

EXPERIMENTAL STUDIES ON EXPECTORANT EFFECT OF EXTRACT FROM *PIMPINELLA SAXIFRAGA* L.

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Abstract

The study was conducted with the aim to further use *Pimpinella saxifraga* L. rhizomes and roots not only in folk medicine used as an expectorant effect but in official medicine. Studies of acute toxicity of thick extracts from the rhizomes and roots of *Pimpinella saxifraga* L. were performed V. B. Prozorovskiy method. The expectorant effect of a thick extract from the rhizomes and roots of the study plant on the influence of the motor activity of the ciliated epithelium and the secretory function of the bronchi were studied. The activity of the extract of *Pimpinella saxifraga* L. raw material was somewhat inferior to the activity of the reference drug, namely Hedelix drops, and were 31.87 % and 40.89 %, respectively. Consequently, it is expedient to continue work on the analysis of rhizomes and roots of *Pimpinella saxifraga* L. in order to manufacture a phytopreparation with expectorant activity.

Keywords: *Pimpinella saxifraga* L., rhizomes and roots, extract, expectorant effect

Introduction

Today, the global pharmaceutical industry widely uses herbal raw materials as a basis for the creation of medicines [1]. Given the ever-growing needs of the industry in herbal raw materials for the manufacture of medicines, an important task of modern pharmaceutical science is to expand existing and search for new sources [2, 3]. The plants often use in the struggle against many diseases [4, 5].

It is known that the flora of Ukraine includes about 1000 species of plants, which are characterized by pharmacological activity. However, most of them need further study. The advent of synthetic drugs, which mainly simulate the biologically active substances of plants, has not diminished the role of natural medicines [6, 7].

Therefore, in order to find new promising medicinal plants, it is important to study an unofficial plant of the family *Apiaceae*, genus *Pimpinella* – saxifrage *pimpinella* (*Pimpinella saxifraga* L.).

Such a promising source of BAS is a plant of the genus *Pimpinella* – *Pimpinella saxifraga* L., which has long been used in folk medicine and according to previous studies contains a number of biologically active substances [8].

The chemical composition of *Pimpinella saxifraga* L. is quite diverse. In the literature there is information that the underground organs of the plant contain up to 0.7 % of essential oil – terpenoids: bisabolol, saxazulene, azulene, polyacetylene compounds, aromatic compounds: propylbenzene, phenolic compounds (isoeugenol, pseudoegenol), up to 0.42 % coumarins (mainly umbelliferone) and furocoumarins (pimpinellin, isopimpinellin, spondin, bergapten, isobergapten, xanthotoxin), tannins, fatty oils, flavonoids, resins, phenolic carboxylic acids (fumanoic, quinic, caffeic), saponins, sugars, pectins, amino acids, benzoic and acetic acids, bitters, gums, vitamins, trace nutrients [9-11].

The above chemical composition of saxifrage *pimpinella* allows us to make assumptions about the presence of antimicrobial, diuretic, expectorant, analgesic, astringent, diaphoretic, litholytic,

antiseptic, sedative action, which is confirmed by the literature data [12, 13].

The use of the roots of saxifrage *pimpinella* is described in the pharmacopoeias of Switzerland, Norway and other countries of Western Europe [14].

On the territory of Ukraine, saxifrage *pimpinella* is used only in alternative medicine. To date, there is enough information about its use in folk medicine for various pathologies. The plant has anti-inflammatory, antispasmodic, diaphoretic, analgesic, diuretic effect. The underground organs of the plant are also used as an antihypertensive and lactogenic agent [15, 16]. The analgesic effect of saxifrage *pimpinella* helps to alleviate the condition of the patient with peptic ulcer disease and gout [17].

Thus, the analysis and generalization of literature data on the experience of using saxifrage *pimpinella* showed that the plant is mainly used in folk medicine. In clinical practice in the treatment of various diseases saxifrage *pimpinella* is rarely used only as an agent of complex therapy of respiratory diseases, although due to the rich phytochemical composition of biologically active substances the range of indications for its use can be quite wide. This allows us to conclude that further in-depth pharmacological study of biologically active substances of the saxifrage *pimpinella* flora of Ukraine in order to create new phytopreparations of multidirectional action is appropriate and promising.

Methods

Plant Materials

The object of the study was to select the rhizomes and roots of the *Pimpinella saxifraga*, which was harvested in the Husiatyn district, Ternopil region, in 2017. The rhizomes and roots were dried using conventional methods and then stored in paper bags in dry place [18-20].

Preparation of extract. About 500 g of dried raw material was powdered with the help of a suitable crusher [21, 22]. It was taken in extractor and extracted using 85 % ethanol as a solvent. The extract was concentrated under vacuum and dried by rotator evaporator under reduced pressure.

Animal models

110 white nonlinear mice of both sexes weighing 27–32 g were used as the experimental animals. The animals were kept in room with a temperature $(22\pm 2)^\circ\text{C}$, and relative humidity of 44–55 % under 12/12 hour light and dark cycle with standard laboratory diet and water given ad libitum.

Pharmacological studies were conducted in accordance with the rules and requirements of the “General Principles for the Work on Animals” approved by the I National Congress on Bioethics (Kyiv, Ukraine, 2001 and agreed with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe No. 123, Strasbourg 1985), and the Law of Ukraine “On the Protection of Animals from Cruelty” of February, 26, 2006 [23-26]. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Acute toxicity studies: Studies of acute toxicity of thick extracts from the rhizomes and roots of *Pimpinella saxifraga* were performed V. B. Prozorovskyi method [27] on white nonlinear mice of both sexes weighing 27–32 g. Animals were divided into groups of 7 animals each. Animals were injected intragastrically with test extracts in the dose range of 500 mg/kg, 750 mg/kg, 1000 mg/kg, 3000 mg/kg and 5000 mg/kg. If the size of the extract exceeded 5 ml, the administration was performed in a fractional manner during the day [28]. At the end of the experiment (14 days), the mortality rate was determined in each group to calculate the average lethal dose (LD_{50}) [29].

Intragastric administration through a metal probe of the studied extracts of *Pimpinella saxifraga* was performed after night (8–12 h) fasting of animals. During the studies, the animals had free access to water; they were allowed to eat only 4 hours after administration [28, 29].

Throughout the experiment, the survival of animals, consumption of food and water by them, as well as clinical manifestations of intoxication (if any): general condition, changes in body position, skin condition, color of mucous membranes and individual symptoms (lacrimation, diarrhea, changes in the color of urine and feces, drowsiness,

convulsions) were observed. In case of death of animals, their autopsy was performed and macroscopic analysis of abdominal organs was performed in order to establish that the lethal outcome of the animal did not occur due to manipulation errors, as well as to determine the probable cause of death.

Investigation of the expectorant effect of a thick extract of rhizomes and roots of *Pimpinella saxifraga*: The effect of *Pimpinella saxifraga* extracts on the secretory function of the bronchi was performed according to the method described in the relevant sources [30-32].

The studied thick extracts of *Pimpinella saxifraga* at doses of 100 and 200 mg/kg and the reference drug Hedelix drops (50 ml, Krewel Meuselbach GmbH.) at the rate of 100 mg/kg and 200 mg/kg were administered intragastrically to mice of both sexes with weight 20–26 g and after 30 min was administered intraperitoneally 500 mg/kg of phenolic red (phenolic red was dissolved in 1–2 drops of dimethyl sulfoxide and adjusted with saline to the required volume). After 30 min, the animals were removed from the experiment by dislocation of the vertebrae in the cervical region, bled by dissection of the abdominal aorta and resection of the trachea was performed. The resulting trachea was placed in 4 ml of saline and washed for 30 min, centrifuged at 8000 rpm at room temperature for 10 min, added 1 N a solution of sodium hydroxide (NaOH) to the supernatant (0.1 ml of 1 N NaOH per 1 ml of supernatant) and then on a photoelectrocalorimeter (PhEC) measured the optical density at a wavelength of 546 nm. Determination of expectorant activity was established by the concentration of phenolic red.

The expectorant effect of the studied thick extracts of *Pimpinella saxifraga* and the reference drug Hedelix drops was studied for their effect on the motility of the ciliated epithelium. This indicator characterizes the evacuation capacity of bronchial secretions. Studies of the expectorant effect were performed on a model of an isolated rat trachea. The studied extracts were administered intragastrically at a dose of 200 mg/kg.

Rats weighing 280–310 g were killed by bloodletting from the abdominal aorta. The trachea

was released, separated between the larynx and its bifurcation and fixed to a plate sized 9×3.7×0.3 cm. The plate was placed in a plastic box of 350 ml with 250 ml of Tyrode's solution and placed 1 cm below the level of the solution. Tyrode's solution was saturated with carbogen while maintaining a constant temperature of 37 °C. The activity of the cilia was determined by counting the time of advancement of poppy seeds, which were located on the opposite edge of the larynx of the tracheal mucosa, at a distance of 5 mm. The basic activity of the cilia was determined in 5 observations using magnification (×20) [33]. The studied compounds were added to Tyrode's solution, where the trachea was located.

Statistical processing of the obtained results was performed in the computer program "Statistica 8.0".

The nonparametric Mann-Whitney U-test was used to estimate the statistical difference in the two independent samples, and the Kruskal-Wallis method was used to compare the independent samples in different groups [34].

Results and Discussion

Study of acute toxicity of a thick extract from the rhizomes and roots of Pimpinella saxifraga

The results of the study of acute toxicity of the studied extract of *Pimpinella saxifraga* when administered intragastrically are presented in Table 1.

The results of observations of animals, which were carried out within two weeks after the introduction of the studied extracts showed that during this period there were no cases of mortality of animals in the experimental groups.

At high doses of the extract (5000 mg/kg), animals appeared lethargic at baseline, their motor activity was slightly reduced, but food and water intake did not change. In the study of low and medium doses, no visible signs of effects on the appearance, appetite or behavior of mice were recorded.

Given that no mortality was observed in animals at high doses of the extract, it can be assumed that LD₅₀ value at enteral administration of the extract

exceeds the maximum dose used in the experiment, ie in mice LD₅₀ > 5000 mg/kg. Thus, the studied extract according to K. K. Sydorov classification can be attributed to class V of toxicity – almost harmless substances [27].

Investigation of the expectorant effect of a thick extract from the rhizomes and roots of Pimpinella saxifraga

A feature that characterizes the expectorant properties of the studied extract is determination of its effect on the secretory function of the bronchi. The research results are shown in Table 2.

The results showed that SPER (a thick extract from the rhizomes and roots of *Pimpinella saxifraga*) has a high ability to secrete sputum, which is not significantly inferior to the ability to compare Hedelix drops (ivy extract) – 144.5 % and 161.0 %, respectively. The best results were shown by the studied extract at a dose of 200 mg/kg.

The expectorant effect of the studied extract and reference drugs was studied for their effect on the motility of the ciliated epithelium. This indicator characterizes the evacuation capacity of bronchial secretions.

Studies of the expectorant effect were performed on a model of an isolated rat trachea. The research results are presented in Table 3.

It was found that a thick extract of *Pimpinella saxifraga* showed the best expectorant effect at a dose of 200 mg/kg, where it showed the ability to increase the secretory function of the bronchi. This dose was chosen to establish the motor function of the airway epithelium. Also, the studied extract showed the ability to affect the motility of the ciliated epithelium of the bronchi.

The results of studies indicate that the size of the expectorant action of SPER at a dose of 200 mg/kg is slightly inferior to the reference drug – Hedelix drops, 31.87 % and 40.89%, respectively.

Conclusions

The acute toxicity of the thick extract from the rhizomes and roots of *Pimpinella saxifraga* was studied. The test extract was determined according to K. K. Sydorov classification class V of toxicity of compounds (almost harmless substances, LD₅₀ ≥

5000 mg/kg). The expectorant effect of a thick extract from the rhizomes and roots of *Pimpinella saxifraga* on the influence of motor activity of the ciliated epithelium and the secretory function of the bronchi were studied. The greatest expectorant activity of the extract was observed in the group of animals administered the extract at a dose of 200 mg/kg. It was found that according to this effect, the activity of *Pimpinella saxifraga* thick extract of rhizomes and roots was somewhat inferior to the activity of the reference drug Hedelix drops (31.87 % and 40.89 %, respectively). In this regard, it is advisable to continue work on the analysis of rhizomes and roots of *Pimpinella saxifraga* in order to manufacture a phytopreparation with expectorant activity based on it.

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Table 1. Acute toxicity study of *Pimpinella saxifraga* extract by intragastric administration to mice

Extract	Way of introduction	Dose, mg/kg (according to active substance)	Number of dead animals/total number of animals in the group	
			Males	Females
SPER	intragastric	500	0/7	0/7
SPER	intragastric	750	0/7	0/7
SPER	intragastric	1000	0/7	0/7
SPER	intragastric	3000	0/7	0/7
SPER	intragastric	5000	0/7	0/7

Note: SPER – a thick extract from the rhizomes and roots of *Pimpinella saxifraga*.

Table 2. The effect of a thick extract of *Pimpinella saxifraga* on the secretory function of the bronchi

Group of animals (n=5)	Dose, mg/kg	Optical density, units of opt. density	The ability to secrete sputum %
Control		0.171±0.037#	100 %
SPER	100 mg/kg	0.383±0.018*#	123.2 %
SPER	200 mg/kg	0.419±0.010*	144.5 %
Hedelix drops	100 mg/kg	0.408±0.043*	137.8 %
Hedelix drops	200 mg/kg	0.447±0.028*	161.0 %

Notes: * – significant differences (p < 0.05) relative to control;

– significant differences (p < 0.05) relative to Hedelix drops;

SPER – a thick extract from the rhizomes and roots of *Pimpinella saxifraga*.

Table 3. The effect of a thick extract of *Pimpinella saxifraga* on the time of passing of poppy seeds on the ciliated epithelium of the trachea of rats

Group of animals (n=5)	Dose, mg per 250 ml of incubation mixture	The time of passing of the poppy seed on the ciliated epithelium of the rat trachea, min
Control (Tyrode's solution)		23.3±0.59
SPER	200	15.9±0.97* (31.87 %)
Hedelix drops	200	13.8±0.58* (40.89 %)

Notes: * – significant differences (p < 0.05) relative to control;

– significant differences (p < 0.05) relative to Hedelix drops;

SPER - a thick extract from the rhizomes and roots of *Pimpinella saxifraga*.