



Investigation on the Expectorant Effect of Extracts from *Primula veris* L.

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Abstract

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BACKGROUND: At present, coughing can be regulated by medications such as dextromethorphan and codeine, which are associated with side effects, including drug drowsiness or dependency. Thus, there is an increasing demand for drugs that promote expectorant activities with fewer adverse effects. The root of *Primula veris* L. (*Primulaceae*) is an herbal medicine that has been used as an expectorant drug for thousands of years in folk medicine.

AIM: The present study aims to create an in-depth pharmacological study of the expectorant activity of *P. veris* to create new drugs in different directions which are appropriate and promising.

MATERIALS AND METHODS: The expectorant effect of thick extracts of the study plant was studied on the influence of the motor activity of the ciliated epithelium and the secretory function of the bronchi. The expectorant activities of the ethanol extracts of leaves and rhizomes with roots from *P. veris* were evaluated using classical animal models. The expectorant assay was performed with phenol red secretion in the mouse trachea. After gastric administration of the test extracts in mice, 2.5% phenol red solution was injected intraperitoneally. The trachea was dissected and the optical density of tracheal secretion was measured.

RESULTS: The results of the studies showed that a thick extract of primrose rhizomes with roots has a high ability to secrete sputum, which is almost not inferior to the Hedelix drops comparator (ivy extract) – 126.6% and 146.4%, respectively. Extract from the leaves of *P. veris* is characterized by less pronounced activity, which, at a dose of 200 mg/kg, was 74.5%.

CONCLUSION: The results of the present study provide evidence that *P. veris* can be used as an expectorant herbal medicine and that triterpene saponins may be the main active ingredients of *Primula veris* responsible for its bioactivities.

Introduction

The creation of new herbal medicines and the improvement of technologies for the production of phytopreparations is one of the most important areas of pharmaceutical science.

Valuable sources of biologically active substances are members of the *Primulaceae* family, in particular the genus *Primula* (*Primula* L.), species of which are used in many countries around the world [1], [2]. Plants of the genus *Primrose* have played an important role in the treatment of diseases of the upper respiratory tract [3], primarily due to the diverse number of active substances: saponins, flavonoids, essential oil, tannins, vitamins, and unlimited base of raw materials [4], [5].

Primrose (*Primula veris* L.; *Primula officinalis* Hill.) is a perennial herbaceous plant, commonly known as cowslip primrose, belonging to the family *Primrose*. Primrose leaves contain triterpene saponins

(about 2%), and flavonoids (kaempferol diramnolide (primula flavonololide), and flowers contain saponins and flavonoids (kaempferol diramnolide, quercetin 3-gentiobioside) [6], [7], as well as essential oil, which includes: 5-methoxymethylsalicylate, 4-methoxymethylsalicylate, primverine, primulaverine; macro- and microelements. Myricetin, quercetin, kaempferol, dolphinidine, and cyanidin from anthocyanins are present in the hydrolyzate of leaf extracts [8]. Saponins and phenolic glycosides were studied in *Primula elatior* and *P. veris* Müller *et al.* [9]. The researchers found that most saponins are in the underground organs of *P. veris*. NMR spectroscopy also revealed the presence of phenolic glycosides in the studied species, primulaverine, and primeverine, of which primeverine dominates quantitatively.

Recent studies concerning medicinal plants unveiled the truth that herbals can be a source of antibacterial activities [10], [11]. Turkish scientists studying the extracts of primrose flowers found antibacterial and antimitotic effects. The extracts had an

inhibitory effect on Gram-positive and Gram-negative microorganisms [12], [13].

Tokalov *et al.* [14] proved that flavonoid drugs from some species of the genus *Primula* are used in epilepsy and convulsions.

The hepatoprotective effect of the therapeutic and prophylactic use of a thick extract of cowslip primrose was studied in experiments on outbred male rats in the model of acute CCL4 hepatitis [15].

Therefore, cowslip primrose is a valuable medicinal plant with sufficient natural resources and centuries of experience in folk and scientific medicine, which mainly uses drugs from underground organs of foreign production plants. Saponins are active components of the primrose that have expectorant action. They stimulate the gastric mucosa, which, due to the vagus reflex, leads to increased bronchial secretion and improves expectoration by diluting sputum in the bronchi.

At present, coughs can be regulated by medications such as dextromethorphan and codeine, which are associated with side effects that include drowsiness or drug dependency [16], [17]. Therefore, there is an increasing demand for drugs that promote antitussive and expectorant activities with fewer adverse effects [18]. In traditional medicine, many natural products have been used to treat cough and sputum for thousands of years and have shown minimal side effects [19], [20]. Therefore, it is valuable to look for effective treatments for both cough and sputum among natural products directed to the characteristics of the patient due to their low toxicity and fewer side effects [21].

Therefore, in this study, an in-depth pharmacological study of the expectorant activity of cowslip primrose to create new phytopreparations with different directions is appropriate and promising.

Materials and Methods

Plant materials

The leaves of *P. veris* L. were collected in the Ternopil region (Ukraine) during the flowering period in April–May 2017. Subterranean organs were collected in September–October, after the aboveground part died. The study raw material was authenticated by Prof. Svitlana Marchyshyn (I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine) [22]. The selected parts of these plants were then dried in the shade, then ground, and stored in paper bags [23], [24].

Preparation of the extracts

About 1000 g of dried *P. veris* leaves and rhizomes with roots were powdered with the help

of a suitable crusher. It used remaceration as the extraction method. Remaceration was performed by dividing the extractant into four equal portions [25]. The extracts were obtained by extraction using 40% ethanol solvent (1 kg of leaves or roots: 10 l of ethanol) and extracted at a temperature of $20 \pm 2^\circ\text{C}$. Each time, the raw material was infused for a day with a fresh portion of the extractant. After the infusion, the extract was drained, and the meal was extracted with a fresh portion of the extractant, namely, 2.5 l. The extraction duration is 4 days. Extracts were concentrated under vacuum to half under volume and dried at a temperature of $50 \pm 2^\circ\text{C}$.

Animal models

In this study, we aimed to investigate whether of cowslip primrose extracts had effects on the tracheal output of phenol red in mice, as well as whether of cowslip primrose extracts could have effect on mucin and lysozyme secretion from the rat tracheal ring explants. Therefore, the study was carried out in non-linear white mice of both sexes weighing 20–24 g and rats of the Wistar strain weighing 280–310 g, which were reared in the Animal House of the Central Research Laboratory of National Pirogov Memorial Medical University, where they were kept under appropriate conditions (at a constant room temperature of $22 \pm 1^\circ\text{C}$, 40–70% humidity conditions and a 12-h light/dark cycle). Throughout the experimental period, the animals received a standard rat diet and water ad libitum.

Pharmacological studies were conducted according to the rules and requirements of the 'General Principles for Animal Work' 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 [26]. All protocols for the animal experiment were approved by the Animal Ethics Committee of National Pirogov Memorial Medical University.

Investigation of the expectorant effect of thick extracts of herb and roots of cowslip primrose

The effect of cowslip primrose extracts on the secretory function of the bronchi was carried out according to the method described in the relevant sources [27], [28].

The thick extracts of cowslip primrose studied at doses of 50, 100, 150, and 200 mg/kg and the reference drugs Hedelix drops (50 ml, Krewel Meuselbach GmbH, Germania) and the "Alteika" syrup (PJSC Pharmaceutical Factory "Viola," Zaporizhzhia, Ukraine) at a rate of 100 mg/kg were administered intragastrically to mice of both sexes with weight 20–24 g

and after 30 min 500 mg/kg of phenolic red (phenolic red was dissolved in 1–2 drops of dimethylsulfoxide 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 measured the optical density at a wavelength of 546 nm. The determination of expectorant activity was established by the concentration of phenolic red.

The expectorant effect of the thick extracts of cowslip primrose studied and the reference drugs Hedelix drops and syrup "Alteika" were 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 isolated rat trachea. The extracts studied were administered intravenously 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 on a plate sized 9 × 3.7 × 0.3 cm. The plate was placed in a 350 ml plastic box with 250 ml of Tyrode solution and placed 1 cm below the level of the solution. The Tyrode 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 the 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) [29]. The compounds studied were added to the Tyrode solution, where the trachea was located.

Statistical analysis

All experimental results are presented with mean standard errors of the mean (n = 5). 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.

Results and Discussion

This study was to report on the potent expectorant effect of *P. veris* using animal models. An

indicator that characterizes the expectorant properties of the extract studied is to determine its effect on the secretory function of the bronchi. The method used was described by Engler and Szelenyi 1984 and Lin *et al.*, 2008 [27], [28].

The results of the studies showed that a thick extract of primrose roots rhizomes has a high ability to secrete sputum, which is almost not inferior to the Hedelix drops comparator (ivy extract) – 126.6% and 146.4%, respectively. The extract of the leaves of cowslip primrose is characterized by a less pronounced activity, which, at a dose of 200 mg/kg, was 74.5% Table 1.

Table 1: Influence of dense extracts of cowslip primrose on the secretory function of the bronchi

Group of animals (n = 7)	Dose, mg/kg	Optical density, unit of optical density	Increased secretory capacity
Control	-	0.266 ± 0.015	100%
TEPL	50 mg/kg	0.313 ± 0.038	+17.7%
TEPRR	50 mg/kg	0.320 ± 0.021*	+20.2%
Hedelix drops	100 mg/kg	0.356 ± 0.050*	+146.4%
Alteika Syrup	100 mg/kg	0.715 ± 0.052*	+168.8%

*Probable differences (p < 0.05) relative to control. TEPL: Thick extract from the leaves of cowslip primrose, TEPRR: Thick extract of rhizomes and roots of cowslip primrose.

The expectorant effect of the studied cowslip primrose extracts and comparison drugs was studied for their effect on the motor activity 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.

Research results are presented in Table 2 and Figure 1.

Table 2: Influence of thick extracts of leaves and rhizomes with roots of cowslip primrose on the time of promotion of poppy seeds on the ciliated epithelium of the trachea of rats

Group of animals (n = 7)	Dose, mg per 250 ml of incubation mixture	The time of advancement of the poppy seed in the ciliated epithelium of the rat trachea, min.
Control (Tyrode solution)	-	22.9 ± 0.44
TEPL	50	21.2 ± 0.81 (7.4%)
TEPRR	50	20.2 ± 0.73* (11.9%)
Hedelix drops	100	13.7 ± 0.96* (40.2%)
Alteika Syrup	100	13.1 ± 1.12* (43.0%)

*Probable differences (p < 0.05) relative to control. TEPL: Thick extract from the leaves of cowslip primrose, TEPRR: Thick extract of rhizomes and roots of cowslip primrose.

Both extracts were found to show a pronounced dose-dependent expectorant effect and showed the ability to increase both the secretory and motor functions of the airway epithelium. The expectorant activity of a thick extract of rhizomes with primrose roots exceeds

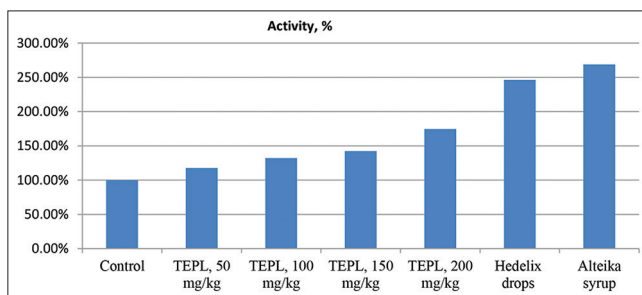


Figure 1: Influence of thick extracts of cowslip primrose leaves on the secretory function of the bronchi (n = 7)

the activity of the extract obtained from the leaves of the plant. In terms of expectorant action, the rhizome extract with primrose roots at a dose of 200 mg/kg was slightly inferior to the activity of Alteika syrup and compared to the activity of Hedelix drops.

These expectorant effects of a thick extract of rhizomes with primrose roots and thick extracts of primrose leaves are most likely due to their major components, such as triterpene saponins, respectively. Saponins (primulagenins A and D), phenolic derivatives (chlorogenic acid), and flavonoid (rutin) are the main components of the thick extracts of *P. veris*. Approximately 15% of the thick extract of primrose roots-rhizome consists of a complex mixture of saponins, and it has been thought that the expectorant activity of saponins is mediated by the gastric mucosa, with reflex stimulation of the bronchial mucous glands through the parasympathetic pathway. Saponins have also been shown to have spasmolytic, bronchodilatory, and antibacterial activities from the leaves and rhizome with roots of *P. veris* [9], [12].

Conclusion

The expectorant activity of extracts of cowslip primrose was studied. Mucus secretion was evaluated in mice by measuring the tracheal output of phenol red. Moreover, the effect of extracts of cowslip primrose on the secretion of mucin and lysozyme was performed in the rat tracheal ring explants. The highest expectorant activity of the extract of rhizomes with primrose roots was observed in the group of animals administered the extract at a dose of 200 mg/kg of 126.6%. The extract of the leaves of cowslip primrose was characterized by less pronounced activity, which, at a dose of 200 mg/kg, was 74.5%. However, the exact mechanism remains elusive and additional studies are necessary in the human population.

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