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Original Research

Hepatoprotective effect of protein extract of the Potamogeton perfoliatus L. against carbon-tetrachloride (CCI4) - induced hepatic injury in mice

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Abstract

This article demonstrates the hepatoprotective action of the protein extract of the aquatic plant *Potamogeton perfoliatus L.* based on the histopathologic changes in the liver of mice with CCl_4-induced liver damage. Using the chromatography, 4 fractions of the protein extract were obtained. It has been suggested that a fourth fraction corresponding to low molecular weight protein should most likely exhibit hepatoprotective properties. SDS-PAGE of *P. perfoliatus L.* revealed a protein with molecular weight of 21 kDa corresponding to the fourth peak of a chromatogram.

Keywords: Potamogeton perfoliatus L., proteins, tetrachloromethane, CCl_4, chromatography, gel filtration, hepatocytes, SDS-PAGE

Introduction

Plants naturally produce a diverse range of bioactive possessing varied therapeutic properties. In addition to polyphenolic compounds, plant proteins are also important. Proteins produced in medicinal plants exhibit antimicrobial, antioxidant, anti-cancerous, neuromodulatory and other properties [9].

The therapeutic potential of aquatic plants have not been properly studied. Potamogetonaceae, or the pondweed family, includes 110 species belonging to six genera,

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where the genus Potamogeton is the largest among them. Twelve species of the genera were revealed in Georgia. The usage of various plants from this genus in folk medicine is reported for curing several pathological conditions [8]. The presence of variety of secondary metabolites, such as glycosides, pentosidases, rosemary acids, quercetin, phenolic acids was demonstrated in P. perfoliatus L [6]. The phenolic extract has a degrading effect on CD68 and GFA expression in the brain stem of rats, indicating its possible neuroprotective properties [4].

Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million due to complications of cirrhosis and 1million due to viral hepatitis and hepatocellular carcinoma [1]. Toxins like carbon tetrachloride (CCl_4) can cause metabolic inefficiency in the liver, which can lead to liver fibrosis, cirrhosis, or in some cases hepatocellular carcinoma [3]. The injections of CCl_4 results in the active generation of reactive molecules with cytochrome P450 and formation of trichloromethyl peroxyl radical [7]. Trichloromethyl peroxyl radical, in turn, causes oxidative stress which promote lipid peroxidation and the damage of hepatocellular membrane and development of inflammation and apoptosis [2]. Despite the large number of hepatoprotective agents, they all have a number of disadvantages, therefore the search for hepatoprotective compounds of natural origin is relevant.

In this study we investigated the hepatoprotective effect of protein extract of the Potamogeton perfoliatus L. based on the histopathologic changes in the liver of mice with CCl_4-induced liver damage.

Material and Methods

Plant Material and Extraction

The whole plant, *P. perfoliatus L.*, was collected from Tbilisi reservoir (coordinates: 41° 44' 58" N, 44° 50' 44" E). The plant was cleaned using distilled water and air dried.15 g of leaves (was extracted using 40% PBS (ratio 1:10). The homogenate was stirred for 60 min using magnetic stirrer and after centrifugated at 3000 rpm for 20 min. The supernatant was used for the protein determination.

Animals

White mice, 4–6-month-old (average weight of 25 ± 0.05 g), were used in the study. The mice were housed under normal light- dark conditions (12 hours light followed by 12 hours dark) for the entire experiment and had access to food and water, *ad libitum*. The animals were randomly assigned to one of 4 groups:

- First group: control animals
- Second group: 10% oil CCl_4 solution;
- Third group: 10% oil CCl_4 solution and *P. perfoliatus L.* extract.
- Fourth group: *P. perfoliatus L.* extract.

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Each group contained 6 mice. The mice of the first group were injected with double distilled water for 30 days. The mice of the second group received a intragastric dose of 10% CCl_4 dissolved in olive oil for 30 days. The mice of the third group received intragastric dose of 10% oil CCl_4 solution and *P. perfoliatus L.* extract (6 g/kg) for 30 days. The mice of the fourth group received intragastric dose of *P. perfoliatus L.* extract (6 g/kg) for 30 days.

Biochemical studies

The protein from the plant extract was concentrated using precipitation with 90% ammonium sulfate solution. The total protein concentration was determined using the Lowry method [5]. The protein solution was subjected to gel filtration on a Sephadex G75 column equilibrated with 5 mM KH_2PO_4. 150 mM NaCl pH 8. Fractions were collected at a flow rate of 0.5 mL/min. Protein content was measured. SDS-PAGE was performed accordingly to Laemmli in a 12% polyacrylamide gel.

Histological staining

Van Gieson's Stain of liver extracted from 6 mice was performed for each group. Mice were anesthetized with chloroform; livers were removed and placed in 15% formalin solution. Liver tissues were sliced into coronal sections using a rotary microtome. Images were obtained using AmScope microscope.

Discussion

Proteins of the *P. perfoliatus L.* extract retained their initial concentration for 30 days after preparation at +4°C (0.038 mg/mL), which allows the use of the protein extracts in experiments for a long period of time (Figs. 1 and 2).

Figure 1. The concentration of proteins of *P. perfoliatus L.* extract (the concentration of 0.038 mg/mL).

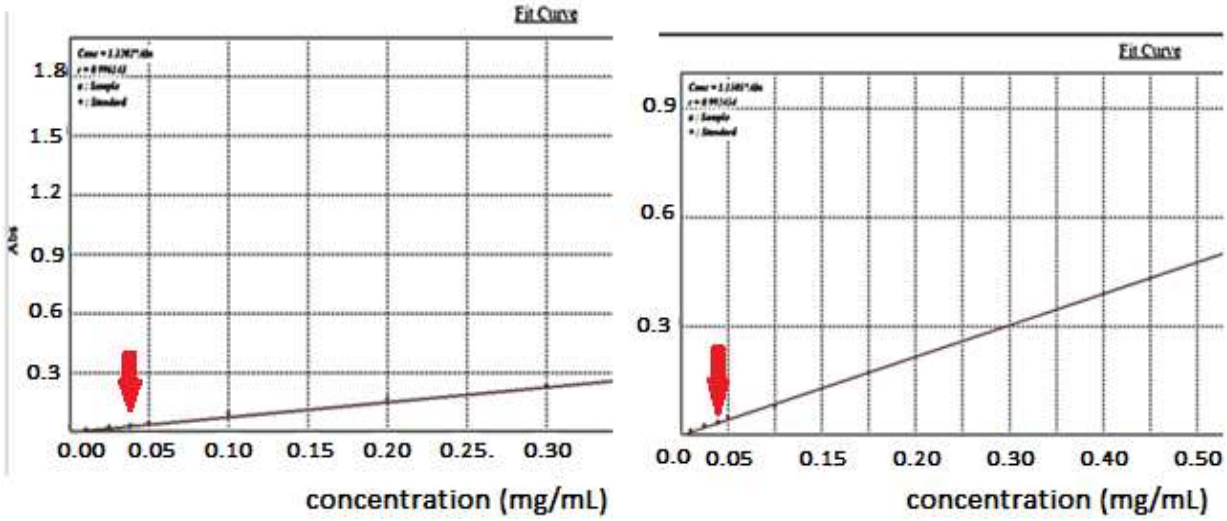

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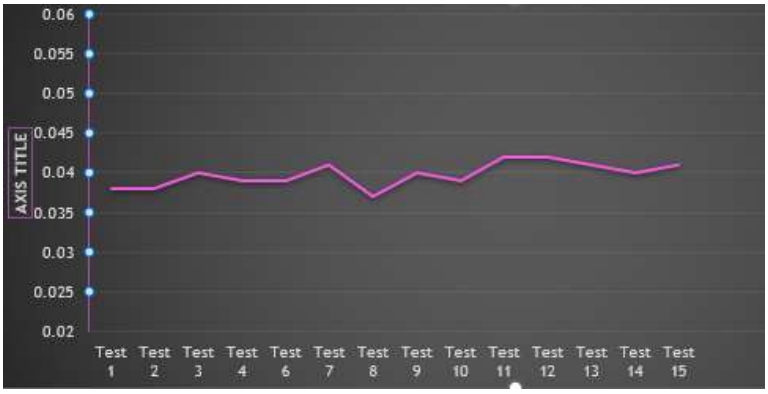
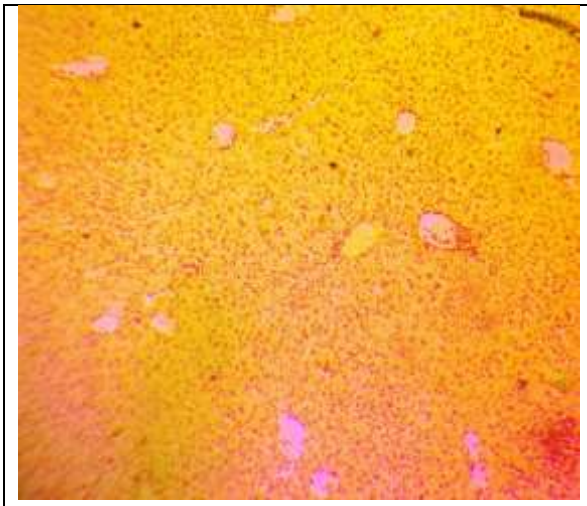
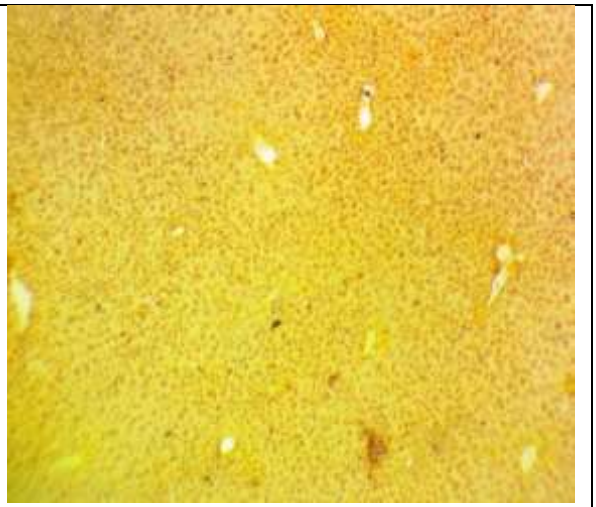
Figure 2. Concentration of of proteins of *P. perfoliatus L.* extract during 30 days after preparation of the extract at + 4°C. Measurements were performed every 48 hours.



Histological analysis of liver tissue morphologies confirmed the protective effect of *P. perfoliatus L.* extract in CCl_4-induced hepatic injury. Liver of mice in the control group (Figs. 3.1 and 3.2) showed normal hepatocytes, obvious sinusoids, and central vein. Liver of mice in the CCl_4 group was characterized by hepatocellular degeneration and substitution of hepatocytes with connective tissue. Liver of mice of CCl_4 + *P. perfoliatus L.* extract group was characterized by less profound injury, as was evident by not significant development of connective tissue. (Figs.4.1-9.2). Liver of mice of *P. perfoliatus L.* extract group showed no pathological changes.



Microphoto 3.1. Liver tissue of mice of control group. Magnification 200x.



Microphoto 3.2. Liver tissue of mice of *P. perfoliatus L.* extract group. Magnification 200x.

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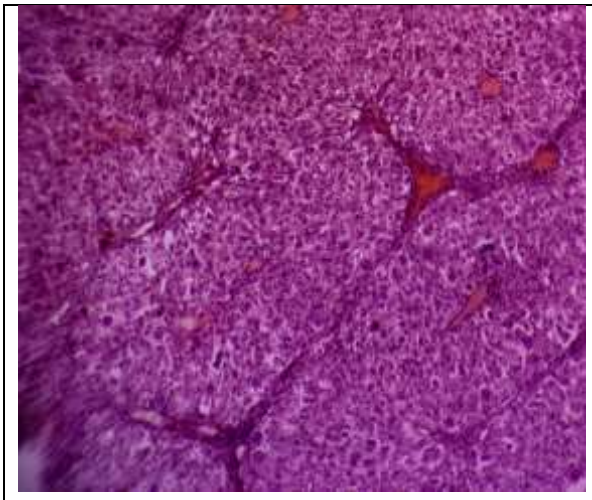
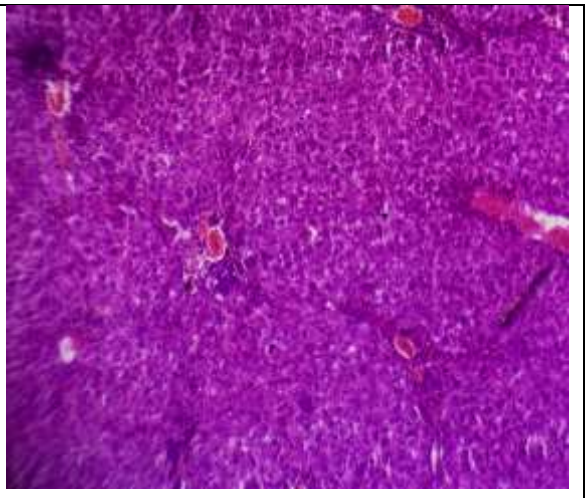
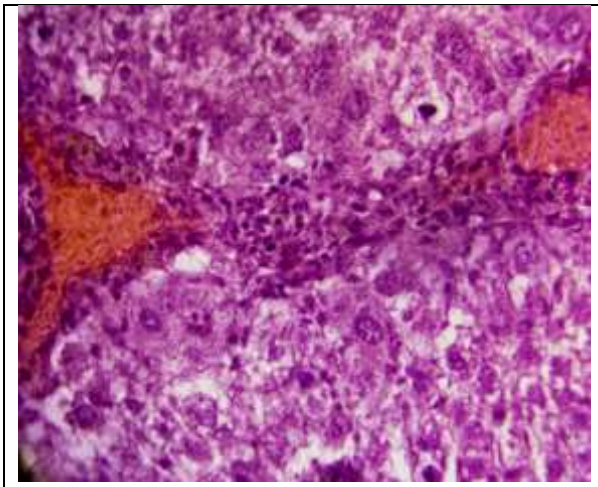
Figure 3.1-9.2. The effect of the P. perfoliatus *L.* protein extract on the liver tissue of mice affected by CCl_4.



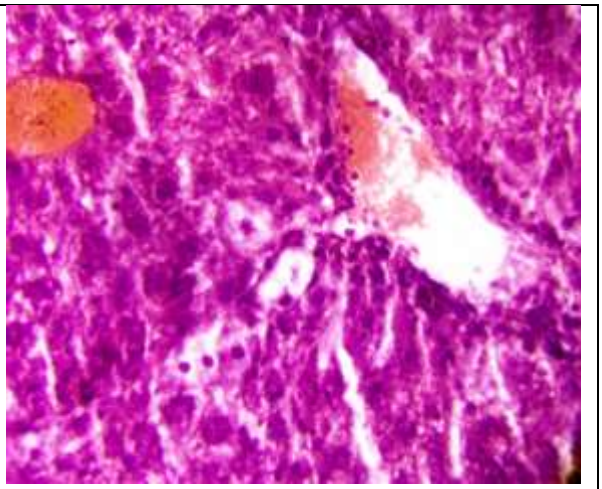
Microphoto 4.1. Liver tissue of mice of CCl_4 group. In the structures of the hepatic lobes, the damage was observed around the central vein and expressed hepatic lobule. Magnification 200x.



Microphoto 4.2. . Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. The connective tissue is less pronounced around the central veins and vessels of the hepatic lobule. There is blood stasis. Magnification 200x.



Microphoto 5.1. Liver tissue of mice of CCl_4 group.
Hepatocytes of damaged mice. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 400x.



Microphoto 5.2. Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. In the same structures
In the same structures there was no connective tissue. Blood stasis was observed in the central vein and hepatic lobule. Blood congestion was observed. Magnification 400x.

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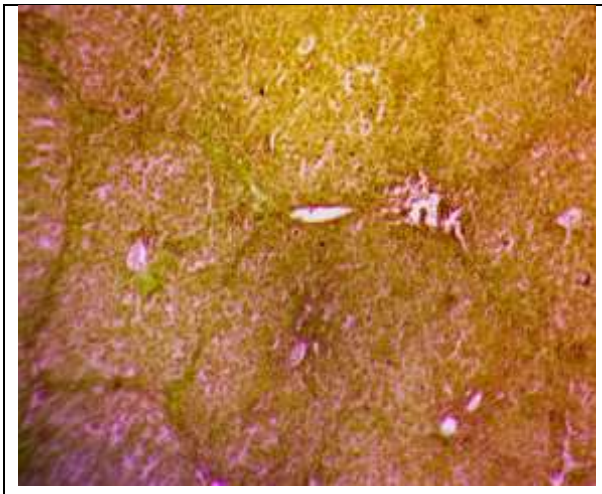
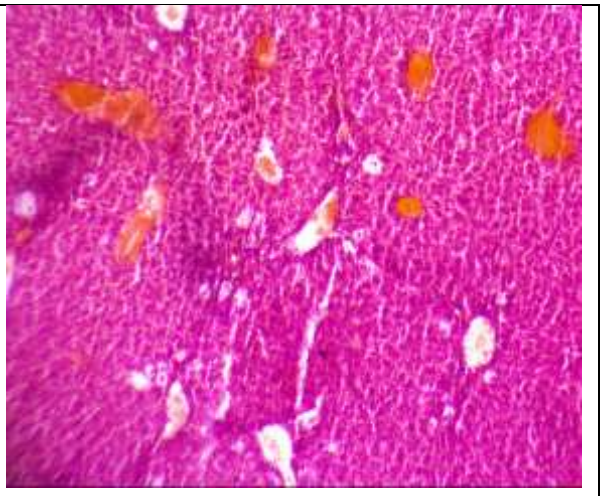
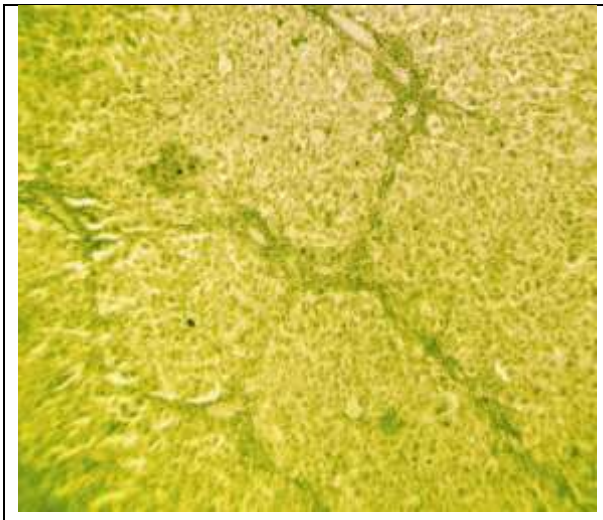


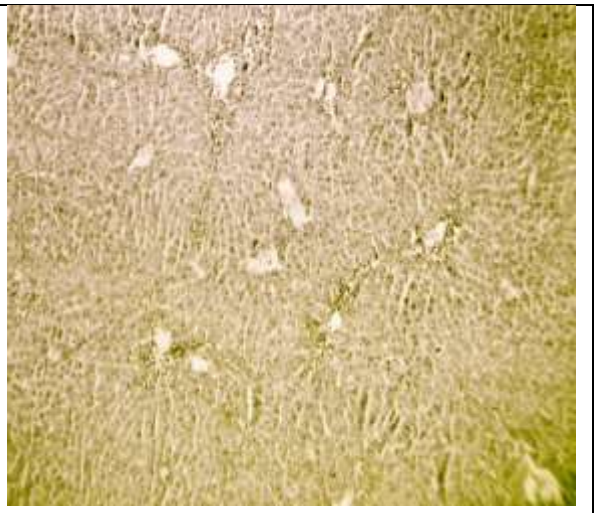
Microphoto 6.1. Liver tissue of mice of CCl_4 group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 6.2. Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. In the same structures connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was observed



Microphoto 7.1. Liver tissue of mice of CCl_4 group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 7.2. Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. Connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.

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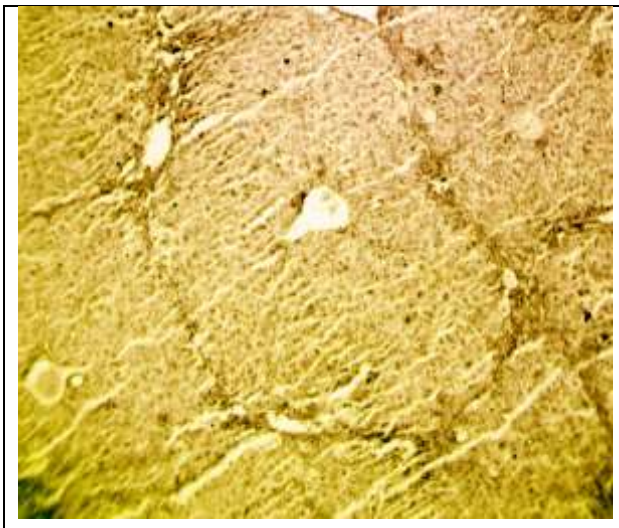
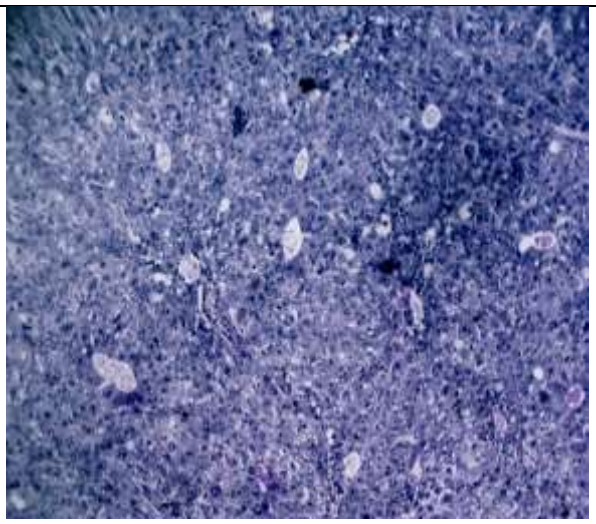
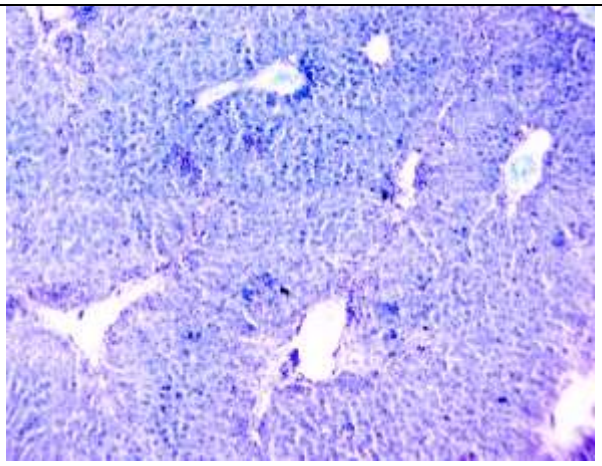


Microphoto 8.1. Liver tissue of mice of CCl_4 group. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 8.2. Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. Connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.

Microphoto 9.1. Liver tissue of mice of CCl_4 group. Hepatocytes of damaged mice. Around the liver lobe, the central vein and the hepatic lobule, connective tissue was well expressed. Magnification 200x.



Microphoto 9.2. Liver tissue of mice of CCl_4 + *P. perfoliatus L.* extract group. In the same structures connective tissue is less expressed. In some central veins and blood vessels of the hepatic lobule, blood stasis was not observed. Magnification 200x.

P. perfoliatus L. extract was subjected to gel filtration on a Sephadex G75 column, which separated the protein solution in 4 fractions (Fig. 10). Fraction IV was of particular

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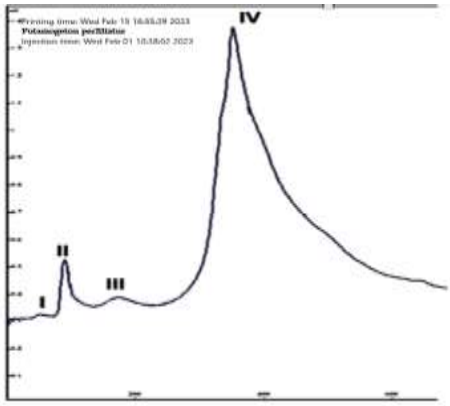
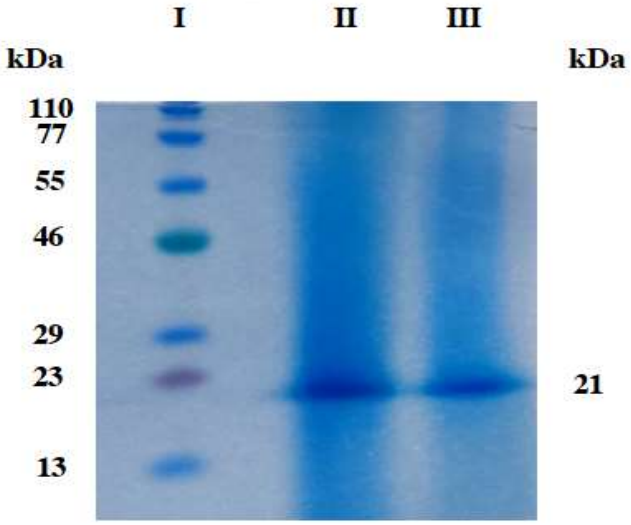
interest since it was present in the highest concentration and most probably the protein of this peak exhibited hepatoprotective properties.

Figure. 10. Elution profile of *P. perfoliatus* L. protein by gel-filtration on Sephadex G-75.



In the later stages of experiments, SDS-PAGE of *P. perfiliatus* L. protein extract revealed one protein fraction with a molecular weight of 21 kDa, which corresponds to the fourth peak of gel-filtration chromatography (Fig. 11.).

Figure. 11. SDS-PAGE of *P. perfiliatus L.* Protein fraction with molecular weight of 21 kDa (lanes II and III). Is shown. Marker proteins (lane I).



Conclusions

1. Proteins of the *P. perfoliatus L.* extract retained their initial concentration for 30 days after preparation at +4°C, which allows the use of the protein extracts in experiments for a long period of time.

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2. *P. perfoliatus L.* protein extract with a concentration of 6 g/kg (0.006 mg/g) exhibited moderate hepatoprotective activity *in vivo* in CCl_4-damaged mice hepatocytes.
3. *P. Perfoliatus L.* protein extract at a concentration of 6 g/kg (0.006 mg/g) had no detrimental effect on the mice hepatocytes.
4. Gel filtration of *P. perfoliatus L.* extract on Sephadex G-75 revealed four fractions, the fourth fraction contained a high concentration of low molecular weight protein.
5. SDS-PAGE of *P. perfoliatus L.* revealed a protein with molecular weight of 21 kDa corresponding to the fourth peak of a chromatogram. With high probability, this fraction exhibited hepatoprotective properties.

წყალმცენარე Potamogeton perfoliatus L-ის ცილოვანი ექსტრაქტის ჰეპატოპროტექტული მოქმედება ტეტრაქლორმეთანით (CCl_4) დაზიანებულ თაგვების ჰეპატოციტებზე

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აბსტრაქტი

სტატიაში მოცემულია წყალმცენარე Potamogeton perfoliatus L.-ის ცილოვანი ექსტრაქტის ჰეპატოპროტექტული მოქმედება თაგვების ტეტრაქლორმეთანით (CCl_4) დაზიანებულ ჰეპატოციტებზე, რომელიც მიკრომორფოლოგიური პრეპარატების ანალიზით გამოვლინდა. გელ-ფილტრაციული ქრომატოგრაფიის საშუალებით მიღებულია, ცილოვანი ექსტრაქტის 4 ფრაქცია. გამოთქმულია მოსაზრება, რომ მეოთხე ფრაქცია, რომელიც დაბალმოლეკულურ ცილას შეესაბამება, დიდი ალბათობით, უნდა ავლენდეს ჰეპატოპროტექტულ თვისებებს. P. Perfoliatus L.-ის ცილოვანი ექსტრაქტის SDS-ელექტროფორეზული ანალიზით გამოვლინდა 21 kDa მოლეკულური მასის ცილა, რომელიც შეესაბამება გელ-ფილტრაციული ქრომატოგრაფიით მიღებულ მეოთხე ფრაქციას.

საკვანძო სიტყვები: Potamogeton perfoliatus L, ცილები, ტეტრაქლორმეთანი, CCl_4, ქრომატოგრაფია, გელ-ფილტრაცია, ჰეპატოციტები, SDS-ელექტროფორეზი.

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