Pharmacological study of thick extracts from the aerial parts of Valeriana species

V. I. Kokitko[B,C,D], V. M. Odyntsova[A,E,F]

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aerial parts of *Valeriana collina* and *Valeriana stolonifera* are considered a promising source of bioactive compounds with antimicrobial and hepatoprotective properties; however, their pharmacological characteristics have not been sufficiently studied.

The aim of the work. To comprehensively evaluate the antimicrobial / antifungal potential, acute toxicity, and hepatoprotective activity of thick extracts from the herbs of *V. collina* and *V. stolonifera*.

Materials and methods. Antimicrobial activity was determined by the agar diffusion method (well method). Acute toxicity was evaluated in Wistar rats according to OECD guidelines. Hepatoprotective activity was studied in a paracetamol-induced hepatitis model by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels; silymarin was used as the reference drug.

Results. Both extracts exhibited a broad spectrum of antimicrobial activity; *V. stolonifera* was more effective against *P. aeruginosa* ($p = 0.0113$), while *V. collina* showed higher activity against *C. albicans* ($p = 0.0080$). Both samples were classified as low-toxicity (GHS Category 5). In the hepatitis model, *V. stolonifera* significantly reduced ALT, AST, and ALP levels, being not inferior – and in the case of ALP, superior – to silymarin.

Conclusions. Both thick extracts of *V. collina* and *V. stolonifera* demonstrated pronounced antimicrobial activity against gram-positive and gram-negative bacteria as well as *C. albicans*. *V. stolonifera* was more effective against *P. aeruginosa*, whereas *V. collina* showed higher activity against *C. albicans*. These differences may be associated with variations in the ratios of biologically active compounds within the extracts, supporting the need for further chemical and pharmacological studies to elucidate mechanisms of action. A 20 % aqueous solution of the thick extract of *V. collina* administered intragastrically can be classified as toxicity class 5, with an LD_{50} ranging from 2000–5000 mg/kg. The solution of the thick extract of *V. stolonifera* caused no mortality in rats, classifying it as a substance with low acute toxicity (GHS Category 5, LD_{50} ≥5000 mg/kg). Further testing of the studied samples is not recommended unless specifically required for regulatory purposes. The studied valerian extracts, especially *V. stolonifera*, exhibited an expressed hepatoprotective effect in a paracetamol-induced hepatitis model in rats. The paracetamol-induced hepatitis led to marked biochemical and morphological signs of liver damage in rats. The *V. stolonifera* extract demonstrated a clear hepatoprotective effect, evidenced by reduced ALT, AST, and ALP levels and a decrease in the necrotic area of the liver. The effectiveness of the *V. stolonifera* extract exceeded that of the reference drug, silymarin.

Keywords: Valeriana collina, Valeriana stolonifera, Valeriana officinalis, aerial plant material, antimicrobial activity, antifungal activity, hepatoprotective activity, acute oral toxicity, paracetamol-induced hepatitis.

Current issues in pharmacy and medicine: science and practice. 2026;19(1):5-12

Фармакологічне дослідження густих екстрактів з надземної частини представників роду Valeriana

В. І. Кокітко, В. М. Одинцова

Надземні частини *Valeriana collina* та *Valeriana stolonifera* є перспективним джерелом біоактивних сполук з антимікробною та гепатопротекторною діями, однак їх фармакологічні властивості вивчені недостатньо.

Мета роботи – комплексно оцінити протимікробний / протигрибковий потенціал, гостру токсичність і гепатопротекторну активність густих екстрактів із трави *V. collina* та *V. stolonifera.*

Матеріали і методи. Антимікробну активність визначено методом дифузії в агарі (метод лунок). Гостру токсичність оцінювали у щурів лінії Wistar за OECD. Гепатопротекторну дію досліджено на моделі парацетамолового гепатиту з визначенням аланінамінотрансферази (АЛТ), аспартатамінотрансферази (АСТ), лужної фосфатази (ЛФ); силімарин – препарат порівняння.

ARTICLE INFO

UDC 615.322:582.971.3:615.451.1.015.11
DOI: 10.14739/2409-2932.2026.1.342449

Current issues in pharmacy and medicine: science and practice. 2026;19(1):5-12

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Received: 28.10.2025 // Revised: 26.11.2025 // Accepted: 17.12.2025

Результати. Обидва екстракти характеризуються широким спектром антимікробної активності; *V. stolonifera* ефективніша проти *P. aeruginosa* (p = 0,0113), а *V. collina* – проти *C. albicans* (p = 0,0080). Обидва зразки належать до малотоксичних (GHS Category 5). На моделі гепатиту *V. stolonifera* достовірно знижувала АЛТ, АСТ і ЛФ, не поступаючись за ефективністю препарату порівняння силімарину, а за ЛФ навіть перевищуючи його.

Висновки. Обидва зразки густих екстрактів *V. collina* та *V. stolonifera* мали виражену антимікробну активність щодо грампозитивних і грамнегативних бактерій, а також *C. albicans*. Встановлено, що *V. stolonifera* ефективніша проти *P. aeruginosa*, а *V. collina* мала вищу активність щодо *C. albicans*. Ці відмінності можуть бути пов'язані з різним співвідношенням біологічно активних сполук у складі екстрактів, що підтверджує доцільність наступних хімічних і фармакологічних досліджень для з'ясування механізмів дії. Водний розчин 20 % густого екстракту *V. collina* при внутрішньошлунковому введенні визначено до 5 класу токсичності з рівнем $ЛД_{50}$ у межах 2000–5000 мг/кг. Розчин густого екстракту *V. stolonifera* не спричинив смертності у щурів, і тому його класифіковано як речовину з низькою гострою токсичністю (GHS Category 5 $ЛД_{50}$ ≥5000 мг/кг). Подальше тестування зразків не рекомендоване без специфічних регуляторних потреб. Досліджені екстракти валеріани, особливо *V. stolonifera*, мали виражену гепатопротекторну дію в умовах парацетамолового гепатиту у щурів. Моделювання парацетамолового гепатиту спричинило розвиток виражених біохімічних і морфологічних ознак ураження печінки у щурів. Екстракт *V. stolonifera* мав виражену гепатопротекторну дію, що підтверджується зниженням рівня АЛТ, АСТ, ЛФ і зменшенням зони некрозу в печінці. Ефективність екстракту *V. stolonifera* вища за ефективність препарату порівняння силімарину.

Ключові слова: Valeriana collina, Valeriana stolonifera, Valeriana officinalis, надземна частина рослини, антимікробна активність, протигрибкова активність, гепатопротекторна активність, гостра токсичність, парацетамолом індукований гепатит.

Актуальні питання фармацевтичної і медичної науки та практики. 2026. Т. 19, № 1(50). С. 5-12

Valeriana collina and *Valeriana stolonifera* are members of the *Valerianaceae* family, known for their medicinal properties and wide geographical distribution. The pharmacological study of liquid and thick extracts of these species aims to explore their therapeutic properties and the composition of their bioactive compounds. Both species have a long history of medical use as natural sedative and anxiolytic agents. They are widespread in Europe and Asia, grow under diverse ecological conditions, and form an integral part of traditional medicine, where they are used to treat insomnia, anxiety disorders, rheumatic pain, and gastrointestinal disturbances [1,2].

Recent studies emphasize the rich phytochemical composition of *V. collina* and *V. stolonifera*, which includes iridoids, flavonoids, alkaloids, and essential oils – each of these classes of compounds making a significant contribution to the pharmacological effects of the plants [3,4]. Iridoids exhibit cytotoxic activity against tumor cells, while flavonoids are characterized by neuroprotective and sedative properties [5].

Despite centuries of use, the efficacy of valerian extracts in various pathological conditions remains a subject of debate. Systematic reviews highlight the need for more detailed clinical studies to confirm their therapeutic potential [6]. Reports of adverse effects – headache, nausea, and gastrointestinal discomfort – indicate the necessity of further safety and long-term exposure studies of valerian preparations [7,8].

Additional attention has been drawn to the potential contamination of medicinal plant material with heavy metals, especially in regions adjacent to industrial areas, necessitating strict quality and safety control [2,9].

Overall, the study of *V. collina* and *V. stolonifera* underscores their significance in phytotherapy. Modern pharmacognostic and pharmacological research focuses on elucidating the mechanisms of action of active substances and optimizing extraction technologies for their application in contemporary medicine.

Traditionally, pharmacognosy has focused primarily on the underground parts of *V. officinalis* and related species officially recognized as medicinal raw materials. However, the aerial parts of *V. collina* and *V. stolonifera* remain insufficiently studied, despite their high biomass and significant content of biologically active compounds – primarily flavonoids and phenolic acids. This opens new prospects for using valerian herb as a novel source of raw material for obtaining extracts with a broad spectrum of pharmacological activity.

Harvesting the aerial part is technologically simpler and less labor-intensive than collecting rhizomes, aligning with the principles of sustainable resource use, as it contributes to the preservation of plant populations and biodiversity. Therefore, studying valerian herb is not only of scientific but also of practical importance – particularly for expanding the raw material base of the domestic pharmaceutical industry.

Species of the genus *Valeriana*, particularly *V. collina* and *V. stolonifera*, are widely distributed throughout Europe and Asia, occurring in a variety of habitats – from meadows and forest clearings to woodland edges and hillside slopes [2]. Their broad geographical range is determined by high ecological plasticity and the ability to adapt to different soil types and climatic conditions.

Within Ukraine, these species are found mainly in the forest-steppe and foothill zones, often forming local populations. They can be cultivated as medicinal plants due to their rapid growth and high biomass yield. The global demand for *Valeriana* raw material continues to increase, driving interest in its pharmacognostic characteristics and potential as a source of biologically active compounds [2].

V. collina and *V. stolonifera* have a long history of application in folk and traditional medicine. The main directions of use include the treatment of neuropsychiatric disorders, insomnia, hysterical and hypochondriacal conditions, migraines, intestinal colic, rheumatic pain, and dysmenorrhea – particularly when associated with increased nervous excitability [10,11].

Traditional medicinal forms included crushed or powdered plant material, decoctions, infusions, and juices from fresh plant parts. Extracts were prepared at a raw material-to-extractant ratio of 1:0.6–0.85, ensuring efficient extraction of active components [1,12].

Centuries of empirical use of these species provide a solid foundation for modern research aimed at pharmacological validation of their traditional effects.

Traditional preparations from these species often encompassed various forms. Common methods of preparation involved the use of crushed or powdered plant material, as well as freshly pressed root juices, with extraction ratios typically ranging from 1:0.60 to 0.85 to ensure effective isolation of beneficial compounds [1,12].

The chemical profile of *V. collina* and *V. stolonifera* is characterized by a wide range of bioactive compounds – iridoids, flavonoids, alkaloids, phenolic acids, and essential oils. Each group of compounds contributes to the pharmacological activity of the extracts.

Iridoids are the main components of the *Valeriana* genus: more than 250 such compounds have been identified to date [3,4,13]. They exhibit cytotoxic effects against several tumor cell lines, including lung adenocarcinoma and metastatic prostate cancer. The most characteristic representatives – valtrate and chlorovaltrate – demonstrate pronounced antitumor and anti-inflammatory activity [5]. The structural diversity of iridoids results from variations in acyl substituents within their molecules, which determines their biological effects [4,5].

Flavonoids play an important role in the sedative, antioxidant, and neuroprotective effects of *Valeriana*. More than 40 individual flavonoids have been identified, including hesperidin, acacetin, apigenin, and luteolin [5]. These compounds ensure the antioxidant activity of extracts and potentiate the effects of other polyphenolic substances [3,5]. The presence of hydroxycinnamic acids further enhances the overall biological activity of plant extracts.

Alkaloids of *Valeriana* occur in smaller quantities but contribute to neuromodulatory and spasmolytic effects. Although research on their composition is limited, it is known that these compounds potentiate the sedative action of iridoids [4].

Essential oils are key components responsible for the sedative, anxiolytic, antibacterial, and anti-inflammatory activity of *Valeriana* extracts. They are dominated by terpenoid and phenolic derivatives, which determine both the aroma and pharmacological effects [4,5]. It has been shown that essential oils prolong sleep duration and exhibit antioxidant and antimicrobial activity.

Chemical analysis of the extracts showed low concentrations of heavy metals: the content of lead (Pb) does not exceed 2 ppm, while cadmium (Cd) and arsenic (As) remain within pharmacopoeial limits [9]. This indicates the safety of the obtained extracts for therapeutic use, provided that technological regulations are properly followed.

V. collin and *V. stolonifera* have been thoroughly studied for their diverse pharmacological properties, demonstrating significant antibacterial, antioxidant, and anticancer effects *in vitro*, as well as neuroprotective potential [14]. Various extracts of these species have been shown to possess anxiolytic, antidepressant, anticonvulsant, and cytotoxic activities, contributing to their potential therapeutic applications [15,16].

Alkaloids and essential oils of *V. officinalis* exhibit activity against gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis*, with minimum inhibitory concentrations ranging from 62.5 μg/Ml to 400 μg/Ml [5]. Similar effects have been demonstrated for *V. jatamansi* extracts against *Pseudomonas aeruginosa* and *S. aureus* [13]. This suggests that other representatives of the genus, including *V. collina* and *V. stolonifera*, potentially possess a comparable spectrum of antimicrobial activity.

Studies have shown that the ethanolic extract of *V. officinalis* improves cognitive functions in animal models of Alzheimer's disease by enhancing antioxidant activity and reducing acetylcholinesterase levels [5]. These findings indicate the potential use of valerian extracts in the prevention of neurodegenerative processes, particularly in Alzheimer's and Parkinson's diseases [5,17,18].

The sedative and hypnotic effects of *Valeriana* extracts are attributed to the presence of iridoids and flavonoids that modulate the activity of the GABAergic system in the brain [5]. These compounds help improve sleep quality, reduce nervous tension, and exert a spasmolytic effect. Experimental studies have also confirmed analgesic properties, expanding the therapeutic potential of valerian preparations for the management of functional nervous system disorders [5,17,18].

In addition to the aforementioned properties, *V. wallichii* and *V. jatamansi* extracts have shown hepatoprotective, anti-inflammatory, and antioxidant activities, explained by their flavonoid and tannin content [9,13]. Iridoids, in turn, contribute to the normalization of gastrointestinal functions through their spasmolytic effects.

Taken together, these data suggest that representatives of the *Valeriana* genus possess a wide pharmacological potential, suitable for the development of new therapeutic agents aimed at treating neurodegenerative, cardiovascular, and inflammatory diseases.

Aim

To comprehensively evaluate the antimicrobial / antifungal potential, acute toxicity, and hepatoprotective activity of thick extracts from the herbs of *V. collina* and *V. stolonifera*.

Materials and methods

Antimicrobial and antyfungal activity. The antimicrobial properties of native thick extracts of *V. collina* and *V. stolonifera* were studied *in vitro* at the Microbiological Laboratory of the Educational and Medical Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University. The tests were performed using the agar diffusion method with wells ("well method") according to the methodological guidelines "Study of Specific Activity of Antimicrobial Drugs" [19] and in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The principle of the method is based on the diffusion of the test substance into the agar medium and inhibition of microbial growth around the wells.

To determine the antimicrobial spectrum, standard reference strains representing different groups of microorganisms were used: *Staphylococcus aureus* ATCC 29213/

NCTC129735 – gram-positive coccus; *Bacillus subtilis* ATCC 6633 – gram-positive spore-forming rod; *Escherichia coli* ATCC 25922 – gram-negative rod, representative of the Enterobacteriaceae family; *Pseudomonas aeruginosa* ATCC 27853 – gram-negative, non-glucose-fermenting rod; *Candida albicans* ATCC 885-653 – yeast-like fungus.

Mueller–Hinton agar was used for cultivating bacteria, while Sabouraud dextrose agar (HiMedia, India) was used for *Candida* species. Bacterial suspensions were prepared in physiological saline to a turbidity standard of 0.5 on the McFarland scale and subsequently diluted to a final concentration of approximately 5×10^5 cells/mL.

Ten milliliters of "base" agar were poured into Petri dishes. After solidification of the surface layer, sterile metal cylinders (10 mm height, 6 mm internal diameter) were placed and filled with molten, cooled (45–50 °C) nutrient agar inoculated with test cultures. For this purpose, 1.5 mL of a 24-hour microbial suspension was added to 13.5 mL of agar. After solidification of the inoculated layer, the cylinders were carefully removed with sterile forceps to form wells.

Each well was filled with 0.30 ± 0.05 mL of the test extract. In each Petri dish, the activity of one extract sample and the control solution (70 % ethanol) were tested in parallel. Each experiment was performed in triplicate.

After inoculation, plates containing bacterial cultures were incubated at 35 ± 1 °C for 18–24 hours, while samples with *Candida albicans* were incubated for 24–48 hours.

The results were evaluated by measuring the presence and diameter of growth inhibition zones around the wells, which reflected the level of antimicrobial and antifungal activity of the tested extracts.

The study of acute toxicity was carried out using thick extracts of *V. collina* and *V. stolonifera*, administered as 20 % aqueous solutions. The identification of the plant raw material had been conducted in advance according to the pharmacognostic standards. The experiments were performed on healthy female Wistar rats aged eight to twelve weeks, weighing 180–210 g [20]. The choice of females was based on literature data indicating their higher sensitivity to toxic effects compared with males.

All animal experiments were conducted in compliance with the principles of humane treatment of animals according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine "On the Protection of Animals from Cruelty" (February 21, 2006, No. 3447-IV). The Bioethics Commission of Zaporizhzhia State Medical and Pharmaceutical University reviewed the materials presented in the article and confirmed the absence of any violations of ethical standards established by applicable regulatory documents, including the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, and other relevant legal instruments (Protocol No. 12, dated October 23, 2025).

The experimental animals were kept in a vivarium at a temperature of 22 ± 3 °C and relative humidity of 50–60 %, under a natural 12-hour light/dark cycle. The rats were fed a standard granulated diet with free access to drinking water. Before the start of the experiment, the animals underwent an acclimatization period in cages for at least five days. Prior to administration, they were subjected to overnight fasting with free access to water, and feeding was restricted for an additional 3–4 hours after dosing [21].

The extract solution was prepared immediately before administration to ensure its stability throughout the experiment. The administration was performed intragastrically using a special probe. To determine acute toxicity, a protocol with a limit dose of 2000 mg/kg body weight was used [22,23], in accordance with OECD methodological recommendations. This dosing regimen was chosen to meet bioethical requirements and minimize mortality.

The study was conducted on several groups of animals, three females per group. The first group received the test sample, the second served to confirm the results, and subsequent groups were dosed only after confirming the survival of the previous group and the absence of pronounced signs of intoxication.

The animals were observed individually during the first thirty minutes after administration, then periodically during the first 24 hours, with special attention to the initial four hours, and subsequently daily for fourteen days. During monitoring, the onset, duration, and nature of possible toxic manifestations, as well as the general condition and behavioral activity of the rats, and their food and water intake were recorded. Clinical signs of intoxication and cases of mortality were also evaluated.

Hepatoprotective activity under experimental toxic liver necrosis induced by paracetamol in rats. The study was conducted on white Wistar rats weighing 190–220 g. The animals were kept under standard vivarium conditions with free access to water and standard food [24].

A model of toxic drug-induced liver necrosis was reproduced by intragastric administration of paracetamol (capsules 500 mg, batch No. 780725, manufacturer: "Pharmaceutical Company Zdorovye", Ukraine) to the rats. The drug was administered at a dose of 1250 mg/kg once daily. For administration, a suspension in 2 % starch gel solution was used. The duration of paracetamol administration was 2 days [25].

Experimental Design. The animals were divided into 5 groups: Group 1 (Intact control) – rats receiving an equivalent volume of solvent; Group 2 (Negative control) – rats with paracetamol-induced hepatitis modeled according to the described protocol; Group 3 (Reference drug) – Rats with modeled hepatitis receiving silymarin at a dose of 20 mg/kg [4] ("Silibor forte", capsules 70 mg, batch No. 10224, manufacturer "Pharmaceutical Company Zdorovye", Ukraine); Group 4 (*V. collina* extract) – Rats with modeled hepatitis receiving *V. collina* extract at a dose of 20 mg/kg; Group 5 (*V. stolonifera* extract) – rats with modeled hepatitis receiving *V. stolonifera* extract at a dose of 20 mg/kg.

The tested substances and reference drug were administered for 2 days. The dosing schedule was 1 hour before and 2 hours after paracetamol administration.

Table 1. Results of the study on the sensitivity of microorganisms to the thick extracts of *Valeriana collina* and *Valeriana stolonifera*

Sample	Microorganism cultures				
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
	Diameters of Growth Inhibition Zones, mm				
V. collina (thick extract)	21.33 ± 1.53	23.00 ± 1.00	12.67 ± 0.58	17.33 ± 0.58*	15.67 ± 0.58*
V. stolonifera (thick extract)	19.33 ± 0.58	22.67 ± 1.53	13.33 ± 0.58	19.00 ± 0.00*	13.67 ± 0.58*

***:** Student's t-test, $p < 0.05$; data are presented as mean values of three independent experiments; statistical significance was determined using Student's t-test; $p < 0.05$ was considered significant.

Evaluation of liver condition was performed 18–20 hours after the last paracetamol administration. Blood samples were collected for biochemical analysis. The following parameters were determined in the blood serum: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and its fractions, and alkaline phosphatase (ALP).

Statistical analysis of the research data was performed using the Statistica software (license No. JPZ804I382130ARCN10-J) and Microsoft Excel (Microsoft Corp., USA).

Results

Antimicrobial and antifungal activity. The sensitivity and resistance of microorganisms to the thick native extracts of *V. collina* and *V. stolonifera* were determined by the presence or absence of growth inhibition zones around the wells in the agar. After incubation, the results were recorded by measuring the diameters of the inhibition zones, including the diameter of the wells themselves. Measurements were taken with an accuracy of 1 mm, based on the complete absence of visible growth in the corresponding zone.

Interpretation of the results was based on the diameter of the inhibition zone, which reflected the level of antimicrobial activity of the tested extracts. In the absence of an inhibition zone or with a diameter up to 10 mm, the culture was considered insensitive to the tested sample. Diameters ranging from 11 mm to 15 mm were interpreted as moderate sensitivity, indicating partial extract activity. Values between 15 mm and 25 mm were considered indicative of pronounced microbial sensitivity, whereas diameters exceeding 25 mm indicated high sensitivity and strong antimicrobial action of the tested extract.

The obtained data on the antimicrobial activity of the thick extracts of *V. collina* and *V. stolonifera* are presented in *Table 1*. The analysis was performed using reference strains of gram-positive and gram-negative bacteria, as well as yeast-like fungi, allowing for a comparative evaluation of the spectrum of activity between the extracts. The results represent mean values of the inhibition zone diameters, accounting for statistical processing.

As a result of the conducted studies, no statistically significant differences ($p > 0.05$) were found in the diameter of the growth inhibition zones between the thick extracts of *V. collina* and *V. stolonifera* against *S. aureus* ($p = 0.1009$), indicating comparable effectiveness of both extracts against this gram-positive bacterial strain. Similarly, no statistically significant differences were recorded for *B. subtilis* ($p = 0.7441$) and *E. coli* ($p = 0.2017$), which indicates a similar level of antimicrobial activity of both extracts toward these test strains.

For *P. aeruginosa*, a statistically significant difference was observed ($p = 0.0113$). The mean diameter of the growth inhibition zone for the thick extract of *V. stolonifera* (19.00 ± 0.01 mm) was significantly higher compared to that of *V. collina* (17.33 ± 0.58 mm). In the case of *C. albicans*, a statistically significant difference was also observed ($p = 0.0080$). However, in this instance, the thick extract of *V. collina* (15.67 ± 0.58 mm) demonstrated a significantly larger mean inhibition zone diameter than the thick extract of *V. stolonifera* (13.67 ± 0.58 mm).

Study of acute toxicity of 20 % solutions of thick extracts of *V. collina* and *V. stolonifera* according to OECD Guideline. According to the OECD Guideline (Acute Oral Toxicity – Acute Toxic Class Method), a model was reproduced to evaluate the acute toxicity of the thick extracts of *Valeriana collina* and *Valeriana stolonifera* after intragastric administration. The methodology is based on a stepwise procedure using a minimal number of animals (three rats per stage) to determine toxicity and classify the substance according to the Globally Harmonized System (GHS) [26].

According to the results, at a dose of 2000 mg/kg, no mortality was observed after administration of the thick extract solution of *Valeriana stolonifera* in either the first or second group. In the case of the *V. collina* extract, one death was recorded in the first group; however, no lethal cases were observed upon repeated administration. Thus, the results confirm the possibility of applying the limit test procedure *(Table 2)*.

During clinical observation, no external pathological signs (lesions of the skin, fur, eyes, or mucous membranes) were detected. The behavioral reactions of most animals corresponded to normal parameters; however, during the first day after administration, a short-term decrease in motor activity and the appearance of drowsiness were noted. Tremor, convulsions, salivation, diarrhea, lethargy, and coma were not observed.

The body weight of the rats was monitored before administration, weekly during the 14-day period, and at the end of the experiment. Variations in body weight did not exceed 5 % and remained within the physiological norm, indicating the absence of toxic effects on the general condition of the animals.

Table 2. Mortality indicators in rats after administration of thick extracts of *V. collina* and *V. stolonifera* (2000 mg/kg, OECD 423)

Name of the preparation	Administration group	Number of animals per group	Number of deceased animals
Solution of thick extract *V. collina*	First administration group	3	1
	Repeated administration group	3	0
Solution of thick extract *V. stolonifera*	First administration group	3	0
	Repeated administration group	3	0

Data are presented for n = 3 animals per group, with observations conducted over a 14-day period.

Table 3. Results of biochemical assessment of ALT, AST, and ALP activity in rat blood serum under paracetamol-induced hepatitis model

Group	ALT (M ± SD)	Δ ALT, %	AST (M ± SD)	Δ AST, %	ALP (M ± SD)	Δ ALP, %
Intact control	64.25 ± 15.17*	-28.81	146.75 ± 34.05	-9.97	78.00 ± 26.20*	-42.86
Negative control (paracetamol)	90.25 ± 14.89	0.00	163.00 ± 8.83	0.00	136.50 ± 22.01	0.00
Silymarin (20 mg/kg)	64.75 ± 8.02*	-28.25	128.50 ± 26.64	-21.17	75.25 ± 17.69*	-44.87
V. collina (20 mg/kg)	74.50 ± 14.39	-17.45	135.25 ± 12.34*	-17.02	97.25 ± 17.91*	-28.75
V. stolonifera (20 mg/kg)	63.75 ± 9.50*	-29.36	128.50 ± 25.67	-21.17	59.25 ± 34.33*	-56.59

*: statistically significant differences compared with the negative control group, $p \leq 0.05$.

Hepatoprotective activity under experimental toxic liver necrosis induced by paracetamol in rats. Drug-induced liver injury is a significant problem in modern medicine. One of the most common hepatotoxic xenobiotics used to model such conditions is paracetamol [27]. Paracetamol overdose leads to severe damage to the liver parenchyma. This occurs due to the formation of its highly toxic metabolite, N-acetyl-p-benzoquinone imine, which forms irreversible bonds with hepatocellular macromolecules and causes hepatic necrosis.

As a result of the study, it was found that in the negative control animals, toxic hepatitis was induced by paracetamol administration. There was an increase in the activity of ALT, AST, and ALP relative to the intact group, indicating pronounced hepatocellular damage *(Table 3)*.

In animals treated with silymarin, a marked decrease in ALT (by 28.25 %), AST (by 21.17 %), and ALP (by 44.87 %) activity was observed, confirming the hepatoprotective effect of the reference drug. The *Valeriana collina* extract reduced the activity of ALT (by 17.45 %), AST (by 17.02 %), and ALP (by 28.75 %) compared with the negative control group. Animals that received the *V. stolonifera* extract showed a more pronounced normalization of biochemical parameters – ALT, AST, and ALP activity decreased by 29.36 %, 21.17 %, and 56.59 %, respectively. These values approached those of the intact animals, indicating a potentially stronger hepatoprotective effect of this *Valeriana* species.

Discussion

The antimicrobial study showed that both species exhibited a broad spectrum of activity against gram-positive and gram-negative bacteria, as well as *Candida* fungi. A species-specific profile was observed: the extract of *V. stolonifera* was significantly more effective against *Pseudomonas aeruginosa* ($p = 0.0113$), while the extract of *V. collina* demonstrated higher fungistatic activity against *Candida albicans* ($p = 0.0080$). This may be explained by differences in the composition of secondary metabolites, particularly the content of flavonoids and essential oils.

Acute toxicity tests confirmed that both extracts can be classified as low-toxicity substances (GHS Category 5). For *V. collina*, a single death was recorded, likely associated with individual sensitivity of the animal. The general condition and body weight of the rats remained within physiological norms, indicating good tolerance of the tested extracts. Thus, the obtained results confirm the safety of the extracts at the studied doses.

In the negative control group of rats that received paracetamol, a significant increase in the activity of hepatic enzymes (ALT, AST, ALP) was observed compared with the intact animals. This confirms that paracetamol at the selected dose caused acute toxic liver injury, validating the chosen model for investigating potential hepatoprotective agents.

Silymarin demonstrated a pronounced hepatoprotective effect. The reference drug effectively counteracted the toxic influence of paracetamol, significantly ($p \leq 0.05$) reducing ALT and ALP activity almost to the levels of the intact animals. Although the decrease in AST (by 21.17 %) was notable, it did not reach statistical significance, which may indicate a specific mechanism of action primarily targeting hepatocyte membranes (reflected by ALT) and the biliary system (reflected by ALP).

The *V. collina* extract exhibited moderate hepatoprotective activity. This extract significantly ($p \leq 0.05$) reduced AST and ALP activity; however, its effect on ALT – a key marker of hepatocellular cytolysis – was less pronounced and statistically insignificant. This suggests that its protective effect is less comprehensive compared with silymarin.

The *V. stolonifera* extract demonstrated the highest hepatoprotective efficacy. It produced the most distinct results, significantly ($p \leq 0.05$) reducing the activity of all three key enzymes: ALT, AST, and ALP. Notably, *V. stolonifera* extract most effectively decreased ALP activity by 56.59 %, which was the highest reduction among all studied groups. This indicates its strong and multifaceted protective effect, which is not inferior to, and in terms of ALP even surpasses, the reference drug silymarin.

Thus, administration of *V. collina* and *V. stolonifera* extracts contributed to a reduction in biochemical markers of cytolysis and cholestasis under toxic liver injury, confirming their hepatoprotective activity.

The obtained results confirm that the aerial parts of *V. collina* and *V. stolonifera* are promising subjects for pharmacological investigation. High levels of antioxidant and antimicrobial activity indicate the presence of a complex of compounds capable of influencing the main pathogenetic pathways of various pathological processes. Considering the substantial biomass of the herb and the ability of plant populations to regenerate regularly, this raw material may be regarded as an additional or even alternative source of biologically active extracts.

It is important to emphasize that the use of aerial parts instead of underground organs is not only economically feasible but also ecologically justified. This approach reduces pressure on natural populations and promotes sustainable models of medicinal plant harvesting. A promising direction for future research is the standardization of valerian herb extracts based on marker compound content and the evaluation of their clinical efficacy in comparison with traditional raw materials (*Valerianae radix*).

Conclusions

1. Both thick extracts of *V. collina* and *V. stolonifera* exhibited pronounced antimicrobial activity against gram-positive and gram-negative bacteria, as well as *C. albicans*. *V. stolonifera* was more effective against *P. aeruginosa*, whereas *V. collina* demonstrated higher activity against *C. albicans*. These differences may be associated with variations in the ratios of biologically active compounds in the extracts, confirming the need for further chemical and pharmacological studies to elucidate the mechanisms of action.

2. The 20 % aqueous solution of the thick extract of *V. collina*, when administered intragastrically, can be classified as toxicity Class 5, with an LD_{50} value in the range of 2000–5000 mg/kg. The solution of the thick extract of *V. stolonifera* did not cause mortality in rats, allowing it to be classified as a substance with low acute toxicity (GHS Category 5; $LD_{50} \geq 5000$ mg/kg). Further testing of the studied samples is not recommended unless required by specific regulatory purposes.

3. The valerian extracts studied, particularly *V. stolonifera*, demonstrated expressed hepatoprotective effects under conditions of paracetamol-induced hepatitis in rats. The experimental model of paracetamol-induced hepatitis led to the development of distinct biochemical and morphological signs of liver injury in animals. The *V. stolonifera* extract exhibited a strong hepatoprotective effect, confirmed by decreased ALT, AST, and ALP levels and a reduction in hepatic necrosis areas. The efficacy of the *V. stolonifera* extract exceeded that of the reference drug, silymarin.

4. The obtained data indicate the promising potential of both *Valeriana* species for further research to confirm their role as sources of natural antioxidants and compounds with antimicrobial activity.

Funding
The study was performed without financial support.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Information about the authors:
Kokitko V. I., Graduate Student of the of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0001-9231-7627
Odyntsova V. M., PhD, DSc, Professor of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-7883-8917

Відомості про авторів:
Кокітко В. І., аспірантка каф. фармакогнозії, фармакології та ботаніки, Запорізький державний медико-фармацевтичний університет, Україна.
Одинцова В. М., д-р фарм. наук, професор каф. фармакогнозії, фармакології та ботаніки, Запорізький державний медико-фармацевтичний університет, Україна.

Valeriia Kokitko (Валерія Кокітко)
valeriakokitko@gmail.com

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