UDC/УДК 582.948.2:615.07
https://doi.org/10.30895/1991-2919-2025-15-6-701-711
Original article | Оригинальная статья

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Pyrrolizidine Alkaloids of *Boraginaceae* Family and Safety Assessment of Acute Toxicity

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ABSTRACT

INTRODUCTION. *Boraginaceae* plants *Pulmonaria mollis*, *Nonea rossica*, *Onosma simplicissima*, and *Cynoglossum officinale* are widespread over the Russian Federation and are promising sources of phytomedicines with important pharmacological properties, such as antimicrobial, antianaemic, anticoagulant etc. Currently, *Boraginaceae* are not classified as officinal plants, presumably due to pyrrolizidine alkaloids (PA) that can cause hepatotoxic effect.

AIM. This study aimed to assess safety using *Boraginaceae* plants based on acute toxicity and content of pyrrolizidine alkaloids.

MATERIALS AND METHODS. The study objects were dried herbs of *P. mollis, N. rossica, O. simplicissima,* and *C. officinale*, collected from flowering plants in Novosibirsk region over 2023-2024. The composition and amount of alkaloids in alcohol extracts were determined by high-performance liquid chromatography with diode array and mass-spectrometry detection with electrospray ionisation. Acute toxicity was tested *in vivo* in 102 mature male and female CD-1 mice weighing 24.0±2.0 g, aged 12 weeks, taken from Conventional Animal Vivarium of Scientific Center of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. The animals received a single dose of dried study extracts diluted in distilled water at 5 g/kg.

RESULTS. The absence of PA in *P. mollis* herb was established, alongside with its trace amounts (0.01 μg/g) in *P. mollis* leaves. In other studied plant species, PA were found, such as enantiomers of intermedine, lycopsamine and their derivatives: *O. simplicissima* herb – 1.07±0.03 μg/g, *N. rossica* herb – 8.25±0.08 μg/g; in *C. officinale* herb, PA content was significantly higher – 676.3±7.4 μg/g. Assessed acute toxicity made it possible to classify dry extracts from *P. mollis* herbs and leaves, *N. rossica* herb, and *O. simplicissima* herb as toxicity class 5, and *C. officinale* herb as toxicity class 4.

CONCLUSIONS. Study doses of extracts taken from herbs and leaves of *P. mollis* are non-toxic. For extracts from *O. simplicissima* and *N. rossica* herb, further research is relevant to determine toxicity in prolonged use. Extracts from *C. officinale* herb are toxic and cannot be used *per os*.

Keywords: *Pulmonaria mollis*; *Nonea rossica*; *Onosma simplicissima*; *Cynoglossum officinale*; HPLC; alkaloids; acute toxicity; safety; pyrrolizidines

For citation: Velichko V.V., Olennikov D.N., Oleshko E.D., Yershov K.I., Kruglov D.S., Koshlich K.A. Pyrrolizidine alkaloids of *Boraginaceae* family and safety assessment of acute toxicity. *Regulatory Research and Medicine Evaluation.* 2025;15(6):701–711. https://doi.org/10.30895/1991-2919-2025-15-6-701-711

Funding. The study was performed without external funding.
Disclosure. The authors declare no conflict of interest.

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Пирролизидиновые алкалоиды растений семейства *Boraginaceae* и оценка безопасности их применения по критерию острой токсичности

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РЕЗЮМЕ

ВВЕДЕНИЕ. Растения семейства бурачниковые (*Boraginaceae*) *Pulmonaria mollis*, *Nonea rossica*, *Onosma simplicissima* и *Cynoglossum officinale* широко распространены на территории Российской Федерации и могут являться источниками лекарственных средств, обладающих противомикробными, противоанемическими, антикоагулянтными свойствами. В настоящее время данные растения не являются официнальными, что может быть связано с содержанием в них пирролизидиновых алкалоидов, способных вызывать гепатотоксические эффекты.

ЦЕЛЬ. Оценка безопасности применения растений семейства *Boraginaceae* по критерию острой токсичности и содержанию пирролизидиновых алкалоидов.

МАТЕРИАЛЫ И МЕТОДЫ. В качестве объектов исследования использовали высушенные надземные части растений *Pulmonaria mollis*, *Nonea rossica*, *Onosma simplicissima* и *Cynoglossum officinale*, заготовленные в период цветения. Состав и содержание алкалоидов в спиртовых извлечениях определяли методом высокоэффективной жидкостной хроматографии с диодно-матричным и масс-спектрометрическим детектированием с ионизацией электрораспылением. Определение острой токсичности проводили на 102 половозрелых мышах обоего пола стока CD-1 весом 24,0±2,0 г в возрасте 12 нед., полученных из ЦКП «Виварий конвенциональных животных» ФГБНУ ФИЦ ИЦиГ СО РАН. Животным однократно внутрижелудочно вводили сухие экстракты изучаемых видов сырья, растворенные в дистиллированной воде, в дозе 5 г/кг.

РЕЗУЛЬТАТЫ. Установлено отсутствие пирролизидиновых алкалоидов в траве *P. mollis*, наличие их следовых количеств (0,01 мкг/г) в листьях *P. mollis*. В других изученных видах растений обнаружены пирролизидиновые алкалоиды — энантиомеры интермедина и ликопсамина и их производные: в траве *O. simplicissima* — 1,07±0,03 мкг/г, в траве *N. rossica* — 8,25±0,08 мкг/г, в траве *C. officinale* — 676,3±7,4 мкг/г. По результатам оценки острой токсичности сухие экстракты из травы и листьев *P. mollis*, травы *N. rossica* и травы *O. simplicissima* отнесены к 5 классу токсичности, экстракт из травы *C. officinale* — к 4 классу токсичности.

ВЫВОДЫ. Извлечения из травы и листьев *P. mollis* в исследуемых дозах являются нетоксичными, для извлечений из травы *O. simplicissima* и *N. rossica* необходимо проведение дальнейших исследований для определения токсичности при длительном применении. Извлечения из травы *C. officinale* токсичны и не могут использоваться для внутреннего применения.

Ключевые слова: медуница мягкая; нонея русская; оносма простейшая; чернокорень лекарственный; *Pulmonaria mollis*; *Nonea rossica*; *Onosma simplicissima*; *Cynoglossum officinale*; ВЭЖХ; алкалоиды; острая токсичность; безопасность; пирролизидины

Для цитирования: Величко В.В., Оленников Д.Н., Олешко Е.Д., Ершов К.И., Круглов Д.С., Кошлич К.А. Пирролизидиновые алкалоиды растений семейства *Boraginaceae* и оценка безопасности их применения по критерию острой токсичности. *Регуляторные исследования и экспертиза лекарственных средств*. 2025;15(6):701–711. https://doi.org/10.30895/1991-2919-2025-15-6-701-711

Финансирование. Работа выполнена без спонсорской поддержки.
Потенциальный конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

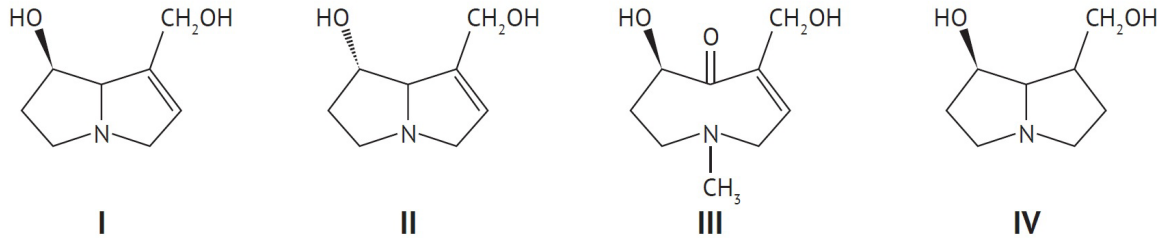
INTRODUCTION

As reported by Angiosperm Phylogeny Group IV (APG IV) [1], *Boraginaceae* family comprises about 90 genera and approximately 1,700 plant species globally, mostly distributed in Europe and Asia. Members of this family contain bioactive compounds such as naphthoquinones, flavonoids, terpenoids, phenylpropanoids, etc. [1–3] that exhibit diverse pharmacological effects, including antibacterial, antiviral, anti-inflammatory, and anti-platelet properties [4, 5]. Research on *Boraginaceae* species is relevant owing to their potential applications in pharmacology and cosmetology.

Several tribes of the *Boraginaceae* family are native to Western Siberia, providing adequate raw material supplies [6]. Notably, some widespread representatives are *Pulmonaria mollis* Wulfen ex Hornem. and *Nonea rossica* Steven from the *Borageineae* tribe, *Onosma simplicissima* L. from the *Lithospermeae* tribe, and *Cynoglossum officinale* L. from the *Cynoglossinae* tribe [7, 8]. *Pulmonaria* is used in the alternative and evidence-based medicine of various countries due to their anti-inflammatory and expectorant properties [9]; *Nonea* plants exert anticoagulant [10], anti-inflammatory, antibacterial, and antifungal effect and inhibit acetylcholinesterase [11]. *Onosma* plants have anti-inflammatory, antibacterial, and antioxidant potential [12]. *C. officinale* has been widely used in traditional medicine: root extracts are more often applied topically as a hemostatic, antibacterial, analgetic, and anti-inflammatory agent; herbal infusions or tinctures are used in gastrointestinal diseases, cough, blood expectoration, purulent infections, and muscle seizures. Homeopathy uses an extract from the fresh flowers against diarrhea, seizures, and joint pains [10, 13].

However, *Boraginaceae* plants typically accumulate pyrrolizidine alkaloids (PAs), known for their potential hepatotoxic effects [10]. Chemical classification divides PAs into four main groups: retronecine (I), heliotridine (II), otonecine (III), and platynecine (IV). Different species exhibit variable compositions and contents of these alkaloids [14], affecting their toxicity profiles.

Since alkaloids were found in *C. officinale*, all plant parts have been prohibited within food supplements[1]. Cases were reported where agricultural animals died after consuming *C. officinale* [15], due to the high content of heliotridine PAs in various parts of the plant [10, 16–18]. However, *Borago officinalis* L. herb is cultivated in certain countries as a vegetable crop and is widely consumed by humans [19].



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[1] Recommendation No. 33 of the Eurasian Economic Commission Board dated November 14, 2023 'On the Guidelines for Handling Laboratory (Experimental) Animals in Preclinical (Non-Clinical) Studies'; Decision No. 81 of the Eurasian Economic Commission's Council dated November 3, 2016 'On Approval of Good Laboratory Practices of the Eurasian Economic Union Regarding Drug Circulation'; World Medical Association's Declaration of Helsinki regarding animal welfare, 1996.

Although currently not recognised as official species in the Russian Federation, phytochemical tests of *P. mollis*, *N. rossica*, *O. simplicissima*, and *C. officinale* [4, 20] have revealed biological significance of their metabolites and defined their pharmacological properties [5]. Preliminary screening of the chosen plants suggests their high potential in the development of novel phytopharmaceuticals [21]. Nonetheless, prior toxicological assessments are warranted because of the typical presence of hepatotoxic PAs [22].

The aim of the study is to assess safety using *Boraginaceae* plants based on acute toxicity and content of pyrrolizidine alkaloids.

MATERIALS AND METHODS

Materials

Raw materials. Dried aerial parts of *P. mollis, N. rossica, O. simplicissima*, and *C. officinale* harvested from flowering and growing plants in Novosibirsk region (Russia) in 2023–2024 were selected as the study object *(Table 1)*.

Extraction. Extract preparation satisfied the pre-established process parameters [23, 24]. The raw materials were finely ground (particle size ⩽3 mm), placed in a flask and mixed with 70% ethanol (1:20 w/v). The vial with the reflux condenser was heated in a water bath at 55 °C for 60 min and subsequently cooled by stirring for another 60 min before filtration. The cooled extract was filtered, the solvent evaporated by convective drying at 30–40 °C.

Reference standards. Reagents supplied by *BioCrick Co., Ltd. (China)* included: 7-acetyllycopsamine (catalogue No. BCN2000, purity ⩾98%); N-oxide of 7-O-acetyllycopsamine (catalogue No. BCN8935, purity ⩾98%); reagents from Sigma-Aldrich catalogue: lycopsamine (catalogue No. PHL89726, purity ⩾95%); lycopsamine N-oxide (catalogue No. PHL83447, purity ⩾90%); reagents from *MedChemExpress (USA)* catalogue: intermedine (catalogue No. HY-113845, purity ⩾99.0%); intermedine N-oxide (catalogue No. HY-W707957, purity ⩾99.0%); reagents from *ChemFaces (China)*: viridiflorine N-oxide (catalogue No. CFN00337, purity ⩾98.0%); 7'-acetylintermedine (catalogue No. CFN00286, purity ⩾98.0%); 7'-acetylintermedine N-oxide (catalogue No. CFN00518, purity ⩾98.0%).

Experimental animals. Acute toxicity tests were performed on 102 adult CD-1 mice (males: 25±2 g; females: 23±2 g; 12 weeks old), sourced from the Conventional Animal Vivarium, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (Novosibirsk). The animals were held under standardised conditions, with *ad libitum* access

Table 1. *Herbal raw material sources*

Herbal raw material	Date of collection, phenophase	Place of growth		
		Description	Coordinates	
			Latitude	Longitude
Pulmonaria mollis herb	15.05.2024 flowering	Novosibirsk region, Vengerovo district, surroundings of Kozlovka village, birch grove	55°60'	76°94'
Pulmonaria mollis leaves	20.07.2024 vegetation	Novosibirsk region, Vengerovo district, surroundings of Kozlovka village, birch grove	55°60'	76°94'
Nonea rossica herb	18.07.2023 flowering	Novosibirsk region, Kolyvan district, surroundings of Vorobyevo village, steppe meadow	55°31'	82°57'
Onosma simplicissima herb	28.06.2023 flowering	Novosibirsk region, Iskitim district, 6.5 km to the east of Lozhok railway station, rocky slope of the Shipunikha river	54°34'	83°21'
Cynoglossum officinale herb	28.06.2023 flowering	Novosibirsk region, Iskitim district, 6.5 km to the east of Lozhok railway station, country roadside	54°34'	83°21'

The table was prepared by the authors using their own data

to food and water. All experimental protocols complied with the standards defined by current guidelines that regulate care and use of laboratory animals. Protocol No. 162 dated November 28, 2024 was approved by the Ethics Committee of Novosibirsk State Medical University.

Methods

Chromatographic analysis. Composition and content of alkaloids in the extracts were determined using high-performance liquid chromatography coupled with mass spectrometry with electrospray ionization (HPLC-ESI-MS). Analyses were carried out on a chromatograph–mass spectrometer system comprising an HPLC unit (LC-20 Prominence, Shimadzu, USA), a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, USA), and a column (ReproSil-Pur 120 C18-AQ, 250×4.6 mm×5 μm, Dr. Maisch GmbH, Germany).

HPLC conditions: mobile phase, eluent A (water) and eluent B (acetonitrile). Gradient program: 0–20 min (2–80% B), 20–30 min (80–100% B), 30–35 min (100% B), 35–40 min (100–2% B). Injection volume: 1 μL; flow rate: 1 mL/min; column temperature: 30 °C.

ESI-MS conditions: Positive ionization mode, electrospray; interface temperature: 300 °C; desolvation line temperature: 250 °C; heating block temperature: 400 °C; nebulizer gas (nitrogen) flow rate: 3 L/min; heater gas (air) flow rate: 10 L/min; collision-induced dissociation gas pressure: 270 kPa; argon flow rate: 0.3 mL/min; capillary voltage: 3 kV; scan range (m/z): 100–1,900.

Quantitative analysis: Individual compounds were quantified following previously described methods [25]. Reference solutions (1–100 μg/mL) of substances (lycopsamine, lycopsamine N-oxide, 7'-acetyl-lycopsamine, 7'-acetyl-lycopsamine N-oxide, intermedine, intermedine N-oxide, 7'-acetyl-intermedine, 7'-acetyl-intermedine N-oxide, viridiflorine N-oxide) were chromatographed three times under the above conditions. Calibration curves correlating compound concentrations with peak areas corresponding to selected ions ([M+H]+) in the mass spectrum (see *Table 2*) were plotted using Advanced Grapher v.2.2 (Alentum Software, Inc., USA). Data were presented as the mean of five replicates.

For quantifying 3,7'-diacetyl-intermedine N-oxide and 3,7'-diacetyl-lycopsamine N-oxide, external calibrators (7'-acetyl-intermedine N-oxide and 7'-acetyl-lycopsamine N-oxide) were used, accounting for molecular mass differences between PA di- and monoacetates via a correction factor (k = 1.117)

Acute toxicity. The study objects in the acute toxicity test included dry extracts from *P. mollis* leaves and herb, as well as *N. rossica*, *O. simplicissima*, and *C. officinale* herbs.

Laboratory animals were divided into 10 groups (six animals per each group) by age, sex, body weight, and randomisation; for probit analysis, seven groups of animals were formed, six animals per each group. The extracts were dosed in mg/kg body weight of the experimental animals. A single dose of the dry extract was administered intragastrically, with the maximum possible dose of 5 mg per kg of animal body weight. The dry extracts were dissolved in the distilled water to obtain 0.6 g/mL; administration volume was 0.17 mL per 20 g of animal body weight.

When evaluating acute toxicity of dry extracts from leaves and herbs of *P. mollis*, *N. rossica*, *O. simplicissima*, and *C. officinale* herbs, general welfare of animals (behavior, appearance, motor performance, food and water intake, response to external stimuli, and weight), clinical symptoms of intoxication, and possible death were considered[2]. Continuous surveillance for the first signs of toxicity was performed for 8 h following the administration. The follow-up was performed daily for 14 days considering normal circadian rhythm of the animals. Acute toxicity was assessed by general welfare and appearance of mice, body weight on Day 14 after administration, the number of surviving and dead animals, and life span.

For animal deaths, additional studies were performed using probit analysis in StatPlus software; major lethal doses, LD_{10}, LD_{50}, LD_{90}

[2] Mironov AN. Guidelines for Conducting Preclinical Studies of Drugs. 2012.

[26], and pathomorphological examinations of internal organs were performed.

Histopathological examination of liver preparations from the dead animals was performed using Nikon Eclipse 200 microscope (Japan) and Levenhuk 1400M camera (US), with ×10–40 magnification. The preparations were stained by standard hematoxylin and eosin method.

Statistical methods. Descriptive statistics (Mean±Standard Deviation, M±SD) was performed using Statsoft Statistica 10.0.1011 software. Normal distribution of quantitative data was tested using Shapiro–Wilk test.

RESULTS AND DISCUSSION

Ethanol extracts from *N. rossica*, *O. simplicissima*, and *C. officinale* contained PAs (*Table 2*) detected by chromatographic separation. Their compositions and contents varied significantly depending on the study object. Reproductive shoots of *P. mollis* lacked detectable alkaloids, although the traces were present in leaf extracts.

Notably, *N. rossica* exhibited the greatest diversity of alkaloids, up to 8.25±0.8 μg/g of air-dry raw materials detected in the herb. *N. rossica* aerial parts predominantly contained intermedine and lycopsamine enantiomers along with their derivatives. Alongside with lycopsamine, the aerial part of *O. simplicissima* additionally contained viridiflorine N-oxide, contributing to a total PA content of 1.07±0.03 μg/g. The highest PA content was detected in *C. officinale* herb (676.3±7.4 μg/g). Chromatography results of the compounds are described in *Table 2*.

Lycopsamine shows antitumor effect in lung cancer; its antiproliferative activity is caused by its autophagic, apoptotic ability and inhibition of IL-2 [27]. Given the substantial amount

Table 2. *Quantitative composition of pyrrolizidine alkaloids in herbs of certain plants*

Compounds	[M+H]+	Identification level*	Content of pyrrolizidine alkaloids, μg/g of air-dried raw material (M±SD)				
			Pulmonaria mollis		*Onosma simplicissima* herb	*Nonea rossica* herb	*Cynoglossum officinale* herb
			herb	leaves			
Intermedine	300	1	–	–	–	0.34±0.01	35.64±0.70
Lycopsamine	300	1	–	–	0.25±0.01	0.19±0.01	22.83±0.44
Viridiflorine *N*-oxide	302	1	–	–	0.07±0.01	–	3.29±0.07
Intermedine *N*-oxide	316	1	–	–	–	2.52±0.05	206.04±4.33
Lycopsamine *N*-oxide	316	1	–	0.01±0.01	0.74±0.02	2.20±0.04	285.39±5.73
7′-Acetyl-intermedine	342	1	–	–	–	0.04±0.01	1.67±0.03
7′-Acetyl-lycopsamine	342	1	–	–	0.01±0.01	0.03±0.01	8.22±0.17
7′-Acetyl-intermedine *N*-oxide	358	1	–	–	–	1.48±0.03	54.67±1.08
7′-Acetyl-lycopsamine *N*-oxide	358	1	–	–	–	1.45±0.03	55.28±1.14
3,7′-Diacetyl-intermedine *N*-oxide	400	2	–	–	–	< 0.01	2.32±0.04
3,7′-Diacetyl-lycopsamine *N*-oxide	400	2	–	–	–	< 0.01	0.93±0.02
		Total content	–	0.01±0.01	1.07±0.03	8.25±0.08	676.3±7.4

The table was prepared by the authors using their own data

Notes. *–, not found.*
** 1, compound identified after UV analysis and mass spectrometry in comparison with the reference compound spectra registered in the same conditions; 2, compound identified by comparison of UV and mass spectra with the reference database.*

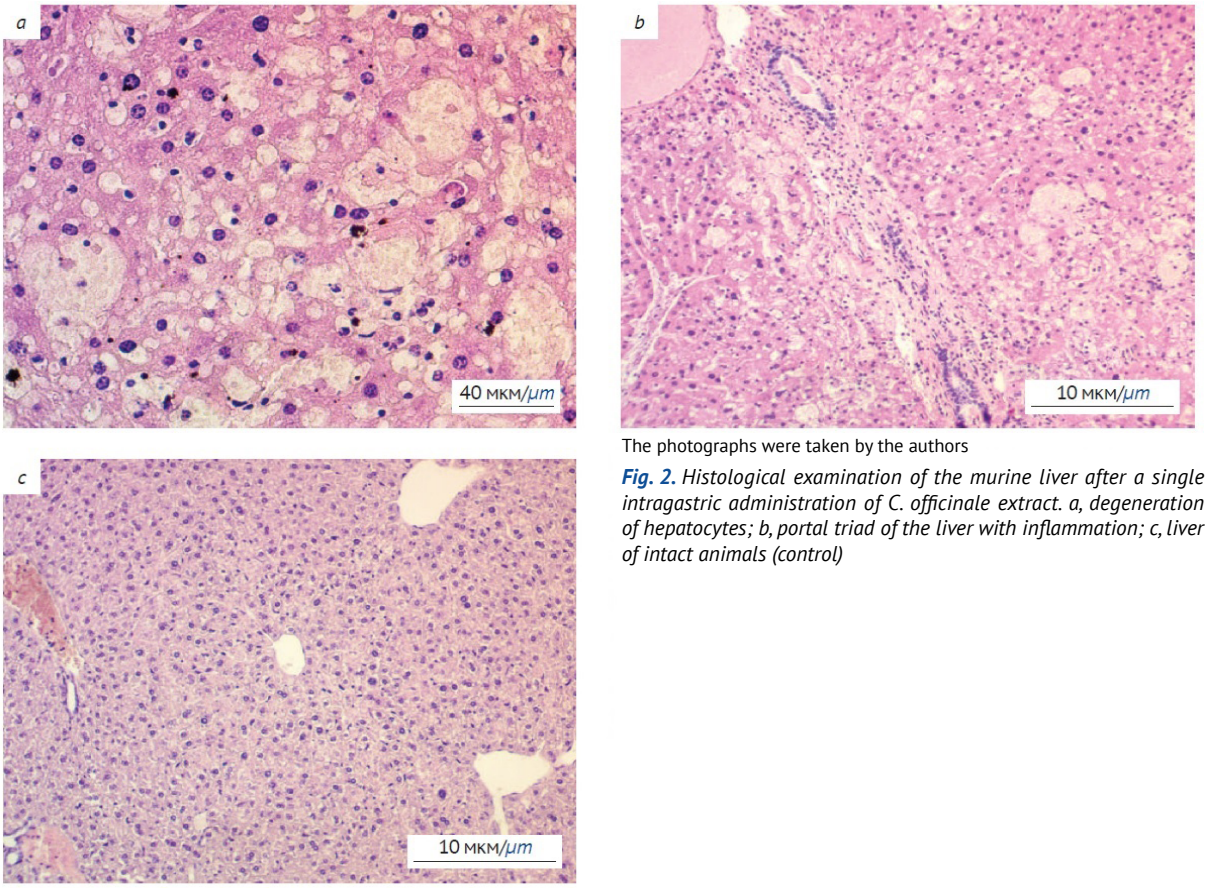
of lycopsamine derivatives in *C. officinale* herb, isolating and evaluating this compound for antiproliferative potential is warranted.

P. mollis does not accumulate alkaloids in its reproductive shoots, while the leaves contain minor content (<0.01 μg/g per 1 g air-dry materials). This finding supports the hypothesis of low toxicity for these extracts. To confirm this hypothesis, subsequent acute toxicity trials were conducted *in vivo* for all raw materials studied.

Pyrrolizidine alkaloids typically induce hepatotoxicity upon chronic exposure and acute intoxication for high doses [28, 29]. In this study, animals received extremely high doses (5,000 mg/kg) intragastrically. Despite this extreme challenge, no deaths occurred among animals treated with extracts from *P. mollis*, *O. simplicissima*, and *N. rossica*.

Most PAs develop hepatotoxicity following their chronic use or acute intoxication following high-dose administration [28, 29]. The experimental animals were administered the extracts intragastrically at high doses (5,000 mg/kg). Zero lethality in animals that were administered *P. mollis*, *O. simplicissima* and *N. rossica* extracts *(Table 3. Change in body weight of mice (g) after single intragastric aministration of certain herbs, X±m* is published on the Journal website[3]) did not allow determining the acute toxicity parameters by probit analysis (LD_{10}, LD_{50}, LD_{90}). The data confirm the safety of these extracts at the above doses.

Since no deaths were registered at the maximum allowable dose, LD_{50} for *P. mollis*, *O. simplicissima* and *N. rossica* dry extracts should be at least 5 g/kg, allowing to classify these extracts as toxicity class 5 (low toxicity), according to current standards[4]. Further studies of subchronic toxicity are warranted that would allow identifying possible dysfunctions of the extract administration.



The photographs were taken by the authors

Fig. 2. *Histological examination of the murine liver after a single intragastric administration of C. officinale extract. a, degeneration of hepatocytes; b, portal triad of the liver with inflammation; c, liver of intact animals (control)*

[3] https://doi.org/10.30895/1991-2919-2025-15-6-701-711-table
[4] GOST 32644-2014. Test methods for the effects of chemical products on the human. Acute oral toxicity — Acute Toxic Class Method.

All animals receiving *C. officinale* herbal extract intragastrically died within the first three days, followed by autopsy and histological sampling.

Morphology of the internal organs showed changes in gastrointestinal tract: flatulent (distended) stomach; pancreas with the signs of acute inflammation, dark brown with small separate necrotic and steatotic foci; liver insignificantly enlarged, of uneven colour, with large light- and dark-brown foci, and ambiguous boundaries; other organs without apparent changes.

Histological liver examination *(Figure 2)* showed signs of damage: centrilobular hepatopathy with massive hemorrhages and expanded plethoric liver sinusoids. Structural morphological changes include hepatic steatosis (*Figure 2a*) and mononuclear (inflammatory) infiltration in the portal triad (*Figure 2b*). *Figure 2c* shows intact liver.

For *C. officinale* herbal extract, dose range that resulted in increasing lethality up to the death of all animals was 2.75 to 4.25 g/kg. Within this range, the animals were administered the studied extracts at intervals of 0.25 mg/g to find out the number of deaths per dose (*Table 4. Animal mortality results after intragastric admninistration of C. officinale extract* is published on the Journal website[5]).

Based on probit analysis, various levels of lethal doses were calculated for 70% *C. officinale* alcohol extract; the 50% lethal dose (LD_{50}) was estimated as 3.51±0.13 g/kg, classifying *C. officinale* herbal extract as toxicity Class 4, according to current standards.

Overall, toxicity of *C. officinale* extract is likely caused by PAs and their derivatives metabolised into the relevant pyrroles [30] that are highly reactive. The pyrroles break the DNA replication, resulting in mutations that cause liver cancer [31, 32].

Some countries monitor and ration PA contents in the products and phytomedicines due to the increasing risk of chronic liver diseases [33]. For allowable PA consumption 1 μg/kg [34], maximum allowable PA dose will reach 70 μg/day.

The findings show that *P. mollis* herb and leaves extraction are non-toxic at the study doses for oral administration. *O. simplicissima* and *N. rossica* herbal extracts warrant further studies to define long-term toxicity, while *C. officinale* herbal extracts are toxic and therefore prohibited for internal use.

CONCLUSIONS

HPLC test has confirmed PA absence in *P. mollis* herb, with trace amounts (~0.01 μg/g) detected only in the leaves. No observable hepatotoxicity occurred when *P. mollis* herb and leaves were administered in mice at high doses (5 g/kg).

Conversely, *O. simplicissima* and *N. rossica* exhibited 1.07±0.03 μg/g and 8.25±0.08 μg/g, respectively, whereas *C. officinale* displayed substantially higher concentrations (676.3±7.4 μg/g).

Different PA compositions and contents triggered the toxic effect after a single intragastric administration of the study extracts in mice. Extracts from *P. mollis* herb and leaves, *N. rossica* herb and *O. simplicissima* herb are marked as toxicity class V (low-toxic substances), *C. officinale* herb – as toxicity class 4[6].

These findings underscore the heterogeneity of PA distribution within the study plants of the *Boraginaceae* family, thus suggesting different treatment prospectives. Specifically, *P. mollis* herb and leaves do not cause any symptoms of acute toxicity following a single administration in laboratory animals. Use of herbal extracts from *O. simplicissima* and *N. rossica* warrants further toxicity studies for long-term oral administration. Finally, *C. officinale* herbal preparations are toxic and should not be used internally.

[5] https://doi.org/10.30895/1991-2919-2025-15-6-701-711-table
[6] GOST 32644-2014. Test methods for the effects of chemical products on the human. Acute oral toxicity — Acute Toxic Class Method.

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Additional information. Tables 3, 4 are published on the website of *Regulatory Research and Medicine Evaluation*.
https://doi.org/10.30895/1991-2919-2025-15-6-701-711-table

Authors' contributions. All the authors confirm that they meet the ICMJE criteria for authorship. The most significant contributions were as follows. *Viktoriya V. Velichko* developed research concept and design, drafted the manuscript, formulated conclusions, and finally approved the manuscript for publication. *Daniil N. Olennikov* obtained and analysed chemical composition, interpreted them and corrected the manuscript. *Egor D. Oleshko* prepared the samples and obtained experimental data. *Konstantin I. Ershov* conducted animal experiments, processed and interpreted statistical data. *Dmitry S. Kruglov* statistically processed experimental data, worked with literature sources, and critically revised the manuscript. *Ksenia A. Koshlich* performed morphological and histochemical study and interpreted the data.

Ethics approval. The research was approved at a meeting of Ethics Committee, Novosibirsk State Medical University (Meeting Minutes No. 162 of 11 November 2024).

Дополнительная информация. Таблицы 3, 4 размещены на сайте журнала «Регуляторные исследования и экспертиза лекарственных средств».
https://doi.org/10.30895/1991-2919-2025-15-6-701-711-table

Вклад авторов. Все авторы подтверждают соответствие своего авторства критериям ICMJE. Вклад авторов распределен следующим образом: *В.В. Величко* — концепция и дизайн исследования, написание текста рукописи, формулировка выводов,утверждение окончательной версии статьи для публикации; *Д.Н. Оленников* — получение, анализ данных химического состава, их интерпретация, редактирование текста рукописи; *Е.Д. Олешко* — пробоподготовка, получение экспериментальных данных; *К.И. Ершов* — проведение экспериментов с животными, статистическая обработка и интерпретация экспериментальных данных; *Д.С. Круглов* — статистическая обработка экспериментальных данных, работа с источниками литературы, критический пересмотр и доработка текста рукописи; *К.А. Кошлич* — морфологическое и гистохимическое исследование, интерпретация данных.

Соответствие принципам этики. Исследование было одобрено на заседании Комитета по этике Новосибирского государственного медицинского университета (протокол № 162 от 28.11.2024).

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Received 23 June 2025
Revised 20 August 2025
Accepted 15 October 2025

Поступила 23.06.2025
После доработки 20.08.2025
Принята к публикации 15.10.2025