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STUDY OF THE UROANTISEPTIC ACTIVITY OF THE COMPLEX OF GLYCOSIDES OF PHENOLIC COMPOUNDS FROM LINGONBERRY LEAVES IN COMPLEX WITH THE ARGININE AMINO ACID

Karyna Tsemenko, Karyna Tolmachova, Igor Kireyev, Inna Vladymyrova, Natalia Zhabotynska

The aim of the work *– study the uroantiseptic activity of a complex of glycosides of phenolic compounds from lingonberry leaves in a complex with the amino acid arginine.*
Materials and methods. *The object of the study was a phytosubstance, which is a complex of glycosides of phenolic compounds from lingonberry leaves in combination with the amino acid arginine (CGPA). Female laboratory rats were given a cleansing enema twice, after which the rats were provided with adequate sedation by injecting IV XYLA 2 % for anesthesia, then rectally injected 1000 μl of Escherichia coli strain at a concentration of 10^9 CFU/ml. The next day after infection, the animals were subjected to cryogenic action – acute cold stress at a temperature of 0+2 °C for 2.5 hours, promoting the development of the infectious and inflammatory process. The uroantiseptic effect was studied according to the following parameters: the number of leukocytes in the urine, the number of colony-forming microorganisms E. coli in 1 ml of urine (CFU/ml).*
Results*. According to the obtained results of the experiment, it was established that the complex of glycosides of phenolic compounds from the leaves of lingonberry in a complex with the amino acid arginine (CGPA) showed a high uroantiseptic effect in the experimental treatment of urinary tract infection caused by E. coli ATCC 25922, which was manifested in the sanitation of the urinary tract from the uropathogen E. Coli.*
Conclusions*. Analyzing the results, it can be concluded that on the model of urinary tract infection against the background of cryogenic exposure, CGPA at a dose of 100 mg/kg showed a uroantiseptic effect, which was stronger than the comparison drug "Inurek" and contributed to a faster disappearance of the titer of colony-forming microorganisms in 1 ml of urine about 2 days earlier than in the group that received dietary supplements of herbal origin "Inurek" and the disappearance of leukocyturia 3 days earlier compared to the group that received therapy with the comparison drug "Inurek". The obtained results indicate the prospects of further research of CGPA with the aim of creating new effective uroantiseptic agents of plant origin based on it*
Keywords*: phytosubstance, leaves, lingonberry, uroantiseptic, rats*

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1. Introduction

Urinary tract infections (UTIs) accompanied by inflammation are one of the most significant problems of practical medicine and rank third among all infectious diseases in general [1]. UTI is a group of diseases of infectious and inflammatory origin, most often found in girls, women and the elderly. In the USA, UTIs account for 100,000 hospitalizations annually, most often for pyelonephritis [1]. Statistical data for Ukraine show that every second woman has encountered a UTI at least once in her life. In 2016, 233.9 thousand cases of cystitis were registered in Ukraine (506.3 per 100 thousand of the total population) [2]. The risk group of such pathologies includes people with weakened immunity, congenital abnormalities of the genitourinary system, and people with diabetes.

For many decades, gram-negative flora, in particular *E. coli*, is considered the main pathogenetic flora cultured from urine in uncomplicated UTIs. The frequency of presence of the causative agent in uncomplicated UTIs is 50–75 % [3, 4], and in cases of out-of-hospital UTIs it reaches 85 % [4, 5].

According to statistics, *Proteus mirabilis* is in second place in terms of seeding frequency. The frequency of sowing of *Proteus mirabilis* reaches 45–48 % [6, 7].

Other pathogenic microorganisms in uncomplicated UTI are cultured much less often and are represented by *Klebsiella spp.*, *Pseudomonas spp.*, *Staphylococcus aureus*, *Morganella morganii*, *Citrobacter freundii*. Thus, *Klebsiella spp.* in the urine of patients with chronic pyelonephritis is found in 2 % to 17.5 % of cases [6].

Today, rather great importance is attached to the causative agents of the genus *Candida*. In recent years, the prevalence of candiduria in urine has increased from 1 % to 8 % [7].

UTI is one of the causes of chronic renal failure, which determines the importance of their timely treatment and effective prevention. Traditional therapy of UTI includes the use of antibiotics and herbal preparations.

Considering the fact that the infectious and inflammatory process in the urinary tract often recurs, patients need repeated courses of antibacterial therapy [7, 8]. Treatment with modern synthetic antibacterial agents is often accompanied by pronounced side effects, and the effectiveness of most of them tends to decrease [9]. This phenomenon is caused by the appearance of antibiotic-resistant forms of pathogens in connection with the active spread of resistant strains of microorganisms, and the introduction of new antibacterial drugs into medical practice is characterized by significant financial and time costs for their acquisition, study of the mechanism of action, preclinical and clinical trials, reducing the relevance of their timeliness [9]. All this determines the constant interest of scientists in improving UTI therapy [10]. One of the possible directions for solving this issue is the search for promising phytosubstances and the further development of uroantiseptics of plant origin, which are used as anti-relapse treatment of UTI [10]. The safety of most herbal remedies makes it possible to prescribe them in long-term courses at the initial stages, at the stages of anti-relapse or rehabilitation treatment [11, 12]. The appointment of herbal remedies to patients with UTI is pathogenetically justified [13], as they have a multimodal effect: they have the ability to limit the spread of inflammatory edema, have antibacterial, antiseptic and diuretic properties, while improving microcirculation and, counteracting the further spread of the pathological process, have an analgesic and immunomodulatory effect and potentiate the effect of antibacterial agents [14]. One of the ways to solve the issue of creating new highly effective remedies of plant origin is their modification, in particular enrichment of BAS by adding amino acids and preclinical study of their pharmacological activity [15]. In modern medicine, phytotherapy is used both as an independent form of treatment and in combination with other medicinal products. At the Department of Pharmacognosy of the NUPh (Kharkov) under the leadership of Professor O. M. Koshovoy, a new modified phytosubstance was obtained – a complex of glycosides of phenolic compounds from lingonberry leaves in combination with the arginine amino acid (CGPA), which became the basis for an experimental study of their pharmacodynamics and safety with the prospect of recommendations for further research and the use of herbal medicines for the prophylactic treatment of recurrent urinary tract infections (RUTIs). The use of the amino acid arginine as an auxiliary substance in addition to the active substance, in particular herbal, leads to an increase in the stability and bioavailability of the active components, as a result of which their pharmacological effect increases [15]. It should also be noted that arginine salts are used to improve the solubility of a number of drugs, for example, as adjuvants in painkillers and antibacterial drugs [16]. Previous studies have demonstrated pronounced antibacterial and diuretic effects in CGPA [16, 17].

The widespread use of herbal preparations recommended for long-term use for the treatment and prevention of UTI relapses necessitated the study of the effectiveness of CGPA on the model of the infectious-inflammatory process (MIIP). Considering the results of previous studies of the pharmacological activity of CGPA [17], which confirmed the presence of an antibacterial effect, which is realized due to the effect on the causative agents of inflammatory processes of the urinary system, in particular, on uropathogenic *E. coli*, it was reasonable to reproduce MIIP on female rats. Modelling on experimental animals' pathological conditions and various diseases that occur under the influence of adverse factors is the main approach at the stage of preclinical research of new drugs. In this regard, studies aimed at modelling the pathology of the urinary bladder and kidneys in laboratory animals continue to be relevant in experimental science [18, 19]. The uroantiseptic effect of CGPA was studied on MIIP, which was caused by acute cold stress, because acute hypothermia, which leads to the development of an inflammatory process in kidney tissue, is a frequent clinical situation. It is for these reasons that MIIP with cold stress was chosen from all the existing ones, according to etiopathogenesis, clinical course and laboratory parameters, MIIP is identical to the inflammatory process in the urinary tract observed in humans [20].

The aim of the work is to study uroantiseptic activity of CGPA.

2. Planning (methodology) of the research

Taking into account the results of previous studies of CGPA on the presence of this phytosubstance with a pronounced antibacterial effect, our goal was to check the presence of uroantiseptic properties of CGPA, in particular, the effect on uropathogenic *E. coli.* Taking into account the literary sources that indicate the ability of phytosubstances from the leaves of common lingonberry to have an antibacterial effect against the main uropathogens, and it is stated above that *E. coli* is the causative agent of UTI in 75–80 %, it is logical to assume the presence of a uroantiseptic effect in relation to the main uropathogen. That is why the experimental MIIP was chosen, because this model, unlike others, is reproduced without technical difficulties, is recommended at the stage of preclinical studies and provides a quick and safe reproduction of the microbial-inflammatory process in the urinary tract and the absence of surgical trauma in laboratory animals. In contrast to the method of modelling pyelonephritis by injecting cultures of pathogenic microorganisms into the area of the kidney projection and applying surgical trauma followed by removing the animals from the experiment, our model of reproduction of urinary tract inflammation is characterized by a number of advantages, the main ones of which are: ease of execution, higher reproducibility by due to the use of standardized museum strains that have virulence and pathogenicity at the same time, the use of veterinary safe XYLA 2 % – a tranquilizer, analgesic and muscle relaxant, which has a minimum of side effects, and the possibility of working with animals after the end of the experiment, since we used to confirm the inflammatory process biological fluid (urine) followed by microscopic and microbiological confirmation of the presence of inflammation.

Stages used:

1. Analysis of literary sources.

2. Preparation of all equipment, setup of centrifuge and microscope with photofixation and software.

3. Distribution of animals into groups.

4. Conducting an experiment.

5. Collection of urine of laboratory animals.

6. Preparation of smears.

7. Analysis of the received data.

3. Materials and methods

The object of the study was a phytosubstance, which is CGPA. The method of obtaining CGPA: 100 g of common lingonberry leaves, crushed to a particle size of 2–3 mm, were placed in a flask, filled with a 50 % solution of ethyl alcohol in a ratio of 1:5, considering the absorption coefficient of the raw material, and extracted for a day at room temperature. The extraction was repeated three times with new portions of the extractant. The obtained extracts were combined, stood for a day, filtered. The filtrate was evaporated using a rotary vacuum evaporator with the addition of arginine in a 3-fold equimolar amount in relation to the total amount of phenolic compounds in terms of gallic acid. The resulting solution was brought to a thick extract. Phytosubstance CGPA is a brown substance that contains a complex of phenolic compounds (hydroxycinnamic acids 1.30 %, flavonoids 4.01 %, hydroquinone derivatives – 8.61 %, the sum of phenolic compounds – 22.03 %) in a complex with arginine – 39 %. Among hydroxy acids: chlorogenic acid – 121.43 mg/100 g, caffeic acid derivatives – 400.87 mg/100 g). Among flavonoids: rutin – 554.72 mg/100 g, quercetin glycoside – 855.22 mg/100 g, quercetin – 27.08 mg/100 g, kaempferol glycoside – 13.35 mg/100 g.

E. coli ATCC 25922 strain obtained at the STATE INSTITUTE "MECHNIKOV INSTITUTE OF MICROBIOLOGY AND IMMUNOLOGY of the National Academy of Medical Sciences of Ukraine (SU "MIMI NAMS"), which was grown on nutrient agar. In each female rat, MIIP was reproduced using a single technique. To create the model, a dose of *E. coli* ATCC 25922 10^9 microbial cells per 1 ml of physiological solution was used. Preparation of suspensions of microorganisms with a concentration of 10^9 microbial cells per 1 ml (optical density) was carried out using a turbidity standard (0.5 units on the McFarland scale). We used a Densi-La-Meter device (manufactured by PLIVA-Lachema, Czech Republic; wavelength 540 nm). The introduction was carried out rectally, because the transurethral introduction of a microbial suspension to laboratory rats, firstly, creates the risk of causing additional injury and requires additional guarantees against conductor contamination and the exclusion of infection of animal urine with microorganisms from the environment. Rectal introduction of *E. coli* probably provided an ascending path for the development of UTI, but the diagnosis of UTI makes sense as a temporary one at the pre-hospital stage of medical care in the process of identification of the nosological form, because in clinical practice the differentiation of the localization can be difficult and establish the level at which the inflammatory process occurs the process, especially in outpatient settings, is simply impossible. To confirm the formation of an inflammatory process of the urinary tract, we used a bacterioscopic or microscopic method of examining urine sediment (a diagnostic method for studying the morphological and tinctorial properties of microorganisms in the studied material using a microscope. In our case, we used the "pressed drop" method, which allowed us to identify the presence of any microorganisms in general, then we used stained urine smears for the presence of leukocytes as a reliable sign of the presence of inflammation) and cultural methods (which allowed us to identify the causative agent of *E. coli* by sowing on Endo medium).

The animals were divided into three groups of 8 rats each: Group 1 group (control pathology) – animals that were not treated; Group 2 (comparison group) – animals that were injected with the comparison drug "Inurek"; Group 3 (main) – animals that were administered CGPA at a dose of 100 mg/kg. The drug "Inurek" (produced by "Farmasierra Manufacturing", Spain) is a biologically active supplement (BAS) in the form of tablets containing American cranberry, used in a dose of 38 mg/kg once a day, we chose it not by chance, because it is standardized by proanthocyanidins and similar in pharmacological action. Groups of animals were formed by the method of randomization. The period of quarantine and acclimatization lasted 7 days. The laboratory rats were twice given a cleansing enema, after which the rats were adequately sedated by injecting IV XYLA 2 % for anesthesia, then rectally injected 1000 µl of *E. coli* strain at a concentration of 10^9 CFU/ml. On the next day after infection, the animals were subjected to cryogenic action – acute cold stress at a temperature of 0–(+2) °C for 2.5 hours, promoting the development of the infectious and inflammatory process [21]. On the third day after infection, animals were placed in diuretic racks for urine collection. To detect the presence of an inflammatory process at the screening stage to select rats for further research, urine analysis was performed using CitoLab test strips (Producer – Farmasco) for clinical urine analysis to detect leukocyte esterase and nitrites. The simultaneous determination of leukocyte esterase, which allows you to detect leukocyturia, and the nitrite test, which is a sign of microbial contamination of urine, are reliable signs of the microbial-inflammatory process of the urinary system (instructions for CitoLab diagnostic test strips for urine analysis). The next step after the selection of all urine samples of laboratory animals in which leukocytes and nitrites were identified was the preparation of a native preparation of urine sediment and staining of smears according to Romanowsky-Giemsa. All studied substances were used in the therapeutic mode of administration – on the third day after the reproduction of the model. The duration of treatment and observation of animals was 14 days. Clinical observation included the study of behavioural reactions (movement, food activity) and evaluation of the dynamics of *E.coli* CFU in urine. On the 3rd, 4th, 5th, 6th, 7th, 10th, 12th, 13th and 14th day after infection of the animals, a comparative assessment of the effectiveness of the treatment was

carried out based on the determination of the quantitative dynamics of microbial insemination of urine in the three groups. Determination of the bacterial load of urine was carried out by bacteriological examination. To collect the material, urine was taken into a sterile container with a catheter. A series of 5-fold dilutions was prepared and 0.1 ml was sown on nutrient agar plates.

All studies were conducted in compliance with the main provisions of the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes of March 18, 1986 [21], Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on protection of animals used for scientific 66 purposes, Order of the Ministry of Health of Ukraine dated December 14, 2009 No. 944 "On Approval of the Procedure for Preclinical Study of Medicinal Products and Examination of Materials for Preclinical Study of Medicinal Products" and the Law of Ukraine dated February 21, 2006 No. 3447-IV "On the protection of animals from cruel treatment". (excerpt from protocol No. 4 of the meeting of the Commission on Bioethics of the National Academy of Sciences dated October 2, 2020).

All studies on the study of CGPA activities were conducted in accordance with the methodological recommendations "Preclinical studies of medicinal products" edited by O. V. Stefanov. [22]. Methods of statistical analysis. Experimental data were processed by the methods of variational statistics using the programs of the license package "Statistica® for Windows" (StatSoft Inc., USA) and "Microsoft Office Excel 2013" (Microsoft, USA) according to the Student's t test in the case of normal distribution, and according to the U Mann-Whitney – in other cases. Differences were considered significant at $p<0.05$.

4. Research results

To confirm UTI, experts of the European Urological Association, first of all, recommend evaluating the number of colony-forming microorganisms in 1 ml of urine (CFU/ml). Therefore, it was advisable to carry out an assessment of the bacteriological load of urine. The results of the evaluation of the bacterial load of urine are given in Table 1.

As a result of the evaluation of the bacterial load of urine in the dynamics for two weeks, different dynamics of CFU *E. coli* ATCC 25922 from urine were observed in all animals. On the third day after infection in the animals of all experimental groups and the control group, the amount of *E. coli* ATCC 25922 in the urine was almost unchanged and amounted to $(5.1\pm0.6)\times10^7$ CFU/ml in the control pathology group, $(7.2\pm0.4)\times10^7$ CFU/ml in the group of the comparison drug and $(6.7\pm0.6)\times10^7$ CFU/ml in the group of the comparison drug. On the fourth day of animal infection, the quantitative CFU of *E. coli* ATCC 25922 was $(3.3\pm0.6)\times10^7$ CFU/ml in the group of control pathology, $(4.7\pm0.4)\times10^7$ CFU/ml in the group of the comparison drug and $(3.7\pm0.6)\times10^7$ CFU/ml in the group of the comparison drug. On the fifth day of infection, the microbial load of *E. coli* ATCC 25922 in urine was $(7.8\pm0.5)\times10^6$ CFU/ml in the control pathology group, $(4.9\pm0.5)\times10^6$ CFU/ml in the drug group comparison and $(5.1\pm0.4)\times10^6$ CFU/ml in the main group of animals that received CGPA. On the sixth day after infection with *E. coli* in animals of all experimental groups and the control group, the number of microbial cells of *E. coli* ATCC 25922 in the urine gradually began to differ in the groups and was equal to $(4.8\pm0.6)\times10^6$ CFU/ml in the control pathology group, $(1.1\pm0.6)\times10^6$ CFU/ml in the group of the comparison drug and $(7.2\pm0.7)\times10^5$ CFU/ml in the main group of animals that received CGPA. On the seventh day of infection in the urine of animals, the quantitative content of *E. coli* ATCC 25922 in the urine was recorded at the level of $(2.3\pm0.7)\times10^6$ CFU/ml in the control pathology group, $(4.9\pm0.5)\times10^5$ CFU/ml in the group of the comparison drug and $(1.2\pm0.4)\times10^4$ CFU/ in the main group of animals that received CGPA. On the eighth day of infection, a variety of the quantitative composition of *E. coli* ATCC 25922 was observed in the urine, where this indicator was $(2.1\pm0.6)\times10^6$ CFU/ml in the control pathology group, $(1.1\pm0.6)\times10^4$ CFU/ ml in the group of the comparison drug and single colonies in the number of – $(3.6\pm0.5)\times10^3$ CFU/ ml in the main group of animals that received CGPA. On the ninth day of observation after *Escherichia coli* infection in animals of all groups, the amount of *E. coli* ATCC 25922 in the urine differed by several orders of magnitude and was equal to $(1.5\pm0.7)\times10^6$ CFU/ml in the control pathology group, $(2.9\pm0.7)\times10^3$ CFU/ml in the group of the comparison drug and in the main group, on the background of CGPA treatment single colonies were removed, which amounted to $(1.4\pm0.6)\times10^2$ CFU/ml. On the tenth day of infection, the microbial load of *E. coli* ATCC 25922 in urine was $(8.9\pm0.8)\times10^5$ CFU/ml in the group of control pathology and $(3.5\pm0.6)\times10^2$ CFU/ml in the group of the comparison drug. In the main group of animals receiving CGPA, the microflora was not removed, which allows us to conclude that the urine is sterile after treatment with CGPA. On the eleventh day after infection with *Escherichia coli*, the number of *E. coli* ATCC 25922 in the urine was $(6.6\pm0.7)\times10^5$ CFU/ml in the control group and in the comparison group single colonies in the amount of $(1.8\pm0.6)\times10^2$ in contrast to the main group, where the microflora was not removed, which makes it possible to conclude about the effectiveness of the CGPA application. On the twelfth day after infection of the animals, the amount of *E. coli* ATCC 25922 in the urine was noted in the control pathology group and was $(5.5\pm0.6)\times10^5$ CFU/ml, while in the group of the comparison drug and the main group, the microflora was not removed.

Also, to confirm the uroantiseptic effect of CGPA, we performed daily smear microscopy and dynamic leukocyte count (Table 2).

According to the obtained results, in the group of animals with control pathology that did not receive the drug, leukocyturia remained at the level of 37 in the field of vision until the end of the experiment ($p<0.05$) (Fig. 1). In the group of animals receiving CGPA, from the 3rd to the 8th day of the experiment, a rapid decrease in the number of leukocytes from 31 to 5 in the field of vision was observed, i.e., on the 6th day of treatment, single leukocytes were detected up to 5 in the field of vision ($p<0.05$), which indicates on the normalization of the laboratory picture (Fig. 2).

Table 1

Bacterial contamination of animal urine after infection with *E. coli* ATCC 25922 culture ($M\pm\sigma$, n=8)

Period of examination of animals, day	Microbial contamination, CFU/ml		
	Control pathology	CGPA, 100 mg/kg	«Inurek», 38 mg/kg
3	$(5.7\pm0.7)\times10^7$	$(6.7\pm0.6)\times10^7$	$(7.2\pm0.4)\times10^7$
4	$(3.3\pm0.6)\times10^7$	$(3.7\pm0.6)\times10^{7\#}$	$(4.7\pm0.4)\times10^{7*}$
5	$(7.8\pm0.5)\times10^6$	$(5.1\pm0.4)\times10^{6*}$	$(4.9\pm0.5)\times10^{6*}$
6	$(4.8\pm0.6)\times10^6$	$(7.2\pm0.7)\times10^{5*\#}$	$(1.1\pm0.6)\times10^{6*}$
7	$(2.3\pm0.7)\times10^6$	$(1.2\pm0.4)\times10^{4*\#}$	$(4.9\pm0.5)\times10^{5*}$
8	$(2.1\pm0.6)\times10^6$	$(3.6\pm0.5)\times10^{3*\#}$	$(1.1\pm0.6)\times10^{4*}$
9	$(1.5\pm0.7)\times10^6$	$(1.4\pm0.6)\times10^{2*\#}$	$(2.9\pm0.7)\times10^{3*}$
10	$(8.9\pm0.8)\times10^5$	Microflora is not obtained	$(3.5\pm0.6)\times10^{2*}$
11	$(6.6\pm0.7)\times10^5$	Microflora is not obtained	$(1.8\pm0.6)\times10^{2*}$
12	$(5.5\pm0.6)\times10^5$	Microflora is not obtained	Microflora is not obtained

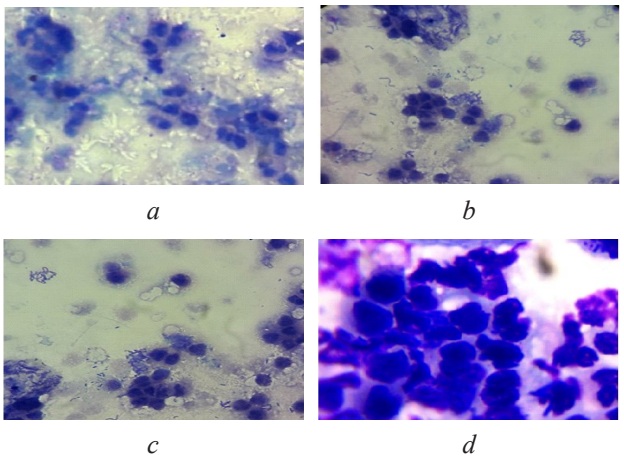
*Note: * – deviations are reliable in relation to control animals; $p<0.05$; # – deviations are reliable in relation to the comparison drug; $p<0.05$; n is the number of animals in the group*

Table 2

Dynamics of leukocyturia on the model of UTI in experimental animals ($M\pm\sigma$, n=8)

Experiment day	Control	CGPA	Inurek
3	37±2.58	31±2.16*	33±1.86*
4	38±2.58	32±2.58*	34±2.04*
5	36±2.0	21±2.04*	27±5.16*
6	37±2.58	16±2.16*	23±2.58*
7	37±4.02	9±0.89*	17±2.58*
8	36±3.76	5±1.03*	14±1.97*
9	36±3.76	4±1.03*	11±1.72*
10	36±3.76	4±0.63*	9±0.84*
11	33±2.74	4±0.52*	8±0.55*
12	36±2.04	3±0.52*	6±0.55*

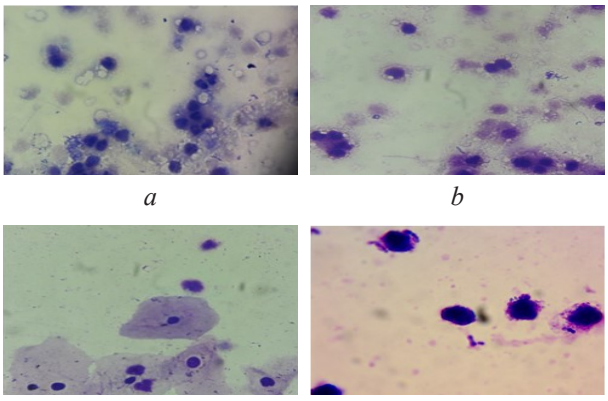
*Note: * – $p<0.05$ in relation to control animals; n – the number of animals in the group*



a *b* *c* *d*

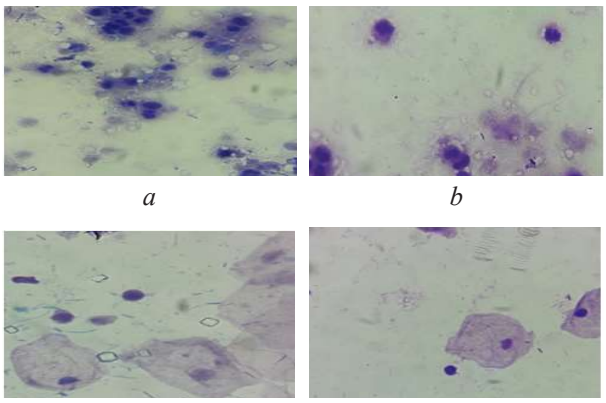
Fig. 1. Microscopy of urine smears of animals in the control pathology group: *a* – on the 3rd day of the experiment; *b* – on the 7th day of the experiment; *c* – on the 9th day of the experiment; *d* – on the 12th day of the experiment

In the group of animals that received the comparison drug "Inurek", on the 8th day of the experiment, it did not lead to a significant decrease in leukocyturia, and it remained at the mark 14 in the field of vision, which is a confirmation of the inflammatory process. Normalization of the level of leukocytes in the comparison group was observed on the 12th day of the experiment and on the 10th day of treatment (Fig. 3).



a *b* *c* *d*

Fig. 2. Microscopy of urine smears of animals in the group receiving CGPA: *a* – on the 3rd day of the experiment; *b* – on the 7th day of the experiment; *c* – on the 9th day of the experiment; *d* – on the 12th day of the experiment



a *b* *c* *d*

Fig. 3. Microscopy of urine smears of animals in the group receiving the comparison drug "Inurek": *a* – on the 3rd day of the experiment; *b* – on the 7th day of the experiment; *c* – on the 9th day of the experiment; *d* – on the 12th day of the experiment

5. Discussion of research results

Analyzing the above, it can be concluded that on MIIP against the background of cryogenic exposure, CGPA at a dose of 100 mg/kg showed a uroantiseptic effect, which was stronger than the comparison drug "Inurek" and contributed to a faster disappearance of the CFU titer in 1 ml of urine for about 2 days earlier than in the group that received the herbal preparation "Inurek" and the control group, where the level of CFU in 1 ml of urine *E. coli* remained at a high level until the end of the experiment and the disappearance of leukocyturia 3 days earlier compared to the group that received therapy with the comparison drug "Inurek".

The presence of uroantiseptic effect of CGPA is because it contains a large number of phenolic compounds, among which there is proanthocyanidin A-type. It is known that lingonberry A-type proanthocyanidins can suppress the attachment of *E. coli* to the epithelium of the urinary tract, the first stage of UTI formation [23]. Also, the additional antibacterial and uroantiseptic effects can be explained precisely by the addition of the amino acid arginine, which is a donor of nitric oxide, which has a bactericidal effect associated with the inhibition of catalase, which leads to the accumulation of hydrogen peroxide and hydroxyl radicals, the formation of peroxynitrite, which is toxic to bacterial and fungal infectious agents [24].

Study limitations. Without further study of the pharmacological activity of the CGPA phytosubstance, it is obviously incorrect to state that the uroantiseptic effect is due to the presence of A-type proanthocyanidins, which prevent the attachment of *E. coli* to the mucous membrane of the uroepithelium.

Prospects for further research. The obtained results are a prerequisite for the creation of an effective domestic phytosubstance from a complex of glycosides of phenolic compounds from lingonberry leaves in combination with the amino acid arginine, and in the future, medicinal forms based on it.

6. Conclusions

Analyzing the above, it can be concluded that on MIIP against the background of cryogenic exposure, CGPA at a dose of 100 mg/kg showed a uroantiseptic effect, which was stronger than the comparison drug "Inurek" and contributed to a faster disappearance of the CFU titer in 1 ml of urine for about 2 days earlier than in the group that received the herbal preparation "Inurek" and the disappearance of leukocyturia 3 days earlier compared to the group that received therapy with the comparison drug "Inurek". The obtained results indicate the prospects of further research of CGPA with the aim of creating new effective uroantiseptic agents of plant origin based on it.

Conflicts of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The manuscript has no associated data.

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