International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 04, 2020
ISSN: 1475-7192

DEVELOPMENT OF THE COMPOSITION AND STUDY OF THE MICROBIOLOGICAL PURITY OF THE ANTI-ANALYZED COLLECTION "MIGLIKAL"

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Abstract*---*In diseases of the gastrointestinal tract, in general, and in peptic ulcer of the stomach and duodenum, in particular, medicinal plants are widely used because of their wound healing, hemostatic, antispasmodic, astringents, which improve digestion function. The problem is successfully solved by the use of multicomponent herbal compositions or fees.At the same time, in the production of medicinal charges, it is necessary to assess the probability of the possibility of producing poor-quality medicinal charges and improve their control system, ensuring quality assurance. The safety and harmlessness of the drug collection directly depends on its microbiological parameters. This article presents the results of a study of the microbiological purity of the anti-ulcer collection "Miglikal" in accordance with the State Pharmacopoeia XI, no. 2, p. 193 and amendments #2.

***Key words*---*drug collections, pharmacological screening, acute toxicity, specific activity, quality control, safety, microbiological purity, anti-ulcer collection "Miglikal"*.**

I. INTRODUCTION

In the last decade, a clear tendency toward an increase in digestive diseases remains. According to the statistics, 5th place in the structure of total mortality is occupied by diseases of the gastrointestinal tract. Thus, this situation dictates the need to create new drugs with a polyvalent therapeutic effect on the main links of the pathological process, including conjugated organ systems with minimal side effects. The problem is successfully solved by the use of multicomponent herbal compositions or fees. In diseases of the gastrointestinal tract, in general, and in peptic ulcer of the stomach and duodenum, in particular, medicinal plants are widely used because of their wound healing, hemostatic, antispasmodic, astringent, which improve digestion function both separately and in the form of fees [1, 2,3].

The modern market of anti-ulcer drugs does not fully meet the requirements of modern medicine and does not keep pace with the ever-growing need of the population for safe, effective and preferably inexpensive medicines. One of the

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DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

possible options for expanding the range of medicines of this therapeutic group is the search and development of new medicines based on local medicinal plant materials.

The basis for obtaining fees are medicinal plants, which are raw materials that are most contaminated with various microorganisms, and can be carriers of various bacteria, fungi and viruses, as well as pollution from animals and insects. At the same time, not only medicinal plant materials can undergo microbial contamination, but also finished dosage forms obtained from them.

We have developed a composition for collecting antiulcer action. The collection included the roots of licorice, flowers of yarrow and meadowsweet flowers and flowers of calendula pharmacy. When developing new drugs, one of the most important criteria for assessing their quality is their microbiological purity. Since medicinal plant raw materials and preparations based on it are subject to excessive contamination by microorganisms and fungi, the task of evaluating and monitoring this indicator is relevant.

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At the present stage of development of pharmaceutical production, it is mandatory to comply with the rules of good manufacturing practice (GMP), which leads to toughening approaches to quality control in the production of medicines. In the pharmaceutical industry, there is a system for ensuring the quality of medicines, one of the most important parameters of which characterizing the quality of any substances and dosage forms is their microbiological purity [4].

Consequently, when developing new medicines, and especially herbal medicines, the assessment of microbiological risks is a prerequisite. Moreover, given the direct relationship between the safety of the drug and the microbiological indicators of its contamination, it is necessary to strictly control the quality of microbiological tests, which should be as accurate and reliable as possible [5].

The purpose of the study: Selection of the composition of anti-ulcer collection based on local medicinal plant materials using pharmacological screening, research on the study of acute toxicity, specific activity and assessment of the microbiological purity of the anti-ulcer collection “Miglikal”.

II. EXPERIMENTAL PART

Materials and methods

To create this collection, medicinal plants have been studied and selected, which are widely used in folk medicine and in medical practice, possessing gastroprotective, wound healing, antimicrobial, astringent and improving digestion properties: licorice roots, yarrow flowers, marigold (marigold) flowers, plantain leaves large, elecampane roots and

DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

chamomile flowers pharmacy [6,7,8]. When choosing medicinal plant materials, the natural reserves and volumes of cultivation of these medicinal plants were also taken into account.

Based on the above medicinal plants, preparations were prepared in 6 different ratios. The composition of the prepared fees are given in table 1.

№	Name of a medicinal plant	Composition of fees, in					
		Collection No. 1	Collection No. 2	Collection No. 3	Collection No. 4	Collection No. 5	Collection No. 6
1.	Flowers of the yarrow meadowsweet	-	45	-	**45**	45	45
2.	Leaves of a large plantain	-	45	45	-	-	10
3.	Licorice roots	45	-	-	**45**	-	-
4.	The roots of elecampane	45	-	45	-	45	45
5.	Calendula (marigold) flowers	10	-	-	**10**	-	-
6.	Chamomile flowers pharmacy	-	10	10	-	10	-

Dry extracts were obtained from the aqueous extracts of the charges given in the table, which were subsequently used to study specific activity using pharmacological screening.

The study of acute toxicity

For pharmacological studies, 1% aqueous solutions were prepared from the obtained dry extracts, which were administered orally to laboratory mice in an amount of 250 mg/kg to 1000 mg/kg.

Animals were observed for 2 days in the laboratory every hour. Moreover, as indicators of the functional state of the animals, survival during the experiment, general condition, possible convulsions, and death were used. From the second day, observation was carried out daily, for 14 days in vivarium conditions. Moreover, as indicators of the functional state of animals used their survival during the experiment, General condition, activity, behavior, response to tactile, pain, sound and light stimuli,the frequency and depth of respiratory movements, the rhythm of heart contractions, the condition of the hair and skin, the position of the tail, the change in body weight, and other indicators. All experimental animals were kept under identical conditions and on a common diet with free access to water and food [9].

After completion of the experiment, the LD50 and the toxicity class of the drug were determined [10, 11].

DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

The study of specific activity

Experiments on the study of antiulcer activity were performed on male rats weighing 180-200 g. The model of acute ulcers in animals was reproduced by administering 0.15 ml of a 2% formalin solution under rat aponeurosis. Before measuring the volume of the hind paw, the animals were divided into 3 groups of 6 animals and 30 minutes before the administration of formalin solution, the animals were orally administered: 1 group of animals - purified water, 2 and 3 group of animals - extracts in the amount of 75 mg/kg and 125 mg/kg, respectively.

The research results are presented in table.2.

№	Dosage of the preparations	Number of animals	Volume of the hind paw of rats	Volume of the hind paw of rats after administration of formalin Anti			Anti-inflammatory property,%
				4 hours after the of introduction formalin	The condition of the back foot in relation to the norm		
					inflammatory property	%	
1.	The control group (1 group)	6	0,75±0,068	1,53±0,158	0,78±0,084	104	--
2.	4 composition 75 mg / kg (2 group)	6	0,74±0,061	1,18±0,116	0,38±0,053	48,7	55,3
3.	4 composition 125 mg / kg (3 group)	6	0,75±0,088	1,04±0,098	0,34±0,042	45,3	58,7

This table shows the data on the study of the anti-inflammatory action of the collection No. 4 selected for screening. Given that the anti-inflammatory effect of fees under №1,2,3,5 and 6 presented in table 1, did not exceed 30-40%, the results of studies on these fees were not listed in Table 2.

The study of microbiological purity

The research on the study of microbiological purity of the Miglikal collection was conducted at the microbiological laboratory of DORI VOSITALARINI STANDARTLASH ILMIY MARKAZI LLC (Tashkent). [12].

Used culture media for microbiological research [13].

Environment No.1- for the cultivation of aerobic bacteria, dry, “HIMEDIA”, India.

Wednesday No.2 - (Saburo agar with glucose and antibiotics) - for the cultivation of yeast and molds, dry, HIMEDIA, India.

Environment No.3 - for enrichment for enterobacteria, dry, HIMEDIA, India.

Wednesday No.4 - for the isolation of enterobacteria, dry, HIMEDIA, India.

DOI: 10.37200/IJPR/V24I4/PR201343
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Environment No.8 - for growing bacteria, HIMEDIA, India.

Wednesday No.10 - for the isolation of Staphylococcus aureus, dry, HIMEDIA, India. Wednesday No. 11 - for preliminary enrichment of enterobacteria, dry, HIMEDIA, India.

Wednesday No.12 - for isolation of salmonella, dry, HIMEDIA, India.

Wednesday No.13 - for identification of salmonella, dry, HIMEDIA, India.

Wednesday No.14 - for identification of E. coli, dry, "HIMEDIA", India.

The study of the microbiological purity of the anti-ulcer collection “Miglikal” was carried out in accordance with the requirements for the microbiological purity of drugs and substances described in the Global Fund XI, issue 2, p. 193 and amendments No. 2 [14, 15].

According to the requirements of the State Pharmacopoeia XI, no. 2, p. 193 and amendments No.2, herbal medicines, depending on the method of application, are divided into categories 3 and 4. The limits of permissible microbiological norms of certain groups of microorganisms were established for them, such as: the total number of bacteria (TNB) and the total number of fungi (TNF), as well as the presence of Escherichia coli, Salmonella, and enterobacteria [14, 15].

According to this classification, anti-ulcer collection belongs to category 4A - herbal medicines or Angro medicinal herbs used in the form of infusions and decoctions prepared using boiling water (Table 1).

Table 1. Requirements for the microbiological purity of drugs

Preparations	Recommended Requirements
Category 4 A - Herbal preparations or Angro medicinal plants used in the form of infusions and decoctions prepared using boiling water	• The total number of aerobic bacteria is not more than 107 CFU per 1 g or 1 ml

The microbiological purity test included preparing various samples before testing, sampling samples for analysis, methods for quantifying viable bacteria and fungi, identifying and identifying certain types of bacteria whose presence is unacceptable or limited in non-sterile drugs. The test was carried out under aseptic conditions in order to prevent contamination of the test samples.

Quantification of microorganisms

The test was carried out by a two-layer method in Petri dishes. Samples of 10 g and 10 ml were mixed or diluted with a pH 7.0 phosphate buffer solution so that the final volume of the solution (mixture) was 100 ml. The results of determining the number of enterobacteria and other gram-negative bacteria in the sample are presented in table. 2.

Table 2. Determination of the number of enterobacteria and other gram-negative bacteria in the samples

Appropriate amount of test sample. Most likely number of bacteria.			The most likely number of bacteria in 1 g of sample
0,1 г	0,01	0,001	
in 1 g of sample	1 ml of homogenate 1 in a dilution of 1:10	1 ml of homogenate 1 diluted 1: 100	
Anti-ulcer collection “Miglikal”			
+	+	+	Over 103
+	+	+	From 102 to 103

DOI: 10.37200/IJPR/V24I4/PR201343
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+	+	-	From 101 to 102
+	-	-	Less than 101

Designations: (+) –positive test; (-) - negative testmecm

The amount of aerobic bacteria. From the prepared solution, 1 ml was added to each of two tubes with 4 ml of medium No. 1 cooled to a temperature of 45 ° C.

Identification of Escherichia coli

The test sample, diluted with sterile 1:10 buffer solution, was transferred in an amount of 10 ml (corresponding to 1 g) in 100 ml of No.8 liquid nutrient medium, mixed and incubated for 18-48 hours. Then, 1 ml of the contents of the vial was transferred to 10 ml of medium No.3. Crops were incubated for 18-24 hours. If there was growth, if the medium was uniformly clouded in tubes, reseeding was performed on medium No.4. Crops were incubated for 18-24 hours. On medium No. 4 E. coli formed - raspberry colonies with a metallic sheen, surrounded by raspberry zones, not mucous. Colonies suspicious of belonging to E. coli on solid media were microscopic.If gram-negative bacilli were detected in smears, individual colonies were sifted onto medium No. 1 beveled in test tubes and incubated for 18-24 hours.

To confirm the results used biochemical tests

From pure culture tubes, reseeds on Simmons agar and soya-casein broth were made (Wednesday No. 15), and a test for the presence of the cytochrome oxidase enzyme was also performed. After 18-24 hours of incubation, bacterial growth or its absence on Simmons agar was noted (Wednesday No. 14). Utilization of citrate was determined by the shift of the pH medium to the alkaline side (color change from green to blue). The presence of indole was determined by the appearance of a red ring on the surface of soya-casein broth with the addition of Kovacs reagent.

If gram-negative non-spore-forming bacilli were found in the sample that did not have the cytochrome oxidase enzyme, did not utilize sodium citrate and formed indole, the drug was considered to be contaminated with E. coli.

Quantification of E. coli

Quantification of E. coli was carried out in the same way as the quantification of other enterobacteria by transferring from homogenate A to tubes with medium No. 3. If the medium was uniformly clouded, the tubes were reseeded with a dense medium to confirm the presence of E. coli. No. 4. Crops were incubated for 18-24 hours. The appearance of gram-negative rods of colonies characteristic of E. coli was a positive test, the absence of growth of these colonies was a negative test. The most probable number of E. coli cells in 1 g of the sample was determined according to table 1.

Identification of bacteria of the genus Salmonella. At the beginning, 10.0 g of the test sample was transferred to 100 ml of medium No. 8, mixed and incubated for 18-24 hours. If there was growth, 1 ml of medium after mixing was transferred to 10 ml of medium No.12 and incubated for 16-24 hours. Then, reseeding was done with a loop on Bismuth sulphite agar and incubated for 24-48 h. On Bismuth sulphite agar, bacteria from the genus Salmonella formed black colonies with a characteristic metallic luster, while the portion of the medium under the colony was stained black. Colonies suspected of belonging to the genus Salmonella were microscopic. If gram-negative rods were found in smears, 2-3 characteristic colonies (each separately) were plated on three-sugar agar with iron salts (medium No. 13), applying a large amount of culture with a loop, first to the mowed part of the agar, and then with a prick in the column, without touching the bottom of the tube. After 24 hours of incubation, a color change from red to yellow was noted in the column of culture medium.

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Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

The blackening of the medium indicated the formation of hydrogen sulfide, a typical feature of species of the genus Salmonella. At the same time, a test for the presence of the enzyme “cytochrome oxidase” was used, using a pure culture from agar medium No. 1 agar. If additional confirmation, appropriate biochemical and serological tests were used.

If gram-negative non-spore-forming bacilli were found in the sample that did not have the cytochrome oxidase enzyme, did not ferment sucrose and lactose, and emit hydrogen sulfide, it was believed that the drug was contaminated with bacteria of the genus Salmonella.

Identification of Staphylococcus aureus. The test sample, diluted with sterile buffer solution 1:10, was transferred in an amount of 10 ml (corresponding to 1 g) in 100 ml of liquid nutrient medium No. 8, mixed and incubated for 24-48 hours. If there was growth, it was reseeded with a loop on medium No. 10 and incubated for 24-48 hours. Golden yellow colonies surrounded by yellow zones on medium No. 10 indicated the presence of S.aureus.

III. RESULTS AND DISCUSSION

Acute toxicity

After administration of the extracts during the day, the mice remained active; no visible changes were observed in the behavior and functional state of the mice. The condition of the coat and skin was normal, without changes, they did not refuse food and water, and the death of mice was not observed.

On the second day and during the entire observation period for 14 days, the mice showed no changes in behavior and other physical indicators, the mice eagerly consumed food and water, the reactions to light and sound stimuli remained normal, the coat and skin were clean, urination and excretion normal, the weight and growth of mice did not lag behind in development. The death of mice was not observed. According to the classification of toxicity of substances, the extracts obtained from the charges are almost non-toxic [16].

Specific activity

Because of the studies, it was found that in the control group of animals, the volume of the hind paw of rats 4 hours after administration of formalin increased by 104% compared with the initial values. In the group of animals to which the drug was administered in an amount of 75 mg/kg, this indicator was 48.7%, and in the group of animals to which the drug was administered in an amount of 125 mg/kg, this indicator was 45.3%.

Microbiological purity

For identification, the plasma coagulation reaction with a pure staphylococcus culture screened on medium No. 1 was used. If gram-positive cocci fermenting mannitol (medium No.10) with the coagulase enzyme were found in the sample, the drug was contaminated with S.aureus.

The results of the study of the microbiological purity of the anti-ulcer collection “Miglikal” are presented in table.

Table 3. The results of the study of microbiological purity of anti-ulcer collection and tincture “Miglikal”

Requirements ND	Test results
Anti-ulcer collection "Miglikal"	
The total number of aerobic bacteria is not more than 106 CFU / g	30 CFU

DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

(colonies forming units in 1 gram)	
The total number of mushrooms is not more than 104 in 1 g	2000 CFU
Enterobacteria, and other g-bacteria, not more than 103 in 1 g	Not detected
The absence of Escherichia coli in 1 g.	Not detected
The absence of Ps.aeruginoza in 1 g.	Not detected
Staphylococcus aureus in 1 g	Not detected
Lack of Salmonella in 10 g	Not detected

IV. CONCLUSIONS

1. The results of pharmacological screening showed that drug collection No. 4 has a higher antiulcer activity compared to other collections.

2. According to the classification of toxicity of substances, the extracts obtained from the charges are practically non-toxic [17].

3. The results of pharmacological studies showed that the extract obtained from collection No. 4 reduces the level of formalin inflammation by 55.3 and 58.7%, respectively, compared with the control group.

4. The results of microbiological studies have shown that the indicators of microbiological purity of the anti-ulcer collection “Miglikal” comply with the requirements of the State Pharmacopoeia XI, no.2, p.193 and changes No.2., presented for the manufacture of non-sterile forms of medicines for internal use, and can be used to ensure quality in the production of anti-ulcer collection “Miglikal”.

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DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020

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DOI: 10.37200/IJPR/V24I4/PR201343
Received: 22 Sep 2019 | Revised: 13 Oct 2019 | Accepted: 15 Jan 2020