late gestosis (Russian). *Akush Ginekolog*, 1989, 65, 37–41.
 135. Shaw D, Leon C, Kolev S, Murray V: Traditional remedies and food supplements. A five year toxicological study (1991-1995). *Drug Saf*, 1997, 17, 342–356.
 136. Singh AK, Jiang Y, Benlhabib E, Gupta Sh: Herbal mixtures consisting of puerarin and either polyenylphosphatidylcholine or curcumin provide comprehensive protection against alcohol-related disorders in P rats receiving free choice water and 15% ethanol in pure water. *J Med Food*, 2007, 10, 526–42.
 137. Singh NK, Prasad RC: A pilot study of polyunsaturated phosphatidylcholine in fulminant and subacute hepatic failure. *JAPI*, 1998, 46, 530–532.

138. Skakun NP, Klimnyuk EV: Experimental pharmacotherapy of indomethacin-induced lesions of the liver (Russian). *Farmakol Toksikol*, 1990, 53, 52–54.
139. Son Y, Lee JH, Kim N-H, Surh N-Y, Kim E-Ch, Chung H-T, Kang DG, Pae H-O: Dilinoleoylphosphatidylcholine induces the expression of the anti-inflammatory heme oxygenase-1 in RAW264.7 macrophages. *Biofactors*, 2010, 36, 210–215.
140. Tanamly MD, Tadros F, Labeeb S, Makld H, Shehata M, Mikhail N, Abdel-Hamid M et al.: Randomised double-blinded trial evaluating silymarin for chronic hepatitis C in an Egyptian village, study description and 12-month results. *Dig Liv Dis*, 2004, 36, 752–759.
141. Théret CG, Alliet J, Comlan G, Köbele H, Gourdiér D: Hepatic steatosis in the malnourished child – protective and curative effects of phosphatidylcholine with highly unsaturated fatty acids, demonstrated with the ultrastructures of 66 biopsies (French). *L'Ouest Méd*, 1977, 22, 1595–1614.
142. Uteshev BS, Baiburin FY, Prokopenko LG: Immunomodulating and antioxidant effect of β -carotene and Essentiale in disturbed lipid metabolism (Russian). *Eksp Klin Farmakol*, 1998, 61, 41–44.
143. Veldhuizen R, Nag K, Orgeig S, Possmayer F: The role of lipids in pulmonary surfactant. *Biochim Biophys Acta*, 1998, 1408, 90–108.
144. Vengerovskii AI, Baturina NO, Chuchalin VS, Saratikov AS: Role of lipid peroxidation in the mechanism of hepatic fibrosis tissue proliferation in experimental chronic hepatitis (Russian). *Patol Fiziol Eksp Ter*, 1996, 40, 37–39.
145. Vengerovskii AI, Ogorodova LM, Perevozchikova TV: Hepatoprotectors containing phospholipids decrease the immunosuppressive effect of prednisolone in rats with experimental toxic hepatitis (Russian). *Eksp Klin Farmakol*, 2004, 67, 50–53.
146. Verma S, Thuluvath PJ: Complementary and alternative medicine in hepatology, review of the evidence of efficacy. *Clin Gastroenterol Hepatol*, 2007, 5, 408–416.
147. Visco G: Polyunsaturated phosphatidylcholine (EPL) in association with vitamin B complex for the treatment of acute viral hepatitis B. Results of a randomized double-blind clinical study (Italian). *Clin Ter*, 1985, 114, 183–188.
148. Vorobjov PA, Avksentieva MV, Sidorova LV, Kuligina YA, Sura MV, Tyurina IV: A clinical-economic study of the application of Essentiale H preparation in the combination with interferon in patients having chronic viral hepatitis B and C infection (Russian). *Biull Clin-Econ Analysis*. In: *The Problems of Standardization in Public Health* (Russian), 2004, 3, 57–62.
149. Wang BE, Li JH, Lin SR, Min J, Zhang LX, Li JH: The multicentre randomized double-blind controlled clinic trial of Essentiale for chronic viral hepatitis. The final report. Research Report 2000.
150. White DA: The phospholipid composition of mammalian tissues. In: *Form and Function of Phospholipids*. Eds. Ansell GB, Hawthorne JN, Dawson RMC, Elsevier, Amsterdam-London-New York, 1973, 441–482.
151. Wojcicki J, Dutkiewicz T, Gieldanowski J, Samochowiec L, Barcew-Wiszniewska B, Rózewicka L, Wira D et al.: Essential phospholipids (EPL) modify immunological functions and reduce experimental atherosclerosis in rabbits. *Atherosclerosis*, 1992, 93, 7–16.
152. Xian C, Tang X, Xiao Z: Essentiale solution for injection in fulminant hepatitis. Research Report 1992
153. Xu Y, Leo MA, Lieber CS: DLPC attenuates alcohol-induced cytotoxicity in HepG2 cells expressing CYP2E1. *Alcohol Alcohol*, 2005, 40, 172–175.
154. Zamaria N: Alteration of polyunsaturated fatty acid status and metabolism in health and disease. *Reprod Nutr Dev*, 2004, 44, 273–282.
155. Zhang X, Gundermann K-J, Wu S: Therapeutic effect of polyene phosphatidylcholine compound in chronic active hepatitis B (Chinese). *Chin J Pharmacoevidemiol*, 1995, 4, 4–6.
156. Zierenberg O, Assmann G, Schmitz G, Rosseneu M: Effect of polyenephosphatidylcholine on cholesterol uptake by human high density lipoprotein. *Atherosclerosis*, 1981, 39, 527–542.
157. Zierenberg O, Grundy SM: Intestinal absorption of polyenephosphatidylcholine in man. *J Lipid Res*, 1982, 23, 1136–1142.
158. Zierenberg O, Odenthal J, Betzing H: Incorporation of PPC into serum lipoproteins after oral or i.v. administration. *Atherosclerosis*, 1979, 34, 259–276.
159. Zverinsky IV, Karpovich VE, Bushma MI: The effect of Essentiale and its combination with cordiamin and vitamin E on the processes of xenobiotic biotransformation, lipid peroxidation, and structure of the liver in rats with cholestasis (Russian). *Eksp Klin Farmakol*, 1998, 61, 33–36.

Received: May 26, 2010; **in the revised form:** December 15, 2010; **accepted:** December 28, 2010.