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## The study of the analgesic activity of the modified extract from *Vaccinium vitis-idaea* leaves

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#### **Abstract**

The aim of the work was to study the analgesic activity of the phytosubstance from the complex of cowberry with arginine in a single intragastric administration. The study object was a modified phytosubstance, a complex of phenolic compounds of *Vaccinium vitis-idaea* leaves with arginine. The analgesic activity was studied on the model of acute thermal pain, which was modeled in a conventional behavioral hot plate test to study the pain sensitivity and the analgesic effect of pharmacological drugs. The appearance of a powerful analgesic effect of the complex of cowberry with arginine can be explained by addition of arginine to the phytosubstance studied, while the removal of the amino acid arginine from the phytosubstance was manifested by a decrease in the analgesic effect by 94% in 30 min, on average by 52% in 60 and 120 min and by 34% in 180 and 240 min after administration of the phytoextract from *Vaccinium vitis-idaea* leaves extracted with 50% ethyl alcohol compared to the group animals receiving the complex of cowberry with arginine. The amino acid arginine itself had no analgesic effect at all compared to the control group.

**Keywords:** Phytosubstances, leaves, cowberry (*Vaccinium vitis-idaea* L.), analgesic activity

#### **1. Introduction**

Urinary tract infections are ones of the most common infections that are widely found in outpatient and inpatient practice [1]. Every year more than 150 million cases of urinary tract infections are registered in the world. According to a number of authors the incidence of this pathology is 40% of all hospital infections. According to the World Health Organization (WHO) data in 2012, acute cystitis in women in Europe was in the second place in frequency after ARVI. It is one of the diseases that are most common and for which patients seek medical care [2]. In the USA there are about 7 000000 references to a doctor per year for urinary tract infections, among them more than 2 million visits to doctors are recorded for cystitis [1, 3].

One of the clinical symptoms of urinary tract infections is acute pain syndrome. Pain is a complex and diverse concept in clinical and pathogenetic aspects [4, 5]. In a general sense, a painful sensation is part of the signaling system that warns the body of emerging disorders and injuries, determining its leading role in the self-preservation of the body. Pain is the most common reason for patients to see a doctor. The exact prevalence of pain is difficult to determine. It has been found that two thirds of the population live with pain for more than 5 years and consulted more than 3 specialists about getting rid of it. In addition, about three-quarters of residents report daily pain, and almost a quarter of people assess the intensity of pain as acute. On the other hand, to eliminate pain the self-use of analgesics by patients is widespread: almost 80% of elderly patients at least once a year resort to the analgesic self-treatment. A wide range of over-the-counter painkillers (analgin, baralgin, paracetamol, etc.) and aggressive advertising of new "extra-potent" analgesics disorient patients. The use of analgesics and non-steroidal anti-inflammatory drugs themselves as analgesics (aspirin, diclofenac, ibuprofen, indomethacin) makes it necessary to assess the risk - benefit ratio of their application. The studies show that non-steroidal anti-inflammatory drugs are taken daily by more than 30 million people in the world, and complications from their use, primarily gastrointestinal ones, are very frequent and constantly increasing [5]. In the presence of a severe pain syndrome in urinary tract infections nonsteroidal anti-inflammatory drugs are prescribed, they suppress the synthesis of prostaglandins and have a pronounced analgesic effect. Indomethacin, diclofenac and others are prescribed. The effect of non-steroidal anti-inflammatory drugs, as a rule, persists for 3-4 months after their withdrawal.

Therefore, today the search for non-synthetic drugs with minimal risk of side effects is an urgent task. Herbal medicinal products using cowberry (*Vaccinium vitis-idaea* L.) extracts are

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of practical interest in the treatment of uncomplicated infections of the genitourinary system. In official medicine, cowberry is used *per os* in the form of an infusion or decoction as a diuretic and disinfectant for inflammatory diseases of the bladder and the urinary tract. Cowberry drugs are recommended by the European Scientific Cooperative on Phytotherapy (ESCOP) and the German Commission E Monographs for the treatment of dysuric disorders and cystitis in cases where there are no indications for the use of antibiotics. Herbal medicinal products based on cowberry in defined therapeutic doses have a pronounced antimicrobial effect on a number of uropathogenic strains of bacteria; they enhance the effect of antibacterial drugs, thereby reducing the risk of developing resistant forms of microorganisms in the process of the antibacterial therapy. In the classic domestic manuals on urology cowberry drugs are mentioned as the most effective herbal products in the treatment of cystitis [6, 7, 8].

At the Pharmacognosy Department of the National University of Pharmacy (NUPh) under the supervision of the head of the Department professor Koshovyi O.M. modified galenical and neogalenical substances from *Vaccinium vitis-idaea* leaves were obtained. According to our previous studies a complex of phenolic compounds with arginine appeared to be the most effective phytosubstance. Therefore, further study of its specific activity is reasonable [9, 10].

The aim of the work was to study the analgesic activity of the phytosubstance from the complex of cowberry with arginine in a single intragastric administration.

**Materials and methods**

The study object was a modified phytosubstance, a complex of phenolic compounds of *Vaccinium vitis-idaea* leaves with arginine [10]. Amino acids affect the solubility, bioavailability and overall pharmacotherapeutic effect of extracts. Therefore, arginine was added to the extract from *Vaccinium vitis-idaea* leaves obtained with 50% ethyl alcohol in a triple equimolar amount in relation to the total amount of phenolic compounds, and the complex of phenolic compounds with arginine was obtained. To compare the analgesic activity of the modified extract from *Vaccinium vitis-idaea* leaves with arginine as a whole, the activity of individual components of this phytosubstance – arginine and a dry cowberry extract – was also studied. The analgesic activity was studied on the model of acute thermal pain, which was modeled in a conventional behavioral hot plate test to study the pain sensitivity and the analgesic effect of pharmacological drugs [11].

The analgesic activity was studied on intact white rats weighing 180-220 g. Animals were kept in the vivarium of the Central Research Laboratory of the National University of Pharmacy under standard conditions at the air temperature of 22–24 °C and a relative humidity of 50-70% with free access

to food and water. Within 3-4 hours before the introduction of the test substance or a solvent animals were kept without food. All studies were performed in compliance with the main provisions of the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes dated March 18, 1986, Directive 2010/63/EU of the European Parliament and of the Council dated September 22, 2010 on the protection of animals used for scientific purposes [12]. Groups of animals were formed by the method of randomization. The period of quarantine and acclimatization lasted 7 days. Animals were divided into 5 groups of 6 rats in each group. The compounds studied were administered intragastrically in the dose of 100 mg/kg in the form of an aqueous suspension 30 min before placing the animals on a metal plate. The studies and analysis of the experimental data were carried out compared to the reference drug Analgin in the dose of 50 mg/kg in the form of 10% solution and Voltaren in the dose of 10 mg/kg. The control group received 0.9% NaCl solution in the dose of 0.1 ml per 1 kg of the body weight. After administration of the phytosubstance studied in 30 min later the animal is gently placed on the hot plate, which temperature was 55°C. The pain sensitivity indicator was duration of the animal staying on the hot plate until the onset of defensive reflexes (licking the limbs, bouncing), which was measured in seconds. The animals were observed in 0.5, 1, 2, 3 and 4 hours. The criterion of the analgesic effect was considered to be a significant increase in the latent period of the response after administration of the substance compared to the control. To prevent thermal damage during the experiment the exposure time of animals in the hot plate test did not exceed 60 seconds. On the hot plate model the analgesic activity was calculated by the following formula:

$$AA = \frac{\Delta T_d - \Delta T_c}{\Delta T_c} * 100\%$$

where AA – is the analgesic activity, %;  
 ΔTd – is the difference in the latent period of the corresponding response in the group of experimental animals before and after administration of a potential analgesic;  
 ΔTc – is the difference in the latent period of the corresponding response in the group of control animals before and after administration of the solvent.  
 Statistical data processing was performed using parametric methods of statistics by Student t-test. The level of statistical significance of differences in results was p<0.05.

**Results and discussion**

The results of the study of the analgesic activity of the phytosubstance under research are presented in Tab. 1.

**Table 1:** The analgesic activity of the complex of cowberry with arginine.

Phytosubstance	The time of discomfort occurrence (seconds) Analgesic activity (% in relation to control)					
	before administration	after administration in				
		30 min	60 min	120 min	180 min	240 min
Control		5.67±0.52*				
Complex of cowberry with arginine	5.33±0.52	17.67±1.03*(+118)	17.17±0.75*(+109)	16.33±0.82*(+94)	16±0.63*(+88)	15.67±0.52*(+82)
Arginine	6±0.63	7.33±0.52*(-77)	6.83±1.17*(-85)	6.17±0.98*(-97)	6.33±1.03*(-94)	6.67±1.03*(-88)
Extract with cowberry leaves	6±0.63	13±0.89*(+24)	14.67±0.52*(+53)	14.50±0.84*(+50)	13.50±0.84*(+32)	13.67±1.03*(+35)
Analgin	6.17±0.41	16.83±0.41*(+88)	21.50±1.38*(+170)	20±0.63*(+144)	18.17±0.98*(+112)	11.50±0.55*(-6)
Voltaren	6.33±1.03	19.00±1.26(+124)	22.83±1.33(+191)	22.33±0.52(+182)	20.50±0.55(+150)	15.67±0.82(+65)

\* - p<0.05 in relation to the control animals.

As a result of the studies in determining the analgesic activity in the group of animals that received the complex of cowberry with arginine it was found that before the experiment the time from the moment of placing a rat on a hot surface until the appearance of a behavioral response to pain irritation was on average 5.3 seconds. The response time after administration of the phytosubstance under research was increased by an average of 3 times compared to the control in 30 min and 1 hour after administration, and 2.8 times in 120 min, 180 min and 240 min after administration. The analgesic effect of the complex of cowberry with arginine increased by 118% in 30 min after administration, by 109% in 60 min, and on average by 88% in 120, 180, 240 min after injection of the phytosubstance compared to the control. The appearance of a powerful analgesic effect of the complex of cowberry with arginine can be explained by addition of arginine to the phytosubstance studied, while the removal of the amino acid arginine from the phytosubstance was manifested by a decrease in the analgesic effect by 94% in 30 min, on average by 52% in 60 and 120 min and by 34% in 180 and 240 min after administration of the phytoextract from *Vaccinium vitis-idaea* leaves extracted with 50% ethyl alcohol compared to the group animals receiving the complex of cowberry with arginine. The amino acid arginine itself had no analgesic effect at all compared to the control group.

The reference drug Analgin had the maximum analgesic effect in 60 min after administration of Analgin; it was higher by 170% compared to the control and did not possess the analgesic effect in 240 min after administration compared to the control group.

The reference drug Voltaren also had the maximum analgesic effect after its administration and was by 191% higher compared to the control and by 21% higher compared to Analgin for this time. Unlike Analgin, which did not possess the analgesic effect in four hours after administration, Voltaren was higher than in the control group by 65% in 240 min after administration, indicating a more prolonged analgesic effect of this drug.

Compared to the reference drug Analgin the analgesic effect of the complex of cowberry with arginine exceeded Analgin by 29% in 30 min after administration, by 90% in 230 min, but was inferior to it in 60, 120, 180 min after administration.

Compared to the reference drug Voltaren the complex of cowberry with arginine was inferior to it in the analgesic effect by 6% in 30 min after administration, by 82% in 60 min.

The results of the experiment conducted indicate an increase in the latent period of the behavioral response to pain stimulation of the rat's limb thermoreceptors under the effect of the substance – the complex of cowberry with arginine under study in the dose of 100 mg/kg and the presence of a pronounced analgesic effect of this phytosubstance.

## Conclusion

1. The analgesic activity of the modified extract from *Vaccinium vitis-idaea* leaves in combination with arginine, as well as the individual components of this phytosubstance, has been studied.
2. It has been found that amino acids in the complex of with phenolic compounds of *Vaccinium vitis-idaea* leaves affects the intensity of the analgesic effect, and potentiates it.
3. The phytosubstance, which is the complex of glycosides of phenolic compounds with arginine, is recommended

for in-depth studies of the analgesic activity on other models of pain stimulation.

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