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DETERMINATION OF ACUTE TOXICITY OF OINTMENT WITH PINUS SYLVESTRIS EXTRACT

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The necessity of search for new biologically active substances among the medicinal plants of Pinus species does not cause any doubts in it. Promising outlook of Pinus sylvestris application is confirmed by many publications of both domestic and foreign researchers. Post the performed study it is known that the obtained dry extract is practically non-toxic and has a wide range of biological effects. One of the main priorities of the new dosage form implementation is its low toxicity. Therefore, purposeful technology of ointment based on dry extract and controlled trial of acute toxicity are the priorities in the pharmacological studies. The purpose of the work was to study the acute toxicity of the ointment based of the Pinus sylvestris dry extract, which was first performed. The acute toxicity was studied on white mice with a single oral administration of the drug. The mice were observed for 14 days. The animals' general condition, mortality, body mass dynamics before and after the experiment were studied. Macroscopic assessment of the internal organs status was performed and their histological structure was studied. As studies have shown, after oral administration of the ointment with the Pinus sylvestris extract in the dose of 0.5 ml, signs of toxicity in mice were not revealed. According to the classification by K. K. Sidorov, the ointment with the Pinus sylvestris extract belongs to practically non-toxic compounds. Thus, the ointment with Pinus sylvestris extract belongs to the fifth class - practically non-toxic when administered orally.

Key words: acute toxicity, ointment with Pinus sylvestris extract, macroscopic analysis of internal organs.

The work is a fragment of the research project "Study of wild and cultivated medicinal plants in the Western region of Ukraine and development of their application technologies with therapeutic purposes", state registration No. 018U003809.

Qualitative therapy requires the deliberate search and creation of new, highly effective medicines without side-effects.

The main task of pharmacy is to find new, modern and high-quality medicines.

A promising group is herbal drugs that are widely used in the medicines market. Broad-spectrum of these drugs is enhanced by the multicomponent composition of biologically active substances and simultaneous presence of different nature compounds. Mild therapeutic effects, low toxicity and minimal side effects, cost-effectiveness are just some of the plant-based drugs benefits.

Pine (Pinus sylvestris L.) of the Pinaceae family is an unofficial medicinal plant in Ukraine. The plant has long been used in medicine in the form of infusion, tincture, decoction, extract, as a diuretic, antiseptic, wound-healing and anti-inflammatory agent. Needles, buds, galipot of Pinus sylvestris L. are used as medicinal plant raw material [2]. Pinus sylvestris L. contains resin, essential oil, vitamins C, B₁₂, K, R, phytoncides, tannins, starch, carotene, mineral salts, turpentine. In general, due to the accumulation of different groups of biologically active substances aerial parts of the plant is promising for integrated pharmacognostical and pharmacological studies aimed at establishing new domestic phytopreparations. Because search and creation of effective and affordable new medicines of plant materials is an important task of the domestic pharmaceutical science and practice, an important step in the new drugs development is to study their safety, namely to establish their class of toxicity and impact on the animals' internal organs [2].

The purpose of the work is to study acute toxicity of the ointment based on the Pinus sylvestris dry extract.

Materials and methods. The study was carried out on outbred white mice males. The experiments were carried out at the Pharmacy Department, Human Anatomy Department, academician G.A. Babenko Biological and Medical Chemistry Department and Bioelementology Center at the Ivano-Frankivsk National Medical University. To study the acute toxicity we selected the methodology approved by the State Pharmacopeia and the MOH of Ukraine on white mice with a single oral administration of the drug. The animals were kept in the same standard conditions and feeding. The mice were observed for 14 days and the animals' general condition, mortality, body mass dynamics before and after the experiment were assessed. Macroscopic assessment of the internal organs status was performed and their histological structure was studied.

The object of research was the ointment with thr Pinus sylvestris extract, obtained on base of the Ivano-Frankivsk National Medical University. As the studies have shown, after oral administration of the ointment with the Pinus sylvestris extract in the dose of 0.5 ml, signs of toxicity in mice were not revealed: the animals were neat and tidy, they reacted to sound and light stimuli, processes of urine and defecation were normal, respiratory failure and convulsions were not observed, eye fissures were narrowed. According to the classification by K. K. Sidorov, the ointment with the Pinus sylvestris extract belongs to practically

non-toxic compounds. Therefore, in the study on acute toxicity of ointment with *Pinus sylvestris* extract, in the dose of 0.5 ml, signs of toxicity were not revealed.

All manipulations were carried out in compliance with the International Convention of working with animals and the Law of Ukraine "On protection animals from brutal treatment".

Data processing calculations were performed using Statistica 6.0.

The instrumental methods of study were applied. The obtained histological sections were studied in light MC300 Micros Austria microscope and photographed with TouPCam figure 5.1 UHCCD M C-Mount camera with Sony adapter TouPCam Photonics AMA075 using TouPCam software v3. (at magnification $\times 400$).

Results of the study and their discussion. Acute toxicity ointment with extract of *Pinus sylvestris* was studied by the method of State Pharmacopeia of Ukraine and the MOH of Ukraine. According to the classification by K.K. Sidorov they are practically non-toxic [6].

As our studies have shown, after oral administration of the ointment with the *Pinus sylvestris* extract in the dose of 0.5 ml, signs of toxicity in mice were not revealed: the animals were neat and tidy, reacted to sound and light stimuli, processes of urine and defecation were normal, respiratory failure and convulsions were not observed, eye fissures were narrowed. The reflex excitability of all the animals was preserved. Death of animals during the whole observation period was not registered.

The results of acute toxicity studies on *Pinus sylvestris* ointment with a single intragastric administration to white mice are presented in table 1.

Table 1

Studies on acute toxicity of *Pinus sylvestris* ointment with a single intragastric administration to white mice

Group of animals	Body weight, g, $\bar{x} \pm \Delta \bar{x}$, n = 10	
	Before the experiment	After the experiment
Group 1, intact animals	18.0 \pm 0.548	19.0 \pm 0.508
Group 2, ointment with <i>Pinus sylvestris</i> extract	19.0 \pm 0.507	20.0 \pm 0.358
Group 3, the ointment base	18.0 \pm 0.547	19.0 \pm 0.359

After 14 days of the experiment a macroscopic analysis of the animals' internal organs was carried out. In the thorax cavity, all organs were anatomically correct. The form, color, size were regular. The surface of the kidney, liver and adrenal glands was smooth, the spleen was full and elastic. The pancreas had a grayish-pink color.

The stomach mucous membrane had a pronounced relief of folds, the intestine mucous membrane was not changed. The heart muscle is dark red colored in the cross-section, lungs airiness was observed, and the pulmonary pleurae was not changed.

At the Department of biological and medical chemistry named after academician G. A. Babenko and Center bioelementology Ivano-Frankivsk national medical University in the serum of mice blood: leukocytes, erythrocytes, hemoglobin, cholesterol, total protein were determined. Biochemical and hematological parameters of animals are given in table. 2.

Table 2

Biochemical and hematological parameters of mice blood in acute intoxication

The studied parameters	Group of animals		
	Intact animals	Ointment basis	Ointment with <i>Pinus sylvestris</i> extract
Leukocytes, $\times 10^9/l$	7.60 \pm 0.33	7.40 \pm 0.38	7.60 \pm 0.24
Erythrocytes, $\times 10^{12}/l$	8.10 \pm 0.24	8.00 \pm 0.32	8.10 \pm 0.32
Hemoglobin, g/l	130.8 \pm 0.61	130.1 \pm 3.31	132.2 \pm 2.47
Cholesterol, mmol/l	2.78 \pm 0.13	2.81 \pm 0.15	2.83 \pm 0.22
Total protein, g/l	54.5 \pm 0.95	55.1 \pm 0.80	56.2 \pm 0.72

Thus, the experimental results show that the ointment with the extract of of *Pinus sylvestris* needles shows no toxic effect on the white mice body. Organs were prepared to the study using conventional methods in morphology. The sections were stained with hematoxylin and eosin (fig. 1).

14 days after the oral administration, in the histological structure of the kidneys (fig. 1 A) moderate blood filling of intraorganic blood vessels was revealed, epithelial cells of the proximal convoluted tubules were slightly swollen. Increased blood filling of the glomeruli capillaries, expansion of Boumen's spaces were observed. The tubules epitheliocytes' cytoplasm was granular. There were no specific findings in distal tubules, only in some of them isolated vacuoles were present.

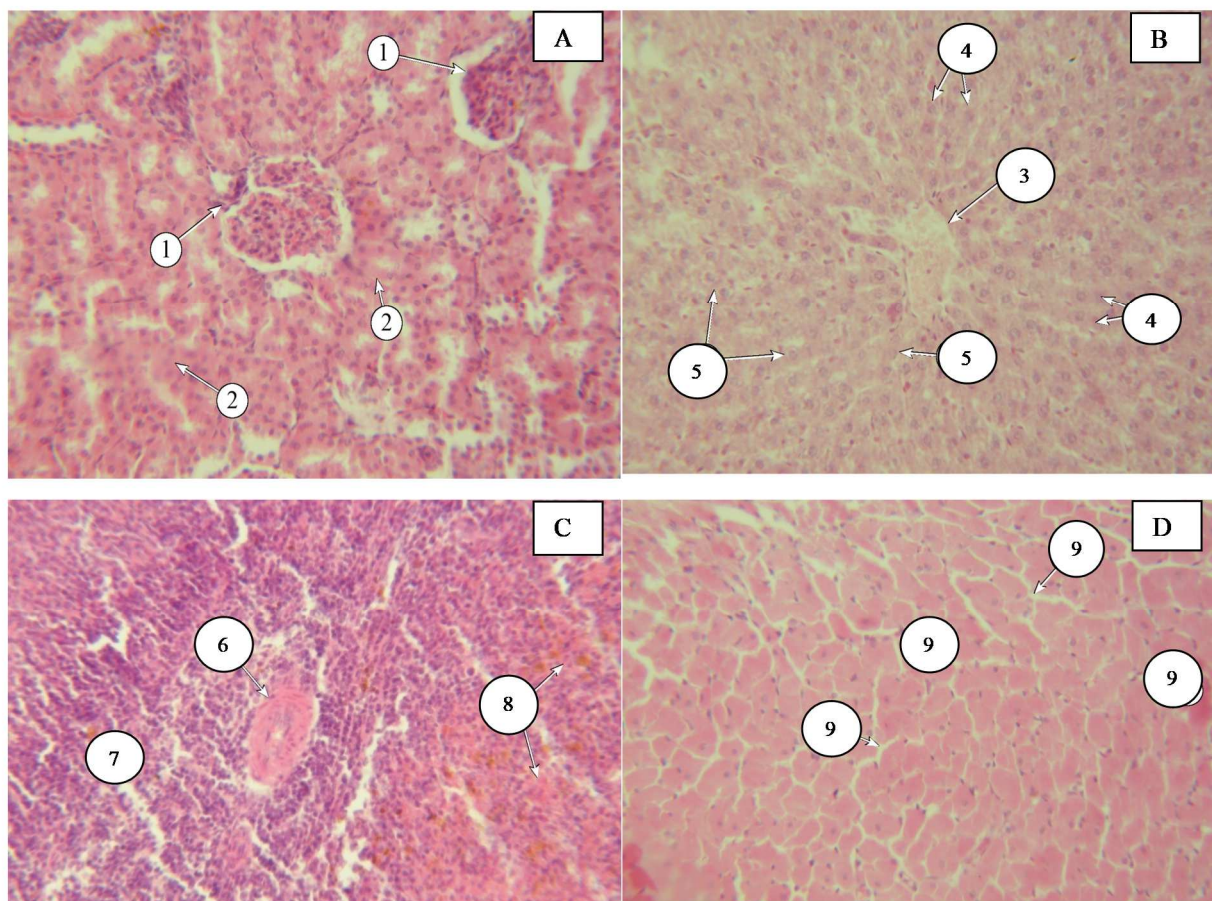


Fig. 1. Histological examination of tissues after 14 days: A - the kidney histological structure (1 – many cells vascular pole, 2 – single cleared epithelial cells of the tubules), B – histological structure of the liver (3 – the central vein of liver, 4 – hepatic plates, 5 – single burned-out hepatocytes), C – the histological structure of the spleen (6 – the central artery, 7 – lymph follicles, 8 – red pulp), D – histological structure of the heart (9 – cardiomyocytes). Staining: hematoxylin and eosin. Increase.: $\times 400$.

Morphological features of the liver (fig. 1 B) under these conditions are characterized by a slight impairment of the hepatic lobules protein structure. Notable alternative processes (signs of protein dystrophy, small vacuolar hydropic vacuolization and pyknosis of nuclei). There are hepatocytes with hypertrophied nuclei. Expansion of Disse's spaces and the plethora of the central veins were observed, sinusoid hemocapillaries having a sludge of erythrocytes in them. There is a moderate lympho-, macrophage infiltration.

The structuredness of lymphatic nodules is traced in the spleen (fig. 1 C). However, the boundaries between the periarterial and marginal zones of the periarteriolar sheath are sporadically erased. In lymph nodules the germinative center is small. Central arteries have narrow lumen and thickened walls. Trabecular vessels are plethoric.

In the study of histological heart sections (fig. 1 D), cross-striation was well pronounced in most fields of vision. A slight dissection of muscle bundles is only observed in the papillary muscles, here the sarcoplasm of cardiomyocytes is unevenly stained with eosin. Blood vessels of the same localization have the deformed wall. Thus, the performed study (table 1, 2 and Fig. 1) indicate that the ointment with the *Pinus sylvestris* L. extract refers to the V class of toxicity “Practically non-toxic”, as confirmed histologically. Our results are consistent with data obtained by other authors [3, 4 - 9], but in this aspect, our results do not coincide with them.

Conclusions

1. In oral administration of the ointment with pine extract in conventional doses any negative effects of their toxicity were not revealed.

2. The ointment with *Pinus sylvestris* extract was assessed according to the classification by K.K. Sidorov and belongs to the V class of toxicity – “practically non-toxic”.

3. The histological structure of internal organs confirms the nontoxicity of the ointment with the *Pinus sylvestris* extract.

References

1. Bazaka HYa, Dukhnytskyi VB, Ishchenko VD. Hostra toksychnist mospilanu dlya laboratornykh tvaryn. Biolohiya tvaryn. 2014; 16(3): 9-16. [in Ukrainian]
2. Kozymenko TM, Dudchenko LG, Hrabova TYu. Zastosuvannya roslyn klasu khvoyni u medytsyni. Rodyna sosnovi (Ohlyad literatury). Fitoterapiya. 2014; 2: 34-39. [in Ukrainian]
3. Marchyshyn SM, Yaroshenko II, Milyan. Vyvchennya hostroyi toksychnosti ta farmakolohichnoyi aktyvnosti sukhoho ekstraktu travy veroniky likarskoyi. Medychna ta klinichna khimiya. 2015; 17(4): 96-100. [in Ukrainian]
4. Marchyshyn SM, Zarichanska OB, Cholach SU. Doslidzhennya hostroyi toksychnosti ta neyrotropnykh vlastyovostey hustykh ekstraktiv kvitok liliynyka buro-zhovtoho (Hemerocallis Fulva L.) i liniynyka hibrydnoho (Hemerocallis hybrida Var. "Stella de Oro"). Farmatsevtichnyy chasopys. 2016; 1: 79-84. [in Ukrainian]
5. Marchyshyn SM, Yaroshenko TY, Milyan II, Nakonechna SS. Vyvchennya hostroyi toksychnosti ta farmakolohichnoyi aktyvnosti sukhoho ekstraktu travy veroniky likarskoyi. Medychna ta klinichna khimiya. 2015; 17(4): 69-100. [in Ukrainian]
6. Sidorov KK. O klassifikacii toksichnosti yadov pri parenteral'nykh sposobakh vvedeniya. 1973; 13: 45-71. [in Russian]
7. Khokhlova KA, Vyshnevskaya LI, Naboka AI. Farmakolohichne vyvchennya protyzapalnoyi aktyvnosti i hostroyi toksychnosti nastoyky skladnoyi «Aterofit-norma». Ukr. med. almanac. 2012; 15(5): 60 – 62. [in Ukrainian]
8. Shanaida MI, Oleshchuk AM. Vyvchennya hostroyi toksychnosti ridkoho ekstraktu travy chaberu sadovoho. Ukrayinskyy biofarmatsevtichnyy zhurnal. 2017; 4(51): 22 – 26. [in Ukrainian]
9. Shtroblya AL. Doslidzhennya hostroyi toksychnosti sukhoho ekstraktu z lystya abrykosa zvychnooho. Farmatsevtichnyy chasopys. 2015; 1: 105-108. [in Ukrainian]

Реферат

ВИЗНАЧЕННЯ ГОСТРОЇ ТОКСИЧНОСТІ МАЗІ
З ЕКСТРАКТОМ СОСНИ ЗВИЧАЙНОЇ

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Пошук нових біологічно-активних речовин серед лікарської рослинної сировини видів роду Сосна не викликає жодних сумнівів щодо її дослідження. Перспективність застосування сосни звичайної підтверджена багатьма публікаціями як вітчизняних, так і закордонних вчених. Після проведеного дослідження відомо, що отриманий сухий екстракт є практично нетоксичним та володіє при цьому широким спектром біологічної дії. Одним з основних пріоритетів впровадження нової лікарської форми є її незначна токсичність. Отже, цілеспрямована технологія з отримання мазі на основі сухого екстракту та орієнтоване вивчення гострої токсичності є першочерговими завданнями на шляху фармакологічних досліджень. Метою роботи було вивчення гострої токсичності мазі на основі сухого екстракту з сосни звичайної, яке було проведено вперше. Гостру токсичність вивчали на білих мишах при одноразовому пероральному застосуванні препарату. За мишами спостерігали протягом 14 днів. Було вивчено загальний стан тварин, смертність, динаміка маси тіла до і після експерименту. Було проведено макроскопічну оцінку стану внутрішніх органів і вивчено їх гістологічну структуру. Як показали дослідження, після прийому всередину мазі з екстрактом *Pinus sylvestris* в дозі 0,5 мл ознак токсичності у мишей не виявлено. Відповідно до класифікації К.К. Сидорова, мазь з екстрактом *Pinus sylvestris* належить до практично нетоксичних сполук. Таким чином, мазь з екстрактом *Pinus sylvestris* відноситься до п'ятого класу - «практично нетоксична при пероральному застосуванні».

Ключові слова: гостра токсичність, мазь з екстрактом *Pinus sylvestris*, макроскопічний аналіз внутрішніх органів.

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ОПРЕДЕЛЕНИЕ ОСТРОЙ ТОКСИЧНОСТИ МАЗИ
С ЭКСТРАКТОМ СОСНЫ ОБЫКНОВЕННОЙ

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Актуальность поиска новых биологически активных соединений среди лекарственного растительного сырья не вызывает никаких сомнений по ее исследованию. Перспективность применения сосны обыкновенной подтверждена многими публикациями как отечественных, так и иностранных ученых. После проведения исследования известно, что полученный сухой экстракт практически нетоксичный и обладает при этом широким спектром биологического действия. Одним из основных приоритетов внедрения новой лекарственной формы является ее незначительная токсичность. Итак, целенаправленная технология по получению мази на основе сухого экстракта и ориентированное изучение острой токсичности - первоочередные задачи на пути фармакологических исследований. Целью работы было изучение острой токсичности экстракта из сырья сосны обыкновенной. Острую токсичность изучали на белых мышах при однократном пероральном применении препарата. За мышами наблюдали в течение 14 дней. Было изучено общее состояние животных, смертность, динамика массы тела до и после эксперимента. Была проведена макроскопическая оценка состояния внутренних органов и изучена их гистологическая структура. Как показали исследования, после приема внутрь мази с экстрактом *Pinus sylvestris* в дозе 0,5 мл признаков токсичности у мышей не обнаружено. Согласно классификации К.К. Сидорова, мазь с экстрактом *Pinus sylvestris* относится к практически нетоксичным соединениям. Таким образом, мазь с экстрактом *Pinus sylvestris* относится к пятому классу - «практически нетоксична при пероральном применении».

Ключевые слова: острая токсичность, мазь с экстрактом *Pinus sylvestris*, макроскопический анализ внутренних органов.

Рецензент Єрошенко Г.А.