Liver is an important organ that metabolizes toxins, synthesizes nutrients, and promotes hematopoiesis. Due to these functions, liver ailments continue to be among the main threats to public health. Nowadays, people have increased liver load because of the work pressure, imbalanced diet, excessive drinking, and viral infections, which causes a series of liver diseases. The metabolism by the liver of various chemical agents such as carbon tetrachloride (CC\textsubscript{14}) cause severe damage to the liver cells over time.

Although liver disease is caused by a variety of different etiologies, the pathogenesis of chronic liver disease is relatively uniform. The healthy liver is prompted by various triggers (extensive alcohol abuse, hepatitis infection, metabolic disorders, genetic diseases, environmental toxins and drug consumption) to undergo hepatic fibrogenesis resulting in an injured, fibrotic liver. The prevalence of liver diseases necessitates effective and cost-efficient treatments.

One of the best candidates for preventing of such liver pathologies could be lipid complexes containing essential phospholipids with fatty acids substitutes represented by the n-3 fatty acids with one of the mechanism of action to be through cellular membrane stabilization.

The marine environment may be explored as a rich source for novel antifibrotic drugs. A number of marine derived compounds are shown to prevent formation of the reactive oxygen species (ROS) and possess anti-inflammatory activity. Marine derived Omega-3 long-chain polyunsaturated fatty acids (Omega-3 PUFAs) especially;
eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pic.1) have long been reported for their valuable hepatoprotective effects and their ability to decrease hepatic injury. They have a variety of proposed mechanisms of action; the most significant of which would be; modulating cell proliferation, regulating fatty acid metabolism, inhibiting lipogenesis as well as suppressing inflammation and oxidative stress.

**The main marine derived omega-3 fatty acids**

![Diagram of fatty acids]

Pic. 1 Marine omega-3 polyunsaturated fatty acids

In the course of searching for hepatoprotective (HP) agents of lipid nature from marine sources, we evaluated the lipid complex of marine red alga, *Ahnfeltia tobuchiensis* (AT) for HP activity. Algae samples were collected in the autumn in coastal waters of Alekseeva bay, Popov Island, Peter the Great bay (Sea of Japan).

![Map of Alekseeva bay and Popov Island]

Pic. 2. Marine red algae *Ahnfeltia tobuchiensis* and the region of its harvesting.

Isolation of the lipid complex was carried out by the standard Bligh and Dyer method. Obtained lipid extract from AT (ATL) contained 56% of membrane active fraction (sum of glyco- (GL)- and
phospholipids (PL)). PL and GL made 25.7% and 30.5% of total lipids accordingly. Fraction of PL consisted of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatydilinosit (PI). PC and PE are among the most important structural bricks of membranes that are providing the spatial organization of the membrane matrix and the functioning of membrane structures. The fatty acids pattern of GL and PL was presented mainly by unsaturated fatty acids where prevailed PUFA of omega-3 (α-linolenic and eicosapentaenoic fatty acids) and omega-6 (linoleic and arachidonic fatty acids) groups. Monounsaturated fatty acids were represented by oleic acid and palmitic was the main among saturated ones.

HP potential of the obtained preparation was assessed using the model of CCl₄ induced liver damage in outbred male mice (25-30g) (Pic.3). All animals were housed in a well-ventilated environment, received standard mice food pellets and water was provided ad libitum throughout the experimental period. Mice were injected in the dorsal neck fold with 2 ml/kg of a 50% solution of CCl₄ in olive oil for 4 days. ATL was administered intragastrically in a dose of 80 mg of total lipids/kg of body mass. Mice were divided into four groups of ten animals. Group 1 was normal control, group 2 – CCL₄ group; group 3 – CCL₄+withdrawal for 7 days; group 4 – CCL₄ + ATL for seven days. After 4 days, the administration of CCL₄ produced severe liver damage characterized by significant elevation (P < 0.001) in serum ALT by 400%, liver Mass Index (LMI) by 35% and total lipids amount in the liver by 3 times, compared with the control group (Pic.4). The neutral lipids pattern in the liver was changed to the direction of an increase by 20-24% (p<0.01) in the amount of triacylglycerols (TAG), cholesterol (Chol), and free fatty acids (FFA) due to enhanced flow of lipids due to the peripheral lipolysis in fat tissue – stress response to toxin.
Pic.3. The model for experimental carbon tetrachloride toxic hepatitis

Experiment design for evaluation of hepatoprotective potential of lipid extract from red algae *Ahnfeltia tobuchiensis* at experimental CCl4 toxic hepatitis in mice

![Diagram of experiment design](image)

- **Group #1:** Normal Control
- **Group #2:** CCl4
- **Group #3:** Negative Control (Withdrawal)
- **Group #4:** Experimental (Ahnfeltia extract)

- Vaseline oil intragastrically for 7 days
- 50% CCl4 2 ml/kg in skin fold for 4 days
- Ahnfeltia. tob. 80 mg of lipids per kg of body weight intragastrically for 7 days

Pic.4 Influence of lipid complex from *Ahnfeltia tobuchiensis* on some liver indices and ALAT activity in blood at acute CCl4 poisoning.

- **Alanine Aminotransferrase (blood)**
- **Liver Mass Index**

- **General Lipids (liver)**

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Pic.5 Influence of lipid complex from *Ahnfeltia tobuchiensis* on Neutral Lipids Pattern at carbon tetrachloride toxic hepatitis. Shown fractions content difference from intact animals.

The mechanism behind CCl₄-hepatotoxicity appears to be oxidative stress initiated by CCl₄-derived reactive free radical metabolites, including trichloromethyl (CCl₃) and trichloromethyl peroxyl (OOCCl₃). The former is responsible for the covalent binding to cell components like reduced glutathione (GSH), resulting in its depletion, whereas the latter initiates the lipid peroxidation process with the consequent formation of by-products such as, Malone dialdehyde (MDA), resulting in loss of membrane integrity and fibrosis development.

We observed double increase in the MDA level (p<0,001). Hepatic GSH content and the level of antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GRx), Superoxide dismutase (SOD) were strikingly depleted (by 25-48%, p<0,01) in CCl₄-intoxicated mice, compared with the normal mice (Pic.6).
Pic.6 Influence of lipid complex from *Ahnfeltia tobuchiensis* on Gluthatone cycle parameters and Malone dialdehyde level in mice’s blood at acute carbon tetrachloride liver damage.

Also, we observed reduction of the levels of the main structural phospholipids PC and PE by 7-12% (p<0.01) with concurrently increasing by 58-70% (p<0.001) of lyso-PC and lyso-PE. This indicated a high level of free radical generation due to reducing dehalogenation of CCl₄ by CYP₂E₁, which are actively included in the phospholipid fatty acid chains, disorganizing the structure of cellular membranes.

After 7 days of CCl₄ withdrawal, most of the controlled biochemical parameters in the mice liver (group 3) did not recover to the control value, indicating a continuing toxic impact and insufficiency of the body resources to counteract the toxic pathology. Furthermore, the level of the antioxidant enzymes GPx, GRx, SOD as well as GSH content were expressed by further reduction. The neutral lipids pattern
remains misbalanced, which indicates further progress of catabolic processes in the liver. Serum ALT remains increased by 2 times (p<0.001), while the level of PC and PE remains reduced and even underwent to the further declining while lyso-forms of these phospholipids continue to grow up, indicating the high level the structure disorder of cellular membranes.

The administration of the ATL during CCl\textsubscript{4} withdrawal (group 4) exhibited significant HP activity by reducing the CCL\textsubscript{4} caused changes and led to the recovery of almost all studied biochemical parameters to the control value. We observed a restoration of the LMI and total lipids amount and neutral lipids pattern balance, which means improvement of fatty liver. MDA level returned to normal and as well as the level of antioxidant enzymes GPx, GRx, SOD and GSH pool together with the level of PC and PE which indicates the reduction of the free radical activity and restoration of the spatial organization of the membrane matrix.

The results obtained by this study suggest that a lipid complex enriched with marine phospholipids and omega-3 fatty acids from AT is a promising source for effective hepatoprotectors of lipid nature.