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Evaluation of the anxiolytic activity of dry extract of *Patrinia scabiosifolia* under acoustic stress

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Abstract. – OBJECTIVE: *Patrinia scabiosifolia* has been used in traditional medicine in East Asia, Africa, and South America for a variety of diseases for more than 2000 years. The purpose of the article is to evaluate the anxiolytic properties of dry extract of *P. scabiosifolia*.

MATERIALS AND METHODS: *In vivo* experiments were performed on outbred white male mice. The psychotropic effect of *P. scabiosifolia* dry extract was assessed using behavioral test systems aimed at identifying changes in the psycho-emotional state of animals under the influence of acoustic stress. In addition, the preparation toxicity was also assessed. HPLC-MS analysis was carried out to confirm the presence of active components in local raw materials.

RESULTS: The article describes the possibility of using dry extract of *P. scabiosifolia* as an anxiolytic and sedative for psycho-emotional stress in experimental animals. Based on comprehensive research results, the effectiveness and safety of the studied herbal preparation have been proven.

CONCLUSIONS: In this study, a dry extract of *P. scabiosifolia* has been proposed as a novel means of combating neuropsychiatric disorders. *P. scabiosifolia* showed efficacy comparable to the reference drug (Mebicar), reducing sleep time and increasing sleep duration. The results obtained can subsequently serve as the basis for clinical trials.

Key Words:
Anxiolytic effect, Behavioral tests, *Patrinia scabiosifolia*, Sedative.

Abbreviations

AS: acoustic stress; CA: central area; CAB: coefficient of asymmetry of behavior; CNS: central nervous system; CS: close (dark) sleeve elevated plus-maze test; EPM: elevated plus-maze test; FA: formic acid; GPA: general physical activity; HA: horizontal activity; LDB: light-dark box test; MR: mink reflex; OF: open field test; OS: open (bright) sleeve elevated plus-maze test; PS: *Patrinia scabiosifolia*; PsDr: *Patrinia scabiosifolia* dry extract; VA: vertical activity.

Introduction

Modern society shows a noticeably increased interest in medicinal plant treatments containing biologically active substances, which are unique sources of healing compounds. Medicinal plants have found their way not only into traditional medicine but also into official one, primarily for the treatment of chronic diseases.

Psycho-emotional stress poses an obvious danger to modern humans. Constantly rising psycho-emotional stress that results from technological progress, human-made and environmental disasters, military conflicts, and extreme living and working conditions causes post-stress disorders, which are one of the leading causes of rising mortality, particularly at an early age, suicides, alcoholism, and drug addiction. Anxiety disorders constitute a part of the structure of many somatic and neurological diseases. As shown by the results of medical and social tests, a higher level of anxiety increases the probabilities of myocardial infarction and sudden death 2.3 times and 4.5 times, respectively[1,2].

A wide range of anxiolytic preparations of synthetic and natural origin is used for the treatment of psycho-emotional disorders accompanied by an increase in anxiety levels. While effectively eliminating anxiety symptoms, synthetic preparations have substantial side effects. Preparations of plant origin are successfully used to correct mild, substandard, and vegetative manifestations of anxiety. Preparations based on valerian, motherwort, Baikal skullcap, and other plant preparations are used in clinical practice to reduce fear and anxiety. According to the World Health Organization data, 80% of the world population prefers preparations of plant origin, while the use of herbal preparations to treat insomnia and Central Nervous System (CNS) hyperexcitability reaches 25% and 21%, respectively[3,4].

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Thus, the search for anxiolytics based on plant materials to prevent light subsyndromal and vegetative manifestations of anxiety is relevant.

One of the recognized plants that are widely used as a sedative is *Valeriana officinalis* (L.). *Patrinia scabiosifolia* (Fish ex Link) (PS) is known in the Russian Federation to a lesser extent and is unofficially used for therapy, while both plants belong to the subfamily (*Valerianoideae Raf.*) of the family *Caprifoliaceae*.

The medicinal properties of the genus *Patrinia* have long been used in traditional Chinese, Korean, and Tibetan medicine. PS has been known as a medicinal plant for more than 2000 years and is used as an anti-inflammatory, analgesic, gastro- and hepatoprotective, detoxifying, antioxidant, and anticancer preparation. The plant in question is used in traditional Chinese medicine to treat sleep disorders and postpartum depression, with the relevant preparation known as Bai Jiang Cao.

In Buryatia, located near Lake Baikal, the plant is used in traditional medicine. It was first developed in the region by presumed ancestors Bayegu and Kurykans during the 6th century. *Patrinia* species are used by indigenous peoples in the Amur River basin (Udege, Nanai, and Taz- In ethnic Buryatia, Tibetan medicine comprises treatment methods and means that have developed over time from different types of pre-scientific medicine practices. The plant is used for treating diseases of inflammatory nature and cancer manifestations, and also as an antimicrobial preparation. PS is used in combination with other plants by peoples of South America and Africa[5-8].

Despite the growing demand for and fascination with using plants for disease treatment, especially chronic illnesses, skepticism persists regarding their effectiveness. A striking example that can dispel such distrust is the use of the decoction Uiin-Buja-Paejangsan comprising *Coix lacrymajobi* (L), *Aconitum carmichaelii* (Debeaux), and *P. villosa* (Thunb) for the treatment of acute appendicitis in the preoperative phase[8].

As has already been noted, neurological pathologies have now become a serious problem that lowers living standards and causes the development and aggravation of related diseases. As tests of *PS* preparations used as a sedative and soporific medicine show, the efficacy of the extract varies depending on the method of preparation. It is also noted that, as a sedative, water-alcoholic extract is more effective than water extract, and its effect is not inferior to pentobarbital[9,10].

The tests previously conducted in the authors'[11] laboratory showed a pronounced sedative, antioxidant, adaptogenic, and hypolipidemic effect of water-alcoholic herbal infusions and the roots of *P. scabiosifolia*.

This study aims to identify the anxiolytic properties of the dry extract of *PS*.

Materials and Methods

Plant Material

Inflorescences and leaves of *P. scabiosifolia* collected during the full bloom phase in Ussuriysk District of Primorsky Region, near Gornotaezhnoye Village (43°43'10.2"N 132° 05'48.5"E) were used as raw materials for preparing a dry extract. The plant was identified by Dr. Olga Zorikova (Laboratory of Medicinal Plants, Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS). The raw materials were then dried in the shade and thoroughly checked to remove any foreign components. A voucher specimen (VLA00002176) was deposited at the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS, Vladivostok, Russian Federation.

Preparation of the Dry Extract

The air-dry raw materials were grinded in a dry sample mill (LZM-1m, OLIS, Moscow, Russia), which was followed by taking 2 mm particle fraction and extracting with 40% ethanol solution in water to achieve optimal extraction through a sieve, with the ratio of raw materials and extractant pf 1:10. The extraction was carried out three times in a water bath with a backflow condenser for 90 min at 60ºC. The combined extract was filtered twice with a vacuum filtration plant (PVF-47/4, BMT, Vladimir, Russia) through a paper filter (Hyundai HM 10, Seoul, Korea) and a finer filter (Hyundai HM 20, Seoul, Korea). The combined filtrate was further vaporized with rotary evaporator (RV-8, IKA, Staufen, Germany) at room temperature and under reduced pressure. The concentrated extract was subjected to lyophilic drying (Martin Christ Alfa 1-2, Osterode, Germany) until it reached the powder state at -55ºC and 0.007 mbar vacuum for 40 min. The percentage share of dry extractive substances was calculated. The resulting dry extract of *P. scabiosifolia* (PsDr) was stored in a sealed dark container at +4°C.

Chemicals

All chemicals were pure and of research-grade quality.

HPLC-MS Analysis

Ethanol was used as a solvent for PsDr compounds at a concentration of 10 mg/ml before High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) analysis. HPLC analyses of PsDr were performed at the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. HPLC with MS and UV detection (HPLC–MS-UV) for the identification and quantification of all components was performed using the 1260 Infinity analytical HPLC system (Agilent Technologies, Santa Clara, CA, USA), equipped with a G1315D photodiode array detector, G1311C quaternary pump, G1316A column oven, and G1329B autosampler. The HPLC system was interfaced with an ion trap mass spectrometer (Bruker HCT ultra PTM Discovery System, Bruker Daltonik GmbH, Bremen, Germany) equipped with an electrospray ionization (ESI) source. The MS analyses were performed with negative ion detection. MS data were collected using the Bruker Daltonics Compass 1.3 esquire control software (v. 6.2.581.3) and processed by the Bruker Daltonics Compass1.3 Data Analysis software (v. 4.0.234.0).

All solvents were of high-performance liquid chromatography (HPLC) grade. An analytical reverse phase column (Zorbax C18, 150 mm, 2.1-mm i.e., 3,5-μm part size, Agilent Technologies, Santa Clara, CA, USA) for separation was applied. Separation was carried out at following conditions: the column temperature was 40°C, the mobile phase consisted of 0.1% aqueous formic acid (A) and acetonitrile (B). The following elution gradient with a flow rate of 0.2 mL/min was used: The mobile phase consisted of a gradient elution of 0.1% aqueous formic acid (A) and acetonitrile (B). The gradient profile with a flow rate of 0.2 ml min/1 was as follows: 0 min 0% B; 35 min 40% B; 40 min 50% B; 50 min 100% B and then eluent B until 60 min.

Experimental Animals

The experiments aimed at evaluating the psychopharmacological profile of PsDr were performed on 288 white outbred mice (♂) with a weight of 20-25 g weight obtained from the vivarium of the Pacific State Medical University. The animals were kept under standard vivarium conditions with a natural 12-h light-dark regime, on a standard food diet, and with free access to water and food (briquetted feed) at a temperature of 22±2ºC and humidity of 55-60%. The animals were kept in accordance with the laboratory practice rules and Order of the Ministry of Healthcare of the Russian Federation No. 267 dated June 19, 2003, "On approval of the laboratory practice rules". During the experiments, the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes were observed. The experimental work was approved by the Biomedical Ethics Commission under the Federal Centre for Biodiversity of the FEB RAS.

To eliminate the impact of transport stress on the tests, all animals underwent a 15-day period of adaptation to new husbandry conditions and daily handling. During this period, the animals were weighed twice. The experimental tests excluded animals with no weight change, altered behavior, or visible physical abnormalities. During the formation of experimental groups, the animals were primarily randomized by age and weight and placed in cells of 8 animals each. To avoid the impact of circadian biorhythms on the course of the test, the experimental work was performed in the same time interval (between 9 and 14 hours) and under the same climatic conditions of autumn-winter period (no experiments were carried out during the periods of sharp weather changes).

Acute Toxicity Assay

The acute toxicity of the preparation was determined by a single intragastric injection. The toxicity parameters were evaluated by Kerber. Doses of the ethanol extract were preliminary dealcoholized and concentrated by steaming in a water bath. The preparation was injected into the animals' stomach in a physiologically permissible volume (for a mouse weighing between 20 and 30 grams, no more than 1 milliliter was administered), whereas high doses were injected fractionally.

To determine the LD_{50} value, the dealcoholized and concentrated extract was injected intraperitoneally once.

Determination of the Optimal Effective Dose

To select the optimal effective dose of the extract, a preliminary assessment of the drug activity in the "open field" was carried out, taking into account the spontaneous motor activity of the animals. Experimental animals were divided into 16 groups and control of 8 animals. The total number of behavioral acts (GPA) was considered in the norm (control) and with preliminary administration (30 minutes before testing) of PsDr in the dose range from 2.5 to 190.0 mg/kg.

Reference Drug

The reference drug Mebicar (Tatkhimfarmpreparaty, Kazan, Russia) was administered at an average therapeutic dose of 100 mg/kg. The solutions were prepared using purified water.

Evaluation of Behavioral Reactions

The basic tests for identifying anxiolytic activity are the open field (OF), elevated plus maze (EPM), and light-dark box (LDB) (RPC Open Science, Krasnogorsk, Russia)[12].

The animals were orally injected with the extract in a minimum active dose of 130 mg/kg established during the preliminary experiment[11] 60 min prior to the experiment. The dry extract was dissolved in purified water. The mice in the control groups received an equivalent volume of purified water.

An 80 dB(A) acoustic stress was used as a stress factor for 10 min at a 5.5-m distance. The illumination of the test sites was 90 lux. The plant floor was thoroughly wiped with neutral wet wipes after each test to eliminate the olfactory signals that affected the animals' behavior.

Open field test

The motor activity, orienting, and investigative behavior were studied. The tests were performed for 5 min to register the horizontal activity (HA): the number of crossed squares (a square was considered to have been crossed if a mouse crossed its boundary with its right foreleg), vertical activity (VA): free stackings and stackings supported with the plant board, mink reflex (MR), grooming, number of defecation boluses, and general physical activity (GPA); the coefficient of behavior' asymmetry was calculated as a ratio of HA to GPA[13].

Elevated plus maze

A mouse was placed in the central area, which was followed by registering the following indicators for 5 min: motor activity (time of stay and number of times a mouse entered open and closed maze sleeves, time of stay in the central area); exploration activity (stackings and dipping); emotional activity (auto-grooming and defecation boluses). The animal's anxiety indices in the EPM were calculated using the following formulas:

$$I_t = T_{open\ sleeve} / T_{open\ sleeve} + T_{closed\ sleeve} \times 100 \quad (1)$$

$$I_n = N_{open\ sleeve} / N_{open\ sleeve} + N_{closed\ sleeve} \times 100 \quad (2),$$

where I_t – index of the time of stay in the open sleeves;

$T_{open\ sleeve}$ – time spent in the open maze sleeves;

$T_{closed\ sleeve}$ – time spent in the closed maze sleeves;

I_n - index of the number of times a mouse entered the open sleeves;

$N_{open\ sleeve}$ – number of times a mouse entered the open maze sleeves;

$N_{closed\ sleeve}$ – number of times a mouse entered the closed maze sleeves;

These indicators are standard in the methodology for evaluating the anxiety of laboratory animals. The ratio between the anxiety index and anxiety level is expressed in reverse proportion, i.e., the lower the index, the higher the anxiety level of the object being studied and vice versa[14].

Light-dark box

During the experiment, the following animal activity indicators were registered for 5 min: the number and time of lookouts from the dark compartment to the light compartment; the number and time of entering the light compartment. The minimum time of a single peeping was taken as 1 s, while a series of consecutive lookouts was considered as several independent acts.

Statistical Analysis

The results were processed using the method of variation statistics together with determining the confidence boundaries (M±m) and calculating the reliability of differences using the Student *t*-test. The software package "Statistica 6.0" was used for data processing. $p > 0.05$ was selected as the point of minimal statistical significance in all analyses.

Results

Content of Extractive Substances in the Preparation

The content of extractive substances calculated by dry residue was 10.2%.

HPLC-MS Analysis

The total ion chromatogram (TIC) of the water-ethanol extract of *P. scabiosifolia* (Figure 1) revealed the compounds shown in Table I.

Acute Toxicity Assay

PsDr belongs to the class of low-toxic substances, whose LD_{50} value for intragastric injection (per os) exceeds 1,000 mg/kg[15]. The maximum injected dose was 2,400 mg/kg, which resulted in the survival of all animals.

In the case of intraperitoneal injection, the lethal dose for mice in 50% of cases (LD_{50}) was 780 mg/kg.

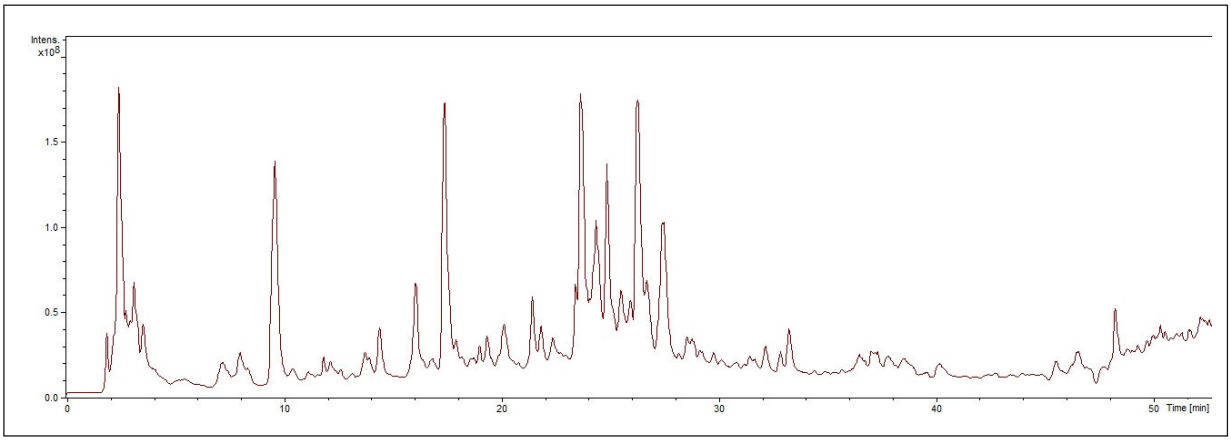

Figure 1. HPLC–ESI-MS total ion current chromatogram of *P. scabiosifolia* dry extract at the negative ion mode.

Table I. Identified *P. scabiosifolia* dry extract (PsDr) compounds.

RT, min	Compound name	m/z	Characteristic ions for confirmation
2.5	Quinic acid	383	383.191
9.6	Synapoil hexoside [M+FA]	415	415.369.207.161
12.7;13,2;13.7; 13.7;15.9	Patrinoside+isomers	407	407.361.199
16.0	Loganic acid	375	375.213.169
17.4	Chlorogenic acid	353	353.191.179
21.4	Flavovilloside	755	755.609
21.8	Deoxyhexose quinic acid	337	337.191.163
23.6	Flavovilloside	609	609.343.301.270
24.3	Quercetine-3-O-glucoside	463	463.301
24.4	Patrinaloside	507	507.461.345.161
24.9	Patriscabride 3 [M+HCOO]	507	507.461.179
25.5	Quercetine-3.7-di-O-glucoside	505	505.463.301
26.2	Chlorogenic acid glucoside	515	515.353
26.4	Loganic acid glucoside	537	537.375
27.3	C-hexoyl flavone	473	473.429
27.4	Dicaffeoyl quinic acid	515	515.353.299.255.203.173
28.5	p-coumaroylquinic acid glucoside	499	499.337.163
32.2	Quercetine	301	301.257.197.151
33.2	Caffeic acid ethyl ester	207	179.161.135

The consequences of a single intragastric injection of the preparation in increasing doses were observed after 14 days. After the animals had been dissected at the end of the experiment, it was found that the internal organs of the animals injected with the preparation did not have any deviations from standard values in terms of external features and weight (Table II).

During the observation period, the animals were weighed, and the body weight of the mice that were injected with the preparation did not significantly differ from that of the control group. By the end of the experiment, increases in body weight in groups with all specified preparation doses were practically equal to that in the control group (Table III).

Open Field Test

As the open field test (Table IV) of orienting and investigative behavior of the animals that received PsDr (group 2) showed, GPA was substantially reduced. The indicators reflecting the

Table II. Weight of internal organs of the mice during intragastric injection of various doses of *P. scabiosifolia* dry extract (PsDr).

Animal group	Organ weight mg/10 g					
	Liver	Kidney	Spleen	Thymus	Adrenal glands	Testis
Control	556.0±17.5	153.0±4.2	111.0±8.7	10.7±0.3	3.3±0.2	67.6±4.6
PsDr dose						
160 mg/kg	558.8±17.3	151.4±8.7	109.1±8.8	10.8±0.6	3.1±0.1	68.2±3.4
240 mg/kg	561.3±14.1	156.9±4.5	114.7±4.8	10.3±0.4	3.7±0.2	67.6±1.1
320 mg/kg	557.3±16.8	154.0±3.2	112.4±6.1	10.5±0.3	3.4±0.1	68.8±2.1
400 mg/kg	558.6±12.2	153.3±4.8	111.8±3.2	10.6±0.1	3.6±0.3	70.1±4.1
480 mg/kg	560.0±15.1	154.7±2.1	114.2±2.8	10.7±0.2	3.3±0.3	71.3±3.8

Table III. Weight of the mouse body during intragastric injection of the *P. scabiosifolia* dry extract (PsDr) preparation, g.

Animal group	Observation days			
	1	5	10	14
Control	20.26±0.5	23.61±0.2	23.91±0.4	24.85±0.8
PsDr dose				
160 mg/kg	21.72±0.4	23.89±0.5	24.54±0.9	25.08±0.4
240 mg/kg	19.84±0.6	22.84±0.7	23.94±0.3	24.79±0.6
320 mg/kg	21.82±0.2	23.05±0.5	24.15±0.3	24.94±0.5
400 mg/kg	22.35±0.8	23.21±0.6	24.26±0.2	24.75±0.4
480 mg/kg	20.56±0.4	23.03±0.4	24.17±0.4	24.86±0.8

Table IV. Weight of internal organs of the mice during intragastric injection of various doses of *P. scabiosifolia* dry extract (PsDr).

Group	Behavioral acts						
	HA	VA	MR	Grooming	Defecation	GPA	CAB
1	29.7±1.8	15.3±0.9	13.6±2.1	3.2±0.5	2.1±0.2	64.3±3.2	0.46
2	21.8±2.0*	10.6±0.4*	9.4±1.7	2.0±0.4	2.0±0.2	42.8±1.2*	0.51
3	51.4±4.6*	1.4±0.3*	4.8±0.5*	6.7±0.7*	8.1±0.4*	71.7±2.5	0.72*
4	27.3±2.2**	11.5±0.6**	12.2±1.8**	3.9±0.5**	1.9±0.3**	56.1±2.3**	0.48**

*$p<0.05$ when comparing groups 1-2 and 1-3; ** $p<0.05$ when comparing groups 3-4. 1: intact control; 2: PsDr; 3: acoustic stress-control; 4: acoustic stress+ PsDr. HA: horizontal activity; VA: vertical activity; MR: mink reflex; GPA: general physical activity; CAB: coefficient of asymmetry of behavior.

emotional state of the animals and the coefficient of asymmetry of behavior did not significantly change compared to control group 1.

After the acoustic stress by the stressor in group 3, there was an increase in total motor activity and a change in the coefficient of behavior's asymmetry toward the horizontal behavioral activity. The investigative component of behavioral acts was substantially reduced, while the indicators reflecting the degree of emotional tension increased.

The animals injected with the preparation against the stress background (group 4) featured the restoration of the behavior pattern specific to intact animals.

Elevated Plus-Maze Test

As the EPM test results (Table V) showed, the impact of the stressor (group 2) on the animal behavior was followed by a significant reduction in the time spent in the OS and CA and, accordingly, an increase in the time spent in the CS.

Table V. Effect of the *P. scabiosifolia* preparation on behavioral reactions in the elevated plus-maze test (EPM).

Indicators	Animal groups		
	Control (1)	AS (2)	AS + PsDr (3)
Time spent in the OS, sec	8.4±0.6	2.7±0.6*	7.7±0.4**
Time spent in the CA	70.9±4.1	52.5±5.5*	75.0±5.7**
Time spent in the CS, sec	220.7±3.7	244.8±5.4*	217.3±4.2**
Number of times a mouse entered the OS	1.6±0.03	0.7±0.06*	1.6±0.04**
Number of times a mouse entered the CS	7.5±0.6	9.6±0.6*	7.3±0.3**
Stackings	1.8±0.4	-	1.3±0.6
Dipping	0.74±0.03	-	0.68±0.4
Grooming	6.8±0.2	1.2±0.9*	7.1±0.3**
Defecation	0.3±0.05	1.8±0.1*	0.5±0.02**

*$p<0.05$ compared to the control group; **$p<0.05$ compared to AS group. AS: acoustic stress; OS: open (bright) sleeve elevated plus-maze test; CA: central area; CS: close (dark) sleeve elevated plus-maze test.

Under the AS conditions (group 3), PsDr normalized the indicators by bringing them to the values close to the intact control and led to the restoration of investigative activity. The indicators of behavior's emotional component changed similarly by approaching the values of the control group.

The anxiety index by the time spent in the sleeves decreased compared to the intact control in group 2 (AS), which reflects an increase in the anxiety level, while *P. scabiosifolia* overcame the stressor's adverse effects by reducing anxiety and increasing the index, while the anxiety index changed in a similar way anxiety by the number of times a mouse entered open and closed sleeves (Figure 2).

Light-Dark Box Test

As the light-dark box test showed, the emotional stress caused by acoustic impact resulted in significant changes in the indicators reflecting the anxiety state among the mice (Table VI).

Injection of the *P. scabiosifolia* preparation to the mice prior to AS reversed anxiety manifestation, which was reflected in the behavioral indicators.

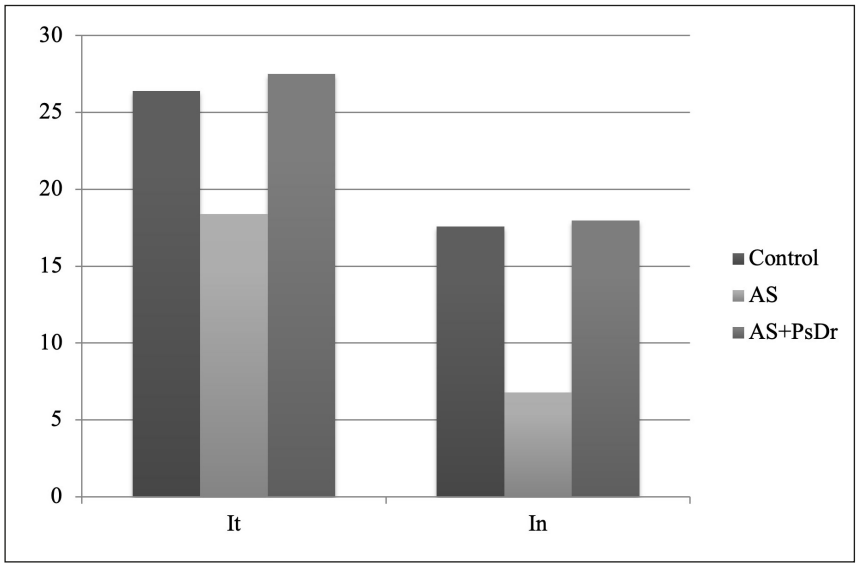

Figure 2. Change in anxiety indices under acoustic stress and dry extract of *P. scabiosifolia*. In: index of the number of times a mouse entered the open sleeves; It: index of the time of stay in the open sleeves; AS: acoustic stress; PsDr: *P. scabiosifolia* dry extract.

Table VI. Effect of the *P. scabiosifolia* preparation in combination with acoustic stress on behavioral reactions in the light-dark box test (LDB), (M±m).

Indicators	Animal groups		
	Control	Acoustic stress	Acoustic stress+ PsDr
Latency period of the first entrance to the light compartment, sec	78.4±2.0	149.0±2.40*	82.5±2.2**
Lookouts from the dark compartment to the light one, n	8.4±0.31	2.67±0.36*	7.85±0.63**
Duration of lookouts from the dark compartment to the light one, sec	62.3	23.7*	69.20**
Number of times a mouse entered the light compartment from the dark one, n	3.2±0.73	1.67±0.45	2.75±0.12
Duration of stay in the light compartment, sec	39.3±0.57	7.3±0.94*	28.3±0.55**
Duration of stay in the dark compartment, sec	198.4±8.3	269.0±10.5*	202.5±5.5**
Defecation boluses, pcs	1.7±0.07	3.65±0.17	0.75±0.01**
Defecation	0.3±0.05	1.8±0.1*	0.5±0.02**

*p<0.05 compared to the control group; **p<0.05 compared to AS group. AS: acoustic stress.

Discussion

The Oriental populations have used this preparation for over 2000 years as a broad-spectrum plant in traditional medicine. A number of papers[5,16,17] report the sedative effect of preparations based on *P. scabiosifolia* and other kinds of the genus in question, while the psychotropic effect of this plant is rarely noted. Hence, the presence of the anxiolytic action of *P. scabiosifolia* preparations needed to be confirmed scientifically.

The conducted phytochemical tests confirmed the presence of known biologically active compounds[18] in the raw materials growing in the Primorsky Region of the Russian Federation, which served as a source for preparing a PsDr. The phytochemical test results showed the presence of glycosides and their derivatives, iridoids, flavonoids, and organic acids in the preparation. Each group of substances is responsible for the manifestation of a particular biological activity; for instance, the plant anti-inflammatory effect is due to the action of several compound classes, namely iridoids, flavonoids, and organic acids. These same components are also responsible for the antioxidant activity of the preparation. The psychotropic activity studied by the authors is attributed to volatile compounds[16,19]. The diverse components explain the plant's therapeutic properties.

A toxicity assessment was carried out to identify potential and real hazards of the *P. scabiosifolia* preparation. As the results showed, the maximum oral dose containing 2,400 mg/kg of the preparation did not cause animal death. According to the classification of toxicity of chemical substances, the *P. scabiosifolia* preparation tested can be referred to as the class of low-toxic substances, whose LD50 value in case of injection to the stomach exceeds 1,000 mg/kg[15]. The animals were reported to have static-dynamic (coordination) disorders, low mobility, and lateral position for 6 to 14 h in 30 to 60 min after the preparation had been injected, but their physiological condition normalized in one day.

In accordance with the "Rules of Preclinical Safety Assessment of Pharmaceuticals", the consequences were monitored after a single intragastric injection of the preparation in its doses of 160, 240, 320, 400, and 480 mg/kg. The animals were observed for 14 days. When the mice were injected with large doses (320 mg/kg or more), a decrease in spontaneous motor activity, muscle tone, dystaxia, and complete immobility lasting 40-50 min were reported in 20-30 min, followed by recovery of the somatic state. During the entire observation period, the animals were weighed on the 5th, 10^{th,} and 14th days. The body weight of the mice injected with the preparation did not significantly differ from that of the control group. By the end of the experiment, an increase in the body weight in the groups with all mentioned preparation doses was almost equal to that of the control (Table II). After the animals were dissected at the end of the experiment, it was found that the organs detoxifying and excreting xenobiotics (liver,

kidneys) and ensuring the adaptation processes (spleen, thymus, adrenal glands) and reproductive function (testis) in all groups of mice, which received the preparation, did not have any deviations from standard values in terms of external features and relative weight.

Since the lethal outcome was not achieved, the acute toxicity of the preparations was determined by intraperitoneal injection. In this case, the dose of the preparations causing the death of 50% of mice was 780 mg/kg. The preparation in question was less toxic compared to Diazepam and some of its derivatives (LD_{50} - 25.5 mg/kg in case of intraperitoneal injection to mice), which is among the main tranquilizers used in Russia and other countries[20,21].

According to the open field test measuring orienting and investigative behavior of the animals that received PsDr, Group 2 experienced a significant (34%) decrease in total motor activity compared to the control group (Table IV). At the same time, there was a reported 26.6% decrease in the HA, a 30.7% decrease in the VA, and a 30.9% decrease in the MR compared to the control. The indicators reflecting the animals' emotional state and the coefficient of asymmetry of behavior did not significantly change compared to the control data. The reported change in motor activity allows us to assume the presence of a sedative effect.

Recording the mice's behavior after stress (group 3) showed an 11.5% increase in the total motor activity and a change in the coefficient of asymmetry of behavior toward the horizontal behavioral activity. The horizontal activity increased by 73% compared to the intact control, which was due to a "circus movement" along the wall without entering the field center, thus showing a pronounced anxious reaction. The investigative component of behavioral acts decreased substantially, with the number of vertical stackings decreasing more than 10 times and the number of lookouts into holes 2.8 times, respectively. The reactions reflecting the degree of emotional tension, namely grooming and defecation, increased by 109% and 285%, respectively.

The animals that received the preparation against the stress background (group 4) featured the restoration of the behavioral pattern characteristic of the intact animals. Compared to the stress-control group (group 3), the preparation reduced the HA by 46.9% and simultaneously increased VA and MR 8.2 times and 2.5 times, respectively, while the number of grooming and defecation acts was reduced by 41.8% and 76.5%, respectively. The literature suggests that the preparations in question may have a psycho-stimulating effect and possible nootropic activity. This is evidenced by the increase in the number of times a mouse entered the field center, as well as the central area which is generally considered aversive for burrow rodents. The increase in the latter also indicates a rise in the anxiety level of the animal. The increase in the number of such acts indicates the preparation of anxiolytic activity[22]. The test results identified the anxiolytic properties of *P. scabiosifolia*.

The EPM method is used in global and domestic experimental psychopharmacology as an adequate test to identify the anxiolytic properties of known and new preparations[23]. In contrast to the OF test, the EPM test gives an animal a choice between more and less stressful areas of the maze. Thus, the presence of animals in the maze and the behavioral patterns demonstrated allow to better evaluate the anxiety level of animals.

According to the analysis of the EPM test results for animals (Table V), the stressor (group 2) caused a significant decrease in the time spent in open spaces (OS) and closed arms (CA) by 67.9% and 26% respectively, which affected the animal behavior. However, the time spent in the sheltered region (SR) increased by 11% due to this stressor. A significant decrease was reported for the number of times a mouse entered the OS (by 56.3%), and grooming (by 82.4%), while the number of times a mouse entered the CS and defecation increased by 20.0% and 500%, respectively.

Such behavioral parameters stackings and dipping were not reported in group 2, which indicates a decline in investigative activity, an increase in anxiety, and a narrowing of the cognitive perception field due to the stressor.

Under the AS conditions (group 3), PsDr normalized the indicators by bringing them to the values close to the contact control. The time spent in the OS and CA in the group increased 2.9 and 1.4 times, respectively. The time of stay in the CS decreased by 11.2%. The number of times a mouse entered the OS increased by 2.3 times, while the number of times a mouse entered the CS decreased by 24%.

Against the preparation's background, the animals in group 3 reported the restoration of investigative activity, vertical stacking, and dipping, with their values close to the intact indicators, which means reduced anxiety level.

A similar pattern is shown by the emotional component of behavioral reactions, namely the number of grooming acts which increased 5.9 times, while the number of defecation acts decreased by 72.2%.

The anxiety level is known to be expressed with the indicators inversely proportional to the anxiety index, i.e., the higher the index, the lower the anxiety level in the animals being studied. The results given in Table V were used to calculate the anxiety indices by the time of stay (It) and the number of times a mouse entered (In) the OS and CS (Figure 2).

The anxiety indices reveal visible changes caused by AS on the animals' anxiety levels, both in terms of the time spent in the maze sleeves (It) and the number of times a mouse entered them (In). The It level decreased by 30.3% compared to the intact control in group 2 (AS), which reflects an increase in the anxiety level, whereas the *P. scabiosifolia* preparation overcomes the stressor's adverse effects by reducing anxiety and increasing It by 49.5% (Figure 2).

The index's indicators by the number of times a mouse entered the sleeves (In) more clearly show an increase in the animals' anxiety in the case of AS, with In reduced by 2.6 times compared to the intact control. By normalizing the mice state, the preparation restores the index almost to its original value. The latter index is a commonly recognized criterion characterizing the presence of the preparation's anxiolytic properties[24].

As the experiment showed, the *P. scabiosifolia* preparation expresses anxiolytic activity contributing to overcoming anxiety-phobic manifestations in animals inside the EPM under stress. The experiment results are consistent with those of the OF tests and confirm the assumption about the presence of anxiolytic activity within the psychopharmacological spectrum of the *P. scabiosifolia* preparation.

The anxiety level of the mice was assessed in the light-dark box (LDB) test, which is based on the rodents' innate desire to avoid brightly lit areas in their habitat. As in other tests that evaluate an animal's behavior during free movement (EPM, OF, etc.), an animal is in a combined state of fear (fear-anxiety) and the desire to obtain information about the environment.

As the results of the light-dark box test showed, the emotional stress of the mice caused by acoustic exposure resulted in significant changes in the indicators reflecting the anxiety state: the time of stay in the dark significantly increased (by 35.6%) and, accordingly, the duration of stay in the lightbox decreased 5.4 times compared to the intact animals; the indicators of "indecisive behavior" decreased with the number of lookouts from the dark box to the light one decreased 3.14 times and the duration of this parameter increased 2.63 times; accordingly, the latent period of entering the light box significantly increased by 90%. The stressed animals preferred to spend most of the time in the dark box, and the number of times a mouse left the dark box decreased almost twice by 47.8% compared to the intact animals. Stress and anxiety impeded normal functioning of the gastrointestinal tract and increased the defecation intensity 2.15 times, as reported in the experiment, which allowed to judge the force of emotional tension in animals (Table VI).

The injection of *P. scabiosifolia* preparation to mice prior to the exposure to AS reversed manifestations of anxiety, which affected the behavioral indicators: the latent period of the first time a mouse entered the lightbox decreased by 44.6%, while the periods of lookouts from the dark box to the light one became more frequent (2.94 times) and lengthened (2.92 times). Compared to the group exposed to acoustic stress, the number of times a mouse entered from the dark box to the light one increased by 64.7%, and the duration of stay in the latter increased by 3.9 times, while the duration of stay in the dark box decreased by 24.7%. The normalization of the emotional pattern was evidenced by a 4.9-fold decrease in the number of defecation boluses.

Conclusions

This study revealed that the proposed *P. scabiosifolia* preparation has low toxicity and expresses pronounced sedative and anxiolytic properties. It has no adverse impact on internal organs and physiological processes, which is confirmed by the integral indicator of body weight gain. The HPLC-MS analysis confirmed the presence in the local materials of *P. scabiosifolia* of secondary metabolites described in the literature which determine the preparation's biological activity.

The study justifies the use of this plant in the traditional medicine of Oriental people and expands the field of its application as a natural safe sedative and anxiolytic preparation.

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Conflict of Interest
The authors declare that they have no conflict of interest to disclose.

Ethics Approval
The research was conducted in accordance with the laboratory practice rules and Order of the Ministry of Healthcare of the Russian Federation No. 267 dated June 19, 2003, "On approval of the laboratory practice rules"; the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Besides, the experimental work was approved by the Bioethics Commission of the Federal Scientific Center of the East Asia Terrestrial Biodiversity of the Far Eastern Branch of Russian Academy of Sciences (FSC EATB FEB RAS) dated April 25, 2022, No. 4-1.

Informed Consent
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Authors' Contributions
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Data Availability
All data generated or analyzed during this study are included in this published article.

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