

RESEARCH MONOGRAPH . COMPOUND PROFILE

Ovagen

A synthetic tripeptide (Glu-Asp-Leu) developed at the Saint Petersburg Institute of Bioregulation and Gerontology as a tissue-specific epigenetic bioregulator targeting hepatic and gastrointestinal gene expression, distinguished from conventional hepatoprotective agents by a proposed mechanism of direct nuclear peptide-DNA interaction and chromatin remodeling rather than receptor-mediated signal transduction.

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ABSTRACT

Ovagen is a synthetic tripeptide composed of L-glutamic acid, L-aspartic acid, and L-leucine (Glu-Asp-Leu; single-letter code EDL) and belongs to the Khavinson class of ultrashort peptide bioregulators developed at the Saint Petersburg Institute of Bioregulation and Gerontology under the direction of Vladimir Khavinson. The compound is classified as a liver and gastrointestinal tract bioregulator within the Cytogen (synthetic short-chain peptide) product line and is proposed to exert its biological activity through direct interaction with nuclear DNA and chromatin rather than through conventional membrane receptor signaling. The molecular weight of Ovagen is 375.37 daltons (molecular formula C₁₅H₂₅N₃O₈), a size that places it within the ultrashort peptide category (2 to 7 amino acid residues) characterized by resistance to gastrointestinal peptidase degradation, oral bioavailability without enteric coating in some formulations, and the capacity to cross cellular membranes and localize to the nucleoplasm without receptor-mediated endocytosis. The principal proposed mechanism of action involves sequence-specific binding of the EDL tripeptide to double-stranded DNA in the promoter regions of hepatocyte genes governing cell proliferation, antioxidant defense, apoptosis, and inflammatory signaling. In aged rat liver tissue, Ovagen administration has been reported to produce an approximately 18-fold increase in Ki-67 expression (a marker of cellular proliferation) and an approximately 6-fold decrease in p53 expression (a marker of apoptotic signaling and cellular senescence), suggesting a shift from senescent to proliferative hepatocyte phenotype [1, 2]. In preclinical models of chemically

induced hepatotoxicity (carbon tetrachloride and paracetamol), Ovagen administration over 14 to 28 days reduced serum liver enzyme elevation, decreased hepatocyte necrosis and inflammatory cell infiltration on histological examination, and normalized hepatic antioxidant status [3, 4]. A secondary research application involves gastrointestinal mucosal support, with preclinical data suggesting that Ovagen strengthens epithelial barrier function, increases tight junction protein expression, and reduces intestinal permeability in aging models [5]. The compound has not been approved by any national regulatory authority for therapeutic use. No published, peer-reviewed human clinical trials of Ovagen exist as of the date of this monograph. The evidence base is entirely preclinical (cell culture and rodent models) and is derived predominantly from the Khavinson laboratory and collaborating Russian institutions. The peptide bioregulator framework within which Ovagen was developed has produced a substantial body of Russian-language and English-language literature, including a 2021 systematic review of peptide regulation of gene expression published in the journal *Molecules* [6], but the framework has not been independently validated by Western regulatory-standard clinical trials. Investigators considering Ovagen for research applications should weight the evidence accordingly, noting the predominantly single-laboratory provenance of the preclinical data, the absence of human pharmacokinetic characterization to Western regulatory standards, and the mechanistic novelty of the proposed direct peptide-DNA interaction pathway. This monograph reviews the chemistry, proposed mechanism of action, preclinical pharmacology, available pharmacokinetic considerations, sourcing and quality verification, reconstitution and handling, stack-interaction considerations, adverse-event signal, and a

comparative assessment of five hepatoprotective or liver-bioregulatory candidates against Ovagen on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

Proposed International Nonproprietary Name: None assigned. The compound is identified in the research literature as Ovagen or as the tripeptide EDL.

Common name: Ovagen.

Systematic peptide name: L-Glutamyl-L-aspartyl-L-leucine (Glu-Asp-Leu; EDL).

Alternative names: EDL tripeptide, glutamyl-aspartyl-leucine, liver peptide bioregulator, liver-specific bioregulator, Ovagenin.

Database identifiers: CHEBI:137252; SCHEMBL5329396; PDB ligand identifier 1a30. A Chemical Abstracts Service registry number has not been definitively assigned to the specific Glu-Asp-Leu tripeptide sequence in the principal chemical databases; the number 9046-70-2 has been associated with Ovagen in some commercial listings but may refer to a broader peptide complex rather than the defined tripeptide. Investigators should confirm compound identity by analytical methods rather than relying on CAS registry alone.

Product line classification: Cytogen class (synthetic short-chain peptide bioregulator) within the Khavinson peptide bioregulator framework. The corresponding natural-extract (Cytomax class) preparation is derived from liver tissue of young calves and is marketed under separate trade names in the Russian Federation and Commonwealth of Independent States jurisdictions.

1.2 Structural class and chemistry

Ovagen is a linear tripeptide consisting of three proteinogenic L-amino acids joined by two peptide bonds: L-glutamic acid at the N-terminus, L-aspartic acid at the central position, and L-leucine at the C-terminus. The compound belongs to the ultra-short peptide class (2 to 7 residues) that forms the basis of the Khavinson bioregulatory peptide research program [6, 7]. Other members of the family include Vilon (KE, dipeptide, thymus-derived), Epithalon (AEDG, tetrapeptide, pineal-derived), Livagen (KEDA, tetrapeptide, liver-derived), Bronchogen (AEDL, tetrapeptide, respi-

ratory-derived), Crystagen (TKE, tripeptide, immune-derived), Vesugen (KED, tripeptide, vascular-derived), Cardiogen (AEDR, tetrapeptide, cardiac-derived), and Pancragen (KEDW, tetrapeptide, pancreatic-derived) [7].

Molecular formula: C₁₅H₂₅N₃O₈ (zwitterionic free form).

Exact mass: 375.1642 g/mol (monoisotopic).

Molecular weight: 375.37 g/mol.

Isoelectric point: Approximately 3.1 to 3.4, reflecting the preponderance of acidic residues (glutamic acid, aspartic acid) with no basic residues other than the N-terminal alpha-amino group. The compound carries a net negative charge at physiological pH.

IUPAC name: (S)-2-((S)-2-((S)-2-amino-4-carboxybutanamido)-3-carboxypropanamido)-4-methylpentanoic acid.

Physical properties: White to off-white lyophilized powder or amorphous solid. Freely soluble in water and in aqueous buffers at physiological pH. Sparingly soluble in apolar organic solvents. The compound contains two acidic side chains (glutamic acid and aspartic acid) and one hydrophobic side chain (leucine), producing a net negative charge at physiological pH (approximately minus two: the glutamic acid gamma-carboxyl, the aspartic acid beta-carboxyl, and the C-terminal carboxyl are deprotonated, while the N-terminal alpha-amino group is partially protonated).

Stereochemistry: All three amino acid residues are in the L-configuration. The compound contains three stereocenters (the alpha-carbon of each residue). No D-amino acid substitutions have been reported in the active form. Investigators acquiring research-grade Ovagen should confirm stereochemical integrity by chiral chromatography or by comparison of optical rotation to a reference standard.

1.3 Synthesis

The synthesis of Ovagen proceeds by standard solid-phase or solution-phase peptide synthesis methods. The tripeptide is sufficiently short that both approaches are practical and economical at scale.

For solid-phase synthesis using fluorenylmethyloxycarbonyl (Fmoc) chemistry, the procedure is: (a) coupling of Fmoc-Leu-OH to Wang resin for a free-acid C-terminus; (b) Fmoc deprotection with 20 percent piperidine in dimethylformamide;

(c) coupling of Fmoc-Asp(OtBu)-OH using HBTU or HATU with N,N-diisopropylethylamine; (d) Fmoc deprotection; (e) coupling of Fmoc-Glu(OtBu)-OH; (f) final Fmoc deprotection; (g) cleavage from resin and simultaneous side-chain deprotection with trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5); (h) purification by reversed-phase high-performance liquid chromatography on a C18 column; and (i) lyophilization [8]. Industrial yields for tripeptide synthesis at this scale are typically greater than 80 percent.

For solution-phase synthesis, the general procedure involves sequential coupling of N-protected glutamic acid to aspartic acid followed by leucine, with appropriate orthogonal side-chain protecting groups (tert-butyl esters for carboxyl groups). Coupling reagents (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide with hydroxybenzotriazole, or benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate) provide efficient amide bond formation. Global deprotection under acidic conditions, purification by chromatography, and lyophilization complete the synthesis. The simplicity of the tripeptide structure and the absence of unusual or modified amino acids render the synthesis straightforward by contemporary peptide chemistry standards.

The product is characterized by electrospray ionization mass spectrometry (expected $[M+H]^+ = 376.17$), amino acid analysis after acid hydrolysis (confirming Glu:Asp:Leu in 1:1:1 ratio), and analytical HPLC (purity greater than 95 percent for research grade, greater than 98 percent achievable with additional purification).

2. Abstract

Ovagen (Glu-Asp-Leu; EDL) is a synthetic tripeptide bioregulator developed within the Khavinson peptide research program at the Saint Petersburg Institute of Bioregulation and Gerontology as a tissue-specific epigenetic modulator targeting hepatic and gastrointestinal cell populations [1, 2]. The compound is distinguished from conventional hepatoprotective agents (silymarin, ursodeoxycholic acid, S-adenosylmethionine, N-acetylcysteine) by its proposed mechanism of action: rather than scavenging reactive oxygen species, modulating bile acid composition, or replenishing glutathione precursors, Ovagen is proposed to penetrate the cell nucleus and interact directly with DNA regulatory sequences in the promoter regions of genes governing hepatocyte proliferation, oxidative stress response, apoptotic signaling, and inflammatory cytokine production [6, 7].

The mechanistic framework derives from a broader body of research on ultra-short peptide-DNA interactions conducted principally by the Khavinson laboratory over approximately four decades. The central hypothesis holds that peptides of 2 to 7 amino acid residues possess sequence-specific affinity for double-stranded DNA, binding in the major groove through complementary electrostatic interactions between the peptide backbone and side chains and the nucleotide bases [6]. This binding is proposed to modulate chromatin condensation state, shifting heterochromatin (transcriptionally silent) toward euchromatin (transcriptionally active), thereby reactivating genes that have been epigenetically silenced by aging, oxidative damage, or pathological chromatin remodeling. The 2021 systematic review by Khavinson and colleagues in *Molecules* documented the evidence base for this mechanism across multiple peptide-tissue pairs [6].

For Ovagen specifically, the preclinical evidence includes: (a) hepatocyte cell culture studies demonstrating prolonged cell survival and increased proliferative activity following EDL tripeptide exposure at nanomolar to low-micromolar concentrations [1, 2]; (b) rodent models of carbon tetrachloride-induced and paracetamol-induced hepatotoxicity in which Ovagen administration reduced serum alanine aminotransferase and aspartate aminotransferase, decreased histological necrosis, and normalized hepatic glutathione and superoxide dismutase levels [3, 4]; (c) aged rat liver tissue studies demonstrating an approximately 18-fold increase in Ki-67-positive hepatocytes and an approximately 6-fold decrease in p53-positive

hepatocytes following Ovagen treatment, consistent with a shift from senescent to proliferative phenotype [2]; and (d) gastrointestinal mucosal models in which Ovagen enhanced tight junction protein expression, reduced intestinal permeability, and supported epithelial cell turnover in aging animals [5].

The compound has not been evaluated in published, peer-reviewed human clinical trials. No regulatory authority has approved Ovagen for therapeutic use. The pharmacokinetic properties of Ovagen in humans have not been characterized to Western regulatory standards, though the ultrashort peptide class as a whole is proposed to exhibit oral bioavailability owing to resistance to gastrointestinal peptidase degradation at the di- and tripeptide level [9]. The compound is supplied commercially as a research-grade lyophilized powder and as an oral capsule formulation (marketed in the Russian Federation and through international peptide suppliers). Investigators should treat the available evidence as preliminary and predominantly single-laboratory in provenance.

3. Discovery and Development History

3.1 Origin of the Khavinson peptide bioregulator program

The peptide bioregulator research program was initiated in the late 1960s and early 1970s at the Military Medical Academy in Leningrad (now Saint Petersburg) by Vladimir Khavinson and Vyacheslav Morozov as part of a Soviet military research initiative to develop protective agents against radiation exposure, laser-induced retinal injury, and extreme environmental stressors affecting military personnel, cosmonauts, and athletes [7, 10]. The initial approach involved extraction of low-molecular-weight peptide fractions from animal organ tissues (thymus, pineal gland, brain cortex, retina, prostate, liver, and other organs) of young calves, followed by assessment of these fractions for tissue-specific biological activity in aged or stressed animal models.

3.2 Development of liver-specific peptide extracts

The liver-specific peptide extract, designated within the Cytomax product line, was prepared from liver tissue of young calves by acid extraction, ultrafiltration to isolate peptide fractions below approximately 10 kilodaltons, and further fractionation by ion-exchange and size-exclusion chromatography [10, 11]. This crude liver peptide complex demonstrated hepatoprotective activity in multiple animal models of liver injury, including carbon tetrachloride-induced hepatitis, ethanol-induced hepatotoxicity, and age-related hepatocyte functional decline. The polypeptide liver complex was evaluated in a 2020 review by Kuznik, Khavinson, and Linkova, which documented efficacy in restoring liver function in experimental models of liver fibrosis, acute hepatitis, and chronic hepatitis [12].

3.3 Identification of the active tripeptide sequence

The transition from crude tissue extract to defined synthetic peptide followed the general strategy employed across the Khavinson peptide program: the active peptide fraction was subjected to sequential chromatographic separation and amino acid sequence analysis to identify the minimal peptide sequence responsible for the observed biological activity. For the liver bioregulator, the tripeptide Glu-Asp-

Leu (EDL) was identified as the active sequence and was designated Ovagen [1]. The related tetrapeptide Lys-Glu-Asp-Ala (KEDA), designated Livagen, was identified from the same liver peptide extract lineage and shares a partially overlapping sequence (the Glu-Asp dipeptide core) but exhibits distinct biological activity, particularly in chromatin remodeling and immunomodulatory endpoints [13, 14].

3.4 Relationship to Livagen and other liver bioregulators

Ovagen (EDL, tripeptide) and Livagen (KEDA, tetrapeptide) represent two synthetic peptides derived from the same liver tissue peptide extract but with proposed complementary mechanisms. Livagen has been more extensively characterized for chromatin remodeling activity, with the landmark 2002 study by Khavinson and Lezhava demonstrating that KEDA induced pericentromeric heterochromatin decondensation and ribosomal gene activation in lymphocytes from elderly donors [13, 15]. Ovagen has been more extensively characterized for hepatocyte proliferation and apoptosis modulation (Ki-67 and p53 endpoints). The two peptides are proposed to have complementary rather than redundant mechanisms, with Livagen operating primarily through chromatin architectural remodeling and Ovagen operating primarily through gene-specific transcriptional regulation in hepatic tissue [14].

3.5 Commercial and regulatory status

Ovagen is marketed commercially in several formulations: as a research-grade lyophilized powder through international peptide suppliers (typically at 20 mg per vial); as an oral capsule formulation containing peptide complex (60 capsules per package) marketed through Russian and international supplement distributors; and as sublingual drops. The compound has not been approved by any Western regulatory authority (United States Food and Drug Administration, European Medicines Agency, Therapeutic Goods Administration of Australia, or equivalent). In the Russian Federation, peptide bioregulators of the Khavinson class have been registered as dietary supplements (*biologicheski aktivnye dobavki*) rather than as pharmaceutical drugs, a regulatory distinction that has limited the requirement for and conduct of formal clinical trials to Western standards [7].

4. Mechanism of Action

Ovagen is proposed to operate through a mechanism fundamentally distinct from conventional hepatoprotective agents. Rather than acting at cell-surface receptors, scavenging free radicals, or modulating enzymatic pathways in the cytoplasm, the EDL tripeptide is proposed to enter the cell nucleus and interact directly with DNA to modulate gene expression at the transcriptional level [6, 7].

4.1 Nuclear penetration and DNA interaction

The ultrashort peptide bioregulator hypothesis, developed principally by the Khavinson laboratory, holds that peptides of 2 to 7 amino acid residues are sufficiently small (molecular weight below approximately 1000 daltons) to cross the plasma membrane and nuclear envelope without requiring receptor-mediated endocytosis, active transport, or nuclear localization signal-mediated import [6, 9]. An alternative uptake pathway is through the proton-coupled oligopeptide transporters PepT1 (SLC15A1) and PepT2 (SLC15A2), which are expressed on intestinal enterocytes and hepatocytes and transport di- and tripeptides with broad substrate specificity [16].

Once within the nucleus, the EDL tripeptide is proposed to bind to specific DNA sequences in gene promoter regions through electrostatic complementarity between the amino acid side chains and the nucleotide bases exposed in the major groove of the DNA double helix [6]. The glutamic acid and aspartic acid residues (both negatively charged at physiological pH) are proposed to interact with regions of the DNA-histone interface, while the leucine residue provides a hydrophobic anchor. The specificity of this interaction is proposed to arise from the three-dimensional fit between the peptide conformation and the local geometry of the DNA groove at specific promoter sequences [6, 17]. Thermal denaturation studies have demonstrated that Ovagen modifies DNA melting profiles in a concentration-dependent manner, consistent with physical interaction with the double-stranded DNA structure [17].

4.2 Chromatin remodeling and epigenetic regulation

A second component of the proposed mechanism involves modulation of chromatin condensation state. The Khavinson laboratory has documented that ultra-short peptides, including the related tetrapeptide Livagen (KEDA), can induce de-heterochromatinization in aging cells, converting transcriptionally silent heterochromatin to transcriptionally active euchromatin [13, 15]. This chromatin remodeling is proposed to reactivate genes that have been epigenetically silenced by age-related chromatin condensation, a process that accumulates progressively in aging tissues and is associated with declining cellular function.

For Ovagen specifically, the proposed chromatin-level activity targets hepatocyte genes involved in: cell cycle progression (Ki-67, cyclins); apoptotic signaling (p53, Bcl-2 family members); antioxidant defense (superoxide dismutase, catalase, glutathione peroxidase); and inflammatory cytokine production (tumor necrosis factor alpha, interleukin-6, interleukin-1 beta) [1, 2, 4]. The net effect is proposed to be a restoration of youthful hepatocyte gene expression patterns in aged or damaged liver tissue.

4.3 DNA methylation modulation

A third proposed mechanism involves regulation of DNA methylation status. Peptide bioregulators have been reported to modulate the methylation state of cytosine residues in CpG dinucleotides within gene promoter regions, influencing whether genes are transcriptionally active (hypomethylated promoters) or silenced (hypermethylated promoters) [6]. For Ovagen, the proposed activity involves selective demethylation of promoter regions of hepatoprotective genes, restoring their expression in aged or damaged hepatocytes. This epigenetic mechanism would complement the direct DNA-binding and chromatin-remodeling mechanisms.

4.4 Downstream biological effects

The downstream consequences of the proposed nuclear mechanisms, documented in preclinical models, include: increased hepatocyte proliferation (Ki-67 upregulation); decreased hepatocyte apoptosis (p53 downregulation); enhanced antioxidant enzyme expression and activity (superoxide dismutase, glutathione peroxidase, glutathione S-transferase upregulation); reduced proinflammatory cytokine pro-

duction; improved hepatocyte protein synthesis capacity; and enhanced gastrointestinal epithelial barrier integrity through tight junction protein upregulation [1, 2, 3, 4, 5].

4.5 Mechanistic caveats

The proposed mechanism of direct peptide-DNA interaction as the primary mode of action for ultrashort peptide bioregulators remains a subject of scientific discussion. The mechanism has not been independently validated outside the Khavinson laboratory network by Western research groups using contemporary structural biology methods (X-ray crystallography, cryo-electron microscopy, or single-molecule fluorescence resonance energy transfer of peptide-DNA complexes). The binding affinities, sequence specificities, and stoichiometries of the proposed peptide-DNA interactions have not been characterized by surface plasmon resonance, isothermal titration calorimetry, or electrophoretic mobility shift assay in independent laboratories. Alternative mechanisms that could account for the observed biological effects include: conventional receptor-mediated signaling through as-yet-unidentified peptide receptors on hepatocyte surfaces; intracellular signaling through peptide transporter-mediated uptake (PepT1, PepT2) followed by cytoplasmic interactions; or indirect effects mediated by peptide-induced changes in intracellular amino acid pools following hydrolysis. Investigators should consider the mechanistic hypothesis as a working framework rather than an established molecular mechanism.

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

Formal pharmacokinetic studies of Ovagen in humans have not been published in peer-reviewed literature. The pharmacokinetic properties are inferred from the general characteristics of ultrashort peptides and from limited data on related Khavinson peptides [9].

Ultrashort peptides (2 to 4 amino acid residues) are proposed to resist gastrointestinal peptidase degradation more effectively than longer peptides, because the principal gastrointestinal endopeptidases (pepsin, trypsin, chymotrypsin) require a minimum substrate length of approximately 4 to 6 residues for efficient cleavage, and because brush-border and cytoplasmic dipeptidases and tripeptidases may not efficiently cleave all tripeptide sequences depending on the amino acid composition [9]. The Glu-Asp-Leu sequence contains two acidic residues and one hydrophobic residue, a composition that may confer partial resistance to aminopeptidase N and other exopeptidases.

Intestinal absorption of di- and tripeptides is mediated principally by the proton-coupled peptide transporter PepT1 (SLC15A1) expressed on the apical membrane of enterocytes [16]. PepT1 transports a broad range of di- and tripeptides with relatively low substrate specificity, and the EDL tripeptide is a plausible PepT1 substrate. Absorbed tripeptides may be further hydrolyzed by cytoplasmic peptidases within the enterocyte or may be transported intact across the basolateral membrane into the portal circulation. The fraction of intact EDL tripeptide reaching the systemic circulation after oral administration has not been quantified. Bioavailability is estimated in the range of 20 to 50 percent for linear tripeptides based on class data, with the remainder subject to luminal degradation by brush-border peptidases and pancreatic proteases [9].

Sublingual administration bypasses gastrointestinal degradation and provides absorption through the oral mucosa into the systemic circulation. Subcutaneous injection provides near-complete bioavailability but is subject to local tissue peptidase degradation at the injection site.

5.2 Distribution

Distribution data for Ovagen in any species have not been published. The small molecular weight (375.37 daltons) and hydrophilic character (two acidic residues, net negative charge at physiological pH) predict limited passive diffusion across the blood-brain barrier and preferential distribution to well-perfused organs including liver, kidney, and gastrointestinal tract. The proposed hepatic tissue specificity of Ovagen may reflect preferential first-pass hepatic exposure following portal venous absorption from the gastrointestinal tract, the high density of peptide transporters on hepatocyte membranes, or a combination of both factors. Plasma protein binding has not been characterized but is expected to be low for a highly charged small peptide.

5.3 Metabolism

Tripeptides in systemic circulation are subject to rapid hydrolysis by plasma peptidases (aminopeptidases, carboxypeptidases, angiotensin-converting enzyme) and by tissue peptidases in the liver, kidney, and other organs. The plasma half-life of unmodified tripeptides is generally measured in minutes, not hours, in mammalian systems [16]. The constituent amino acids (glutamic acid, aspartic acid, leucine) are proteinogenic and enter standard amino acid metabolic pathways following hydrolysis: glutamic acid enters the tricarboxylic acid cycle via alpha-ketoglutarate; aspartic acid enters via oxaloacetate; leucine enters via acetoacetyl-CoA and the branched-chain amino acid catabolic pathway. No pharmacologically active metabolites of Ovagen have been identified or proposed.

5.4 Elimination

Elimination is expected to proceed via metabolic hydrolysis to constituent amino acids, which are then incorporated into endogenous amino acid pools or excreted as urea (nitrogen) and carbon dioxide (carbon skeleton). Renal excretion of intact tripeptide is expected to be minimal at physiological doses. No formal elimination studies have been conducted.

5.5 Routes of administration

Three principal routes are used in research and commercial contexts: oral (capsule formulations containing peptide complex, typically 10 to 20 mg per capsule, administered 1 to 2 capsules daily on an empty stomach to maximize PepT1-mediated absorption); sublingual (liquid drops, typically 1 to 5 mg per milliliter, 5 to 6 drops held under the tongue for 30 to 60 seconds, 3 to 4 times daily); and subcutaneous injection (research-grade lyophilized powder reconstituted in bacteriostatic water or sterile saline, administered subcutaneously at doses in the range of 100 to 500 micrograms per injection in research protocols) [18]. The oral route is the most commonly used in commercial formulations. The subcutaneous route is used in research settings where systemic bioavailability of intact peptide is desired.

5.6 Special populations

No formal studies in hepatic impairment, renal impairment, pediatric, or geriatric special populations have been published. The preclinical evidence base predominantly employs aged animals (18 to 24 month rats, corresponding to human ages of approximately 60 to 80 years). The age-specific design does not imply dose adjustment in aging; rather, the biological effect is proposed to be more pronounced in aged tissues where the target gene silencing is most extensive.

6. Preclinical Pharmacology

6.1 Hepatocyte culture studies

The foundational preclinical evidence for Ovagen derives from organotypic culture experiments using liver tissue fragments from young (3 to 4 month) and aged (18 to 24 month) rats [2, 19]. In organotypic cultures, liver tissue explants are maintained in culture medium for 3 to 10 days, and the area of the explant zone of cell growth is measured as the primary endpoint of tissue viability and proliferative capacity. Ovagen at concentrations of 10 to 100 nanomolar in the culture medium increased the explant growth zone area by 20 to 30 percent in aged liver tissue cultures compared to untreated controls, while producing minimal effect in young liver tissue (less than 5 percent increase) [2]. This age-dependent response is characteristic of the Khavinson bioregulatory peptides and is interpreted as reflecting the greater degree of age-related gene silencing available for reversal in aged tissue.

In cultured hepatic cells, Ovagen at nanomolar to low-micromolar concentrations prolonged cell survival and increased proliferative activity, as measured by cell counting, MTT assay, and BrdU incorporation [1]. The effect was dose-dependent within the tested range and was not observed with scrambled-sequence control peptides of the same amino acid composition.

6.2 Ki-67 and p53 modulation

Immunohistochemical analysis of organotypic cultures treated with Ovagen demonstrated an approximately 18-fold increase in Ki-67-positive hepatocytes (from approximately 1 to 2 percent in untreated aged tissue to approximately 20 to 36 percent in Ovagen-treated aged tissue) and an approximately 6-fold decrease in p53-positive hepatocytes (from approximately 30 percent in untreated aged tissue to approximately 5 percent in Ovagen-treated aged tissue) [2]. These changes are quantitatively large and statistically significant, and they indicate a fundamental shift in hepatocyte fate from the senescent-apoptotic phenotype characteristic of aged liver toward active proliferative renewal. The Ki-67 and p53 modulation data have been extended to human hepatocyte cultures from surgical resection speci-

mens, where qualitatively similar though somewhat attenuated effects (approximately 8- to 12-fold Ki-67 increase, approximately 3- to 4-fold p53 decrease) were observed in liver tissue fragments from patients aged 60 to 75 years [2].

6.3 Gene expression profiling

Quantitative polymerase chain reaction and microarray studies of hepatocytes treated with Ovagen have demonstrated selective upregulation of liver-specific genes [3]. The principal gene expression changes observed include upregulation of cytochrome P450 family enzymes (CYP1A2, CYP2E1, CYP3A4; 1.5- to 4-fold), glutathione S-transferases (GSTA1; 2- to 3-fold), mitochondrial superoxide dismutase (SOD2; 2- to 4-fold), glutathione peroxidase (GPX1; 1.5- to 3-fold), and albumin (ALB; 1.5- to 2-fold) [3]. These gene expression changes are observed at 24 to 72 hours after peptide treatment and are sustained for the duration of culture (up to 10 days with repeated dosing). The selectivity for liver-specific genes (as opposed to ubiquitously expressed genes) supports the tissue-specific regulatory mechanism proposed by the Khavinson hypothesis.

6.4 Carbon tetrachloride hepatotoxicity model

Carbon tetrachloride (CCl₄) is a standard experimental hepatotoxin that produces centrilobular hepatocyte necrosis through CYP2E1-mediated bioactivation to the trichloromethyl radical, which initiates lipid peroxidation and oxidative damage. In rat models of CCl₄-induced hepatotoxicity, Ovagen administration (0.1 to 1 mg/kg subcutaneously for 14 to 28 days) produced significant hepatoprotection compared to vehicle-treated controls [3, 4]: serum alanine aminotransferase levels were reduced by 40 to 60 percent; aspartate aminotransferase levels were reduced by 35 to 55 percent; histological necrosis scores were reduced by approximately 50 percent; and inflammatory cell infiltration was reduced by approximately 40 percent. The hepatoprotective effect was dose-dependent within the range of 0.1 to 1 mg/kg. Pre-treatment (beginning 24 hours before CCl₄) provided greater protection than concurrent or post-treatment administration, consistent with a mechanism requiring gene expression changes (which require hours to manifest) rather than immediate receptor blockade [3].

6.5 Paracetamol hepatotoxicity model

Paracetamol (acetaminophen) hepatotoxicity is mediated by CYP2E1 and CYP1A2-dependent formation of N-acetyl-p-benzoquinone imine (NAPQI), which depletes hepatic glutathione and produces mitochondrial dysfunction and hepatocyte necrosis. In rat models of paracetamol-induced liver injury, Ovagen administered at 0.5 mg/kg subcutaneously for 5 days before paracetamol overdose produced significant hepatoprotection with reduction of ALT and AST by approximately 45 to 55 percent compared to paracetamol-only controls [3]. The protective effect was attributed to Ovagen-mediated upregulation of glutathione S-transferases and glutathione peroxidase, which conjugate the toxic metabolite NAPQI and reduce oxidative damage.

6.6 Gastrointestinal mucosal effects

Preclinical data from the Khavinson laboratory indicate that Ovagen supports gastrointestinal epithelial barrier function in aging models. The reported effects include: increased expression of tight junction proteins (occludin, claudins, zonula occludens-1) in intestinal epithelial cells; reduced intestinal permeability as measured by FITC-dextran translocation assays; enhanced epithelial cell turnover and regenerative capacity in the intestinal crypt-villus axis; and increased mucin production by goblet cells [5]. These effects are proposed to arise from the same nuclear gene-regulatory mechanism that drives the hepatoprotective activity, operating on intestinal epithelial cell gene expression rather than hepatocyte gene expression.

6.7 Aging models

In naturally aged rats (24 months), chronic Ovagen administration (0.1 mg/kg subcutaneously, three times per week for 3 months) produced measurable improvements in liver function biomarkers including serum albumin (increased by approximately 15 percent), total protein (increased by approximately 10 percent), and hepatic glutathione content (increased by approximately 25 percent) compared to age-matched vehicle controls [19]. The functional improvements were accompanied by histological evidence of reduced lipofuscin accumulation, reduced periportal fibrosis, and increased hepatocyte mitotic index.

6.8 Murine cirrhosis model

In murine models of experimentally induced cirrhosis (chronic CCl₄ administration over 8 to 12 weeks), Ovagen treatment was reported to increase the proportion of dividing hepatocytes, reduce fibrotic tissue deposition, and partially restore liver architecture [2, 4]. The antifibrotic effect is proposed to reflect both the direct hepatocyte proliferative stimulus (replacing damaged cells) and indirect effects on hepatic stellate cell activation through modulation of inflammatory cytokine signaling.

7. Clinical Evidence Base

7.1 Absence of formal clinical trials

No published, peer-reviewed, randomized controlled clinical trials of the synthetic EDL tripeptide have been identified in PubMed, Scopus, Web of Science, or the Cochrane Library as of the date of this monograph. The compound has not been registered in ClinicalTrials.gov, the EU Clinical Trials Register, or equivalent trial registries for any therapeutic indication.

7.2 Ex vivo human tissue studies

The Ki-67 and p53 modulation data described in the preclinical section have been extended to human hepatocyte cultures from surgical resection specimens [2]. In human liver tissue fragments from patients aged 60 to 75 years (obtained during hepatic surgery with informed consent), Ovagen at 20 nanomolar in culture medium produced qualitatively similar effects to those observed in aged rat tissue: increased Ki-67 expression (approximately 8- to 12-fold increase) and decreased p53 expression (approximately 3- to 4-fold decrease). The magnitude was somewhat lower than in the rat model but the direction and statistical significance were preserved, supporting translational relevance.

7.3 Observational and anecdotal evidence

The parent polypeptide complex preparation from which Ovagen was derived has been used in Russian gerontological clinical practice for age-related hepatic dysfunction, metabolic syndrome, and chronic hepatitis sequelae [11, 20]. Published observational reports (primarily in Russian-language journals) describe improvements in liver function tests, reduction of hepatic steatosis by ultrasound assessment, and improved subjective well-being in elderly patients receiving bioregulatory peptide therapy courses including liver-directed peptides. These reports lack the methodological rigor (randomization, blinding, placebo control, adequate sample size) required for regulatory-grade evidence.

The broader Khavinson peptide bioregulator class includes compounds with limited clinical evaluation. Thymalin (thymic peptide extract) and Epithalamin (pineal peptide extract) were evaluated in a 15-year cohort study of elderly subjects in Saint Petersburg, with the treated cohort showing reduced mortality compared to controls [10]. These data provide indirect support for the biological activity of peptide bioregulators as a class but do not constitute direct clinical evidence for Ovagen specifically.

7.4 Human safety in commercial use

No serious adverse events attributable to Ovagen have been reported in publicly accessible pharmacovigilance databases. The oral capsule formulations marketed as dietary supplements have been in commercial use for approximately two decades in the Russian Federation and through international suppliers. The absence of reported adverse events may reflect a favorable safety profile, the low doses used in commercial formulations, the limited pharmacovigilance infrastructure for dietary supplements, or a combination of these factors.

7.5 Current clinical development state

Ovagen is not in active pharmaceutical development in any Western jurisdiction. No investigational new drug (IND) application or clinical trial application (CTA) has been submitted for the synthetic EDL tripeptide. The compound remains in the pre-clinical and research-use phase outside of Russian gerontological practice. The prospects for Western clinical development are constrained by the absence of a conventional pharmacological mechanism (receptor binding, enzyme inhibition) that would support a traditional regulatory pathway, and by the challenge of demonstrating clinical efficacy for an epigenetic gene-regulatory mechanism with endpoints (liver function improvement, anti-aging) that require long-duration trials.

8. Sourcing and Quality Verification

Research-grade Ovagen is supplied by multiple international peptide synthesis companies (Peptide Sciences, BioLongevity Labs, NuScience Peptides, Dragon Pharma, Liberty Peptides, Core Peptides, PrymaLab, and others) at stated purity of 98 to 99 percent by high-performance liquid chromatography. Supply is typically as a lyophilized powder in 20 mg or 50 mg vials. Pricing is moderate, in the range of \$30 to \$80 per 20 mg vial of research-grade material at the time of monograph preparation.

Oral capsule formulations are marketed through Russian and international supplement distributors (CosmicNootropic, iPept, QI Supplements, Limitless Longevity, and others) as dietary supplement products containing liver peptide complex. The capsule formulations may contain the defined EDL tripeptide, a broader liver peptide fraction, or excipients that vary by manufacturer; investigators requiring the defined tripeptide sequence should use research-grade lyophilized material rather than capsule formulations.

Investigators should confirm compound identity and purity on every research-grade lot through:

- Reversed-phase high-performance liquid chromatography area-percent purity, ideally greater than 98 percent
- Electrospray ionization mass spectrometry confirmation of molecular weight (expected $[M+H]^+ = 376.17$ for the protonated free acid)
- Amino acid analysis after acid hydrolysis to confirm the presence of glutamic acid, aspartic acid, and leucine in equimolar ratio
- Assessment of residual trifluoroacetic acid or acetate counterion content by ion chromatography or ^{19}F nuclear magnetic resonance spectroscopy, relevant for accurate concentration calculation in bioassays
- Endotoxin testing (Limulus amebocyte lysate assay) for material intended for in vivo administration
- Karl Fischer or loss-on-drying analysis for residual water content
- Residual solvent analysis by gas chromatography (acetonitrile, dimethylformamide, and other peptide synthesis solvents)

Third-party analytical laboratories (Janoshik Analytical, Vanta Bioscience, and others) provide independent confirmation of vendor certificates of analysis. The research-grade Ovagen market is established but smaller than the market for longer peptides (such as BPC-157, thymosin alpha-1, or epithalon), and lot-to-lot variability in purity, counterion content, and residual solvents is observed. On-receipt analytical confirmation is appropriate for any investigation where dose-response precision is important.

9. Reconstitution and Handling

Ovagen lyophilized powder is soluble in water, normal saline (0.9 percent sodium chloride), and phosphate-buffered saline at room temperature. The compound is acidic in solution (owing to the two dicarboxylic amino acid residues) and may require pH adjustment to physiological range (pH 7.2 to 7.4) with dilute sodium hydroxide or buffered diluent for cell culture and in vivo applications.

Reconstitution for injection studies: Add 1 to 2 milliliters of bacteriostatic water for injection (containing 0.9 percent benzyl alcohol as preservative) to a 20 mg vial to produce a stock solution of 10 to 20 mg/mL. Direct the stream of diluent against the glass wall of the vial rather than directly onto the powder to minimize foaming. Allow the powder to dissolve without vigorous agitation; gentle swirling for 30 to 60 seconds is sufficient. The resulting solution should be clear and colorless; discard any vial producing turbidity or particulate matter.

Reconstitution for in vitro studies: Dissolve in sterile phosphate-buffered saline (pH 7.4) or in cell culture medium (DMEM, RPMI 1640, Williams E medium for hepatocyte culture) at the desired concentration. For concentrated stock solutions, dissolve in sterile water at 10 to 50 mg/mL and dilute to working concentration in culture medium. Dimethyl sulfoxide is acceptable for stock solutions at concentrations up to 100 mg/mL but should be diluted to less than 0.1 percent final DMSO concentration in cell culture to avoid solvent cytotoxicity.

Storage conditions: Store lyophilized powder at minus 20 degrees Celsius for long-term storage (greater than 6 months) or at 2 to 8 degrees Celsius for short-term storage (less than 3 months). Reconstituted solutions in bacteriostatic water should be stored at 2 to 8 degrees Celsius and used within 14 to 28 days. Reconstituted solutions in sterile water (without preservative) should be used within 24 hours or aliquoted and frozen at minus 20 degrees Celsius for single-use.

Stability considerations: The tripeptide is stable in the solid state for at least 24 months at minus 20 degrees Celsius and at least 12 months at 2 to 8 degrees Celsius. In aqueous solution at pH 5 to 8, stability is maintained for at least 30 days at 2 to 8 degrees Celsius based on HPLC monitoring. Light protection is not required but is reasonable practice. Freeze-thaw cycles should be minimized; aliquoting for single-use is recommended if repeated access to the stock is required.

Filter sterilization: For cell culture applications, filter sterilization through a 0.22-micrometer syringe filter is required; peptide loss on syringe filters is generally minimal for tripeptides at the concentrations used in cell culture.

10. Stack Interactions and Combinations

Ovagen operates through a proposed mechanism (direct nuclear peptide-DNA interaction) that is distinct from the mechanisms of most conventional pharmaceuticals and research compounds. Consequently, pharmacodynamic interactions with conventional drugs are not well characterized. The following considerations apply to research applications involving combination use.

Other Khavinson peptide bioregulators: Ovagen is commonly combined with Livagen (KEDA tetrapeptide) in research protocols targeting liver function, on the basis that the two peptides have proposed complementary mechanisms (Ovagen targeting gene-specific transcription, Livagen targeting chromatin remodeling) [14]. The combination has not been evaluated in controlled comparative studies. Other Khavinson peptide bioregulators targeting non-hepatic tissues (Epithalon for pineal, Thymalin for thymus, Cortexin for brain cortex, Vesugen for vasculature, Crystagen for immune system) are used concurrently in multicomponent bioregulatory protocols; interactions between these peptides have not been characterized.

Conventional hepatoprotective agents: Concurrent use of Ovagen with silymarin (milk thistle extract), ursodeoxycholic acid (UDCA), S-adenosylmethionine (SAME), or N-acetylcysteine (NAC) is pharmacologically plausible, as the proposed mechanisms are non-overlapping (epigenetic regulation versus antioxidant, cytoprotective, or choleretic mechanisms). The combination of Ovagen with N-acetylcysteine is particularly rational for oxidative hepatotoxicity given the complementary mechanisms (Ovagen upregulates glutathione synthetic enzymes; NAC provides direct glutathione precursor). No interaction data are available.

CYP450 interactions: Ovagen, as a tripeptide composed of proteinogenic amino acids, is not expected to inhibit or induce cytochrome P450 enzymes at pharmacologically relevant concentrations through acute mechanisms. However, because Ovagen is proposed to upregulate CYP450 expression over days to weeks, concurrent use of drugs metabolized by CYP1A2, CYP2E1, or CYP3A4 could theoretically be affected by altered hepatic clearance capacity. This interaction has not been studied and warrants consideration in research design.

Peptide transporter competition: Concurrent administration of Ovagen with other di- or tripeptide substrates of the PepT1 transporter (including the antibiotic cephalexin, the angiotensin-converting enzyme inhibitor prodrugs enalapril and captopril, and the antiviral prodrug valacyclovir) could theoretically result in competitive inhibition of intestinal absorption. The clinical significance of this interaction at typical Ovagen doses is expected to be negligible.

Immunomodulatory interactions: The proposed anti-inflammatory activity of Ovagen (reduction of proinflammatory cytokine expression through gene-regulatory mechanisms) could theoretically interact with immunosuppressive agents (corticosteroids, calcineurin inhibitors, anti-TNF biologics) or with immunostimulatory agents. No data are available to characterize these interactions.

Anticoagulant and antiplatelet agents: No interaction is expected on theoretical grounds.

Chemotherapy agents: Ovagen may provide hepatoprotective benefit during chemotherapy courses that produce hepatotoxicity (methotrexate, cyclophosphamide, doxorubicin). However, because Ovagen upregulates CYP enzymes involved in drug metabolism, it could theoretically alter the pharmacokinetics of chemotherapy agents. This interaction has not been studied and warrants caution.

11. Adverse Events and Safety Signal

11.1 Preclinical safety

No significant toxicity has been reported in preclinical studies of Ovagen at doses up to 10 mg/kg (approximately 20 times the typical research dose) administered subcutaneously for up to 30 days in rats [3, 19]. Acute toxicity studies have not identified a lethal dose within practicable dosing ranges. The compound degrades to the naturally occurring amino acids glutamic acid, aspartic acid, and leucine, all of which are present in normal dietary protein at gram quantities daily. Formal toxicology studies meeting International Council for Harmonisation (ICH) guidelines have not been published. The theoretical acute toxicity risk is negligible.

11.2 Reported adverse events

No serious adverse events have been reported in published literature or in observational clinical reports [11, 20]. The reported adverse-event profile is limited to:

- Transient mild injection site discomfort (subcutaneous route, approximately 5 to 10 percent, consistent with any subcutaneous injection)
- Mild gastrointestinal discomfort (oral route, less than 5 percent, principally mild nausea or epigastric sensation)
- Transient headache (less than 3 percent, not clearly distinguished from placebo-rate headache)

These adverse events are self-limiting, mild, and do not require treatment discontinuation.

11.3 Theoretical safety considerations

The principal theoretical safety concern for a compound that promotes hepatocyte proliferation and suppresses p53 is the potential for promotion of pre-existing hepatic neoplasia. If hepatocytes carrying oncogenic mutations are stimulated to proliferate while p53-mediated apoptosis is suppressed, the theoretical consequence is accelerated growth of pre-malignant or malignant clones. This concern has not

been demonstrated experimentally (no studies have reported increased hepatocellular carcinoma incidence in Ovagen-treated animals), but it represents a theoretical risk that should be considered in research design. Investigators should exercise caution in applying Ovagen in animals or subjects with known hepatic neoplasia, viral hepatitis with high hepatocellular carcinoma risk, or cirrhosis with established dysplastic nodules.

11.4 Pregnancy and lactation

No reproductive toxicity or teratogenicity data are available. The compound is a naturally occurring tripeptide sequence, and the constituent amino acids are essential or semi-essential nutrients. Nevertheless, the proliferative and gene-regulatory mechanism warrants caution during pregnancy and lactation until formal reproductive safety studies are conducted.

11.5 Long-term safety

No long-term (greater than 12 months) safety data are available from controlled studies. Russian gerontological practice reports use of bioregulatory peptide courses (typically 10 to 20 day courses repeated 2 to 4 times per year) for periods of years to decades without reported cumulative toxicity [10, 20]. These observational data provide some reassurance but do not substitute for controlled long-term safety assessment.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five hepatoprotective or liver-bioregulatory candidates against Ovagen, evaluated against five competency standards: novelty, effect size, promising potential (current research state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected to represent the principal categories of hepatoprotective agents with which Ovagen might be compared in research contexts, spanning conventional small molecules, peptide bioregulators, and cytoprotective peptides.

12.1 Livagen (KEDA; Lys-Glu-Asp-Ala)

Mechanism: Synthetic tetrapeptide bioregulator from the same Khavinson class as Ovagen, targeting liver tissue through chromatin remodeling (pericentromeric heterochromatin decondensation and ribosomal gene activation) and enkephalinase inhibition [13, 14, 15].

Development trajectory: Developed at the St. Petersburg Institute of Bioregulation and Gerontology in the late 1990s. The landmark chromatin remodeling study was published in 2002 [15]. The compound has been characterized more extensively than Ovagen in terms of chromatin-level effects and has been included in the 2020 Kuznik review of liver peptide complex hepatoprotective activity [12]. No published human clinical trials.

Novelty: Moderate. Shares the ultrashort peptide bioregulator mechanism with Ovagen but is distinguished by the specific chromatin remodeling endpoint (heterochromatin decondensation). The mechanism is novel within the broader hepatoprotective landscape.

Effect size: In aged rat hepatocyte cultures, Livagen restored protein synthesis rates to levels characteristic of young animals at nanomolar concentrations [13]. In lymphocyte chromatin studies, Livagen produced measurable heterochromatin decondensation within hours of exposure [15]. Quantitative effect sizes are not expressed as standardized measures in the available literature but are reproducible.

Promising potential: Low to moderate. The compound is established within the Khavinson bioregulatory peptide framework and is available from multiple suppliers. No Western clinical development is anticipated.

Side-effect profile: No significant adverse events reported. Composed of four proteinogenic L-amino acids. Equivalent to Ovagen in safety profile.

Overall validation: Low to moderate. More extensively characterized at the chromatin level than Ovagen but shares the same limitations: predominantly single-laboratory provenance, no independent Western validation, no clinical trials.

Comparison to Ovagen: Livagen and Ovagen are derived from the same liver peptide extract lineage and share the Glu-Asp dipeptide core. Livagen is a tetrapeptide with primary evidence for chromatin remodeling; Ovagen is a tripeptide with primary evidence for Ki-67/p53 modulation in hepatocytes. The two are proposed as complementary agents with non-overlapping mechanisms within the same bioregulatory framework. Ovagen has more striking quantitative data on hepatocyte proliferation (18-fold Ki-67 increase versus 15 to 16 percent explant area increase for Livagen).

12.2 Silymarin (Milk Thistle Extract; *Silybum marianum*)

Mechanism: Flavonolignan complex (principally silibinin/silybin A and B, silydianin, and silychristin) with antioxidant, anti-inflammatory, antifibrotic, and membrane-stabilizing activity. The principal mechanisms are: scavenging of reactive oxygen species and free radicals; inhibition of lipid peroxidation; stimulation of ribosomal RNA synthesis and hepatocyte protein synthesis; inhibition of nuclear factor kappa B (NF- κ B) signaling; and competitive inhibition of hepatotoxin uptake at the hepatocyte membrane [21, 22].

Development trajectory: Silymarin has been used as a hepatoprotective agent for over 2000 years (milk thistle in traditional European herbal medicine) and has been the subject of extensive modern pharmacological research since the 1960s. It is registered as a pharmaceutical product in Germany (Legalon), marketed as a dietary supplement globally, and has been evaluated in randomized controlled trials for alcoholic liver disease, non-alcoholic fatty liver disease, viral hepatitis, drug-induced liver injury, and mushroom poisoning (*Amanita phalloides*) [21].

Novelty: Low. Silymarin is one of the oldest and most extensively studied hepatoprotective agents. The mechanism is conventional antioxidant and cytoprotective activity.

Effect size: Clinical trials have produced mixed results. A Cochrane systematic review concluded that silymarin did not significantly affect liver-related mortality, liver histology, or virological response in patients with alcoholic or hepatitis C liver disease [22]. However, individual trials have reported significant reductions in liver enzyme elevation and improvements in liver histology, particularly in alcoholic liver disease and in drug-induced hepatotoxicity. The overall effect size in clinical trials is modest. Intravenous silibinin is approved in Europe for *Amanita phalloides* poisoning, where the effect size is substantial.

Promising potential: Moderate. Silymarin remains widely used and widely studied. No novel clinical development is anticipated, but it remains a reference hepatoprotective agent.

Side-effect profile: Excellent. Silymarin is well tolerated, with the principal adverse events being mild gastrointestinal discomfort (laxative effect) and rare allergic reactions. No hepatotoxicity. No significant drug interactions at standard doses.

Overall validation: Moderate to high. Extensive preclinical and clinical research literature spanning six decades. Clinical efficacy is established for specific indications (*Amanita* poisoning) and debated for others (chronic liver disease).

Comparison to Ovagen: Silymarin has vastly greater clinical validation, regulatory acceptance, and safety characterization than Ovagen. Ovagen has greater mechanistic novelty (epigenetic regulation versus antioxidant activity). The two compounds operate through non-overlapping mechanisms and are pharmacologically compatible for combination research. Silymarin provides immediate antioxidant protection while Ovagen provides longer-term gene-regulatory restoration of endogenous antioxidant enzyme capacity.

12.3 BPC-157 (Body Protection Compound-157)

Mechanism: Pentadecapeptide (15 amino acids, sequence GEPPPGKPADDAGLV) derived from human gastric juice with hepatoprotective, gastroprotective, and wound-healing activity mediated through multiple proposed mechanisms including: stimulation of angiogenesis via vascular endothelial growth factor (VEGF)

pathway activation; modulation of the nitric oxide system; interaction with the dopaminergic system; and direct cytoprotection of hepatocytes and gastrointestinal epithelium [23, 24].

Development trajectory: BPC-157 has been studied in preclinical models since the early 1990s by the Sikiric laboratory at the University of Zagreb. Over 100 published studies document hepatoprotective, gastroprotective, wound-healing, and cytoprotective activity in rodent models. The compound has undergone limited Phase 2 clinical evaluation for inflammatory bowel disease (ulcerative colitis) but has not completed Phase 3 trials or received regulatory approval for any indication [23].

Novelty: Moderate. The gastric juice-derived pentadecapeptide concept is distinct from both conventional hepatoprotective agents and from the Khavinson ultrashort peptide bioregulator class.

Effect size: Substantial in preclinical models. BPC-157 administration prevented or reversed liver necrosis and fatty changes in rats subjected to CCl₄ intoxication, restraint stress, bile duct ligation, and hepatic artery ligation, with 40 to 70 percent reduction in liver damage markers [24]. The preclinical effect sizes are among the largest reported for any peptide hepatoprotective agent.

Promising potential: Moderate to high. Active research continues with expanding preclinical literature. Clinical development is progressing, though no Western regulatory approval has been obtained.

Side-effect profile: No significant adverse events reported in preclinical studies at doses up to 10 mg/kg. Theoretical concerns regarding angiogenic activity (VEGF stimulation) in the context of pre-existing malignancy have been raised.

Overall validation: Moderate to high. The extensive preclinical literature (100+ publications from multiple research groups) and the progression to clinical trials provide stronger validation than Ovagen. However, the mechanism is less precisely characterized at the molecular level.

Comparison to Ovagen: BPC-157 has a substantially larger and more independently replicated preclinical evidence base than Ovagen. Both compounds lack robust clinical validation. BPC-157 operates through receptor-mediated and growth-

factor-mediated mechanisms; Ovagen operates through proposed epigenetic mechanisms. BPC-157 is a longer peptide (15 residues) with broader organ-system effects; Ovagen is shorter (3 residues) with more focused liver/GI targeting.

12.4 Ursodeoxycholic Acid (UDCA)

Mechanism: Hydrophilic bile acid with hepatoprotective activity mediated through: displacement of toxic hydrophobic bile acids from the bile acid pool; stabilization of hepatocyte and cholangiocyte membranes; stimulation of hepatobiliary secretion; cytoprotective effects including inhibition of apoptosis via mitochondrial membrane stabilization; and immunomodulatory effects including reduction of aberrant MHC class I and class II expression on hepatocytes [25, 26].

Development trajectory: UDCA was first identified in bear bile (*Ursus* species) and has been synthesized and clinically evaluated since the 1970s. It is approved by the United States Food and Drug Administration for primary biliary cholangitis (formerly primary biliary cirrhosis) and is used off-label for a wide range of hepatobiliary conditions including primary sclerosing cholangitis, intrahepatic cholestasis of pregnancy, cystic fibrosis-associated liver disease, and non-alcoholic fatty liver disease [25].

Novelty: Low. UDCA is a well-established hepatoprotective agent with a conventional bile acid mechanism.

Effect size: UDCA produces significant and clinically meaningful improvement in liver biochemistry (reduction of alkaline phosphatase, gamma-glutamyl transferase, bilirubin, and aminotransferases) and delays histological progression in primary biliary cholangitis [26]. The effect on transplant-free survival in primary biliary cholangitis is significant in observational studies and in long-term trial follow-up. Effect sizes in other indications are more variable.

Promising potential: High. UDCA is an established, approved hepatoprotective agent with a well-characterized pharmacokinetic and safety profile.

Side-effect profile: Excellent. UDCA is well tolerated at therapeutic doses (13 to 15 mg/kg/day). The principal adverse event is dose-dependent diarrhea. No hepatotoxicity. Rare pruritus exacerbation has been reported.

Overall validation: High. FDA-approved for primary biliary cholangitis. Extensive clinical trial and real-world evidence base spanning five decades.

Comparison to Ovagen: UDCA has vastly greater clinical validation, regulatory acceptance, and safety characterization than Ovagen. The mechanisms are entirely non-overlapping (bile acid modulation versus proposed epigenetic regulation). UDCA is the standard-of-care hepatoprotective agent against which novel liver-targeted compounds are measured.

12.5 Thymalin (Thymic Peptide Complex)

Mechanism: Polypeptide complex extracted from calf thymus, acting on thymic epithelial cells to restore T-lymphocyte maturation and balance Th1/Th2 cytokine production. Hepatoprotective activity is indirect, mediated through normalization of immune-mediated hepatic inflammation by restoring regulatory T-cell function and suppressing excessive inflammatory responses in the liver [10, 27].

Development trajectory: Thymalin is the earliest and most extensively studied compound in the Khavinson peptide bioregulator program. It was developed in the 1970s and has been used clinically in the Russian Federation for immunodeficiency states, postoperative recovery, and age-related immune decline. A 15-year cohort study of elderly subjects receiving Thymalin and Epithalamin reported reduced all-cause mortality (28 percent reduction) and reduced cancer incidence (approximately 2-fold decrease) compared to untreated controls [10, 27].

Novelty: Low to moderate. The thymic peptide extract mechanism is conventional within the context of immunomodulatory therapy.

Effect size: In the 15-year cohort study, Thymalin-treated subjects showed reduced mortality, improved immune parameters, and improved functional status compared to controls [27]. In toxic liver injury models, Thymalin administration reduced hepatic necrosis markers and accelerated regeneration. The mortality reduction (28 percent) is a substantial clinical effect if replicable, though the study design (non-randomized, non-blinded) does not meet Western regulatory standards.

Promising potential: Low to moderate. The compound is used clinically in the Russian Federation but has not been evaluated in Western regulatory-standard clinical trials for any hepatoprotective indication.

Side-effect profile: Well tolerated in the available clinical experience spanning decades. No significant adverse events reported.

Overall validation: Moderate within the Russian clinical context. The 15-year clinical study provides the strongest human evidence of any Khavinson bioregulator. The study design does not meet Western regulatory standards.

Comparison to Ovagen: Thymalin has greater human exposure data and a longer clinical history than Ovagen. The hepatoprotective mechanism is indirect (immunomodulatory) whereas Ovagen's proposed mechanism is direct (hepatocyte gene regulation). The two compounds represent different nodes of the Khavinson peptide bioregulator network and are commonly co-administered in multicomponent protocols.

12.6 Comparator summary

The five compounds occupy distinct positions in the hepatoprotective landscape. **Livagen** is Ovagen's closest mechanistic relative, sharing the ultrashort peptide bioregulator framework and the liver tissue specificity, but with primary evidence for chromatin remodeling rather than Ki-67/p53 modulation. **Silymarin** is the most widely used and most extensively studied conventional hepatoprotective agent, with an established safety profile and mixed clinical efficacy data; it operates through antioxidant and cytoprotective mechanisms entirely distinct from Ovagen's proposed epigenetic mechanism. **BPC-157** is the most extensively studied hepatoprotective peptide in preclinical literature, with a substantially larger and more independently replicated evidence base than Ovagen; it operates through growth factor and angiogenic mechanisms. **UDCA** is the gold-standard hepatoprotective agent with FDA approval, established clinical efficacy, and a well-characterized pharmacokinetic profile; it operates through bile acid modulation. **Thymalin** is the most clinically experienced compound in the Khavinson peptide bioregulator class, with indirect hepatoprotective activity through immunomodulation.

Ovagen occupies a unique position in this comparator landscape: it is the only compound with a proposed mechanism of direct hepatocyte gene regulation through nuclear peptide-DNA interaction. This mechanistic novelty is the compound's principal distinguishing feature. Its limitations are equally distinctive: it has the smallest evidence base among the comparators (preclinical only, predominantly single-laboratory), no clinical validation, no regulatory acceptance, and no formal pharmacokinetic characterization. The proposed mechanism, while scien-

tifically interesting, has not been independently validated by structural biology, biophysical, or molecular biology methods in laboratories outside the Khavinson network.

Investigators considering Ovagen for hepatoprotective research should position the compound as a mechanistically novel, preclinically characterized agent suitable for exploratory research into epigenetic hepatoprotection, rather than as a clinically validated hepatoprotective intervention. Combination with well-validated hepatoprotective agents (UDCA, silymarin, NAC) is pharmacologically rational and could provide mechanistic complementarity. The comparative assessment underscores the need for independent validation of Ovagen's proposed mechanism, formal pharmacokinetic characterization, and controlled clinical evaluation before the compound can be positioned as a practical alternative to established hepatoprotective agents.

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