



Assessment of toxic liver cirrhosis and its prevention through herbal hepatoprotectors with a proposed innovative strategy

Lilit Arshakyan

Scientific Technological Center of Organic and Pharmaceutical Chemistry, National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia.

***Corresponding Author:** Lilit Arshakyan, Laboratory of Pharmacology and Histopathology, Scientific Technological Center of Organic and Pharmaceutical Chemistry, National Academy of Sciences of the Republic of Armenia, 26 Azatutyun Avenue, Yerevan, 0014, Armenia

Submission Date: October 24th, 2025; **Acceptance Date:** December 28th, 2025; **Publication Date:** January 2nd, 2026

Please cite this article as: Arshakyan L. Assessment of toxic liver cirrhosis and its prevention through herbal hepatoprotectors with a proposed innovative strategy. *Functional Foods in Health and Disease* 2026; 16(1): 1 – 14.

DOI: <https://doi.org/10.31989/ffhd.v16i1.1822>

ABSTRACT

Background: In contemporary medicine, effective and nosologically less harmful approaches for the prevention and treatment of liver cirrhosis remain insufficient, as indicated by the projected increase in mortality over the next decade. In recent years, the discovery and development of herbal medicine have surged. Therefore, a critical need of our time is to identify more comprehensive and novel strategies for studying liver cirrhosis to develop more effective prevention and therapy, such as those in this scientific paper.

Objective: This study aims to observe the progressive dynamics of toxic liver cirrhosis using a novel preliminary diagnostic approach centered on the morphofunctional interrelationship of the liver, lungs, and kidneys and subsequently to formulate therapy strategies employing the proposed Flasil herbal combination.

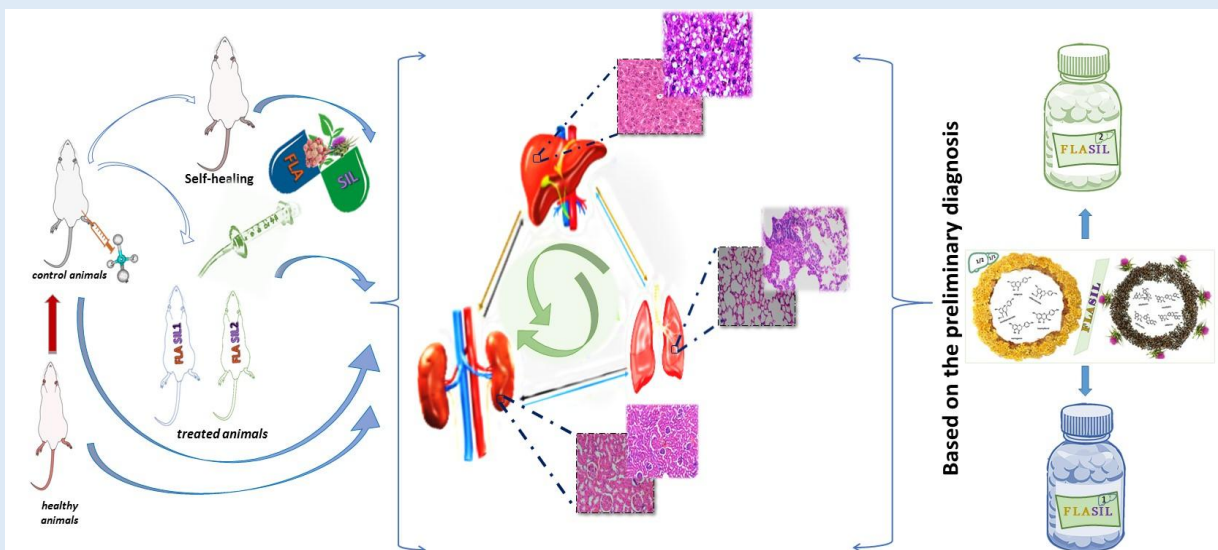
Methods: CCl₄-induced liver cirrhosis model was used in this prospective investigation. Mixtures (Flasil 1 and 2) prepared from active compounds of *Helichrysum rubicundum* (C. Koch.) flowers and *S. marianum* seeds grown in Armenia. At the end of the experiment, H&E staining was used to assess histological abnormalities in the liver and adjacent organs.

Results: Research has demonstrated that toxic liver disease can manifest signs of pulmonary and renal disorders in the pre-cirrhotic state. Histopathological analysis showed that both Flasil formulations exhibited hepatoprotective effects while concurrently reducing pathological changes in pulmonary and renal tissues. Flasil 2 was more effective in promoting liver and kidney repair through enhanced hepatocyte regeneration, whereas Flasil 1 demonstrated greater efficacy in supporting lung tissue regeneration during the acute-to-chronic transition of liver cirrhosis. However, non-invasive tests are needed to demonstrate both the feasibility of the proposed strategy and the different biological activities of the new Flasil combination according to the dose.

Novelty: A liver-lung-kidney morphofunctional correlation tactical approach for the initial diagnosis of cirrhosis was assessed for the first time. FLASIL is the first natural product shown to affect liver-lung-kidney dysfunction in a CCl₄ model.

Conclusion: This new approach (diagnosing through the liver-lung-kidney morphofunctional linkage) enables early detection and, based on it, prevention of cirrhosis by regulating its dynamics and giving varying dosages of the Flasil combination as an innovative herbal hepatoprotector. However, they necessitate the investigation of further diagnostic criteria.

Keywords: liver cirrhosis, new approach, liver-lung-kidney linkage, herbal medicine, Flasil



Graphical abstract: Assessment of Toxic Liver Cirrhosis and Its Prevention Through Herbal Hepatoprotectors with a Proposed Innovative Strategy.

©FFC 2025. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

INTRODUCTION

At the functional intersection of organ systems, the liver plays a crucial role in maintaining dynamic homeostasis.

Despite continuous exposure to internal and external toxins, the liver preserves its structural and functional integrity through its regenerative capacity. However,

persistent neglect can cause liver damage, eventually leading to the development of liver cirrhosis [1, 2]. In contemporary medicine, effective and, according to nosology, less harmful approaches for the prevention and treatment of liver cirrhosis remain insufficient, as indicated by the expected increase in cirrhosis-related mortality over the next decade [3, 4]. Therefore, a critical requirement of our time is the advancement of more comprehensive investigations into cirrhosis and its prevention, enabling the development of new and more effective strategies for its prevention and treatment, including the approach described in this work. The advantage of the proposed approach is the evaluation of the functional condition of functionally related organs within the dynamics of liver cirrhosis, primarily the lungs and kidneys, based on the portal system's interconnections.

Liver cirrhosis, as the terminal stage of chronic liver illnesses, can arise from multiple etiologies and present a whole range of complications, highlighting its interconnection with other organ systems [5-6]. As a result, it was considered vital in this study to investigate the liver's causal-consequential relationship with other organs throughout illness, which enabled the development of the most effective treatment strategies.

In recent years, people have preferred using edible plants over synthetic drugs for the prevention and treatment of diseases. These plants are rich in bioactive compounds (BC), such as vitamins, carotenoids, proteins, carbohydrates, organic acids, flavonoids, tannins, and more. Humans have used them for thousands of years as both food and medicine [7-10].

The use of medicinal plants as raw materials for the production of new drugs is increasing, particularly for the treatment of liver disease. This trend reflects the growing relevance of herbal medicine in the management of liver cirrhosis, as also reported in our previous research [11-13].

Helichrysum and *Silybum marianum* are examples of such plants, with their medicinal properties largely attributed to the biophenolic compounds they contain. The term "biophenols" is commonly used to broadly describe plant-derived phenolic compounds. Biophenols constitute the largest group of plant secondary metabolites and are characterized by their prevalence and broad spectrum of biological activities [14].

Among the hundreds of *Helichrysum* species, the most well-known are *Helichrysum arenarium* and *Helichrysum italicum*. Comprehensive research has been conducted on their composition and many biological characteristics. [15, 16] Preparations developed from *Helichrysum arenarium* include the medicine "Flamin" (a combination of flavonoid chemicals), whose raw material is *Helichrysum arenarium* (L.) Moench flowers. It is well-known for its choleric, anti-inflammatory, antibacterial, spasmolytic, and wound-healing properties [17].

Silymarin is an isomeric mixture of the flavonolignans (silydianin, silychristin, silybin, and isosilybin) derived from the seeds of the *Silybum marianum* (L.) Gaertn, a plant known for its hepatoprotective, antioxidant, antiviral, antifungal, and other properties [18-20]. The combination of the active components of the two indicated plants is expected to enhance hepatoprotective efficacy, which was defined as a primary objective of this study. To summarize the aforementioned arguments, the primary goal of this research is to investigate the combination of flavonoid extracts from Armenian subspecies of these two plants as a novel hepatoprotective medication, referred to as Flasil, and to determine the most effective ratio using a new strategic approach for evaluating liver cirrhosis.

MATERIAL AND METHODS

Sigma-Aldrich supplied CCl_4 ($\geq 99.5\%$ purity). Hematoxiline, Eosine, Ethyl alcohol, and all other reagents were obtained from Sigma-Aldrich.

Plant material: The raw materials (dried extracts) obtained from *Helichrysum rubicundum* (C. Koch.) flowers (FLR or flamin) and from seeds of *Silybum marianum* (silymarin), which were grown in Armenia, were provided by the Laboratory of Medical Herbs Chemistry at STCOPC NAS RA.

To achieve a homogeneous mixture of flamin and silymarin, FLR and silymarin were mixed in olive oil at 1:1 and 1:2 ratios, respectively, and named Flasil 1 and Flasil 2. The mixtures were made immediately before administration. The animals received Flasil 1 or 2 orally at a dose of 300 mg/kg three times weekly for 8 weeks.

Animals: All procedures involving animals were conducted in accordance with ethical principles outlined in EU Directive 2010/63/EU and were approved by the Bioethical Committee of Mkhitar Heratsi Yerevan State Medical University. (minutes № 8-5/2,2021).

The experiment was conducted on 30 non-linear male white rats, weighing 180-210 g (5 groups, six animals in each group). The STCOPC NAS RA provided rats. The animals without any signs of disease were selected for the study and underwent a 2-week quarantine before the experiment. They were placed in a room at 21±2 °C and a 12:12-hour diurnal cycle with humidity levels of 40–43%. Food and water were given ad libitum. Three animals were placed in cages of 1600 cm².

Grouping of animals:

Group 1: Intact (healthy animals)

Group 2: CCl₄ (2ml/kg of 30% CCl₄ i.p. for 2 weeks)

Group 3: Self-recovery (CCl₄ + 8 weeks' self-recovery period)

Group 4: Flasil 1 (300 mg/kg p.o. for 8 weeks)

Group 5: Flasil 2 (300 mg/kg p.o. for 8 weeks)

Modeling of the research strategy: The animals were randomly divided into five groups, with six male rats per group. Group 1 consisted of normal untreated animals,

while Groups 2, 3, 4, and 5 were intraperitoneally (i.p.) injected with 2 mL/kg of 30% CCl₄ in olive oil two times per week for 2 weeks to induce the acute phase of liver cirrhosis. Additionally, Group 3, after 2 weeks of poisoning, had a self-recovery period of 8 weeks; Groups 4 and 5 were administered 300 mg/kg of Flasil 1 and Flasil 2, respectively, in olive oil by oral gavage (p.o.) 3 times a week for 8 weeks. On the final day of the experiments, the animals were sacrificed under anesthesia using intraperitoneal injection of 40 mg/kg Nembutal Sodium. The livers, lungs, and kidneys were excised, followed by fixation of the tissue specimens in 10% buffered formalin.

Total serum protein analysis: Sample preparation: Blood samples were collected via cardiac puncture without anticoagulant on the final day of the experiment. This method provides a more informative assessment of liver functional properties, as blood enriched with liver-derived nutrients passes directly to the heart, and allows collection of relatively large volumes (up to 1 ml in some rats). To separate the sera, blood samples were stored at room temperature for 30 minutes, then at 4 °C for 20-30 minutes.

To determine the total protein content in blood serum samples, a spectrophotometric method was used with a SPECORD 250 PLUS spectrophotometer from *Analytik Jena*. The protein concentration was determined by the Wartburg-Christian formula. The absorbance of the samples was measured at both 280nm and 260nm wavelengths, and the concentration was calculated using the following formula:

$$\text{Concentration (mg/ml)} = ((1.55 \times A_{280}) - (0.76 \times A_{260})) \times \text{dilution factor}$$

Gross examination and weighing data: On the last day, after sacrifice, the livers were dissected and weighed. The organs and tissues were examined macroscopically to establish a diagnosis and select relevant portions for subsequent microscopic examination. Thereafter, livers

and other organs were photographed with a digital camera.

Accordingly, liver indexes (LI) were calculated according to the following formula:

$$LI = \frac{\text{liver weight}}{\text{body weight}} * 100$$

Histopathological analysis: After fixation, liver, lung, and kidney tissues were dehydrated, embedded in paraffin, and 3–5 μm microtome sections were prepared. For standard histological examination, sections were stained with H&E [21]. It was performed by examining tissues under a light microscope (Jenaval) for the qualitative histopathological characterization of the organs. Photomicrographs from histological slides were obtained at 125X magnification.

Statistical analysis: All the data were presented as the mean \pm SD. One-way ANOVA was used to determine statistical significance by comparing the means of the acquired data. Following the one-way ANOVA test, the Tukey post hoc test was employed. All values were analyzed using GraphPad Prism 8, with P-values < 0.01 or < 0.05 considered significant.

RESULT AND DISCUSSION

Neglected persistent metabolic disorders of the liver, often associated with toxic phenomena, can eventually lead to liver cirrhosis, which primarily results from advanced fibrosis and represents the end stage of all chronic liver failures, as evidenced by the increasing trend in related deaths. A significant characteristic of the liver is its sequential connection to the lungs and kidneys via the portal system, allowing venous blood components from the liver to directly impair the pulmonary vascular endothelium and subsequently the kidneys [22]. This is evidenced by the fact that pulmonary and renal

complications may occur in liver diseases of any etiology, particularly in liver cirrhosis, which represents a large group of heterogeneous conditions. It may elucidate the increased mortality rate associated with liver illnesses, which may arise if their consequences are neglected. This study suggests a new approach: investigating the pathological interconnections among the liver, lungs, and kidneys during the preliminary diagnosis of liver cirrhosis, which is crucial for early detection and subsequent prevention and therapy.

Herbal medicines have been used for various therapeutic purposes since ancient times. In recent years, the use of medicinal plants as raw materials for the production of new drugs has increased, particularly for the treatment of liver disease [23-25]. Conventional synthetic drugs often used to manage the pathological conditions can sometimes exacerbate other functional deviations in the liver, potentially leading to conditions that have not yet manifested as pathology. Liver cirrhosis, being a multifactorial illness, exhibits a variety of symptoms; thus, for its prevention and/or treatment, it is prudent to employ complex herbal preparations. Based on the principles presented above, this research study was proposed to evaluate therapeutic strategies for preventing and treating liver cirrhosis using the novel Flasil herbal combination. Flasil is a mixture of flavonoids from *Helichrysum rubicundum* flowers and flavonolignans of *Silybum marianum* (L.) Gaertn seeds growing in Armenia.

Laboratory animal models are crucial for advancing our understanding of human pathogenesis, such as liver cirrhosis, as they enable researchers to identify therapeutic targets and evaluate novel therapeutics. Carbon tetrachloride (CCl_4) is the most often utilized chemical for rodent liver fibrosis and cirrhosis models. It has well-known effects on the liver, including severe

inflammation and periportal and septal fibrosis. Because of its remarkable repeatability, many researchers use it as the preferred model for studying liver fibrosis [26-28].

To validate the suggested tactical approach, the rat model of carbon tetrachloride-induced liver cirrhosis was chosen as the most effective and advantageous model.

Body weight changes: Examining the dynamics of the body weight changes, weight loss was predominantly noticed after 2 weeks of CCl₄ intoxication. (in the pre-cirrhotic stage), which is consistent with data in other articles [29].

The weight changes, suggesting weight gain in the two treated groups, were observed following treatment, especially when compared with the self-recovery group (CCl₄+8 weeks), where the weight change dynamics following poisoning were irregular (Figure 1). Throughout self-recovery, a low body weight was maintained despite fluctuations; however, the animals administered Flasil exhibited a significant weight gain following poisoning. It demonstrates the comprehensive therapeutic impact of the suggested combinations on the body, as evidenced by liver index and total protein calculations.

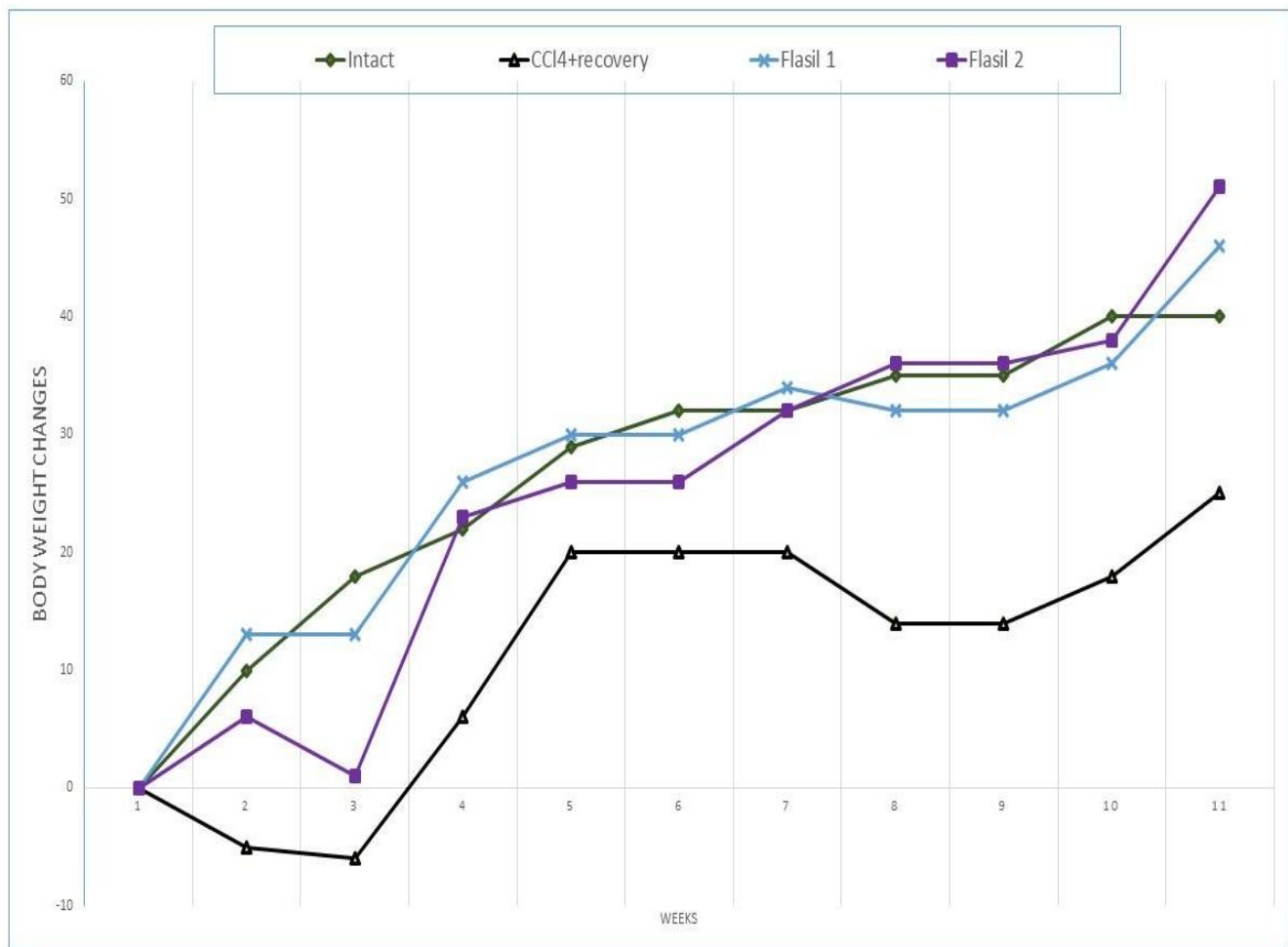


Figure 1: Dynamic changes of the body weight in experimental rats.

Liver index (LI): Comparative investigations of liver index (LI) indicators revealed that the LI was greater in the CCl₄-treated group compared to the intact group. The liver

index was significantly lower in both the self-recovery and therapy groups after poisoning compared to the CCl₄ group (Group 2). Furthermore, the two groups that

received Flasil had lower indicators than the group that did not receive treatment after being poisoned (Figure 2).

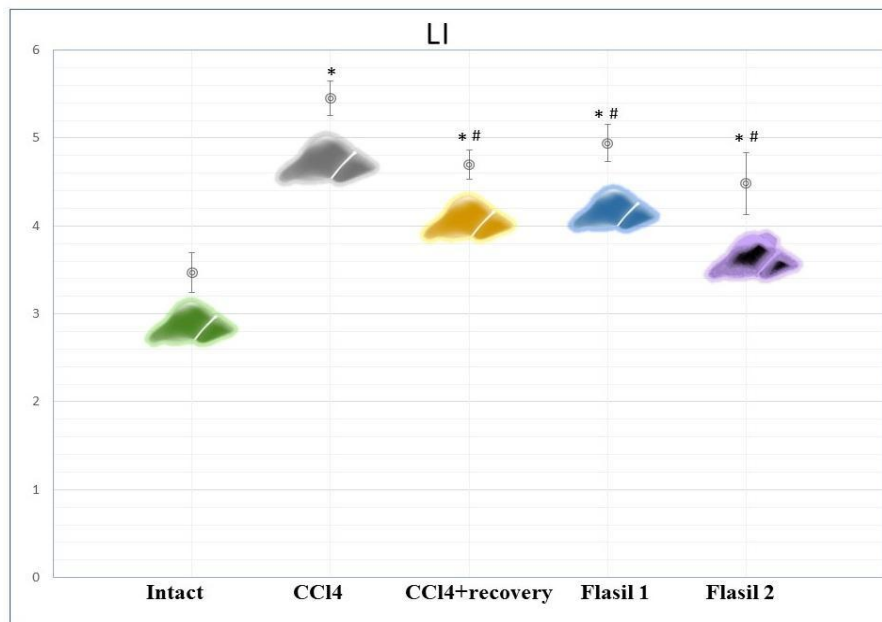


Figure 2. Liver index (LI) of experimental animals. * significantly different from the intact group (healthy control, group 1) at p.0.01; # significantly different from the CCl₄ group (group 2) at p.0.01.

Total protein (TP): The results of the study on serum total protein (TP) concentrations are depicted in Figure 3. After 2 weeks of CCl₄ poisoning, total protein concentration decreased significantly compared with the intact group, but the other three groups showed a rise after recovery and therapy. The group treated with Flasil 2 exhibits a statistically significant increase in protein levels compared to both the pre-cirrhotic (CCl₄ group) and self-healing groups.

The liver plays a significant role in producing total serum proteins that circulate throughout the body and are an essential indicator of the organism's overall functional state [30]. The decrease in its level after poisoning is the key indicator of a deterioration in overall functional ability, which consistently increases both during self-recovery and therapy. The administration of the recommended combined hepatoprotectors comprehensively alters the body's recovery process, taking cause-and-effect relationships into account,

resulting in higher protein concentrations than in the self-recovery group. The results of general macroscopic and microscopic examination prove this conclusion.

Macroscopic examinations: In pathology, macroscopic examination of organs and tissues is critical for establishing a diagnosis and selecting relevant sections for subsequent microscopic evaluation and particular investigations [30]. It was an essential prerequisite for developing the research strategy, enabling the demonstration of the viability of the proposed novel tactical strategy, specifically the reflection of pathological correlations among organ systems during the initial diagnosis of liver cirrhosis.

Macroscopic examinations of the experimental groups showed that the animals in the control groups had perihepatic and abdominal adhesions. Liver granuloma and hemangioma, enlarged kidneys, and obesity, as well as shriveled lungs, have been noticed. After an 8-week

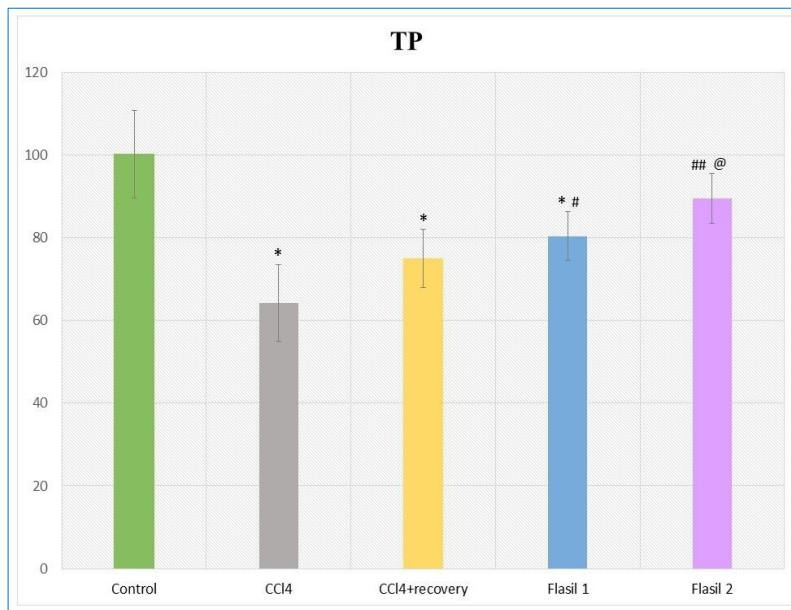


Figure 3. Serum total protein (TP) levels in the experimental rats. * significantly different from the healthy control group (group 1) at p.0.01; # significantly different from the CCl₄ group (group 2) at p.0.05, ## at p.0.01; @significantly different from the CCl₄-recovery group (group 3) at p.0.05.

recovery period, macroscopic examination revealed that the liver tissue was friable and that adhesions between the liver and the diaphragm were present. There are exudates, inflammation, and visible granulation in the periphery of the liver. In this group of animals, the lungs showed signs of atrophy and hemorrhage. In the CCl₄ and CCl₄+8week recovery

groups, a little fibrous layer and tiny abscesses were seen in the kidneys. Macroscopic observation of the liver noted that the Flasil 1-treated group had small granulation and some exudates; no fibrous septa were observed in the liver tissue. The lungs are healthy. In the Flasil 2-treated group, no visible macroscopic changes were observed in the liver and other organs (Figure 4).

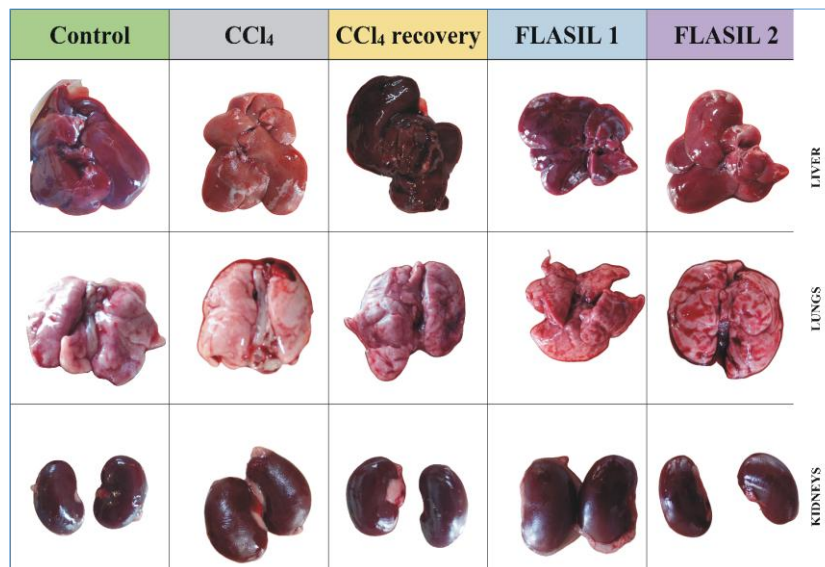


Figure 4. Gross anatomical examination of the liver, lungs, and kidneys.

Histopathological examination: Comparative photomicrographs of rats' tissue from the liver, lungs, and kidneys are shown in Figure 5. The histopathological findings revealed that the control (healthy animals) group exhibited a typical lobular architecture characterized by central veins and radiating hepatic cords. The group that received 2-week CCl_4 showed a tendency to develop cirrhosis (pre-cirrhotic state): fatty dystrophy, pseudo-lobule development, and perivascular infiltration, culminating in fibrosis in specific locations. Some of the aforementioned pathogenic processes are exacerbated in the self-recovery group, including sinusoidal dilatation, mild congestion, and Kupffer cell hyperplasia. Unlike the cirrhotic groups, the therapy groups (Flasil 1 and Flasil 2) have restored liver tissue, particularly in Flasil 2 (Group 5), where pathological changes are more significantly decreased and nearly reach the levels of the healthy animals (G 1).

There were no significant histological alterations in the lungs and kidneys of the intact group. The CCl_4 group showed thickening of the interalveolar septa, an irregular distribution of air spaces, pulmonary interstitial edema, and inflammatory cell infiltration. All these pathogenic

processes intensify following an 8-week self-healing period, manifesting foci of interstitial pneumonia, indications of consolidation, and pulmonary hypertension. In rat lung tissue treated with Flasil following CCl_4 poisoning, characteristic pathological signs were significantly reduced compared with the two cirrhotic groups. However, hemorrhage was observed in some cases in animals treated with Flasil 2.

Histopathological examinations of renal tissue reveal the destruction of the juxtaglomerular apparatus in the early stages of cirrhosis (Group 2) (mainly in the peripheral sections of the cortical layer); the majority of the proximal and distal tubules are necrotic, and there are noticeable hemorrhages. In the acute-to-chronic stage (Group 3), many of the aforementioned pathologies are intensifying; in addition, marked glomerulonephritis features are evident, including dilation of Baum's capsule and tubular degeneration. Figure 5 shows that both Flasil 1 and 2 exert a beneficial effect on kidney tissue regeneration, with the tubular apparatus primarily exhibiting a standard histological structure.

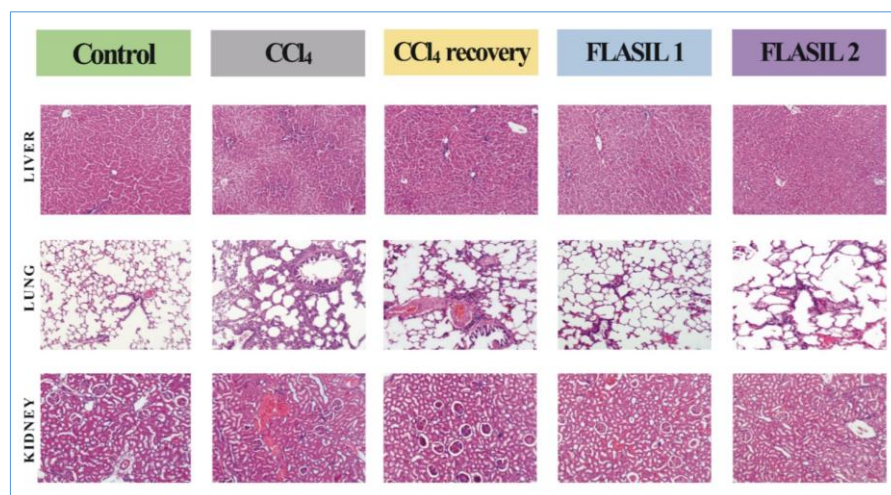


Figure 5. Photomicrograph of experimental rats' lung, liver, and kidney sections; H&E staining; X125.

Comparative studies correlating liver-lung-kidney histological pathologies with total protein measurements and liver index indicate that a pre-cirrhotic condition (2-

week CCl_4 intoxication) in liver tissue is linked to a significant increase in liver index, fatty degeneration, the development of pseudolobules, and a decrease in

hepatocyte synthetic function, reducing total protein levels [31-32].

They are accompanied by several disorders in lung and kidney tissues, such as inflammatory cell infiltration, pulmonary fibrosis, and in the kidney's Juxtaglomerular apparatus. The latter can lead to decreased EGFR and GFR [33].

Already in the self-recovery period (acute-chronic stage, CCl₄ + 8-week recovery), Kupffer cell hyperplasia is observed in the liver, interstitial inflammation and consolidation in the lungs are exacerbated, and the renal tubula function is compromised. Although in this period, blood total protein levels have slightly increased, possibly due to higher creatinine, γ -GT (Gamma-Glutamyl Transferase), C-reactive protein, M2BPGi (Mac-2 Binding Protein Glycosylation Isomer), and IL-6 indicators. This highlights the significance of the new tactical strategy for early cirrhosis diagnosis, which requires additional biomarker testing (AST/ALT, AGR, BUN, etc.) [34 - 41].

This experiment demonstrates the reasonableness of the Flasil combination: on one hand, the antioxidant, choleric, and spasmolytic properties of Helichrysi flowers flavonoids (naringenin, apigenin, salipurposide, isosalipurposide, kaempferol, quercetin, etc) [17, 42] that prevent stagnation, and on the other hand, the potent antioxidant and antifibrotic effects of Silymarin, restoring the structural integrity of hepatocytes and

enhancing their functional capabilities, containing four main flavanolignans: silybin, isosilybin, silychristin, and silydianin [43 - 45]. (Figure 6)

Our previous research indicates that the combination of flamin and silymarin has significant hepatoprotective effects. The present study introduces a novel strategic approach that enables the identification of effective systemic therapeutic options by selecting the optimal dosage based on initial diagnosis. For instance, due to its high silymarin content, Flasil 2 is better at healing liver and kidney tissue by increasing hepatocyte regeneration and may reduce serum creatinine levels [46].

Whereas Flasil 1, in addition to its hepatoprotective properties, is more preferable for lung tissue regeneration during the acute-to-chronic transition phase of liver cirrhosis. This is supported by the higher flavonoid concentration in Flasil 1, corroborated by scientific evidence on the efficacy and molecular mechanisms of quercetin and naringenin in lung diseases [47, 48].

To sum up, implementing the proposed tactical approach to liver cirrhosis research will facilitate the diagnosis of the disease at various stages and enable the development of treatment strategies utilizing the new Flasil combined herbal mixture, considering the organism holistically.



Figure 6. The main chemical components of FLASIL.

Scientific Innovation: A concept for the initial diagnosis of liver cirrhosis and preventative strategies utilizing diverse combinations and doses of active compounds extracted from selected plants.

Practical implication: Despite the progressive advancement of sophisticated therapies for liver cirrhosis in contemporary medicine, it remains classified as an incurable disease. One of the most significant challenges of modern hepatology is the search for new combination drugs for liver cirrhosis. The suggested strategy of predicting the development of cirrhosis based on causative links (primary liver-lung-kidney)—treating the patient rather than the illness—may be crucial to advancing contemporary medicine. Flasil at varying doses, dependent on dynamic circumstances, may be an effective systemic combination hepatoprotector in the prevention and treatment of liver cirrhosis.

CONCLUSIONS

The suggested new tactical model (liver-lung-kidney morphofunctional linkage) demonstrates that cirrhosis, even in the pre-cirrhotic stage, can be multifactorial and multiconsequence. The newly proposed combination medication, Flasil, exhibits both preventative and protective effects on the liver-lung-kidney morphofunctional axis during the pre-cirrhotic stages in the toxic model of liver cirrhosis.

With this new approach, it will be possible to detect and prevent cirrhosis early by controlling its dynamics and administering the different dosages of the Flasil mixture as a new herbal hepatoprotector. However, they require investigation of additional diagnostic markers.

Author Contributions: The author contributed to the study design and conceptualization, data interpretation, and manuscript drafting and approved the final version of the article.

Abbreviations: CCl₄, carbon tetrachloride; TP, total protein; LI, liver indexes; i.p., intraperitoneal; p.o., peroral; AST/ALT, serum aspartate to alanine aminotransferase levels; AGR, albumin-to-globulin ratio; BUN, blood urea nitrogen; M2BPGi, Mac-2 Binding Protein Glycosylation Isomer; γ -GT, Gamma-Glutamyl Transferase; H&E, hematoxylin and eosin.

Conflict of Interest: There are no conflicts of interest.

Funding: This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgment: I am especially grateful to my supervisor, Dr. Hrachik Gasparyan, for their support and encouragement and for making the time to discuss my research, as well as my colleagues in the Department of Pharmacology and Pathohistology STCOPC NAS RA, for their assistance throughout the animal experiments, and Dr. Hayk Harutyunyan, for conducting the blood sampling. Great thanks to Dr. Hrach Ananikyan for the provision of the dried extracts of *Helichrysum rubicundum* (C. Koch.) and *Silybum marianum*. I would also like to thank the head of soil, sediment, and hydrobiological monitoring services of HMC SNPO, Vardan Karyan, for assistance with the spectrophotometric analysis.

REFERENCES

1. Gan C, Yuan Y, Shen H, Gao J, Kong X, Che Z, et al. Liver diseases: epidemiology, causes, trends and predictions. *Signal Transduction and Targeted Therapy* 2025;10(1):33. DOI: <https://doi.org/10.1038/s41392-024-02072-z>
2. Hora, Shainan, and Torsten Wüestefeld. "Liver Injury and Regeneration: Current Understanding, New Approaches, and Future Perspectives." *Cells*, vol. 12, no. 17, 22 Aug. 2023, pp. 2129–2129, DOI: <https://doi.org/10.3390/cells12172129>.

3. Younossi ZM, Wong G, Anstee QM, Henry L. The global burden of liver disease. *Clinical Gastroenterology and Hepatology*. 2023; 21(8):1978–91.
DOI: <https://doi.org/10.1016/j.cgh.2023.04.015>
4. Tapper EB, Parikh ND. Diagnosis and management of cirrhosis and its complications. *JAMA* 2023; 329(18):1589.
DOI: <https://doi.org/10.1001/jama.2023.5997>
5. Fadlallah H, El Masri D, Bahmad HF, Abou-Kheir W, El Masri J. Update on the Complications and Management of Liver Cirrhosis. *Medical Sciences* 2025;13(1):13.
DOI: <https://doi.org/10.3390/medsci13010013>
6. Liu, Yuan-Bin, and Ming-Kai Chen. "Epidemiology of Liver Cirrhosis and Associated Complications: Current Knowledge and Future Directions." *World Journal of Gastroenterology* 2022; 28 (41), 5910–5930.
DOI: <https://dx.doi.org/10.3748/wjg.v28.i41.5910>.
7. Ghalachyan L, Daryadar M, Matevosyan A, Roosta HR, Ghorbanpour M, Ghahramanyan A et al. The effect of different growth conditions on the content of bioactive compounds and gross β -radioactivity of some wild edible plants in the Republic of Armenia. *Functional Foods in Health and Disease* 2025; 15(9): 596–609.
DOI: <https://doi.org/10.31989/ffhd.v15i9.1748>
8. Son J, Martirosyan D. Salient Features for GRAS Status Affirmation. *Functional Food Science* 2024; 4(8): 299-308.
DOI: <https://doi.org/10.31989/ffs.v4i8.1417>
9. Martirosyan D. M., Hayes A. M., Shen N. Phenolic compounds and their potential in cancer management. *Agriculture and Food Bioactive Compounds* 2025; 2(3): 47-61
DOI: <https://www.doi.org/10.31989/afbc.v2i3.1570>
10. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. *Frontiers in Immunology* 2025. 16:1491777.
DOI: <https://doi.org/10.3389/fimmu.2025.1491777>
11. Riaz, Muhammad, et al. Phytobioactive Compounds as Therapeutic Agents for Human Diseases: A Review. *Food Science & Nutrition* 2023, 11(6).
DOI: <https://doi.org/10.1002/fsn3.3308>.
12. Xu Y, Guo W, Zhang C, Chen F, Tan HY, Li S, et al. Herbal Medicine in the Treatment of Non-Alcoholic Fatty Liver Diseases-Efficacy, Action Mechanism, and Clinical Application. *Frontiers in Pharmacology* 2020; 11:601.
DOI: <https://doi.org/10.3389/fphar.2020.00601>.
13. Mancak M, Altintas D, Balaban Y, Caliskan UK. Evidence-based herbal treatments in liver diseases. *Hepatol Forum*. 2024 Jan 16;5(1):50-60.
DOI: <https://doi.org/10.14744/hf.2022.2022.0052>.
14. Obied HK. Biography of biophenols: past, present and future. *Functional Foods in Health and Disease* 2013; Jun 24;3(6):230. DOI: <https://doi.org/10.31989/ffhd.v3i6.51>
15. Badalyan A, Abrahamyan S, Abovyan A, Badalyan A, Semerjyan G, Hovhannisyann N. Helichrysum arenarium as a source of flavonoids: Evaluation of antimicrobial activity and flavonoid content of extracts of Helichrysum flowers in vitro. *Functional Foods in Health and Disease* 2024;14(1):51.
DOI: <https://doi.org/10.31989/ffhd.v14i1.1257>
16. Furlan V, Bren U. *Helichrysum italicum*: From Extraction, Distillation, and Encapsulation Techniques to Beneficial Health Effects. *Foods*. 2023; 12(4):802.
DOI: <https://doi.org/10.3390/foods12040802>
17. Pljevljakušić D, Bigović D, Janković T, Jelačić S, Šavikin K. Sandy Everlasting (*Helichrysum arenarium* (L.) Moench): Botanical, Chemical and Biological Properties. *Frontiers in Plant Science* 2018; 9:1123.
DOI: <https://doi.org/10.3389/fpls.2018.01123>
18. Zhang X, Liu M, Wang Z, Wang P, Kong L, Wu J, et al. A review of the botany, phytochemistry, pharmacology, synthetic biology and comprehensive utilization of *Silybum marianum*. *Frontiers in Pharmacology* 2024; 15:1417655.
DOI: <https://doi.org/10.3389/fphar.2024.1417655>
19. Fallah M, Davoodvandi A, Nikmanzar S, Aghili S, Mirazimi SMA, Aschner M, et al. Silymarin (milk thistle extract) as a therapeutic agent in gastrointestinal cancer. *Biomedicine & Pharmacotherapy* 2021; 142:112024.
DOI: <https://doi.org/10.1016/j.biopha.2021.112024>.
20. Shahsavari K, Ardekani SS, Ardekani MRS, Esfahani MM, Kazemizadeh H, Jamialahmadi T, et al. Are alterations needed in *Silybum marianum* (Silymarin) administration practices? A novel outlook and meta-analysis on randomized trials targeting liver injury. *BMC Complementary Medicine and Therapies* 2025; 25(1):134.
DOI: <https://doi.org/10.1186/s12906-025-04886-y>.
21. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harbor Protocols* 2008; 2008(5):pdb. prot4986.
DOI: <https://doi.org/10.1101/pdb.prot4986>
22. Premkumar M, Anand AC. Overview of complications in cirrhosis. *Journal of Clinical and Experimental Hepatology* 2022;12(4):1150–74.

- DOI: <https://doi.org/10.1016/j.jceh.2022.04.021>
23. Petrovska B. Historical review of medicinal plants' usage. *Pharmacognosy Reviews/Bioinformatics Trends/Pharmacognosy Review* 2012;6(11):1. DOI: <https://doi.org/10.4103/0973-7847.95849>
 24. Chaachouay N, Zidane L. Plant-Derived Natural Products: a source for drug discovery and development. *Drugs and Drug Candidates* 2024;3(1):184–207. DOI: <https://doi.org/10.3390/ddc3010011>
 25. Dehghania P., Masjedi M., Shariati L., Vaseghi G., Dana N., Zeinalian M., Asgary S. Exploring the Potential of Pomegranate (Punica granatum) Peel Extract and Punicalagin as Novel Anti-Hypercholesterolemic Agents for managing LDL Levels through Decreasing PCSK9 Expression Levels. *Functional Foods in Health and Disease* 2024; 14(4): 270-281. DOI: <https://doi.org/10.31989/ffhd.v14i4.1320>
 26. Lee YS, Seki E. In vivo and in vitro models to study liver fibrosis: Mechanisms and limitations. *Cellular and Molecular Gastroenterology and Hepatology* 2023;16(3):355–67. DOI: <https://doi.org/10.1016/j.icmgh.2023.05.010>
 27. Faccioli LAP, Dias ML, Paranhos BA, Goldenberg RCDS. Liver cirrhosis: An overview of experimental models in rodents. *Life Sciences* 2022; 301:120615. DOI: <https://doi.org/10.1016/j.lfs.2022.120615>
 28. Das M, Boerma M, Goree JR, Lavoie EG, Fausther M, Gubrij IB, et al. Pathological Changes in Pulmonary Circulation in Carbon Tetrachloride (ccl4)-Induced Cirrhotic Mice. *PLoS ONE* 2014;9(4):e96043. DOI: <https://doi.org/10.1371/journal.pone.0096043>
 29. Geller SA, Horowitz RE. Gross examination. *Methods in Molecular Biology* 2014; 1180:3–19. DOI: https://doi.org/10.1007/978-1-4939-1050-2_1
 30. Hashim IA. Liver function. Elsevier eBooks. 2024 Jan 1;103–27. DOI: <https://doi.org/10.1016/B978-0-12-822949-1.00008-5>
 31. Huang, Huang Y, Wang J, Dai Z, Hu B, Chen S, Chen X, et al. Low total protein predicts adverse outcomes in patients with chronic obstructive pulmonary disease. *Sleep and Breathing* 2025;29(3):203. DOI: <https://doi.org/10.1007/s11325-025-03376-2>
 32. Huang Y, Liangpunsakul S, Rudraiah S, Ma J, Keshipeddy SK, Wright D, et al. HMGB2 is a potential diagnostic marker and therapeutic target for liver fibrosis and cirrhosis. *Hepatology Communications* 2023;7(11). DOI: <https://doi.org/10.1097/hc9.000000000000299>
 33. Pieters TT, Besseling PJ, Bovée DM, Rookmaaker MB, Verhaar MC, Yard B, et al. Discrepancies between transcutaneous and estimated glomerular filtration rates in rats with chronic kidney disease. *Kidney International* 2024;105(6):1212–20. DOI: <https://doi.org/10.1016/j.kint.2024.02.020>
 34. Lai X, Chen H, Dong X, Zhou G, Liang D, Xu F, et al. AST to ALT ratio as a prospective risk predictor for liver cirrhosis in patients with chronic HBV infection. *European Journal of Gastroenterology & Hepatology* 2024;36(3):338–44. DOI: <https://doi.org/10.1097/meg.0000000000002708>
 35. Cottin V, Valenzuela C. C-reactive protein as a candidate biomarker in fibrotic interstitial lung disease. *Respirology*. 2024; 29(3): 195–198. DOI: <https://doi.org/10.1111/resp.14666>
 36. Zhou HH, Tang YL, Xu TH, Cheng B. C-reactive protein: structure, function, regulation, and role in clinical diseases. *Frontiers in Immunology* 2024; 15:1425168. DOI: <https://doi.org/10.3389/fimmu.2024.1425168>
 37. Ananchuensook P., Moonlisarn K., Boonkaew B., Bunchorntavakul C., & Tangkijvanich P. (2025). Diagnostic Performance of Serum Mac-2-Binding Protein Glycosylation Isomer as a Fibrosis Biomarker in Non-Obese and Obese Patients with MASLD. *Biomedicine*, 13(2), 415. DOI: <https://doi.org/10.3390/biomedicine13020415>
 38. Tobing, E., Tansol, C., & Tania, C. (2024). Albumin–globulin ratio (AGR) as independent predictor of poor survival in renal cell carcinoma: A systematic review and meta-analysis. *Arab Journal of Urology*, 22(4), 219–226. DOI: <https://doi.org/10.1080/20905998.2024.2352954>
 39. El-Khouly N., Bayoumy E. S. M., Ali W. E., Eid A. M. M. A., Sofy M. R., Fakhrelden S. M., Marmoush S. M. H., Elmohaseb G. F., Khlifa E. A., Youssef E. M. I. Vitamin D Levels in Non-alcoholic Fatty Liver Disease in type II diabetic and non-diabetic Patients. *Bioactive Compounds in Health and Disease* 2023; 6(9):202-214, DOI: <https://www.doi.org/10.31989/bchd.v6i9.1128>
 40. Ramadan O., Abuamara T., Taha R., Awad M., Mohammed M., Omar N., Fayyad R., Darwish A., Eltantawy W., Babiker M., Elsharkawy S., Alqassimi S. Alleviation of the arsenic induced hepatotoxicity in rats by ginger or omega-3: a histological and biochemical study. *Bioactive Compounds in Health and Disease* 2024; 7(4): 221-232. DOI: <https://www.doi.org/10.31989/bchd.v7i4.1339>
 41. Brennan, P.N., Dillon, J.F. and Tapper, E.B. 'Gamma-glutamyl transferase (γ-gt) – an old dog with new tricks?'. *Liver International* (2021); 42(1), pp. 9–15. DOI: <https://www.doi:10.1111/liv.15099>.
 42. Dănăilă-Guidea SM, Eremia MC, Dinu LD, Miu DM. Helichrysum arenarium: From Cultivation to Application. *Applied Sciences* 2022;12(20):10241.

- DOI: <https://doi.org/10.3390/app122010241>
43. Mihailović, V., Srečković, N., Popović-Djordjević, J.B. (2023). Silybin and Silymarin: Phytochemistry, Bioactivity, and Pharmacology. In: Xiao, J. (eds) *Handbook of Dietary Flavonoids*. Springer, Cham.
DOI: https://doi.org/10.1007/978-3-030-94753-8_20-1
44. Frounchi N, Mahmoodpoor F, Zakavi SS, Eyvazova K, Yagubova S, Ardalan M, et al. Nephroprotective Effects of Silymarin: A Systematic Review and Meta-Analysis. *Biochemistry (Moscow)* 2025;90(8):1140–52.
DOI: <https://doi.org/10.1134/s0006297925600565>
45. Fazeli M, Sarvazad H, Rahnejat N, Rostampour R, Rad MG, Eskandari-Roozbahani N. Investigation of potential antiviral natural products with an effect on HPV18 E6 protein by molecular docking method. *Functional Foods in Health and Disease* 2021;11(11):586.
DOI: <https://doi.org/10.31989/ffhd.v11i11.840>
46. Huang M, Liu X, Ren Y, Huang Q, Shi Y, Yuan P, et al. Quercetin: A Flavonoid with Potential for Treating Acute Lung Injury. *Drug Design Development and Therapy* 2024; Volume 18:5709–28.
DOI: <https://doi.org/10.2147/dddt.s499037>
47. Huang H, Han J, Liu Y, Zhang Q, Zhou Y, Zheng S, et al. Exploring the molecular mechanism of apigenin in treating bronchiectasis based on network pharmacology and molecular docking. *Scientific Reports* 2025;15(1):39161.
DOI: <https://doi.org/10.1038/s41598-025-24377-x>