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STRESS-PROTECTIVE ACTIVITY OF THE DRY EXTRACTS FROM RHIZOMES AND ABOVE-GROUND PART OF RHAPONTICUM UNIFLORUM L.

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The article presents the results of a study of the stress-protective activity of extracts obtained from the rhizomes and above-ground part of Rhaponticum uniflorum L., in which phytoecdysteroids and flavonoids are the main active ingredients. The developed extracts are practically non-toxic substances. Their course administration in an experimental therapeutic dose of 100 mg/kg has the stress-protective effect in acute emotional stress, decreasing the manifestation of the Selye's triad signs due to inhibition of hyperactivity of central stress-realizing systems of the body. Peripheral mechanisms of adaptogenic action of R. uniflorum extracts are associated with inhibition of free radical oxidation processes.

Keywords: medicinal herbs, *Rhaponticum uniflorum,* phytoecdysteroids, dry extracts, stress-protective activity

Currently, there is an interest in ecdysteroid-containing herbs as sources of new adaptogenic medicines that increase the body's non-specific resistance. Phytoecdysteroids have a wide range of pharmacological properties: they regulate metabolism, have anti-inflammatory, antioxidant, antitumor, immunomodulatory, nootropic, stress-protective, and anabolic effects [1,2]. At the same time, protein synthesis under their influence is not associated with the hormonal effect of animal-derived and synthetic anabolics, which provides that there are no life-threatening side effects when taking them [3]. In this regard, they are used to correct body weight during the training process and achieve high performance in professional sports. In Russia, the only ecdysteroid-containing herb included in the State Pharmacopoeia of the Russian Federation and the State register of medicinal products is a maral root (*Rhaponticum carthamoides* (Willd.) Iljin.).

In recent years, active study of another species of *Rhaponticum – Rhaponticum uniflorum (L.)* DC has been provided. Unlike *R. carthamoides,* this species is more widely distributed, growing in the steppe and mountain regions of Eastern Siberia and the Russian Far East. The content of ecdysteroids in the underground organs of the herb varies from 0.023 to 0.85% according to various sources [4]. Preparations of *R. uniflorum* are widely used in traditional medicine of the East: Tibetan, Mongolian and Chinese [5].

The purpose of this work is to determine the stress-protective activity of dry extracts made of the aboveground and underground parts of *R. uniflorum* in case of emotional stress.

MATERIALS AND METHODS

Dry extracts are obtained from underground (rhizomes with roots) and aboveground (herb) parts of *R. uniflorum*. Plant raw materials were harvested during the period of mass flowering in 2015–2016 in the Republic of Buryatia and the Trans-Baikal territory. The method for obtaining the extracts consists of three-time extraction with 60% ethanol and purified water, followed by filtration, evaporation and drying in a vacuum-drying apparatus. A method for obtaining the dry extract from rhizomes with the roots of *R. uniflorum* is patented [6]. Biologically active substances of the obtained extracts are represented by ecdysteroids, flavonoids, phenolcarbonic acids, triterpene saponins, amino acids, etc. The content of the sum of ecdysteroids in terms of ecdysterone in dry extract is 3.9% [7].

Experimental work was performed on white male and female Wistar rats weighing 180–200 g. The model of psychoemotional stress was reproduced by immobilization of animals in metal cases submerged in water (25°C) for 4 hours [8]. To the rats of the experimental groups the intragastric extracts in doses of 100 mg/kg in a volume of 10 ml/kg of aqueous solution were administrated prophylactically for 7 days prior to stress exposure. A de-alcoholized extract of *R. carthamoides* at a dose of 5.0 ml/kg was used as a comparator. On the 7th day of the experiment, the animals were subjected to psychoemotional stress and the severity of stress injuries was assessed. To do this, the indicators of the Selye's triad were determined: adrenal hypertrophy, thymus and spleen involution, the number of injuries in the gastric mucosa with the calculation of the Pauls index. In serum, the intensity of free radical oxidation processes and the activity of endogenous AOS were determined by the content of malondialdehyde [9], catalase activity [10] and superoxide dismutase [11], as well as by the concentration of reduced glutathione [12]. The plasma and serum levels of epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), corticosterone and aldosterone were determined using standard Tri Cat ELISA immunoassay kits and a DSX analyzer (USA). Statistical processing of the obtained data was performed using the Student's t-test.

RESULTS AND DISCUSSION

Determination of acute toxicity showed that extracts of rhizomes and herbs of *R. uniflorum* are practically non-toxic substances in accordance with the current classification [13].

It was found that course prophylactic administration of rhizome and herb extracts of *R. uniflorum* to animals in doses of 100 mg/kg against the background of 4-hour emotional stress had a pronounced antistress effect, as evidenced by a significant decrease in the severity of signs of the Selye's triad (Table 1).

According to Table 1, the course administration of *R. uniflorum* extracts was accompanied by decrease in the severity of signs of stress reaction: adrenal hypertrophy in rats receiving *R. uniflorum* root and herb extracts was by 27 and 20% less,

respectively, than in the control group; the weight of the thymus by 35 and 42% and the spleen – by 14 and 18%, respectively, more than in the control group of rats. Along with this, the products to be examined had a gastroprotective effect, reducing the severity of ulcerative lesions of the animal mucosa, as evidenced by decrease in the Pauls index for spot hemorrhages and erosions. In rats receiving extract of *R. uniflorum* roots, the stripe-like ulcers were not observed; in animals receiving extracts of *R. uniflorum* and *R. carthamoides* the stripe-like ulcers were observed in one rat in the group, while in the control group, these ulceres were observed in 80% of animals. In general, according to the Selye's triad, the effectiveness of *R. uniflorum* extracts was comparable to that of the comparator, i.e. *R. carthamoides* extract, and exceeded that in a number of parameters.

It was found that the stress-protective activity of *R. uniflorum* extracts is due to the restriction of hyperactivation of the central stress-relieving systems such as sympathetic-adrenal and hypothalamic-pituitary-adrenal systems (Table 2).

According to the data presented in Table 2, administration of extracts of *R. uniflorum* is accompanied by decreased activity of a trigger of the stress response – sympathetic-adrenal system, as evidenced by reduction in concentration of catecholamines in blood of animals in the experimental groups: with administration of extracts of *R. uniflorum* roots and herbs, the concentration of adrenaline is reduced respectively by 23 and 30%; the content of norepinephrine is reduced by 20 and 25% compared with those of rats in the control group. Along with this, due to administration of the phyto-medicines to

Table 1

EFFECT OF R. UNIFLORUM EXTRACTS ON THE DEGREE OF ADRENAL HYPERTROPHY, INVOLUTION OF IMMUNE-COMPETENT ORGANS, AND THE PAULS INDEX IN WHITE RATS UNDER EMOTIONAL STRESS

Parameters	Groups of animals				
	Intact, n=8	Control (stress + H_2O), n=10	Experimental 1 (stress + *R. uniflorum* roots), n=10	Experimental 2 (stress + *R. uniflorum* herb), n=10	Experimental 3 (stress + *R. carthamoides),* n=10
Weight (mg/100 g)					
atrabiliary capsules	16,0±1,08	25,0±2,51	18,3±1,34*	20,2±1,95*	16,3±1,62*
thymus	57,3±2,23	33,5±3,16	45,4±3,47*	47,6±4,03*	49,5±2,53*
spleens	458,0 ±14,5	359,2±20,5	408,5±23,4	425,3±16,8*	430±26,2*
PI for hemorrhagic diseases		6	2,8	3,2	4,3
PI for erosion		3	0,65	0,55	0,75
PI for chancres		1,25	0	0,01	0,01

*Note: * – Hereinafter these are values that differ significantly from the data for animals in the control groups at p≤0.05.*

be examined the decrease in the activity of the hypothalamic-pituitary-adrenal system is observed, as indicated by the decreased concentration of adrenocorticotropic hormone by 30 and 40%, respectively, corticosterone by 22 and 24%; aldosterone – by 12 and 24% in comparison with the similar data for rats of the control group. It was shown that the stress-protective activity of the extract of *R. uniflorum* herb was slightly higher than that of the extract of *R. uniflorum* roots, as well as the activity of the comprator, i.e. the extract of *Rhaponticum carthamoides*.

The data presented in Table 3 show that the course administration of *R. uniflorum* extracts is accompanied by decrease in induction of free radical oxidation (FRO) processes, which are the universal leading molecular-cellular mechanism of cell membrane damage in stress injuries. In particular, this is indicated by decrease in the concentration of MDA in the blood of rats in experimental groups 1 and 2 on average by 30% compared to the data of the control group. It was found that restriction of FRO processes is due to increase in the activity of the endogenous antioxidant system (AOS) of the body, as evidenced by increase in the concentration of reduced glutathione by 2.6 times – with administration of *R. uniflorum* root extract and by 3 times – with administration of *R. uniflorum* herb extract. Also, against the background of administration of test extracts, the activity of enzymes of antioxidant protection such as catalase and SOD, increases with administration of the extract of *R. uniflorum* roots – by 18 and 82%, respectively, and with administration of the extract of *R. uniflorum* herb – by 24 and 57% compared to similar data of control group rats. At the same time, the antioxidant activity of the tested products was similar to that of the comparator such as of *Rhaponticum carthamoides*.

It can be assumed that the stress-protective activity of these extracts is due to the high content

Table 2

EFFECT OF R. UNIFLORUM EXTRACTS ON THE CONTENT OF PITUITARY AND ADRENAL HORMONES IN THE BLOOD OF WHITE RATS UNDER EMOTIONAL STRESS

Parameters	Groups of animals				
	Intact, n=8	Control (stress + H_2O), n=10	Experimental 1 (stress + *R. uniflorum* roots), n=10	Experimental 2 (stress + *R. uniflorum* herb), n=10	Experimental 3 (stress + *R. carthamoides*), n=10
Adrenaline, nM/l	8,5±0,59	37,8±0,35	29,3±0,85*	26,5±1,24*	31,6±0,51*
Noradrenaline, nM/L	64,1±0,27	120,6±4,71	96,3±4,21*	87,7±5,35*	111,3±3,77
Adrenocorticotropic hormone, pg/mL	15,8±1,69	51,0±4,27	35,7±2,06*	31,2±0,86*	42,6±2,10*
Corticosterone, nM/L	44,3±3,74	65,7±3,80	51,6±1,83*	50,5±2,45*	54,7±4,38
Aldosterone, pg/mL	271,8±10,45	296,1±11,74	263,0±15,8	226,0±12,4*	257,3±16,62

Table 3

EFFECT OF R. UNIFLORUM EXTRACTS ON FREE RADICAL OXIDATION PROCESSES AND THE STATE OF THE ANTIOXIDANT SYSTEM OF WHITE RATS UNDER EMOTIONAL STRESS

Groups	Parameters			
	MDA, nM/mL	VH, mmol/L	Catalase, mcat/L	Total radiation dose (TRD), activity unit
Intact, n=8	12,2±1,03	3,1±0,16	8,3±0,61	15,6±1,08
Control (stress + H_2O), n=8	24,7±1,41	0,8±0,12	5,9±0,48	6,2±0,57
Experimental 1 (stress + *R. uniflorum* roots), n=8	15,3±1,04*	2,1±0,04*	7,0±0,22*	11,3±0,94*
Experimental 2 (stress + *R. uniflorum* трава), n=8	14,8±1,05*	2,3±0,17*	7,3±0,46*	9,74±0,14*
Experimental 3 (stress + *R. carthamoides*), n=8	14,3±0,92*	1,5±0,09*	6,7±0,72*	10,9±0,86*

of ecdysteroids, as well as such compounds as flavonoids, amino acids, etc., enhancing their biological effects, which ultimately provides inactivation of free radicals, leading to a violation of the functional and structural consistency of biological membranes under emotional stress. Thus, the data obtained indicate that *R. uniflorum* is a promising ecdysteroid-containing plant raw material for obtaining new adaptogenic agents. Taking into consideration that the content of ecdysteroids in the herb of the plant is 1.3 times higher than in underground organs, and the pharmacological activity of the extract of the herb is similar to that of the extract of rhizomes, the use of the aboveground part of *R. uniflorum* is important for the rational use of medicinal plant raw materials.

CONCLUSION:

1. Extracts of *R. uniflorum* roots and herbs with course administration in doses of 100 mg / kg have a stress-protective effect in emotional stress, preventing the development of signs of the Selye's triad.

2. Stress-protective activity of *R. uniflorum* extracts is caused by restriction of hyperactivation of central stress-realizing systems such as sympathic-adrenal and hypothalamic-pituitary-adrenal ones.

3. Peripheral effects of adaptogenic action of *R. uniflorum* extracts are associated with inhibition of free radical oxidation processes and increased activity of the endogenous antioxidant system of the body.

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REFERENCES

1. *Volodin V.V. Ecdysteroid-containing plants – sources of new adaptogens / V.V. Volodin, S.I. Mataev // Yu.A. ц Bulletin of biotechnology and physical and chemical biology. – 2011. – Vol. 7, № 2. – pp. 52–59.*

2. *Dinan L. Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals / L. Dinan, R.J. Lafont // Endocrinol. – 2006. Vol. 191. – P. 1–8.*
3. *Todorov I.N. Influence of ecdisterone on biosynthesis of proteins and nucleic acids in the organs of mice / I.N. Todorov // Chemical and pharmaceutical journal. – 2000. – Vol. 34, № 9. – pp. 3–5.*
4. *Timofeev N.P. Industrial sources for obtaining ecdysteroids. Part II. Ecdysterone: Plants of the Rhaponticum genus (review) // Non-traditional natural resources, innovative technologies and products. – M., 2007. – pp. 8–49.*
5. *Zhang Y. H, Wang H.Q. Ecdysteroids from Rhaponticum uniflorum // Phamazie, 2001. Vol. 56. Is. 10. – P. 828–829.*
6. *Patent №2582282 Russian Federation, MPK A 61 K. Method for obtaining a medicine that has stress-protective and antihypoxic activity / Nikolaev S.M., Nikolaeva I.G., Shantanova L.N., Nikolaeva G.G., Garmaeva L.L., Tatarinova N.N., Razuvaeva Ya.G., Matkhanov I.E., Seng Li; Song Pang; applicant and patent holder – Institute of General and Experimental Biology of SB RAS (RU) – №2015107164; applied on 2.03.2015; published on 20.04.2016, Bulletin №11.*
7. *Garmayeva L.L. Pharmacognostic study of Fornicium uniflorum L. and development of a medicine with stress-protective and antihypoxic activity / L.L. Garmayeva. Dissertation for the degree of Candidate of Agricultural Sciences – Ulan-Ude, 2016. – 209 p.*
8. *Overmier J. The ulcerogenic effect of a rest period after exposure to water-restraint stress / J. Overmier, R. Murison, H. Ursin // Behav. Neural. Biol. – 1986. – Vol. 46. – P. 372–382.*
9. *Temirbulatov R.A. Method of increasing the intensity of free radical oxidation of lipid-containing blood components and its diagnostic value / R.A. Temirbulatov, E.I. Seleznev // Laboratory science. – 1981. – № 4. – pp. 209–211.*
10. *Korolyuk M.A. Methods for determining catalase activity / M.A. Korolyuk, L.I. Ivanova, I.G. Mayorova // Laboratory science. – 1988. – № 1. – pp. 16–19.*
11. *Chewari S. Role of superoxide dismutase in cell oxidative processes and the method of determining it in biological materials / S. Chewari, I. Chaba, Y. Sekey // Laboratory science. – 1985. – №11. – pp. 678–681.*
12. *Anderson M.E. Glutathion: chemical, biochemical and medicinal aspects / M.E. Anderson / Pt. Ann. – N. Y. – 1989. – pp. 333–405.*
13. *Hodge H. Clinical Toxicology of Commercial Products. Acute Poisoning / H. Hogde, R. Sterner. Ed. IV. – Baltimore, 1975. – 427 p.*

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ANTHELMINTIC ACTIVITY OF MONOSUBSTITUTED AMIDES AND HYDRAZIDES OF 1,4-DICARBOXYLIC ACIDS

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The anthelmintic activity of 21 derivatives of 1,4-dicarboxylic acids in experiments on earthworms was studied in comparison with the anthelmintic agents such as Levamisole and Pyrantel. It was concluded that phenoxyacetylhydrazide and 4-antipyrylamide of citraconic acid, imidazolylamide and methoxyacetylhydrazide of maleic acid and isonicotinoylhydrazide of phthalic acid have a more pronounced anthelmintic effect in comparison with Pyrantel and the effect of phenoxyacetylhydrazide of citraconic acid is comparable with Levamisole.

Keywords: monosubstituted amides and hydrazides, 1,4-dicarboxylic acids, anthelmintic activity

Helminth infections are among the most common infections in the world and most often affect the poorest and most socially deprived communities. According to the World Health Organization, approximately 1.5 billion people in the world are infected with helminths. According to official data, the incidence of helminthiases in Russia is about 1%, and children are most often affected by this disease.

The search for compounds with high anthelmintic activity is important, as far as many anthelmintic agents are toxic to humans and cause severe adverse reactions, including disorders of the gastrointestinal tract (GI), central nervous system (CNS), cardiovascular system (CVS) and hemic system; in addition, their prolonged use often develops insensitivity of helminths to these agents [1,2].

Previously, when studying the antihelmintic activity of a number of 1,4-dicarboxylic acid heterylamides, compounds with an antihelmintic effect were detected, moreover, activity of some compounds exceeded the activity of Levamisole and Pyrantel [3]. For the first time, the antihelmintic effect of 1,4-dicarboxylic acid monohydrazides was studied for derivatives of itaconic and dimethylmaleic acids, but no antihelmintic effect was found [4]. Later, compounds with antihelminthic effect were found among monosubstituted hydrazides of 1,4-dicarboxylic acids [5]. However, antihelmintic activity was studied for a narrow range of 1,4-dicarboxylic acid derivatives.

The purpose of this work is to search for compounds with pronounced antihelmintic activity among monosubstituted amides and hydrazides of 1,4 – dicarboxylic acids.

MATERIALS AND METHODS

The objects of study of biological activity were monosubstituted amides and hydrazides

of succinic, maleic, citraconic, phthalic and tetrachlorophthalic acids, as well as salts of phthalic hydrazide synthesized at the Department of Analytical Chemistry of the Perm State Pharmaceutical Academy using well-known methods [6–9]. The formulas of the studied compounds are shown in the figure

The antihelmintic activity of compounds was studied by the method of M.P. Nikolaev [10] using earthworms 5–8 cm long and 3–5 mm in diameter, purchased in the retail network "Pet Shop" in Perm. 5 ml of 0.5% aqueous solution of the studied compounds was placed and 5 worms were immersed in a Petri dish, then the time of death of each worm was recorded upon the loss of motion activity in response to mechanical irritation. The life time of control worms in purified water is about a day (24±1 h). As a comparator agent, we used antihelmintic agents such as Pyrantel (Ozon LLC, Russia) and Levamisole (Gedeon Richter, Hungary) with a valid expiration date, purchased in a pharmacy chain.

The experimental results were processed using the Fischer – Student's method of variation statistics [11].

RESULTS AND DISCUSSION

The anthelmintic activity of 9 monosubstituted amides (compounds 1–9), 10 hydrazides (compounds 10–16, 19–21) and 2 hydrazide salts (compounds 17 and 18) of 1,4-dicarboxylic acids was studied. Two 1,4-dicarboxylic acid heterylamides (compounds 5 and 8) showed an antihelminthic effect: maleic acid imidasolylamide (compound 5) and 4-antipyrylamide of citraconic acid (compound 8) are 2 and 1.8 times more active than Pyrantel, respectively. Substitution of an acid fragment in 2-pyrimidylamide of tetrachlorophthalic acid [3] with a maleic acid residue (compound 4) leads to the loss of anthelmintic effect. Among the monosubstituted 1,4-dicarboxylic acid hydrazides, three compounds (14, 16, and 20) with anthelmintic activity were

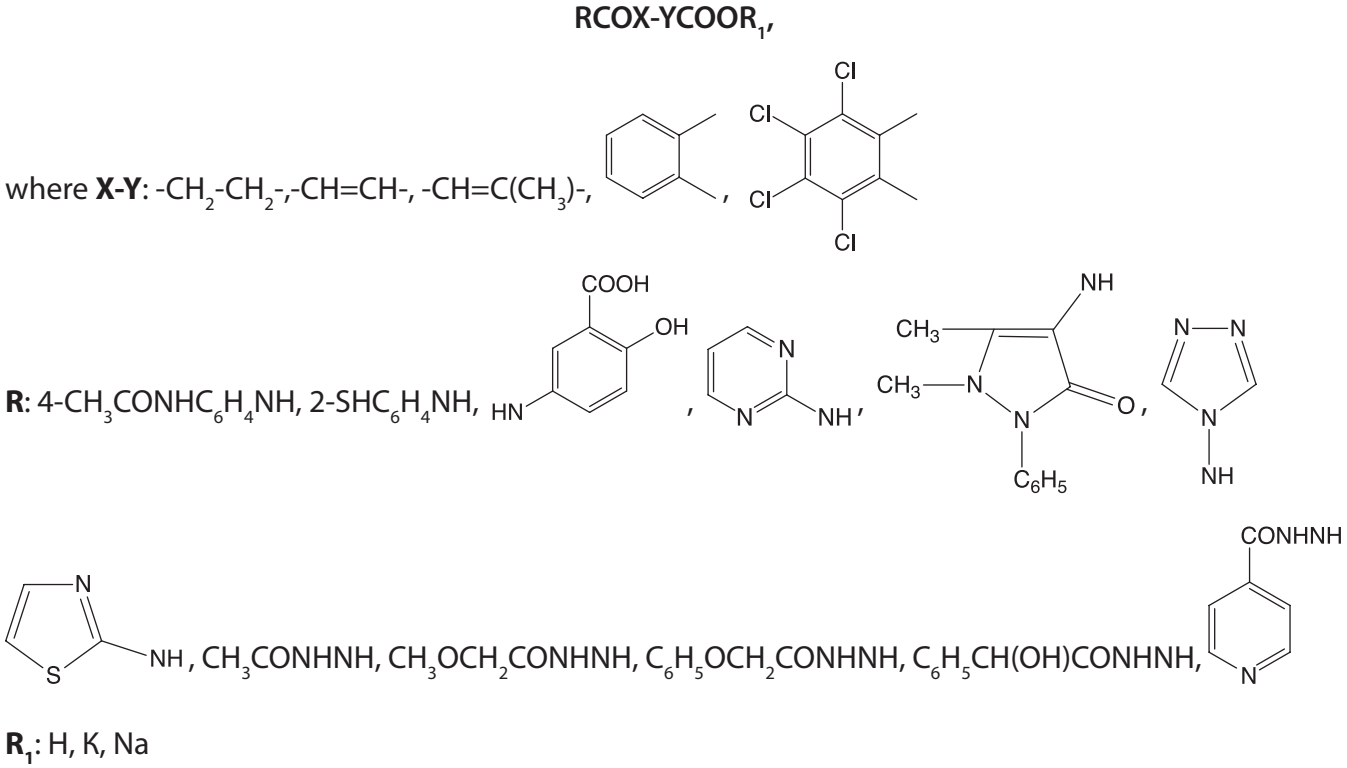

FIG. 1. *Chemical formulas of the studied compounds*

found. Phenoxyacetylhydrazide of citraconic acid (compound 16) has an anthelmintic effect equal to the action of Levamisole, but a similar derivative of succinic acid (compound 12) does not have anthelmintic activity. The effect of methoxyacetylhydrazide of maleic acid (compound 14) is 2.5 times higher than the effect of Pyrantel, while the same hydrazides of succinic (compound 11) and phthalic (compound 19) acids do not affect the life time of worms. Anthelmintic activity of phthalic acid isonicotinohydrazide (compound 20) is 2 times higher than activity of Pyrantel. Substitution of the phthalic acid residue in this hydrazide with a fragment of succinic acid (compound 13) leads to the loss of activity.

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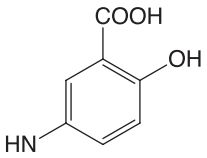
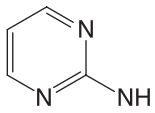
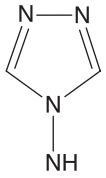
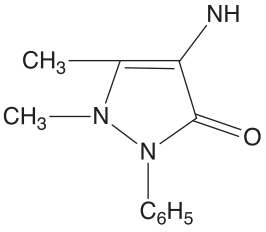
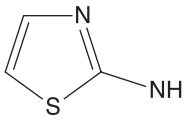
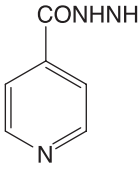
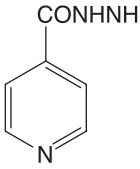
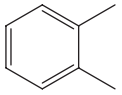
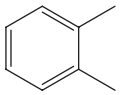
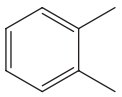
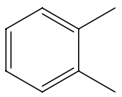
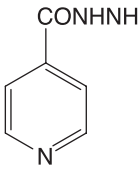
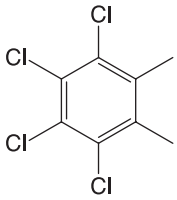
ANTHELMINTIC ACTIVITY OF MONOSUBSTITUTED AMIDES AND HYDRAZIDES OF SUCCINIC, MALEIC, CITRACONIC, PHTHALIC AND TETRACHLOROPHTHALIC ACIDS

	Compound	X-Y	R	Life time of worms, minutes (experiment)*
1.	4-acetamino-phenyl-amide of succinic acid	$-CH_2-CH_2-$	$4\text{-}CH_3CONHC_6H_4NH$	more than 200
2.	2-thio-phenyl-amid of maleic acid	-CH=CH-	$2\text{-}SHC_6H_4NH$	more than 200
3.	3-carboxy-4-hydroxy-phenyl-amide of maleic acid	-CH=CH-		more than 200
4.	2-pyrimidyl-amide of maleic acid	-CH=CH-		more than 200
5.	1-triazolyl-amide of maleic acid	-CH=CH-		101,3±0,67
6.	2-thio-phenyl-amide of citraconic acid	-CH=C (CH_3)-	$2\text{-}SHC_6H_4NH$	more than 200
7.	4-acetyl-phenyl-amide of citraconic acid	-CH=C (CH_3)-	$4\text{-}CH_3COC_6H_4NH$	more than 200
8.	Antipyril-amide of citraconic acid	-CH=C (CH_3)-		121,0±9,99

Продолжение таблицы

	Compound	X-Y	R	Life time of worms, minutes (experiment)*
9.	2-thiazolyl-amide of citraconic acid	-CH=C (CH_3)-		more than 200
10.	Acetyl-hydrazide of succinic acid	-CH_2-CH_2-	$CH_3CONHNH$	more than 200
11.	Methoxy-acetyl-hydrazide of succinic acid	-CH_2-CH_2-	$CH_3OCH_2CONHNH$	more than 200
12.	Phenoxy- acetyl-hydrazide of succinic acid	-CH_2-CH_2-	$C_6H5OCH_2CONHNH$	more than 200
13.	Iso-nicotinoyl-hydrazide of succinic acid	-CH_2-CH_2-		more than 200
14.	Methoxy-acetyl-hydrazide of maleic acid	-CH=CH-	$CH_3OCH_2CONHNH$	85,4±10,32
15.	Iso-nicotinoyl-hydrazide of maleic acid	-CH=CH-		more than 200
16.	Phenoxy- acetyl-hydrazide of citraconic acid	-CH=C (CH_3)-	$C_6H5OCH_2CONHNH$	18,5±2,97
17.	Sodium salt of phthalic acetyl-hydrazide		$CH_3CONHNH$	more than 200
18.	Potassium salt of phthalic acetyl-hydrazide		$CH_3CONHNH$	more than 200
19.	Phthalic methoxy-acetyl-hydrazide		$CH_3OCH_2CONHNH$	more than 200
20.	Phthalic iso-nicotinoyl-hydrazide			104,7±10,90

Окончание таблицы

	Compound	X-Y	R	Life time of worms, minutes (experiment)*
21.	Hydroxyphenyl acetyl-hydrazide of tetrachlorophthalic acid		C_6H5CH (OH) CONHNH	more than 200
Comparator agents			Pyrantel	215,0±0,37
			Levamisole	20,2±2,08

*Note: * – the life time of control worms was about a day (24±1 h)*

CONCLUSION

1. The anthelmintic activity of 21 1,4-dicarboxylic acid derivatives was studied using a model with earthworms. Five compounds (phenoxy-acetyl-hydrazide and 4-antipyril-amide of citraconic acid, imidazolyl-amide and methoxy-acetyl- hydrazide of maleic acid, phthalic acid iso-nicotinoyl-hydrazide) showed more pronounced anthelmintic activity in the experiment in comparison with an anthelmintic agent such as Pyrantel.

2. Anthelmintic activity of phenoxy-acetyl-hydrazide of citraconic acid is comparable with effect of Levamisole.

REFERENCES

1. *Polyakov V.E., Lysenko A.Ya. Helminthiases of children and adolescents. – M.: Medicine, 2003. – 256 p.*
2. *Lukshina R.G., Lokteva M.M., Pavlikovskaya T.N. Under the general editorship of R.G. Lukshina. Human parasitic diseases: Monograph. – 2nd ed., revised and amended. – H.: Publishing house "INJEK". 2005. – 472 p.*
3. *Kolotova N.V., Starkova A.V. Anthelmintic activity of heteryl amides of 1,4-dicarboxylic acids // Development and registration of medicines. – 2017. – №1 (18). – pp. 136–138.*
4. *Kolotova N.V., Starkova A.V., Chashchina S.V. Synthesis and biological activity of monosubstituted itaconic and dimethylmaleic acid hydrazides // Issues of ensuring the quality of medicines. – 2016. – №3 (13). – pp. 15–23.*
5. *Kolotova N.V., Starkova A.V. Biological activity of 1,4-dicarboxylic acids aroyl hydrazides// Development and registration of medicines. – 2017. – №2 (19). – pp. 167–170.*
6. *Dolzhenko A.V., Kolotova N.V., Kozminykh V.O. and others. Substituted amides and hydrazides of dicarboxylic acids. Message 14*. Synthesis, antimicrobial and anti-inflammatory activity of 4-antipyril-amides, 2-thiazolyl-amides and 1-triazolyl-amides of certain dicarboxylic acids // Journal of Pharmacy and Chemistry. – 2003. – Vol. 37. -№3. – pp. 42–44.*
7. *Kolotova N.V., Kozminykh E.N., Kolla V.E. and others. Substituted amides and hydrazides of 1,4-dicarboxylic acids. Message 7*. Synthesis and pharmacological activity of some acyl hydrazides of maleic, succinic and phthalic acids // Journal of Pharmacy and Chemistry. – 1999. – Vol. 33. – №5. – pp. 22–28.*
8. *Dolzhenko A.V., Kolotova N.V., Kozminykh V.O. and others. Substituted amides and hydrazides of dicarboxylic acids. Message 15*. Synthesis*

and hypoglycemic activity of some amides and acyl hydrazides of tetrachlorophthalic acid // Journal of Pharmacy and Chemistry. – 2003. – Vol. 37. – №4. – pp. 186–188.

9. *Kolotova N.V., Skvortsova N.L., Kozminykh V.O. and others. Synthesis of biologically active acyl hydrazides of maleic, succinic and phthalic acids // The manuscript of the department of VINITI 29.09.1997. №2940-B97. – Perm. – 1997. – pp. 1–31.*
10. *Nikolaev M.P. Experimental bases of pharmacology and toxicology. Practical guideline / M.P. Nikolaev. – Moscow-Leningrad: Medgiz. 1941. – 195 p.*
11. *Prozorovsky, V. V., Statistical processing of the results of pharmacological studies / V.V. Prozorovsky // Psychopharmacology and biological narcology. – 2007. – Vol. 7. Issue 3–4. – pp. 2090–2120.*

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CHOLERETIC EFFECT OF EXTRACTS OF CARTHAMUS TINCTORIUS L., TAGETES ERECTA L. AND CALENDULA OFFICINALIS L.

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The aim of the study was to determine the choleretic effect of extracts of safflower dye (Carthamus tinctorius L.), African marigold (Tagetes erecta L.) and pot marigold (Calendula officinalis L.) in experiments with intact white male rats of the Wistar line initially weighing 180–200 g. The obtained extracts in experimental therapeutic doses of 50–200 mg/kg were injected as aqueous solution into the duodenum of anesthetized animals (40 mg/kg of sodium thiopental, abdominally). For control group the equi-volume amount of distilled water was administered. It was established that extracts of safflower and pot marigold have a pronounced choleretic effect when administered once, due to the significant content of phenolic substances i.e.flavonoids.

Keywords: safflower dye (Carthamus tinctorius L.), African marigold (Tagetes erecta L.), pot marigold (Calendula officinalis L.), extracts, choleretic effect

One of the ways to supplement domestic herbal medicines is to study the pharmacological effects of well-known plants that are often used by the public, as well as pharmacopoeial species used in clinical practice for restricted indications. The presence of a wide range of biologically active substances in them implies a multi-sided effect on the human body, affecting many functional systems. In this regard, additional studies of their chemical composition and pharmacological action are required, which will allow to identify the additional properties and to expand the indications for their use in clinical and preventive medicine. In this aspect, safflower dye (Carthamus tinctorius L.), African marigold (Tagetes erecta L.) and pot marigold (Calendula officinalis L.) are of interest.

The purpose of this study was to determine the choleretic effect of extracts of Carthamus tinctorius L., Tagetes erecta L. и Calendula officinalis L. In experimental conditions.

MATERIALS AND METHODS

Dry extracts made of these types of plant raw materials were obtained in the laboratory of chemical and pharmaceutical studies of the Institute of General and Experimental Biology of the SB RAS by Doctor of Pharmaceutical Sciences Olennikov D.N. and Candidate of Pharmaceutical Sciences Kashchenko N.I. in 2017 by extracting

the plant material (above-ground part) with 70% ethanol in the "raw material: extractant" ratio equal to 1: 15 using ultrasonic treatment for 60 minutes with sequential concentrating and drying in a vacuum drying cabinet.

The experiments were performed using intact white male Wistar rats with an initial weight of 180–200 g, obtained from the Scientific Center for Biomedical Technologies of the Russian Federation. The animals were kept in a certified vivarium of the Institute of General and Experimental Biology SB RAS with free access to water and food. When studying, we were guided by the requirements of the following regulatory documents: Guidelines for pre-clinical studies of medicinal products (2012); Order of the Ministry of Health of the Russian Federation No. 199n "On approval of the rules of good laboratory practice" dated 01.04.2016; Rules for performance of work using experimental animals; Rules adopted by the European Convention for protection of vertebrates used for experimental and other scientific purposes (Strasbourg, 1986). Bile was collected from anesthetized animals (sodium thiopental, 40 mg/kg, abdominally) every hour for 4 consecutive hours. Dry extracts made of these types of plant raw materials were administered to rats of the corresponding group directly into duodenum at doses of 50, 100 and 200 mg/kg in the form of aqueous solution. In the control group, the equi-volume amount of distilled water was injected to animals into the duodenum. The severity of the choleretic effect of these extracts was estimated by the rate of secretion and the total amount of bile secreted, as well as by the content of its main ingredients such as bile acids, cholesterol and bilirubin [2,4,5]. Significance of differences in the data of experimental and control groups of animals was evaluated using the nonparametric Mann-Whitney U test. The study protocol was approved by the Ethics Committee of the Institute of General and Experimental Biology SB RAS (Protocol No. 3 of 02.02.2018).

RESULTS AND DISCUSSION

The data obtained during the study is presented in tables 1 and 2. Based on this data, it follows that the extract of calendula officinalis at a dose of 50 mg/kg increases the rate of bile secretion in rats by 16.0–44.0%; at a dose of 100 mg/kg – by 28.0–44.0% with increase in secerned bile by 35.0%. When administered to rats at a dose of 200 mg/kg, the rate of bile secretion increased by 33.0–36.0%, and the content of cholates exceeded the control data by 17.5–30.0% with increase in the excretion of cholesterol with bile (Tables 1, 2). The obtained data on the choleretic effect of the obtained extract of calendula officinalis are consistent with the works [1, 3], testifying to the choleretic properties of extracts from this raw material.

Administration of safflower dye extract to another group of rats was also accompanied by acceleration of bile secretion in animals. Thus, at a dose of 100 mg/kg, the rate of secretion increased by 24.0–29.3%, and at a dose of 200 mg/kg – up to 31.0%. Along with this, in this experiment, there was a tendency for active synthesis of cholates by hepatocytes, and at a dose of 50 mg/kg, increase in concentration of cholesterol in the secerned bile in rats was observed (Tables 1, 2).

The dry extract of African marigold in our experiments did not show a significant effect on the rate of bile secretion in intact rats, as well as on the total content of cholates in bile. It was noted only a moderate effect on the synthesis by hepatocytes and emission of cholates in bile. To some extent, there was acceleration of cholesterol excretion with bile in animals when African marigold extract was administered at the specified doses (Tables 1, 2).

Thus, the administration of pot marigold extract in experimental therapeutic doses of 50–200 mg/kg into duodenum of white rats anesthetized with sodium thiopental is characterized by a pronounced choleretic effect with increase in the rate of bile secretion

Table 1

EFFECT OF PLANT EXTRACTS ON THE RATE OF BILE SECRETION IN WHITE RATS

Experimental conditions	The rate of bile secretion for 4 hours, mg/min per 100 g			
	1 h	2 h	3 h	4 h
1. Control (H_2O), n=8	5,2+0,3	5,3+0,4	5,0+0,3	5,2+0,3
2. African marigold:				
50 mg/kg, n=8	5,6+0,4	5,6+0,4	5,1+0,3	4,5+0,2
100 mg/kg, n=8	5,5+0,3	5,5+0,2	5,2+0,3	4,7+0,2
200 mg/kg, n=8	6,0+0,3	6,0+0,3	6,2+0,4	5,7+0,3
1. Control (H_2O), n=8	6,2+0,3	5,8+0,4	5,2+0,4	5,2+0,3
2. Safflower dye:				
50 mg/kg, n=8	6,2+0,3	6,1+0,3	5,3+0,2	5,3+0,3
100 mg/kg, n=8	6,4+0,2	7,5+0,1*	6,4+0,2*	6,2+0,2*
200 mg/kg, n=8	6,6+0,4	7,8+0,2*	6,3+0,3*	6,0+0,3
1. Control (H_2O), n=8	5,7+0,2	5,0+0,2	4,8+0,2	4,1+0,3
2. Pot marigold:				
50 mg/kg, n=8	5,1+0,1	5,8+0,2	6,6+0,5*	5,9+0,4*
100 mg/kg, n=8	5,8+0,4	6,4+0,4*	6,5+0,4*	5,9+0,4*
200 mg/kg, n=8	5,2+0,2	5,9+0,4	6,4+0,4*	5,6+0,4*

*Note: * – Hereinafter it means that the difference between the experimental and control data is significant at P<0.05; n is the number of animals in the group.*

and increase in the concentration of cholates in the secret. The identified choleretic effect of the resulting extract is due to the content of a complex of biologically active substances, primarily flavonoids. Thus, the pot marigold extract contains up to 40.0% of flavonoids, carotenoids, terpenoids, as well as other natural compounds [1], which provide its choleretic effect. The resulting dry extract of safflower dye shows the presence of luteolin, neocartamine and kaempferol derivatives, as well as contains halcones, which, along with other natural compounds [6], stipulate the observed choleretic effect in white rats. The dry extract of African marigold showed a moderate choleretic effect in our experiments, despite the significant content of flavonoids such as quercetagetrin, patuletin, patulitrin, quercetagetin [7].

As you know, most flavonoids are characterized by a wide range of their effects on the body's functions. In particular, they stimulate the choleretic reaction, have an anti-inflammatory effect, reduce the tone of smooth muscles, provide energy production in cells due to stabilization of membrane formations against the inhibition of free radical processes and mobilization of the endogenous antioxidant defense system [3]. Obviously, the content of significant amounts of flavonoids in extracts of pot marigold and safflower dye provides a pronounced choleretic effect in white rats when the extract is directly administrated into duodenum. The obtained study results can serve as a basis for expanding the indications for the use of extracts of pot marigold and safflower dye in clinical and

Table 2

EFFECT OF PLANT EXTRACTS ON THE TOTAL AMOUNT AND BIOCHEMICAL COMPOSITION OF BILE IN WHITE RATS

Experimental conditions	Total amount of bile for 2–4 hours of experiment	Bile acids	Bilirubin	Cholesterol
	mg/100 g	mg%		
1. Control (H_2O), n=8	930+36,1	507,3	14,0	54,5
2. African marigold:				
50 mg/kg, n=8	912+35,7	587,1	13,0	69,6
100 mg/kg, n=8	924+35,3	559,1	14,0	52,8
200 mg/kg, n=8	1074+56,4	564,3	10,0	56,3
1. Control (H_2O), n=8	972+37,0	832,2	16,0	85,1
2. Safflower dye:				
50 mg/kg, n=8	1002±38,6	934,8	17,0	115,9
100 mg/kg, n=8	1206±30,4*	877,8	15,0	97,2
200 mg/kg, n=8	1194+40,1*	866,4	17,0	74,3
1. Control (H_2O), n=8	834+36,0	552,9	24,0	22,8
2. Pot marigold:				
50 mg/kg, n=8	1098+34,0*	649,8	21,0	25,1
100 mg/kg, n=8	1128+39,0*	718,2	21,0	35,0
200 mg/kg, n=8	1056+41,0*	706,8	22,0	26,6

preventive medicine, as well as for their use at the stage of rehabilitation treatment in health resort organizations.

CONCLUSION

1. Pot marigold extract has a pronounced choleretic effect at experimental therapeutic doses in intact white rats due to significant content of flavonoids and other natural compounds.

2. Administration of safflower dye extract to white rats at experimental therapeutic doses is accompanied by acceleration of the choleretic reaction with increase of cholates in the secerned bile, which is due to the content of a complex of biologically active substances, including substances of phenolic nature.

3. The African marigold extract in our experiments did not show a statistically significant effect on the course of the choleretic reaction in intact white rats; there was a certain tendency to increase of bile acids and cholesterol in the secerned bile, which requires additional studies.

REFERENCES

1. *Kashchenko N.I. Phytochemical study and improvement of methods of standardization of pot marigold flowers and herbs (Calendula officinalis L.). Abstract of Cand. Pharmaceutical Sc. Dissertation. – Ulan-Ude, 2014. – 22 p.*
2. *Miroshnichenko V.P., Gromashevskaya L.L., Kasatkina M.G. Determination of the content*

of bile acids and cholesterol in bile // Laboratory science. 1978. – №3. – pp. 149–153.

3. *Nikolaev S.M. Herbal medicines for damage to the hepatobiliary system. – Novosibirsk: Nauka, 1992. – 131–140 pp.*
4. *Skakun N.P., Oleinik A.N. Comparative effect of atropine and metacin on the external secretory function of the liver // Pharmacology and toxicology. 1967. – Vol. 30. – №3. – pp. 334–337.*
5. *Khabriev R.U. (ed.). Guidelines for conducting the preclinical studies of medicinal products. – M., 2012. – pp. 712–720.*
6. *Wagner H. Chromatographic fingerprint analysis of herbal medicines // Flor. Carthamus. – Wien. 2011. – P. 475–483.*
7. *Xu L., Chen J., Qi H., Shi Y. Phytochemicals and their biological activity of plants in Tagetes L. // Chinese Herbal medicines. 2012. – Vol. 4. – P. 103–117.*

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STUDY AND DEVELOPMENT OF HEPATOPROTECTIVE AGENT

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A multicomponent plant agent under the conditional name "Pentafit" has been developed. As a result of the experiments carried out, it was found that its course administration per os at a dose of 300 mg/kg to nonlinear rats with experimental liver injuries has antihepatotoxic, hepatoprotective and membrane stabilizing effect.

Keywords: antihepatotoxic plant agent, toxic liver injury, preclinical studies, antihepatotoxic action, hepatoprotective activity

Toxic liver injuries hold a leading place in the structure of morbidity and mortality of the population, primarily due to increase of the number of alcohol intoxications, uncontrolled large-scale use of medicines, environmental pollution, including water and food, with foreign chemical compounds [1].

The market for herbal medicines with proven anti-hepatotoxic activity is currently not too large, and the problem of effective therapy is far from being resolved. Despite the use of sufficiently active preventive measures and constantly improved treatment methods, even with the complex use of highly effective anti-hepatotoxic agents, complicated forms of toxic hepatitis are found in 26–42% of cases, and 15–25% of patients have a problem of resistance of toxic hepatitis to the most modern therapeutic effects [2].

In this regard, it is important to search for means that can increase the liver's resistance to the damaging effects of toxins and stimulate detoxification processes [3].

The purpose of the study is to determine the pharmacological activity in the development of an optimal method for obtaining a medicine that has antihepatotoxic activity.

MATERIALS AND METHODS

The object of the study was a plant composition under the conditional name "Pentafit", consisting of roots and rhizomes of elfwort (*Inula helenium* L.), common centaury herb (*Centaurium erythraéa* Rafn.), flowers of common tansy (*Anacétum vulgáre* L.), fruits of sweet-brier (*Rosa* sp.) and hawthorn berries (*Crataegus* sp.).

The components of the medicinal plant collection were selected taking into account the multifactorial mechanisms of the development of diseases of the hepatobiliary system and correspond to the principles of pharmacological regulation of the digestive system functions [3–5].

According to the literature, there is information about the anti-inflammatory, antispasmodic, choleretic and hepatoprotective effects of biologically active substances that are part of the roots and rhizomes of elfwort, common centaury herb, flowers of common tansy, fruits of sweet-brier and hawthorn berries [4–7]. In connection with the above, within the frames of the set task, the study of the anti-hepatotoxic

activity of this plant composition is promising and predictable.

To confirm the anti-hepatotoxic effect, based on data on the chemical composition, we have proposed the following method of obtaining. The medicinal plant collection containing 15% of common centaury herb, 10% of flowers of common tansy, 25% of roots and rhizomes of elfwort, 27.5% of fruits of sweet-brier and 22.5% of hawthorn berries is extracted triply with 45–55% ethyl alcohol while constantly stirring at temperature of 60–70°C for 2 hours. The combined extracts are evaporated under vacuum, separated and dried.

The resulting extract contains polysaccharides, flavonoids, carotenoids, organic acids, vitamins, macro – and microelements, essential oils and other natural compounds. **Pentafit** was standardized by the sum of flavonoids, equivalent to luteolin-standard. The content of the sum of flavonoids is regulated as at least 1%. The presence of this spectrum of biologically active substances proposes the potential anti-hepatotoxic activity of the resulting extract. The method for obtaining an agent that has anti-hepatotoxic activity is protected by patent No. 2689379 [8].

The work was performed in accordance with the Federal law "On medicines" and the Guidelines for conducting preclinical studies of medicines. Experiments were performed on 120 non-linear male rats with initial weight of 180–200 g. The animals were obtained from the Scientific Center for Biomedical Technologies of Russia and kept in a vivarium with free access to food and water. Pharmacological studies were performed according to the Rules of works using experimental animals, the Rules adopted by the European Convention for protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Order of MOH No. 199н dated 01.04.2016 "On approval of rules of good laboratory practice". The study design was approved by the VILAR Bioethical Commission (Protocol No. 7 dated October 1, 2018).

The hepatoprotective activity of the extract under the conditional name "Pentafit" at a previously selected dose of 300 mg/kg and the comparator agent, Karsil (Sofarma JSC, Bulgaria) at an isoeffective dose of 50 mg/kg was studied in conditions of a model of chronic experimental tetrachloromethane hepatitis.

To assess the antitoxic function of the liver the duration of hexenalum sleep was recorded as the duration of rats staying in the lateral position after abdominal injection of hexenal (MedPro Inc. Ltd, Latvia) at a dose of 60 mg/kg weight under the recommendation of Hatsura V.V. [9].

Liver damage was caused by intragastric administration of 50% oil solution of carbon tetrachloride (CCl4) to rats (Reachim, Russia) in the amount of 0.4 ml per 100 g of animal weight once a day for 4 days [10].

The functional validity of the rat liver monooxygenase system was evaluated by the amount of cytochrome P450 in the microsomal fraction of the liver. The content of this enzyme was measured using a Shimadzu spectrophotometer (Japan) by the Omura T. and Sato R. method [11]. Microsomes from animal liver tissue were isolated under the recommendation of I.I. Karuzina and A.I. Archakov [12]. The amount of protein in microsomes was determined using the Lowry method [13]. The rate of inactivation of reduced cytochrome P_{450} was recorded at temperature of 37°C every 3 minutes for 30 minutes.

Statistical processing of the obtained data was performed using the Statistica 10.0 software package (USA) [14]. The differences were considered as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Therapeutic efficiency of "Pentafite" was determined with intragastric course administration (1 per day) of the extract in form of aqueous

solution at a previously established experimental therapeutic dose of 300 mg/kg for 10 days in case of tetrachlormethane hepatitis in rats starting from the 2nd day after the first administration of the damaging agent. As a comparator agent, the plant hepatoprotector Carsil was used at an isoeffective dose of 50 mg/kg.

The influence of "Pentafit" on the duration of hexenalum sleep in rats with carbon tetrachloride hepatitis was studied. The results of the experiments are presented in Table 1.

Previously, non-linear rats were divided into groups: intact (20 rats); control (20 rats); experimental 1 (20 rats); experimental 2 (20 rats). "Pentafit" at a dose of 300 mg/kg was injected to Experimental 1 animals into the stomach via a probe for 10 days in case of carbon tetrachloride hepatitis in rats, starting from the 2nd day after the first administration of the damaging agent. Experimental 2 rats were treated with the reference standard Carsil at an isoeffective dose of 50 mg/kg according to a similar scheme. Animals of the control group took equi-volume amounts of water purified according to a similar scheme. Animals of the intact group served as additional control.

From Table 1 it follows that when administrating "Pentafit" the duration of hexenalum sleep in rats was decreased on the 7th and 14th days of the experiment by 29% and 27%, respectively, indicating a stimulation of the liver detoxification function with a studied extract in the conditions of the tetrachlormethane hepatitis model. The comparator agent Carsil had a less pronounced effect, reducing the duration of hexenalum sleep on the 7th and 14th days of the experiment by 14%.

In case of tetrachloromethane damage to the liver of rats, the administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg had a favorable effect on the detoxifying function of the liver.

The effect of course administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg on the state of the liver monooxygenase system of non-linear male rats with toxic hepatitis was studied. The results of the experiments are presented in Table 2.

Experiments were performed on nonlinear rats, which were divided into groups: intact (10 rats); control (10 rats); experimental 1 (10 rats); experimental 2 (10 rats). "Pentafit" at an experimental therapeutic dose of 300 mg/kg was injected to Experimental 1 animals into the stomach via a probe for 7 days in case of carbon tetrachloride hepatitis in rats, starting from the 2nd day after the first administration of the damaging agent. Experimental 2 rats were treated with

Table 1

THE INFLUENCE OF "PENTAFIT" ON THE DURATION OF HEXENALUM SLEEP IN RATS WITH TOXIC CCL_4-HEPATITIS, M±M

Groups of animals	Duration of hexenalum sleep, s	
	7 days	14 days
Intact (H_2O), n=20	838±75	1103±78
Control (CCl_4 + H_2O), n=20	1470±118	1294±55
Experimental 1 (CCl_4 + "Pentafit" 300 mg/kg), n=20	1038±73*	943±70*
Experimental 2 (CCl_4 + Carsil 50 mg/kg), n=20	1268±212	1121±102

*Note: hereinafter: * – differences are statistically significant between the data of the control and experimental groups when P≤0,05*

Table 2

EFFECT OF "PENTAFIT" ON THE STATE OF THE LIVER MONOOXYGENASE SYSTEM IN CASE OF EXPERIMENTAL CCL_4-HEPATITIS IN RATS (DAY 7)

Groups of animals	Content of Cytochrome P_{450} in nmol/mg of protein	% inactivation of Cytochrome P_{450} for 30-minute incubation	The amount of MDA in μM /mL of serum x min.
Intact (H_2O), n=10	0,79±0,04	21,2±2,0	3,99±0,40
Control (CCl_4 + H_2O), n=10	0,39±0,06	58,7±1,3	5,76±0,10
Experimental 1 (CCl_4 + "Pentafit" 300 mg/kg, n=10	0,60±0,08*	18,1±0,9*	3,89±0,60*
Experimental 2 (CCl_4 + Carsil 50 мг/кг), n=10	0,53±0,07*	18,1±1,1*	4,49±0,40

the reference standard Carsil at an isoeffective dose of 50 mg/kg according to a similar scheme. Animals of the control group took equi-volume amounts of water purified according to a similar scheme. Animals of the intact group served as additional control.

When evaluating the state of the liver monooxygenase system on the 7th day of the experiment in case of toxic hepatitis in rats, it was found that the use of "Pentafit" at the specified dose significantly increased the amount of cytochrome P450 in the liver microsomes.

54% increase in the key enzyme of the monooxygenase system responsible for liver detoxification function was accompanied by slowdown in the rate of inactivation of this enzyme due to stabilization of membrane structures. The comparator agent Carsil also had an effect on the state of the liver monooxygenase system in toxic hepatitis. "Pentafit" reduced the amount of MDA in the blood serum of rats by 32%, which indicates its membrane-stabilizing activity due to the content of BAS of phenolic nature.

Thus, the course administration of "Pentafit" at an experimental therapeutic dose of 300 mg/kg to rats with tetrachloromethane hepatitis has an anti-hepatotoxic and membrane-stabilizing effect.

CONCLUSION

According to the results of the experiments, it was found that the course administration per os of the received multicomponent agent under the conditional name "Pentafit" at an experimental therapeutic dose of 300 mg/kg to non-linear rats with experimental liver damage has an anti-hepatotoxic, hepatoprotective and membrane-stabilizing effect. The pharmacotherapeutic effect of "Pentafit" in toxic liver damage is due to the presence of a complex of biologically active substances, primarily compounds of phenolic nature [3,5].

The obtained study results prove the feasibility of using the resulting multi-component agent "Pentafit", containing biologically active substances of phenolic nature, in the prevention and complex treatment of liver diseases.

REFERENCES

1. *Ivashkin V.T. Gastroenterology: national guide / V.T. Ivashkin- M.: GEOTAR-Media, 2008. – 704 p.*
2. *Javakhyan M.A. Analysis of the market of modern hepatoprotective agents / M.A. Javakhyan,*

Yu.S. Kanunnikova // Questions of Biological, Medical and Pharmaceutical Chemistry. – 2012. – №11. – pp. 63–65.

3. *Nikolaev S.M. Phytopharmacological therapy and Phytopharmacological preventive treatment of diseases / S.M. Nikolaev. – Ulan-Ude: Publishing house of the BSU, 2012. – 286 p.*
4. *Sokolov S.Ya. Phytotherapy and phytopharmacology. Guide for doctors / S.Ya. Sokolov. – Moscow: MIA, 2000. – 976 p.*
5. *Lubsandorzhieva P.-N.B. Development and standardization of herbal remedies for the treatment and prevention of the digestive system diseases / P.-N.B. Lubsandorzhieva. – Ulan-Ude: Publishing house of the BSC, SB RAS, 2016. – 280 p.*
6. *Sokolov S.Ya. Handbook of medicinal plants / S.Ya. Sokolov, I.P. Zamotaev. – M.: Medicine, 1990. – 510 p.*
7. *Vichkanova S.A. Medicinal products made ofplants.Scientificpublication/S.A.Vechkanova, V.K. Kalher, T.A. Sokolskaya, etc. – M: ADRIS, 2009. – 432 p.*
8. *Patent 2689379 of the Russian Federation. Method for obtaining an agent that has antihepatotoxicactivity/FerubkoE.V.,NikolaevS.M., Dargaeva T.D. – No. 2019106135/19, applied on 05.03.2019, published on 28.05.2019. – Bulletin No. 16.*
9. *Sernov L.N. Elements of experimental pharmacology / L.N. Sernov, V.V. Gatsura. – M.: Medicine, 2000. – 352 p.*
10. *Vengerovsky A.I. Methodological recommendations for the study of hepatoprotective activity of medicinal products / A.I. Vengerovsky, V.V. Udut, D.V. Reichart // Guidelines for preclinical studies of medicinal products. – Moscow: Grif and Co., 2012. – Part one. – 832 p.*
11. *Omura A.T., Sato R. The carbon monoxide binding pigment of liver microsomes. Solubilization, purification and properties / A.T. Omura, R. Sato // J. Biol. Chem. – 1964. – V. 239. – №7. – P. 2379–2385.*
12. *Karusina I.I. Isolation and properties of cytochrome P450 from rabbit liver microsomes / I.I. Karusina, G.I. Bachmanova, D.E. Mengazetdinov, and others. Biochemistry. – 1979. – №6. – Pp. 1049–1057.*
13. *1Avdeev V.G. Methods for determining protein concentration // Questions of medical chemistry. – 1977. – №4. – Pp. 562–571.*
14. *1Borovikov V.P. Popular introduction to modern data analysis in the Statistica system / V.P. Borovikov. – Moscow: Hotline. Telekom, 2014. – 288 p.*

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FEATURES OF TWO-DIMENSIONAL PRINTING OF DOSAGE FORMS IN PHARMACEUTICAL TECHNOLOGY

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This review of the literature describes the main techniques used in the method of two-dimensional printing. Various types of inkjet and rotary printing technologies are presented, as well as features, advantages and disadvantages of various types of two-dimensional printing, such as continuous inkjet or print-on-demand. Features of production and application of 2D printed dosage forms (oral dispersible, mucoadgesive films, etc.) are shown. Examples of used substrates and their production technologies with specified characteristics are given, as well as requirements and formulations of solutions with pharmaceutical substances used in printing. The prospects for development of 2D printing technology and its application, in particular in the form of QR-encoded dosage forms, are described.

Keywords: two-dimensional printing of dosage forms, inkjet printing, rotary printing, oral-dispersible films

Currently, with development of therapeutic drug monitoring methods, it is possible to accurately determine for patients the dosage of many medicines that have a narrow therapeutic "window". In addition, a number of pharmaceuticals have accurate data on the relationship between the concentration of medicines in the blood and the pharmacological effect, which allows you to adjust the drug therapy for each patient individually. In particular, these pharmaceuticals include cytostatics, aminoglycoside antibiotics, anticonvulsants [1,2,4].

In addition to improving the methods of diagnosis and control of drug therapy of patients, the development of personalized medicine requires the implementation of a production method that has the ability to manufacture individual dosage forms (DF). The most promising methods in this direction are two-dimensional (2D) and three-dimensional (3D) printing technologies for production of medicines [1,3]. 2D-printing has a more flexible, cheap, easy-to-implement and easy-to-maintain technology compared to 3D-printing. Versatility and accuracy of placement of liquids with pharmaceutical substance (PS), depending on the application, the relative ease of the process control (using the simplest software), and the repeatability of distribution of volumes of liquids are also noted.

2D-PRINTING TECHNOLOGY

2D-printing is a method of producing personalized dosage forms, often oral films, by applying the solutions with pharmaceutical substance (printed "ink" or impression) to a soluble or biodegradable substrate before use.

2D-printing technologies have several production methods, they can be divided into inkjet printing technologies and rotary printing methods. The most common methods are inkjet printing ones, because they allow you to produce medicines on a substrate, using a small amount of solution with pharmaceutical substance, depending on the requirements of the recipe. In turn, inkjet printing technology is divided into continuous inkjet printing and print-on-demand [5,6].

Due to its relative simplicity, low cost, and high accuracy, print-on-demand is more preferable than continuous inkjet printing in the desktop printer markets, and it is this technology that is most commonly used in 2D-printed dosage forms. Two main technologies of the presented printers are piezoelectric and thermal (or bubble) printing.

Thermal inkjet printing uses short thermal pulses generated by a resistive element for the jet liquid. Each printhead contains a micro-resistor that quickly heats up when electrical impulses are received, forming bubbles of superheated steam, as shown in Fig. 1.

The vapor bubble expands, forcing the liquid out of the nozzle and creating a drop. The vapor bubble then collapses, creating partial vacuum that pulls the liquid out of the solution tank, re-filling the thermal inkjet printing chamber. The temperature on the surface of the resistor can reach 300°C, but such high temperatures only exist for a few milliseconds only near the microresistor. Only 0.5% (by volume) of the sample is affected, so the technology usually does not destroy thermolabile components.

In piezoelectric printing, each nozzle is surrounded by a piezoelectric element,

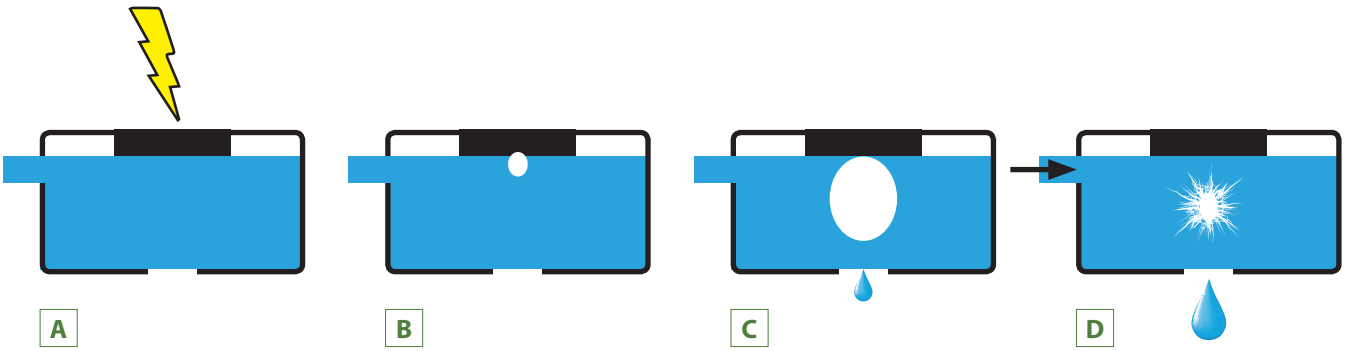

FIG. 1. *Thermal inkjet printing by stages (A) resistor temperature rise; (B) superheated steam bubble formation; (C) bubble growth and droplet deposition; (D) bubble collapse and filling*

usually made of lead zirconate titanate. During the application of voltage to the element, it deforms, creating pressure that leads to the release of liquid. As soon as the element returns to its normal shape, the nozzle is filled again with the solution, ready for repeated ejection (Fig. 2).

Regardless of the technology, inkjet printers emit a precisely controlled volume of solution with the specific coordinates on the substrate. The amount of the precipitated medicine depends on varying the volume of the sprayed solution and /or changing the concentration of the initial solution [14,16].

PREPARATION OF 2D-DOSED DOSAGE FORMS

The main stages of preparation of 2D dosage forms are: preparation of solutions and substrates, determining the size and density of the drawing on the substrate, the printing process and packaging of the dosage forms. The resulting 2D-printed films have an area of approximately 5 to 20 cm^2, in which the pharmaceutical drug is introduced into a matrix containing a hydrophilic polymer. The maximum concentration of medicines is 30–40 mg per dose. Correctly selected or manufactured substrates for printing are very important for preparation of 2D dosage form [3].

SUBSTRATES

Substrates are portable media on which the solution of the medicine is printed. Studies often focus on the practical and technical aspects of 2D printing of specific recipes with less attention to the substrate. However, studies and development of suitable substrates are important tasks, since the nature of the substrate can specify the polymorphic shape of any crystals formed when the solvent evaporates. For example, in the study of Hsu et al. (2013) it was noted that the substrate affected the crystallization of naproxen when printing on various solid amorphous dispersions [15]. In Table 1 the main substrates used according to literature data are listed [10,11,12,14].

Table 1 contains information on the use of a number of different substrates, including food substrates such as sugar sheets, polymer and starch films, and non-food substrates such as paper and acetate films. The use of ready-made food and pharmaceutical substrates, the development and manufacture of new types of them are becoming urgent tasks that should be solved together with the

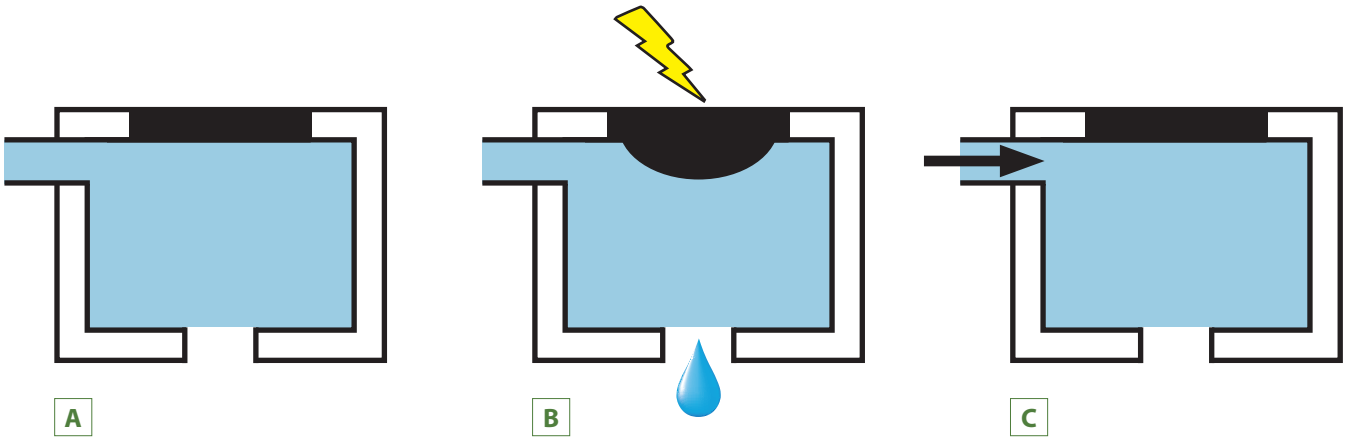

FIG. 2. *Piezoelectric printing: (A) inactive state; (B) movement of the piezoelectric element; (C) re-filling of the chamber*

Table 1

SUBSTRATES USED IN 2D PRINTING OF DOSAGE FORMS

Studies (references)	Substrates
Hirshfield et al. (2014)	Hydroxypropyl methyl cellulose (HPMC) films
Raijada et al. (2013)	Food sugar paper
Sandler et al. (2011)	Uncoated paper, coated paper and polyethylene terephthalate films (PET)
Genina et al. (2013a)	Orodispersible film, waterproof transparent film, cellulose paper
Genina et al. (2013b)	Food paper, PET film, HPC film
Buanz et al. (2011)	Starch film, purified acetate film
Genina et al. (2012)	Uncoated woodfree paper, three times and twice coated paper
Melendez et al. (2008)	PTFE film, coated with clear transparent film
Takala et al. (2012)	Copy and photo paper

implementation of 2D printing technology. The use of various substrates with specified quality characteristics, such as release modification, adsorption, and taste masking, increases interest in substrate manufacturing methods. Some of the substrate preparation technologies are discussed below.

SOLVENT CASTING OF POLYMER FILMS

When casting in a solvent, the substrate is made by casting and distributing a homogeneous layer of a polymer solution on the inert surface. Solution preparation, deaeration, casting or molding, drying, cutting and packaging of substrates are the main six stages of preparing the polymer films by casting in solvent for further 2D printing of dosage forms. As an alternative to film casting, a solvent-free hot melt extrusion method can be used. Substrates (polymer films) obtained in this way are often used for preparation of dosage forms rapidly disintegrated in the oral cavity or compositions for buccal use with prolonged release [13,14].

THE SUBSTRATES OBTAINED BY THE METHOD OF ELECTROSPINNING

Electric spinning (electroforming) is a process that leads to the formation of a polymer fiber as a result of action of electrostatic forces on an electrically charged jet of a polymer solution or melt (Fig. 3).

When electrostatic forces overcome the force of surface tension and charge, the liquid or melt forms a Taylor cone (a cone formed at the tip of a capillary or needle where a jet of liquid polymer is ejected during electrical spinning or spraying). This effect causes the drop to deform and the charged jet to be ejected in the direction of the grounded counterelectrode (drum or plate collector). The solvent evaporates during the transfer of the solution from the ejector to the collector, and finally, continuous solid fibers are collected on a grounded metal drum or plate. Typically, the nonwoven fibrous substrates with random alignment of fibres are obtained by quick whipping during the formation of fibers. However, substrates with parallel aligned fibers can be obtained using other fiber collection methods [14,15].

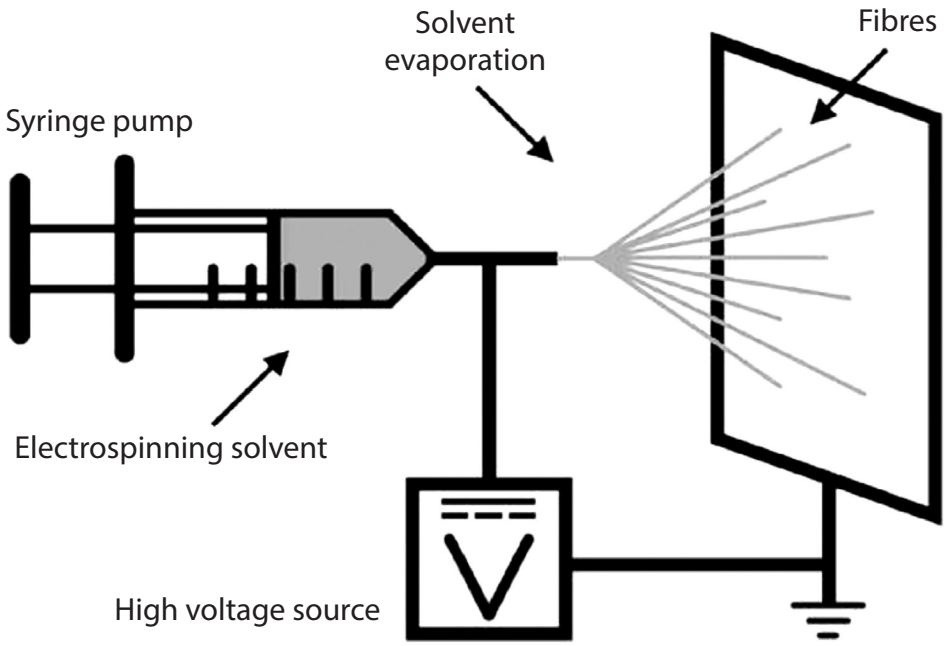

FIG. 3. *Electroforming system*

Electrical spinning is an easy-to-use, but complex-to-standard method in which the formation of fibers strongly depends on the properties of the polymer solution or melt and the parameters and conditions of the electrical spinning process. However, due to the adjustability of the process, electroforming allows you to change and optimize the properties of fibers and substrates for universal use. For example, the diameter, shape, surface topography, and internal structure of electroformed fibers depend to a large extent on the properties of the materials and the polymer solution /melt (molecular weight, concentration, additives, viscosity, surface tension, conductivity), process parameters (applied voltage, solution flow rate, distance between the ejector tip and the collector), and environmental conditions (humidity, temperature). In addition, the fiber morphology depends on the properties of the solvent, such as steam pressure and boiling point, which specify the evaporation rate of the solvent (s) and drying time. By optimizing the composition of the solution/melt and the electrical spinning conditions, it is possible to obtain fiber matrices with modified morphology and structure.

Fiber substrates have a large surface area-to-volume ratio and have the ability to incorporate an increased amount of pharmaceutical substance into their structure, which is an advantage when developing a dosage form. Electroformed substrates can acquire specific properties during the manufacturing process or through subsequent processing, which improves their mechanical strength and elasticity. Electric spinning is an attractive method in the industry due to the simplicity of the process and the possibility of large-scale production. However, this method has significant limitations in production due to the small amount of non-toxic solvents and the problem of ensuring uniformity of electroformed fibers.

In recent years, fiber structural frames containing drug such as antibacterial, anticancer agents, growth factors, and other biologically active molecules for wound healing, chemotherapy, or implants have been thoroughly studied. Electric spinning allows you to produce fibers with a diameter from nano-to micrometers from natural (for example, derivatives of cellulose, chitosan, collagen, gelatin, elastin, silk protein) and synthetic (for example, poly (lactic acid), polycaprolactone,

copolymer of poly (lactide-co-glycolide)) polymers, polymer mixtures, non-polymer materials and multicomponent systems (Fig. 4).

Porous matrices provide increased stability of metastable forms of drug molecules in the structure of substrates and reduce the surface roughness of printed dosage forms. Consequently, the fibrous structure of electroformed substrates demonstrates high suitability for their use in inkjet printing [14,15].

PEPARATION OF SOLUTIONS FOR 2D-PRINTING

The optimized composition of printing solutions is one of the key components of 2D- printed dosage form. "Inks" are divided into solutions with pharmaceutical substances (the most common type for inkjet printing), (nano) suspensions and biofunctional "inks" used in cell engineering. In addition, inkjet printing can be used to form in situ solid dispersions, microcapsules, socrystals, or co-amorphous systems [6,14].

The properties of the solvent and dissolved pharmaceutical substances, excipients, and other additives determine the viscosity and surface tension of solutions, which are critical characteristics for 2D-printing.

The dosage of the printed dosage form directly depends on the concentration of a pharmaceutical substance in the solution. Water-based solutions are preferred due to their non-toxic nature and suitability for thermal inkjet and piezoelectric printing. In aqueous solutions, the concentration of water-soluble pharmaceutical substances can be easily changed to adjust the amount of printed medicine. However, many pharmaceutical substances have certain limitations in solubility. Unlike thermal inkjet printing, piezoelectric printing is applicable for solutions with non-aqueous solvents such as ethanol or dimethylsulfoxide. However, the use of organic solvents should be limited, as this requires the removal of residual solvents after printing. In addition, solvents with low evaporation temperature can cause the nozzle to be clogged and affect the print quality. Therefore, the concentration of solutions strongly depends on the solvent used and/or the addition of solubilizing co-solvents. To modify the viscosity, glycerin, propylene glycol, polyethylene glycols and

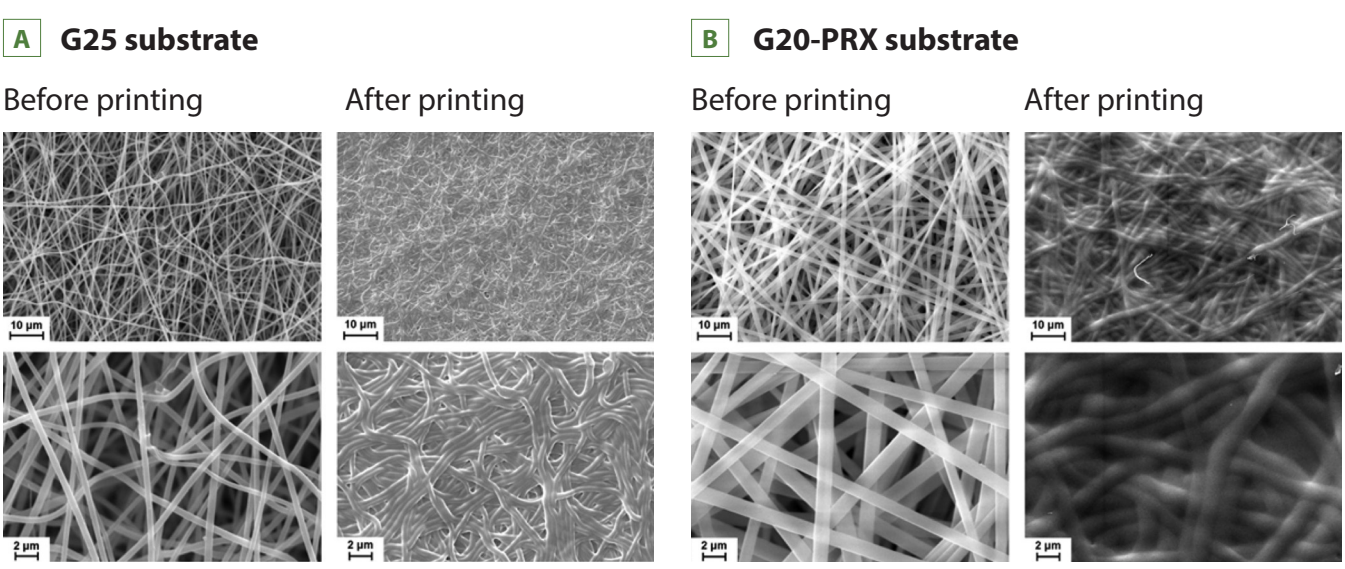

FIG. 4. *Scanning electron microscopy of cross-linked gelatin substrates G25 (A) and gelatin substrates with pyroxicam G20-PRX (B) before and after printing of lidocaine hydrochloride (3000× and 10,000×, from top to bottom) [15]*

Table 2

TYPICAL COMPOSITION OF 2D-PRINTED ORAL DISPERSIBLE FILM

Components	Content
Pharmaceutical substance	5–30%
Water-soluble polymer	45%
Plasticizing agenta	0–20%
Cosolvents	If required
Sweetening agents	3–6%
Excipients as salivants	2–6%
Coloring materials, flavorings, etc.	If required

hydroxypropylcellulose are used, by adding them to various pharmaceutical substances. Other components of solutions include excipients dyeing and masking the taste, the final composition is determined based on the properties of the pharmaceutical formulation and the requirements of the printing system. In general, the resulting composition of 2D-dosage form is shown in Table 2 [3,5,14,18].

TECHNOLOGICAL PARAMETERS OF 2D-PRINTED DOSAGE FORMS

The technological properties of 2D-printed dosage forms are determined by three main aspects: the suitability of the pharmaceutical substances for printing, interaction of solutions with the substrate, and printing parameters. Optimization of printing parameters allows you to provide fine tuning of the printed dosage form within certain limits of the printing system.

When using piezoelectric printing systems, the geometry and behavior of the drop can be optimized by adjusting the applied voltage, waveform, printing frequency, and /or temperature (Fig. 5) [9,16].

However, the influence of these parameters on characteristics of solutions with the pharmaceutical substances has not been systematically studied. The volume, speed and angle of the jet droplet trajectory directly depend on the nozzle diameter (usually 30–60 microns). Creating of smaller droplets allows you to produce 2D-printed films with higher resolution. Inkjet printing is limited by the volume of the applied drop of 1 microL with a corresponding diameter of approximately 12 microns. In this regard, the main problem associated with nonconformity of print quality is clogging of the nozzles. For comparison, the flexography print resolution is approximately 30–75 microns. The accuracy of solution deposition is also determined by the substrate feed system and the overall design of the composition (for example, print pattern, resolution, overlayering).

The assessment of suitability for printing the solutions is based on their physical properties: viscosity, surface tension and density. These properties affect the formation of droplets and the stability of the jet [14,17].

In inkjet printing, optimal viscosity and surface tension ensure uniform spherical droplet formation and solution deposition, avoiding clogging of the nozzles or unwanted leakage. By adjusting these parameters, you can get a trickle of solution drops without tails and satellite drops. In flexographic printing, uniform transfer of solutions to the substrate is achieved using viscous solutions or suspensions with values from 50 to 500 MPa-s.

INTERACTION OF SOLUTIONS WITH THE SUBSTRATE

In 2D-printed dosage forms, where solutions are applied to a supporting matrix, their physical interactions with the substrate affect their own drying mechanism. These interactions can be classified as the spreading of droplets upon

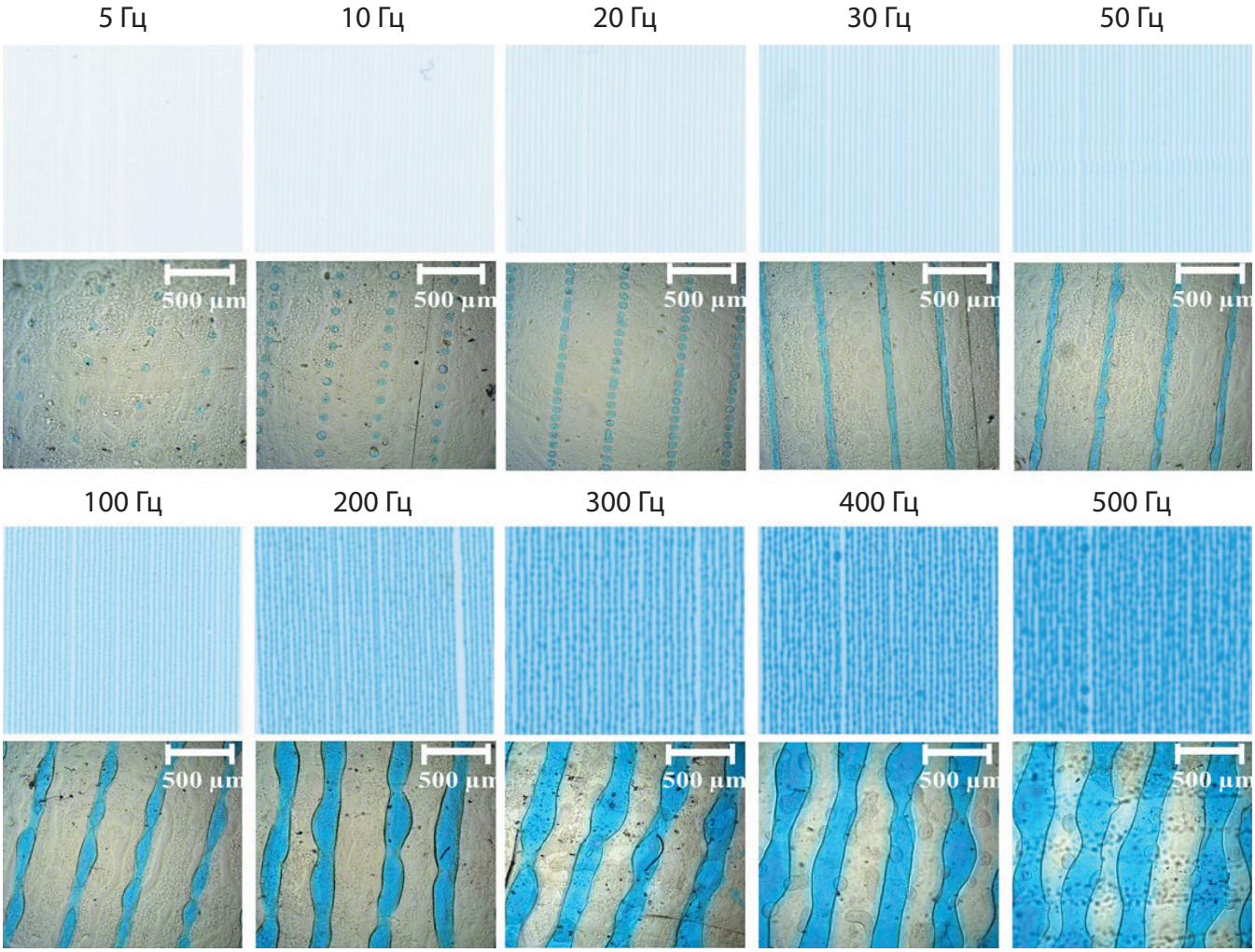

FIG. 5. *2D printing with colored solutions having different frequency of droplet formation*

impact, formation of a pattern on non-porous and porous substrates and solidification of the droplets. After the droplet is ejected from the print head, contact with the substrate is caused by an inertial shock and capillary forces [7]. The contact angle of the droplets determines the shape of the droplet and the printed pattern caused by coagulation. Thus, the pattern of solidified particles varies depending on the wettability of the surface and hydrodynamic flows. Inside the drop, these hydrodynamic flows, including convective flow and surface tension-driven Marangoni flow, attempt to compensate for solvent evaporation during drying. The wettability of the substrate is affected by its roughness, surface energy, and porosity (pore size, volume, and geometry). A reduced distribution over the surface of solutions is observed on porous substrates, but on homogeneous non-porous materials, the droplets tend to merge into larger balls rather than form a uniform layer.

A homogeneous printed pattern is obtained when the dissolved substance is sufficiently distributed during drying. Print quality is degraded due to excessive and/or uneven distribution of solution drops on the substrates. According to the theory of adsorption infiltration, the localization of droplets can be controlled by increasing the affinity of solutions for the substrate, which causes the adsorption retention of droplets on the surface.

Layered systems are produced by printing the multiple layers of ink on top of each other with an intermediate drying stage. Problems with smearing and erosion can be avoided by using printing systems with stationary substrate

holders, by separate solidification of the layers (for example, under UV irradiation or heat treatment), and/or by using porous substrates to increase the absorption capacity. In 2D-printed dosage forms, the penetration of solutions into porous substrates contributes to high accuracy of dosing. When the solution is applied onto impermeable surfaces, the ink layers are combined by re-dissolving, re-suspending, or re-melting the dried layers after applying an additional layer [18–20].

The coated substrates provide additional flexibility for adjusting the characteristics of the surface print. Some studies showed that the hydrophilic coating on porous rice paper makes the surface smoother, but the coating disintegrates or dissolves after printing due to pentration of solutions into the surface layer. In addition, by adding layers of substrate and / or coating, it is possible to change the behavior of pharmaceutical substance release.

Control of the crystal state characteristics of printed solid particles is also of great importance in the development of 2D- dosage forms. During preparation of solid dosed dosage forms, properties of the substrates and interaction of solutions with them affect the solidification and crystallization of printed pharmaceutical substances after the solvent has evaporated. Therefore, additional studies of the interaction of solutions, substrates and the technological process are necessary.

QR-ENCODED SMART DOSAGE FORMS

Personalized 2D-dosage forms themselves represent a very promising area of pharmaceutical technology, but there are many current concepts for the use of two-dimensional printing that expand the possibilities of medicine provision

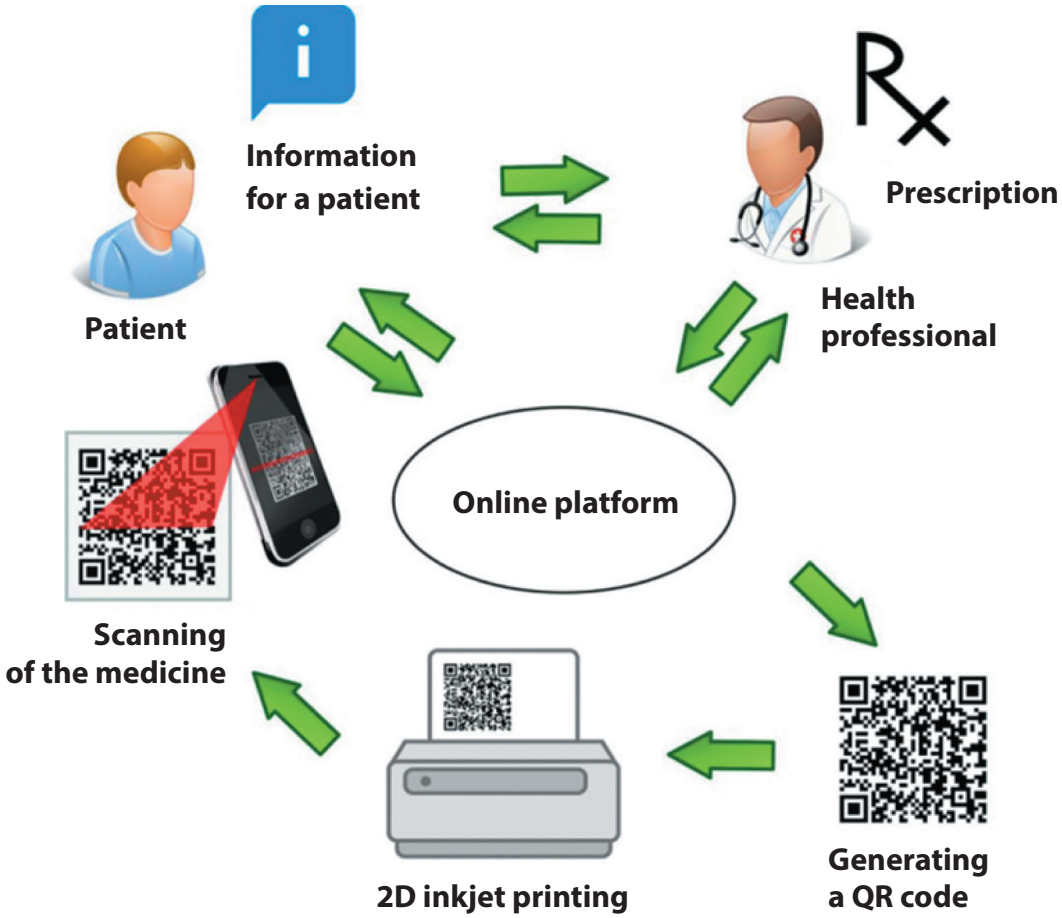

FIG. 6. *Graphical representation of implementation of the idea of QR-encoded dosage form*

for patients. One of these technologies is QR-encoded smart dosage forms.

A QR code is a two-dimensional version of barcode encoding information that can be read using a scanner, such as a smartphone with a QR code reader application. This concept involves combining the individualized medicines with encoding the information in the dosage form itself to ensure that the patient correctly administrate the medicine at the proper time. All necessary information can be included in the QR code in the format specified by national authorities. The possible implementation of the idea is shown in Fig. 6 [8].

This concept has been implemented in some studies. In particular, the University of Copenhagen and ABO University obtained a 2D dosage form as a QR code containing haloperidol [8]. However, the implementation of the concept in practice has multiple difficulties, for example, it is easy to falsify the dosage forms presented in studies, that is, to print QR codes using a regular office printer without pharmaceutical substances in solutions. In addition, the implementation of such coding requires the presence of electronic devices and mobile applications, which is not always feasible. During storage, the QR code may fade or the dye may be transferred. Therefore, in addition to proper packaging for 2D printed dosage forms, the light-, moisture-, and temperature-resistant but edible dyes/pigments should be used. Despite the limitations, 2D printing is being developed and optimized, which in the near future may allow you to arrange the production of innovative medicines to improve the patient adherence to treatment [8,16].

CONCLUSION

2D printing as a method of manufacturing the personalized medicines has a great potential for development in pharmaceutical technology, because it has a number of exceptional advantages, such as simplicity and speed of production, low cost of equipment, high accuracy of dosing, the ability to combine the pharmaceutical substances in a single dosage form. The process proceeds in such a way that after entering the required parameters into the printer software, the required dose can be applied onto a substrate suitable for administration to human within a few minutes. However, medicine production is a complex and regulated process that includes a number of key elements, including ensuring stability, uniformity of dosing, and compliance with regulatory documentation. The 2D printing process must be carried out in good manufacturing practice (GMP) and have appropriate documentation and production conditions. The key stages of the 2D printing process need to be reviewed and adjusted within these production structures and regulatory requirements.

REFERENCES

1. *Blynskaya E.V., Tishkov S.V., Alekseev K.V. Three-dimensional printing technologies for creating solid dosage forms. Development and registration of medicines. 2018. 3 (24): 20–29.*
2. *Dedov I.I., Tulpakov A.N., Chehonin V.P., and others. Personalized medicine: current state and prospects // Bulletin of the Russian Academy of Medical Sciences. – 2012. – Vol. 67. – №12.*
3. *Ahmed R. Effects of different polymers on thermal inkjet printing of oral thin films: a novel approach. – 2018.*
4. *Alomari M., Mohamed F.H., Basit A.W. et al. Personalised dosing: printing a dose of one's own medicine // International journal of pharmaceutics. – 2015. – Vol. 494. – №2. – Pp. 568–577.*
5. *Boehm R.D., Miller P.R., Daniels J. et al. Inkjet printing for pharmaceutical applications // Materials Today. – 2014. – Vol. 17. – №5. – Pp. 247–252.*

6. *Daly R., Harrington T.S., Martin G.D. et al. Inkjet printing for pharmaceutics – A review of research and manufacturing // International journal of pharmaceutics. – 2015. – Vol. 494. – №2. – Pp. 554–567.*
7. *Derby B. Inkjet printing of functional and structural materials: fluid property requirements, feature stability, and resolution // Annual Review of Materials Research. – 2010. – Vol. 40. – Pp. 395–414.*
8. *Edinger M., Bar-Shalom D., Sandler N. et al. QR encoded smart oral dosage forms by inkjet printing // International journal of pharmaceutics. – 2018. – Vol. 536. – №1. – Pp. 138–145.*
9. *Eleftheriadis G.K., Monou P.K., Bouropoulos N. et al. In Vitro Evaluation of 2D-Printed Edible Films for the Buccal Delivery of Diclofenac Sodium // Materials. – 2018. – Vol. 11. – №5. – Pp. 864.*
10. *Genina N., Fors D., Palo M. et al. Behavior of printable formulations of loperamide and caffeine on different substrates – Effect of print density in inkjet printing // International journal of pharmaceutics. – 2013. – Vol. 453. – №2. – Pp. 488–497.*
11. *Goodall S., Chew, N., Chan K. et al. Aerosolization of protein solutions using thermal inkjet technology // Journal of aerosol medicine. – 2002. – Vol. 15. – №3. – Pp. 351–357.*
12. *Hirshfield L., Giridhar A., Taylor L.S. et al. Dropwise additive manufacturing of pharmaceutical products for solvent-based dosage forms // Journal of pharmaceutical sciences. – 2014. – Vol. 103. – №2. – Pp. 496–506.*
13. *Janßen E.M., Schliephacke R., Breitenbach A. et al. Drug-printing by flexographic printing technology – A new manufacturing process for orodispersible films // International journal of pharmaceutics. – 2013. – Vol. 441. – №1–2. – Pp. 818–825.*
14. *Palo M. et al. Design and development of personalized dosage forms by printing technology. – 2017.*
15. *Palo M., Kogermann K., Laidmäe I. et al. Development of oromucosal dosage forms by combining electrospinning and inkjet printing // Molecular pharmaceutics. – 2017. – Vol. 14. – №3. – Pp. 808–820.*
16. *Preis M., Breitkreutz J., Sandler N. Perspective: concepts of printing technologies for oral film formulations // International journal of pharmaceutics. – 2015. – Vol. 494. – №2. – Pp. 578–584.*
17. *Scoutaris N., Ross S., Douroumis D. Current trends on medical and pharmaceutical applications of inkjet printing technology // Pharmaceutical research. – 2016. – Vol. 33. – №8. – Pp. 1799–1816.*
18. *Thabet Y., Breitkreutz J. Printing pharmaceuticals by inkjet technology: Proof of concept for stand-alone and continuous in-line printing on orodispersible films // Journal of manufacturing processes. – 2018. – Vol. 35. – Pp. 205–215.*
19. *Vakili H., Kolakovic R., Genina N et al. Hyperspectral imaging in quality control of inkjet printed personalised dosage forms // International journal of pharmaceutics. – 2015. – Vol. 483. – №1–2. – Pp. 244–249.*
20. *Wickström H., Palo M., Rijckaert K. et al. Improvement of dissolution rate of indomethacin by inkjet printing // European journal of pharmaceutical sciences. – 2015. – Vol. 75. – Pp. 91–100.*

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BRINGING THE REGISTRATION DOSSIER FOR A MEDICINAL PRODUCT TO CONFORMITY WITH THE REQUIREMENTS OF THE EURASIAN ECONOMIC UNION

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At the moment, the pharmaceutical market is gradually moving from the circulation of medicines according to national requirements to the common market of the Eurasian Economic Union (EEU). Until December 31, 2020, the applicant can register a medicinal product according to both national requirements and the rules of the EEU. All registration dossiers (RD) for the medical products must be brought to conformity with the requirements of the EEU by December 31, 2025. The article analyzes the registration procedures and regulatory documents that explain the requirements for the design and content of the RD, which will facilitate the process of bringing the RD to conformity with the requirements of the EEU for Market Authorization Holders (MAH). Thus, during the analysis, the documents necessary for adding to the registration dossier (RD) were identified when it is brought to conformity with the EEU rules in modules 1–5 of the Common Technical Document (CTD).

Keywords: medicinal product, medicine, marketing authorization, Eurasian Economic Union, general pharmaceutical market, registration dossier, common technical document, Market Authorization Holder

One of the functions that form the basis of state regulation of medicines is marketing authorization. Marketing authorization of a medicinal product is the process of obtaining a permit for medical use of a medicinal product, carried out in accordance with current legislation. "For the state registration of a medicinal product, the applicant shall submit to the appropriate Authorized Federal Executive body carrying out state registration of medicinal products, the marketing authorization application for a medicinal product and in accordance with the corresponding procedure of the Authorized Federal Executive body, the necessary documents which form the registration dossier for the medicinal product" [1]. In accordance with Art. 18, p. 3 [1], the registration dossier (RD) for medicinal products for medical use is provided in the form of a Common Technical Document (CTD). The principle of documentation is specified in all good practices. Documenting is a great way of self-reflection. Comprehensive documentation is always required for audit and inspection control. Unlike inspection purposes, for marketing authorization and examination purposes the comprehensive documentation is not required. The registration dossier (RD) does not include all documents that are required by the rules of good practice, called GxP.

One of the main provisions of the Agreement on common principles and rules for the circulation of medicines within the EEU dated December 23, 2014 is the smooth transition of marketing authorization of medicines from national requirements to supranational requirements of the EEU. To solve this problem, the Council of the Eurasian Economic Commission made Decision No. 78 dated 03.11.2016 "On the rules for marketing authorization and examination of medicines for medical use" [4], which will become mandatory for all states-members of the EEU from January 1, 2021. Until December 31, 2020, you can register a medicinal product according to both national requirements and the rules of the EEU. From January 1, 2021 the marketing authorization shall be provided only under the legislation of the EEU, but medical products previously registered under national rules will circulate until the expiration of the marketing authorization certificate (but no later than December 31, 2025) on the territory of the country under rules of which they are registered. Accordingly, all RDs for medicinal products that are registered before December 31, 2020, must be brought conformity with the requirements of the EEU by December 31, 2025. There are two registration procedures within the European Economic Union for new medicinal products that are not registered in the EEU. One of them is the procedure of mutual recognition, when the medicinal product is successively submitted for registration in several states, first to the reference state, and then to the member states concerned (if desired, it can be registered only in the reference state). Another procedure is a decentralized registration procedure, when there is a simultaneous filing of RD for the medicinal product in several countries with selection of the reference state.

For medicines that are registered in the EEU States according to national requirements, there is a procedure for bringing the RD documents to conformity with the requirements of the EEU, making amendments to the RD and confirming registration. In this regard, a number of problems arise, including such as the purchase or creation of software XML to create an electronic common technical document (eCTD) [5,6], re-provide clinical and preclinical studies, for which there is a need for the development and making of organizational and managerial decisions both at the level of various departments (e.g. Registration Department) and senior management of the company.

For Russian pharmaceutical companies, this transition of registration is complicated by new regulations that will allow the circulation of medicines. The search for inconsistencies and differences between the EEU and the Russian Federation takes a sufficient amount of time, because it is necessary not only to identify differences in the rules of the EEU, but also to apply the rules and eliminate inconsistencies in your company.

The purpose of this study is to review the documents of regulatory authorities to resolve the problems that may arise in the Market Authorization Holder (MAH) in the process of bringing the RD for registered medicinal products to conformity with the rules of the EEU.

MATERIALS AND METHODS OF THE STUDY

The main method used in this study is a comparative analysis of registration procedures between the Russian Federation, the Eurasian Economic Union, and the European Union (EU). In the course of this analysis, both the main differences and certain similarities in the procedures were identified, which served as a starting point in searching for problems that may arise for pharmaceutical manufacturers in the process of mandatory re-registration under the requirements of the EEU.

RESULTS AND DISCUSSION

The Market Authorization Holder (MAH) must perform work until December 31, 2025 to provide the RD in the format of a common technical document (CTD) of the EEU to the authorized bodies of the EEU member-states. When bringing the documents to conformity with the requirements of the Union, the applicant can simultaneously amend the registration dossier. The duration of this procedure is no more than 100 calendar days. The applicant independently selects the reference state, then in the Member State concerned brings the registration dossier to confirmity with the type of mutual recognition. Required documents [4]: application in hard copy and (or) in the form of an electronic document in accordance with Annex 2; state fees in accordance with the procedure specified in the reference state and the Member State concerned; RD (modules 1–3) in electronic form. Module 1 is provided on paper, if the medicine is intended for circulation in the territory of the member-state in which it was registered, and modules 4–5 (without necessarily bringing them to conformity with the requirements of the Union for preparation of reports on preclinical studies and clinical trials). The risk management plan, the main dossier (master file) of the production site (s) and the pharmacovigilance master file are also provided in electronic form. When conducting an examination of the registration dossier as part of its harmonization with the requirements of the Union, the "benefit – risk" ratio is not re-evaluated [4]. An exception can only be made if the medicinal product is in the future applied for registration under the mutual recognition procedure in member -states where it was not registered before the entry into force of the Agreement, or before December 31, 2020 after bringing its registration dossier to conformity with the requirements of the Union.

In addition to significant changes in the authorisation procedure and quality evaluation, significant changes also affected the structure of the RD. The requirements for the execution of the dossier and its content have expanded. The contents of the CTD of the Russian Federation and the EEU differ significantly. For example, the administrative part of the EEU dossier does not belong to the CTD, unlike the Russian Federation. Also, the CTD of the Russian Federation does not include the content and introduction to the CTD, reviews and summaries of preclinical and clinical studies. The differences in the authorisation procedures for medicinal products in the Russian Federation, the European Union and the Eurasian Economic Union are due to the fact that the Russian Federation is a separate state that has its own authorized bodies, each of which fulfils a certain role in the authorisation process. As for the EU and the EEU, they are unions of several countries, so the authorisation process differs depending on the chosen procedure. Thus, it follows that in a decentralized procedure or under the procedure of mutual recognition in both the EU and the EEU, the authorized registration body is the national agency (in the EU) or the authorized body of the member state (in the EEU). In addition, the EU has a centralized procedure, unlike the EEU. The duration of the authorisation procedure for a medicinal product in the Russian Federation is 160 working days. But in the EU and the EEU the durations of the authorisation are the same and in the decentralized procedure the duration is 210 working days, and in the mutual recognition procedure it is 90 days. In case of authorization according to the rules of the EEU and the EU it is necessary to be more careful with post-marketing studies of the medicinl product, than in Russia, as they can not in every case issue a perpetual license upon the expiration of five-year period of validity of marketing authorisation.

The range of subjects of circulation of medicines involved in obtaining of marketing authorization has expanded. Subjects of circulation of medicines participating in obtaining of marketing authorization within the EEU are the Eurasian

Economic Commission; authorized (regulatory) bodies of the EEU member-states, including the Ministry of Health of the Russian Federation; expert institutions of the member-states, in particular NC ESMP of the Ministry of Health of the Russian Federation.

Marketing authorization application forms also differ between the Russian Federation and the EEU: the Russian Federation form is the same regardless of the type of a medicinal product, and the EEU form contains different information depending on the type of a medicinal product. In the Russian Federation, the application includes the minimum required amount of information that allows you to subsequently form a marketing authorization and release the medicinal product into circulation, and in the Eurasian economic Union, the application contains a significant amount of additional administrative information that is responsible for the safety of the medicine in circulation in all member-states.

For example, according to the rules of the EEU [4], only module 1 (administrative information) of the registration dossier for a medicinal product is provided on paper. The main file, modules 2–5 are available only in electronic format in accordance with the requirements of [4] to the structure of electronic folder, that for the Russian Federation is an innovation, because in Russia dossier is submitted entirely in electronic and in paper forms [1]. This fact may be a failure for applicants who have only one attempt to correct errors within the framework of an additional request, whereas in Russia the number of additional requests was unlimited. And the EEU procedure provides for only one request at the stage of evaluating the completeness and reliability (validation) of the registration dossier for a medicinal product, and one additional request during the examination process. At the same time, the applicant is limited to 90 calendar days for providing a response. If the response is not provided or is incorrect, the application will be rejected in accordance with the established procedure during the dossier validation stage. There is no legal restriction on the refiling of RD to the regulatory authority. In case of refusal to review the documents, the state fee is not returned, and if you re-submit the documents, you will need to pay it again.

According to [4], it is possible to submit a simplified RD for certain types of medicines. For herbal and homeopathic medicines, it is possible to submit a simplified type of dossier. There are also special requirements for medicines with well-studied medical applications [4].

Differences in the structure of the registration dossier (RD) are shown in the diagram. According to the diagram, the content of the Russian CTD has 4 sections, while the EU and EEU CTDs have 5 modules each, which are similar to each other in content and name, because the structure of the EEU CTD was based on Directive 2001/83/EC [7]. In the CTD structure in the Russian Federation there are only 4 sections and 58 sub-sections, whereas in the EEU, the CTD has 5 modules, which contain 91 subsections that requires thorough study of composition of the dossier for the EEU in order to correctly complement the CTD with missing documents to bring the medicine to conformity with the rules of the Union. Module 1 of the EEU RD is similar to section 1 of the Russian dossier, but contains more items. Significant changes are associated with the introduction of new module 2 "Summary of the Common technical document" in the EEU, which includes brief information on preclinical and clinical studies, as well as on quality. The information should reflect the summary actual data of modules 3–5. Module 3 of the EEU CTD is similar to section 2 of the Russian dossier, but contains more documentation. Modules 4–5 of the Union dossier include the results of preclinical and clinical studies confirming the effectiveness and safety of human use of the medicine.

In the Russian Federation, the presence of preclinical and clinical studies of the medicinal product is mandatory when submitting the RD

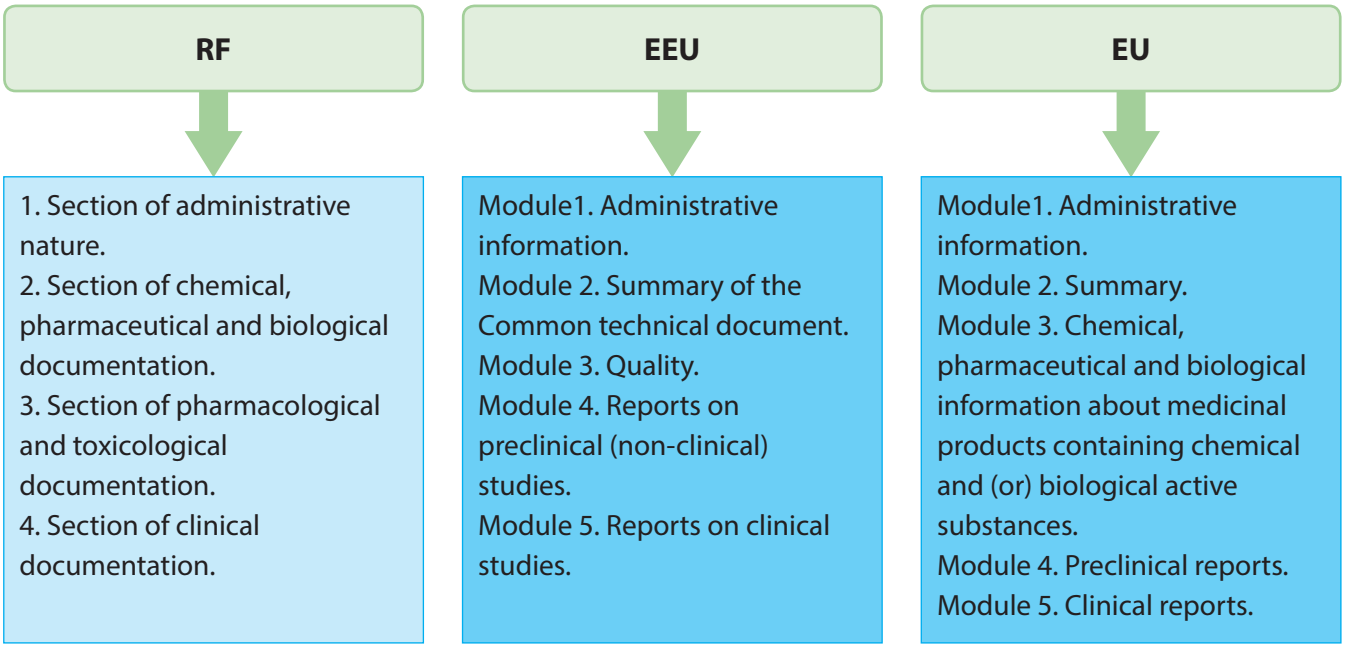

DIAGRAM. *Structure of the registration dossier (CTD) for medicines in the RF, EEU and EU countries*

[8]. However, for generics (other than biological ones) that have been on the market for more than twenty years, the manufacturer can apply reviews of scientific papers, as well as experience of their post-authorization use, instead of reports on preclinical and clinical studies. In addition, when authorizating the generics, it is allowed to apply instead of medicine manufacturers' reports on clinical studies, the reviews of scientific works based on the reference standard, and instead of reports on clinical studies, bioequivalence studies [1]. However, this report is not required to be attached in accordance with article 18 of Federal law No. 6-FZ [1] if the certain conditions are met.

In the EEU, pre-clinical and clinical studies are also required when authorizating a medicinal product. Just like in the Russian Federation, the EEU has a simplified version of submitting a package of documents for clinical trials, for example, bioequivalence studies are not required for certain medications [7]. For the rest of medicinal products, preclinical studies must comply with the GLP (Good laboratories practice) of the EEU, and clinical studies must comply with GCP (Good clinical practice) of the EEU. Preclinical and clinical studies of medicinal products that have been conducted in non-EU countries are considered in the course of medicine examination, if these studies were conducted and described in the report on preclinical and clinical studies in accordance with the requirements of GLP and GCP, as well as the requirements of the EEU (or not lower) [4]. In the RD for homeopathic medicines, modules 4 and 5 are missing, and modules 1–3 must be attached, but not in full.

So, during analysis the following documents which are required for adding to the RD were identified in accordance with the EEU rules.

Module 1. Administrative information

- draft general characteristic of the medicinal product;
- letter of a holder of the Master-file for active pharmaceutical ingredients (API) with obligation to inform on any changes both the medicine manufacturer and authorized body of member-state before introduction of any significant amendments;
- GMP-certificate issued under the EEU rules;
- letter of an quality authorized person on compliance of conditions of production of the

medicinal product presented for authorisation with the requirements of the EEU Guidelines of Good Manufacturing Practice;
- information about claims regarding the quality of themedicinal products for the last 3 years;
- consent of the applicant to conduct the pharmaceutical inspection for compliance with the requirements of the EEU Guidelines of Good Manufacturing Practice (GMP);
- a copy of the Site Master File;
- diagram of production stages with indication of all production sites involved in the process of production and quality control of medicinal products;
- a brief summary of the professionals who prepared a summary on the quality, preclinical and clinical data;
- a letter stating that the Market Authorization Holder (MAH) is a qualified person responsible for pharmacovigilance on the territory of a member-state of the EEU;
- risk management plan for the medicine in accordance with the requirements of the EEU Guidelines of good practice of pharmacovigilance.

Module 2. Summary of the Common technical document

Seven subsections that must be attached in accordance with Appendices 1 and 5 [4] are completely absent in the Russian Federation.

Module 3. Quality

- specifications and their justifications related to the medicine;
- summary of stability studies and conclusion;
- post-authorisation stability studies program and stability commitment;
- production facilities and equipment;
- evaluation of safety regarding extraneous agents;
- regional information;
- copies of the literature sources used.
- In this module, it is necessary to finalize the following documents:
- description of pharmaceutical development of the medicine.

Module 4. Reports on preclinical (non-clinical) studies

- copies of the literature sources.

Module 5. Reports on clinical studies

- list of all clinical studies in the form of a table;
- biopharmaceutical study reports;
- post-authorisation use experience reports;
- case record forms and patient lists;
- copies of the literature sources.

After bringing the registration dossier documents to conformity with the requirements of the EEU, if the medicinal product has been authorised in three states for 5 years or more, a perpetual marketing authorisation certificate is issued. If the medicinal product has been authorised in three countries for less than 5 years, a marketing authorisation certificate is issued for 5 years, and then the re-authorisation procedure must be carried out.

CONCLUSION:

Due to the transition of the medicine market from national requirements to supranational ones, the Market Authorization Holder (MAH) has the opportunity of circulation of medicinal products in the common market of the EEU. When bringing the RD to conformity with the requirements of the EEU the Market Authorization Holder must take into account the following:

1. The need to update documentation according to the requirements of the EEU.

2. Inability to submit the RD on paper, except for the 1st module.

3. Possibility of a simplified RD for a medicine when submitting it for authorisation/re-authorisation.

4. Possibility of scientific advice from the authorized body of the EEU member -state.

5. Expertise on the "benefit-risk" ratio is not required when bringing the RD to conformity with the requirements of the EEU.

6. Possibility of making changes to the RD before submitting it to the authorized body of the reference state

REFERENCES

1. *Federal law "On circulation of medicinal products" dated 12.04.2010 №61-FZ.*
2. *Agreement on common principles and rules for the circulation of medicines within the framework of the Eurasian Economic Union (concluded in Moscow on 23.12.2014).*
3. *Tsyndymeyev A.G., Yagudina R.I., Abdrashitova G.T., Krupnov P.A. Improving the system of market authorisation of medicines in the Russian Federation // Development and market authorisation of medicines. 2017, №2 (19), pp. 284–287.*
4. *Decision of the Council of the Eurasian Economic Commission dated 03.11.2016 No. 78 (ed. of 14.06.2018) "On the Rules for market authorisation and examination of medicinal products for medical use". URL: https://docs.eaeunion.org/docs/ru-ru/01411969/cncd_21112016_78 (access: 23.01.2020).*
5. *Olefir Yu.V., Merkulov V.A., Koshechkin K.A. Transition to electronic market authorisation of medicines for the common pharmaceutical market of the Eurasian Economic Union // Remedium, 2017, №6, pp. 42–46.*
6. *Decision of the Board of the Eurasian Economic Commission of June 30, 2017 No. 79 "On requirements for the electronic type of applications and documents of the registration dossier submitted during the market authorisation and examination of medicinal products for medical use" URL: https://docs.eaeunion.org/docs/ru-ru/01414223/clcd_04072017_79 (access: 23.01.2020).*
7. *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medical products for human use // Official Journal of the European Union. L. 311, 28.11.2001, p. 67.*
8. *Romanov B.K., Sakaeva I.V., Bunyatyan N.D., Vasiliev A.N., Bondarev V.P., etc. The algorithm for evaluating the interchangeability of medicines // Russian Medical Journal. 2015, №21 (5), pp. 43–47.*

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DEVELOPMENT OF AN ALGORITHM FOR COMPILING A STANDARD OPERATING PROCEDURE (SOP) IN THE CONDITIONS OF COMPOUNDING PHARMACIES

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The article presents the algorithm for creating a standard operating procedure (SOP) for pharmacy organizations that carry out quality control of prepared medicines. The relevance of developing the SOPs is determined by the implementation of international industry standards in pharmaceutical field. In order to ensure the uniformity of the documentation system of the pharmacy organization, we suggested the general structure of the standard operating procedure for the quality control of dosage forms prepared at the pharmacy. The main stages of the development of the SOP are specified, recommendations are given on the execution of the document, its updating, making amendments. The development of templates for standard operating procedures will allow pharmacies to create their own SOPs that take into account the features of a particular organization.

Keywords: standard operating procedure, quality control, pharmaceutical organization

One of the priorities of the national healthcare system is to improve the quality and accessibility of medical care, which also includes the availability of medicines. The current trend of reducing the number of compounding pharmacy organizations does not contribute to this task solution.

Compounding pharmacies are a necessary part of the medicine supply system, since they allow to meet the needs of healthcare in dosage forms that do not have commercial analogues, to provide individual dosage of medicinal substances, as well as to manufacture dosage forms without preservatives and other non-indifferent additives [1,4].

Pharmacies engaged in the manufacture of medicines have a number of unresolved problems. Primarily, they are organizational and legal (legislative) issues. The current Federal law No. 61-FZ "On circulation of medicines" and the Guidelines of GMP GOST R 52249–2009 set high requirements for personnel, premises and equipment of compounding

pharmacies, manufacturing of medicines and the system of quality control as wellas for documentation maintained in the pharmacy organization. Another document regulating the activities of pharmacy organizations is the Order of the Ministry of Health of the Russian Federation No. 647n dated 31.08.2016 "On approval of the Guidelines of Good Pharmacy Practice of medicines for medical use". This document defines the transition of pharmacy organizations to GPP (Good Pharmacy Practice) standards [2]. In accordance with this concept, all the activities of pharmacy organizations are considered as a set of processes that should be described in detail in internal controlled documents specifying the sequence of actions when performing any work, in the so-called SOPs – standard operating procedures.

Thus, compounding pharmacies, usually with small staff, must fulfill not only their main responsibilities for the production and quality control of dosage forms, but also develop a number of documents of the quality management system.

In this regard, it is relevant to develop templates of SOPs, which will allow employees of compounding pharmacies to use them as a sample when developing their own documents.

Currently, quite a large number of SOPs have been developed that describe the activities of pharmacies when ordering and accepting inventory, when working with customers, selling goods, and on labor protection. The section of activity of pharmacy organizations on quality control of prepared dosage forms is not sufficiently covered. Quality control of manufactured dosage forms and dosage forms prepared in the pharmacy are significantly different. For manufactured dosage forms pharmacopoeial monographs have been developed, which are actually legally approved methods for quality control of the dosage form or substances. The development of SOPs describing the procedure for quality control of dosage forms prepared in a pharmacy is associated with a number of difficulties. In a pharmacy, the pharmacist-analyst must independently develop methods for analyzing dosage forms, guided by the current State Pharmacopoeia and other legal acts. At the same time, it is necessary to take into account the material and technical equipment of the pharmacy, which, as a rule, significantly limits the possibility of using a number of pharmacopoeial methods of analysis.

Thus, standard operating procedures developed for compounding pharmacies will also be documents containing step-by-step instructions for performing analysis of the prepared dosage form. SOPs should make the process of medicine quality control in the pharmacy to be consistent, coordinated, predictable and reproducible. It is necessary the SOPs to be compiled according to a standard scheme, unambiguously, clearly, so that pharmacy employees, including newly recruited ones, could easily orientate themself and perform the analysis without the help of colleagues.

In connection with the above, the **purpose** of the work is to develop an algorithm for compiling a standard operating procedure (SOP) in the conditions of compounding pharmacies.

MATERIALS AND METHODS

The algorithm for compiling SOP for quality control of dosage forms prepared in compounding pharmacies was developed in accordance with the current regulatory documentation regulating the activities of pharmacy organizations [2,3].

RESULTS AND DISCUSSION

The first stage in the development of SOP for any type of activity is to determine its structure, form, procedure for approval, approval, making amendments, updating, cancellation. When

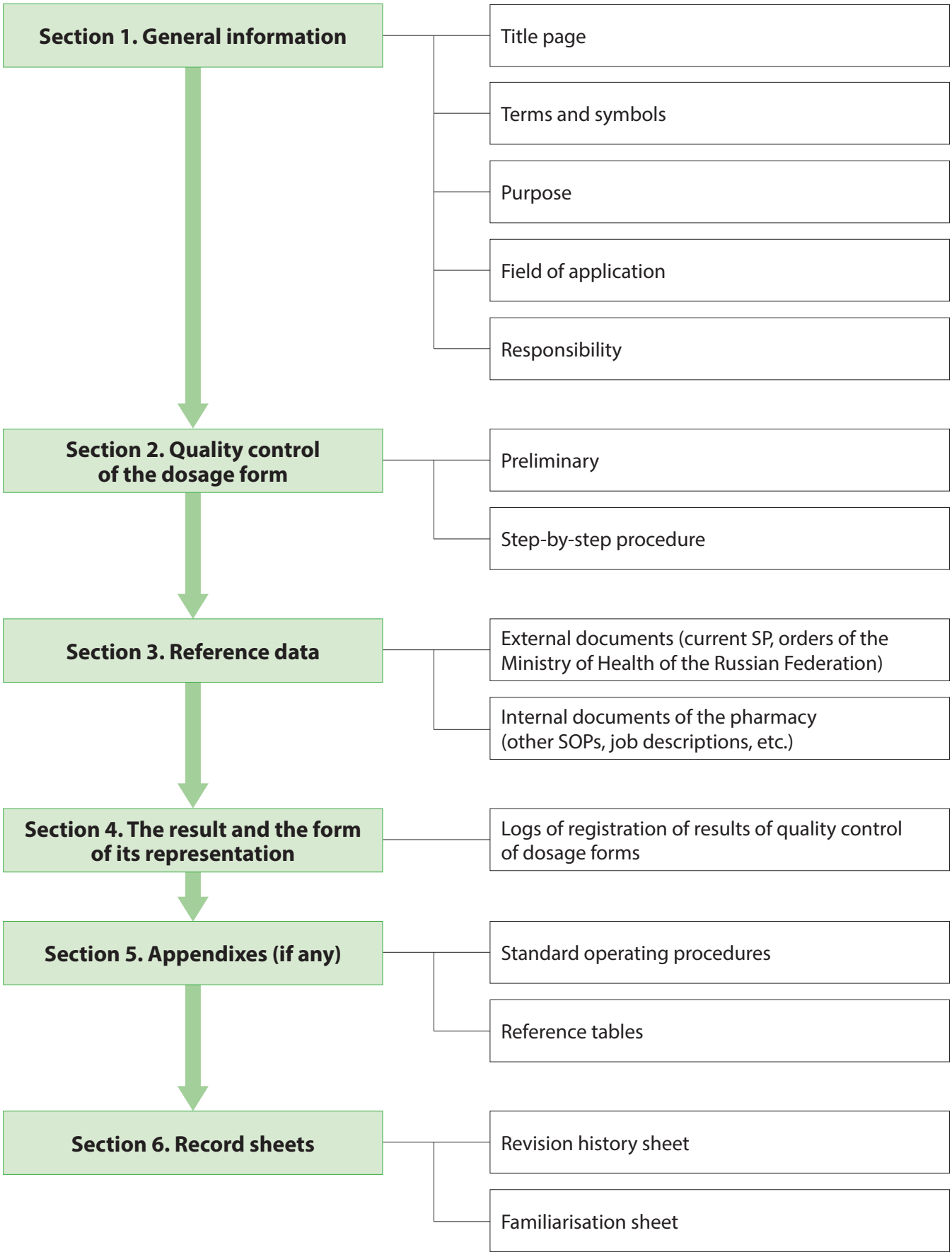

FIG. 1. *Content of sections of the SOP on quality control of dosage forms prepared in compounding pharmacies*

the SOP is first implemented, the SOP version number is assigned as 1, and the version number increases with subsequent revisions.

When making a SOP, it is necessary to develop the document structure, which can be presented in the following form (Fig. 1):

Section 1. General information

Execution of the "Title page" subsection

The title page of the document may contain the columns shown in Table 1.

A unique code is assigned to each SOP:

СОП-XX.YY,

where

СОП (SOP) – a letter combination that defines the document type (short for "Standard operating procedure");

XX – a combination of digits that defines the scope of the document (for example,

01 – SOP, describing the analysis of purified water and/or water for injections;

02 – SOP, describing the analysis of eyedrops;

03 – SOP, describing the analysis of liquid dosage forms;

04 – SOP, describing the analysis of concentrated solutions);

YY – sequential number of the document in the classification group.

The developed SOP should be updated. The SOP is usually updated every three years [5]. After updating, a mark is made on the title page (date, signature of the person who updated) of the original and copies of the document.

Execution of subdivision "Purpose"

Specify the main purpose of using the procedure. For example: standardization of the procedure for monitoring the quality of purified water.

Execution of subdivision "Responsibility"

Specify the positions of employees responsible for the implementation of the developed SOP. If necessary, specify the required level of competence of the executors, conditions for preliminary training. Specify the person responsible for monitoring the procedure. If necessary, specify the official responsible for timely revision and updating of the SOP text.

Execution of subdivision "Terms and Symbols"

Specify all the terms that are necessary for the SOP executor to correctly interpret the SOP text. Pay special attention to special terms and give them clear and concise definitions. Decode specific acronyms and/or abbreviations. Check the consistency of terms and definitions in the

Table 1

TITLE PAGE OF SOP

<table>
<tr><td colspan="4">Name of SOP: _____</td></tr>
<tr><td colspan="4">Name of the pharmacy organization _____</td></tr>
<tr><td colspan="4">Code (number) of document: СОП-XX.YY
Version №</td></tr>
<tr><td colspan="2">Compiled:
Pharmaceutist-analyst

_____ _____
full name signature</td><td colspan="2">Approved:
Head

_____ _____
full name signature</td></tr>
<tr><td colspan="2">The document put into effect:
“____” _____ 20____</td><td colspan="2">Document updated:
“____” _____ 20____</td></tr>
</table>

developed SOP with other documents of the pharmacy quality system.

Execution of subdivision "Field of application"

This section can contain information about the place where the procedure is performed and description of the situations in which it is performed. In addition, this section should specify the types of dosage forms the quality control of which is described in this procedure.

Section 2. Quality control of the dosage form

Execution of subdivision "Preliminary measures"

List the laboratory utensils and auxiliary materials required to perform quality control of a single dosage form. Specify all the necessary reagents and their quantity for performing a single analysis. If necessary, specify the procedure for preparing devices and equipment, specify their operating conditions. Describe the procedure for sampling the dosage form for quality control.

Execution of subdivision "Step-by-step procedure"

Step by step, in a clear sequence, exhaustively and realistically describe the actions that must be performed in the quality control of the dosage form specified in the name of the SOP.

The description of actions should be divided into several main parts:

- In accordance with current legislation, describe in detail all types of control that should be subjected to the dosage form specified in the name of the SOP.
- In cases where chemical control is mandatory, perform preliminary calculations of the required amount of the dosage form that will be used for the analysis. At the same time, it is necessary to take into account the sensitivity of the reactions carried out, the norms of permissible deviations, and to provide for the need for further release of this dosage form to the pharmacy customer. It is also necessary to make preliminary calculations of the volume of the titrated solution, which will be spent on titrating the sample weight of the studied dosage form. In this section, it is possible to draw up chemical equations that underlie the reactions, calculate equivalence factors, and titrate titrants for the substances to be determined.
- Describe in detail and step-by-step the implementation of the main operations for quality control of the dosage form: determining the authenticity of the incoming ingredients and their quantitative content.
- Final operations (evaluation of the quality of the dosage form).

When describing all stages of quality control of dosage forms, the safety requirements of the work performed must be specified or references to the relevant internal documentation.

Section 3. Reference data

Provide references to external documents used in the development of SOP (laws and regulations, state standards, reference books, etc.).

Specify the internal documents of the pharmacy organization that should be taken into account when performing the developed SOP.

The developed SOPs must not contradict the requirements of the legislation regulating pharmaceutical activities in the territory of the Russian Federation.

Section 4. The result and the form of its representation

Specify what is the result of performing the SOP and how it is registered, which logs are filled in. Keep in mind that any analysis, control, or verification can have at least two results – positive and negative. It is required to describe the sequence of actions when receiving each of the results, and specify the persons responsible for making decisions.

Section 5. Appendixes (if any)

Appendices may contain an operating instruction that briefly describes the tests being performed. The operating instruction can be

Table 2

REVISION HISTORY SHEET

Revisions of SOP-XX.YY	Basis for revisons in СОП-XX.YY	Executor (full name)	Signature	Date
1	2	3	4	5

Table 3

FAMILIARIZATION SHEET

Number of version of SOP-XX.YY	Full name of an employer	Signature	Date
1	2	3	4

presented as a visualized card or table and placed directly on the workplace of the pharmacist-analyst.

Appendices may also contain reference tables (refractometric, standards of acceptable deviations, etc.) that can (or should) be used by the executor when performing the SOP.

Section 6. Record sheets

Amendments to the SOP may be made in connection with changes in the regulatory framework or in the activities of a pharmacy organization. The developer makes amendments to the SOP and registers them in the Revision history sheet. The head of the pharmacy or the responsible person must inform on the amendments made the employees who participate in this procedure. After reviewing the employee puts his signature in the Revision history sheet. When a large number of amendments are made, a new version of the SOP is published.

The Revision history sheet and the Familiarization sheet can be presented in the form of tables (table 2 and 3).

The required number of copies of a particular SOP in a pharmacy is determined based on the number of employees who use this SOP in their activities, but there must be at least two copies. All first copies of SOP are marked as "Control copy", these samples are stored by the Head of Pharmacy. The remaining copies of SOPs are stored at the workplaces of pharmacists who perform quality control of dosage forms.

CONCLUSION

The proposed structure and algorithm for creating SOPs can be used as a template for developing own standards in compounding pharmacies. The implementation of SOPs into the activities of pharmacies will ensure a logical sequence of actions in the process of quality control of medicines, clearly distribute tasks by competence, and increase the responsibility of employees for performing certain operations. All this will eventually ensure that the availability and quality of medicines will increase.

REFERENCES

1. *Gachenko R.A., Goryachev A.B. Modern requirements for industrial production and pharmaceutical preparation of infusion medicines in the Russian Federation: unity and differences // Pharmacy and pharmacology. – 2018. – №6 (3). – Pp. 214–228.*
2. *Rules of good pharmacy practice of medicines for medical use: order of the Ministry of Health*

of the Russian Federation dated 31.08.2016 No. 647n // Official Internet portal of legal information. URL: http://www.pravo.gov.ru (access date: 30.04.2019).

3. *Federal law of the Russian Federation No. 61-FZ of 12.04.2010 "On circulation of medicinal products". – M., 2010.*
4. *Nikulina M.A., Voronkina I.V., Pudovkina T.V., Grigorieva I.V. Problems of the pharmacy compounding department in modern conditions // International journal of applied and fundamental studies. 2016. – №2. – Pp. 265–269.*
5. *Strelkov S.V., Podushkin V.Yu., Kotova N.I. Formation of internal normative documentation of the pharmacy organization according to the basic principles of the quality management system // Advances of modern science. 2017. – Vol. 5. №1. – Pp. 112–116.*

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MORPHOLOGICAL AND ANATOMICAL STUDY OF EUROPEAN VERBENA HERB (VERBENA OFFICINALIS L.)

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Morphological and anatomical study of a new promising domestic type of medicinal plant raw materials – European verbena herb was carried out according to the thematic plan of the R&D works of VILAR No. 0576–2019–0010. As a result of study, morphological and anatomical features were established that have diagnostic significance and allow to determine the authenticity of this type of medicinal plant raw materials. The obtained data will be used in the development of the draft regulatory documentation for a new type of medicinal plant raw materials – European verbena herb.

Keywords: European verbena (Verbena officinalis L.), herb, morphological and anatomical features

European verbena in the Middle ages was considered as a universal remedy for all diseases. In Greece, it is still considered as a sacred plant that brings happiness. European verbena has a tonic, antipyretic, anti-inflammatory, anti-edema effect. Verbena is often used in homeopathy: the homeopathic medicine Verbena is recommended for insomnia, nervous disorders, kidney and gall bladder stones. It is also widely used in Chinese, Tibetan and Korean medicine as a sudorific agent for malaria and fever [1, 2, 4, 6].

The above-ground part of European verbena contains glycosides (verbenalin, verbenin), triterpenoids, steroids, tannins, small amount of camphor and essential oils, bitterness, mucus [2,4,6].

European verbena – *Verbena officinalis L.* of the Verbenaceae family is perennial herb of height of 30–70 cm with a straight branching stem four-sided in the upper part. Lower leaves are pinnated-incised, middle leaves are trehyadernye, upper leaves are inciso-crenate or smooth-margin, sessile. The flowers are small, light purple, collected in spike-shaped inflorescences on the tops of the stems. Verbena blooms in June and August. European verbena is widespread in the temperate zone in Europe, Asia and North America; the plant grows in meadows, clearings, forest edges, along rivers and seas, on vacant lots or hills, on roadsides, as a weed in gardens, vegetable gardens, among crops [1–3,6].

The European verbena herb is included in the European, British herbal, American herbal, and Chinese Pharmacopoeia [5]. In Russia, this type of medicinal plant raw materials is not official and there is no regulatory documentation for it.

Currently, VILAR is studying the possibility of using the aboveground part of European verbena as a raw material for development of a new medicine. In this regard, the need arose

to standardize this type of raw material. One of the stages of standardization of medicinal plant raw materials is the establishment of morphological and anatomical characteristics as one of the indicator of identity.

The purpose of this work was studying the morphological and anatomical structure of the aboveground organs of European verbena (leaves, stems, flowers) and identifying their characteristic diagnostic features.

MATERIALS AND METHODS

The object of study is a dried whole herb of European verbena, harvested in 2018 on the territory of the Botanical garden of VILAR.

Morphological and anatomical study of raw materials was carried out according to the pharmacopoeial monographs of the State Pharmacopoeia of the Russian Federation, XIV edition: OFS.1.5.1.0002.15 "Herbs" [7] and OFS.1.5.3.0003.15 "Technique of microscopic and microchemical study of medicinal plant raw materials and herbal medicinal products" [8]. Microscope slides were studied using a biological microscope "Altami BIO 2 LED" with a digital ocular USB camera 3.1 Mpix (Russia). The photos were processed on computer in Adobe Photoshop 7.0.

RESULTS AND DISCUSSION

Morphological study. Macroscopic examination revealed morphological features of the European verbena herb. It is established that the raw material is whole and partially crushed frondose stems with flowers and buds, individual leaves, flowers, buds. Stems are straight, branched, four-sided, longitudinally ribbed, along the edges of the sides covered with pressed hairs. Leaves are ovoid, ovoid oblong, oblong-lanceolate or oblong, 4–8.5 cm long, up to 4 cm wide, wedge-shaped narrowed at the base, sessile, pinnated-incised, with prominent veins on the lower side, pubescent. The middle leaves are trehyadernye, large-toothed, with blunt teeth, the upper leaves are oblong, inciso-crenate or smooth-margin. Flowers are small, numerous in apical, thin axillary spikes, collected in a large panicle; bracts are ovoid or lanceolate, sharp, shorter than the calyx. Calyx is pubescent, 2.5 mm long, 1.5 mm wide, short, sharp teeth. Corolla is five-lobed, almost twice as long as the calyx; the lobes are unequal: 3 lobes are larger and 2 lobes are slightly smaller. There are 4 stamens. Upper leaves are green, lower leaves are lighter, the stems are green, calyx is green, corolla is light purple.

Anatomic study. Microscopic examination revealed anatomical features of the European verbena herb. When examining the leaf surface (Fig. 1–8), cells of the upper and lower epidermis with sinuous walls are visible. Stomata on both sides of the leaf, more numerous on the lower surface, are surrounded by 3 stomatal cells, one of which is much smaller than the other two (anisocyte type), and another 3–5 (6) cells (anomocyte type). On the entire surface of the leaf, especially on the veins on the lower side, there are two types of hairs: simple and glandular. Simple hairs are single-celled thick-walled, with warty surface, having an expanded base and a pointed tip. At the base, simple hairs are surrounded by a single row of rounded polygonal cells that differ from other epidermal cells, i.e. they have a characteristic rosette. Glandular hairs are of two types: on a long thin-walled leg, often collapsed, with a flattened head consisting of 4–8 radially arranged cells, and on a short single-celled leg with an ovoid head consisting of 4 radially arranged cells.

The epidermis of the stem (Fig. 9–11) consists of polygonal and slightly elongated cells with straight, often beaded-thickened walls and cuticle folding, the epidermis cells along the edges are longer and narrower. Stomata are numerous, of a characteristic structure, oriented along the length of the stem. The hairs have the same structure as on a leaf; simple hairs are found

only along the edges of the stem faces. Around the stomata and articulations of glandular hairs, the radiant folding of the cuticle is clearly visible.

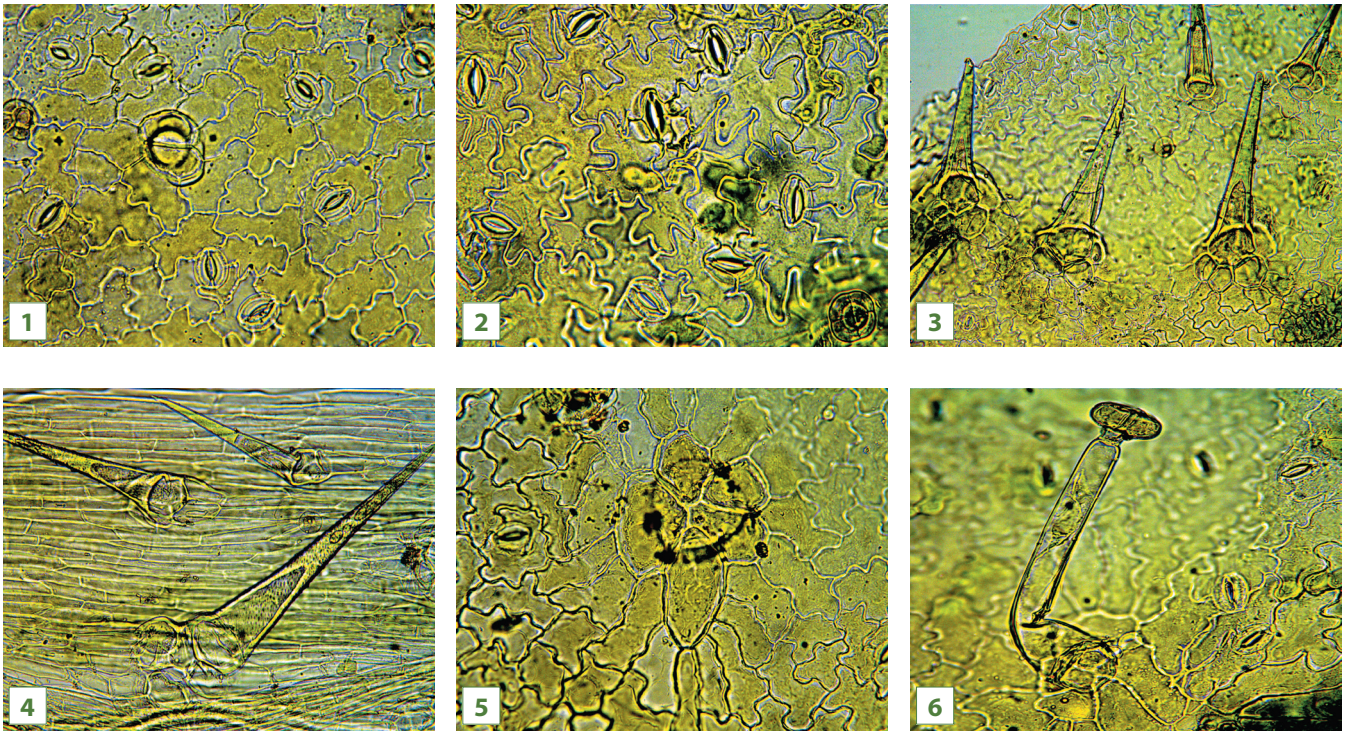
When viewing the calyx from the surface (Fig. 12–15), longitudinally elongated cells of the inner and outer epidermis with strongly branched walls are visible; on the outer epidermis, there are clear beaded thickenings of the cell walls and cuticle folding. Stomata of a characteristic structure are located on the outside, on the inside they are met on the teeth. On the surface of the calyx, there are hairs of the same structure as on the leaf. On the outside, there are numerous simple large hairs, on the inside – they are smaller and fewer. Glandular hairs are found on the outside of the calyx.

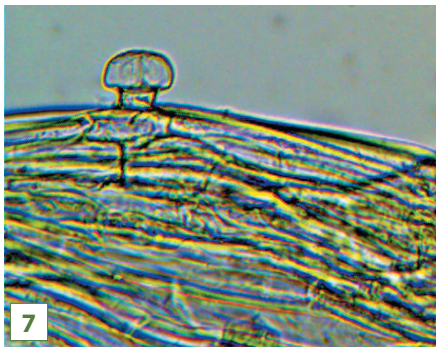
The corolla epidermis (Fig. 16–18) is covered with papillary outgrowths on the inner side and along the edge, and the epidermis cells on the outer side have sinuous walls. The corolla is pubescent with simple single-celled thin-walled hairs with a pointed end, covered with soft-warty cuticle; long simple single-celled hairs with rough-warty cuticle, having a strongly branched shell, the hairs are often twisted together. In addition, there are papillary outgrowths of the epidermis with a rough-warty cuticle and head hairs on a 1–2-cell leg with a rounded 1–2-cell head. Pollen is tricolpate-porous with a smooth exine.

CONCLUSION

Based on the study of European verbena herb, morphological and anatomical features were

FIG. *Diagnostic signs of the anatomical structure of European verbena herb (1–3, 5, 6, 8, 9, 11–13, 15–18 – ×400; 4, 7, 10, 14 – ×200): 1 – upper leaf epidermis; 2 – lower leaf epidermis; 3 – simple hairs on the leaf surface; 4 – simple hairs along the leaf vein; 5 – rosette at the base of simple hairs; 6 – glandular hair on a long leg (side view); 7 – glandular hair on a short leg (side view); 8 – glandular hairs (top view); 9 – stem epidermis; 10 – simple hair; 11 – glandular hair on a long leg; 12 – inner calyx epidermis; 13 – outer calyx epidermis; 14 – hairs on the edge; 15 – hairs on the epidermis of the calyx: 15a – on the inner epidermis, 15b, 15c – on the outer epidermis; 16 – inner epidermis of the corolla; 17a – simple hairs with a soft-warty cuticle; 17b – simple hairs with a rough-warty cuticle; 18 – papillary outgrowths*

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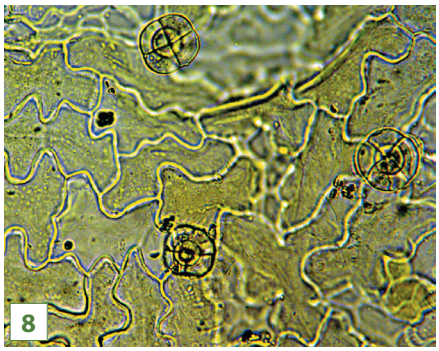
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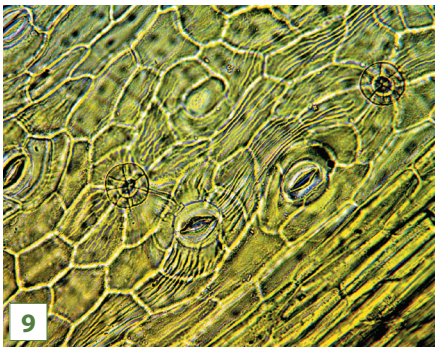
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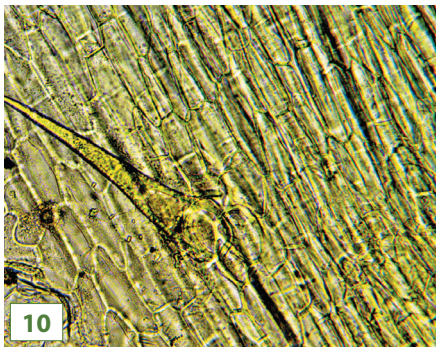
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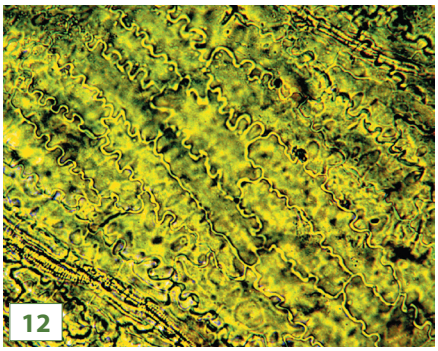
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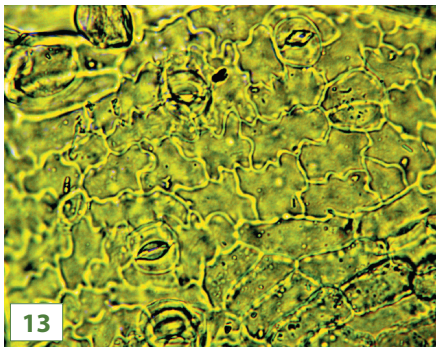
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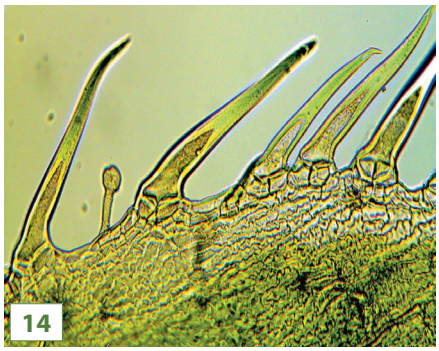
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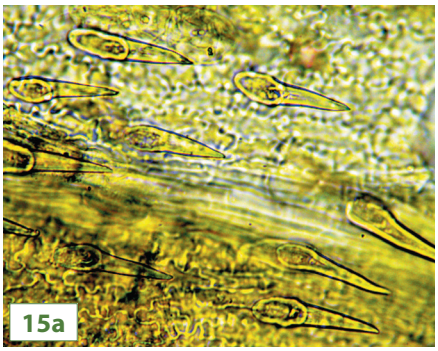
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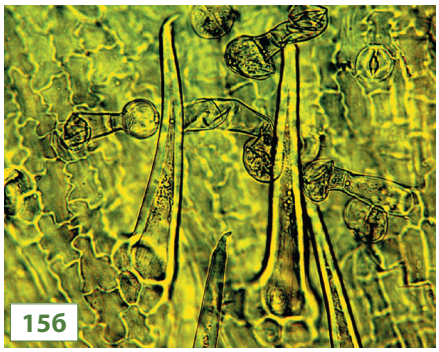
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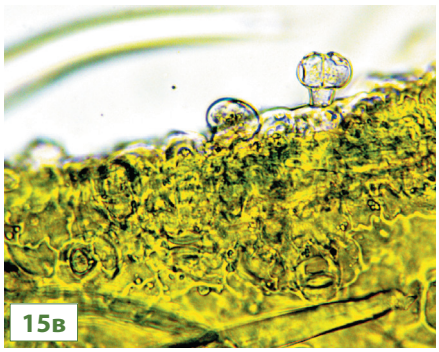
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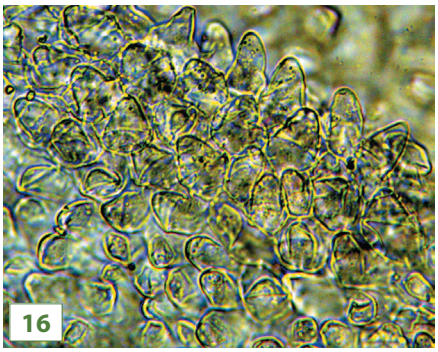
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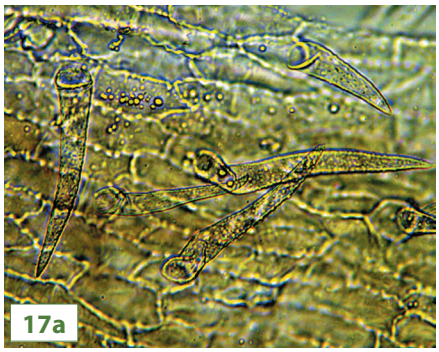
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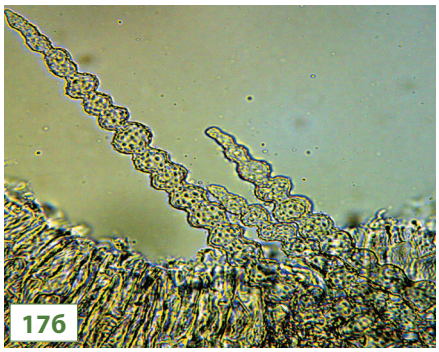
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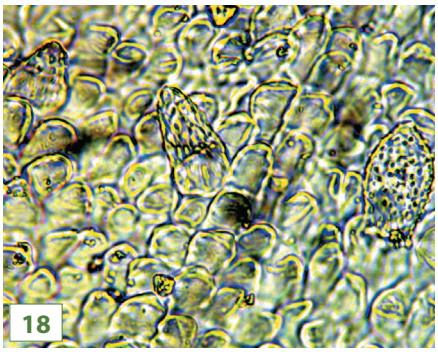
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determined that have diagnostic significance and allow to establish the identity of this type of raw material, which will be included in the regulatory documentation for a new promising type of domestic medicinal plant raw material such as European verbena herb.

REFERENCES

1. *All about medicinal plants. – Vilnius: UAB, Bestiary, – Saint Petersburg: SZKEO, 2014; – 33.*
2. *Cjosev P.A. Complete Handbook of medicinal plants. – M.: EKSMO-PRESS, 2001; – 921.*
3. *Flora of the USSR. – M.-L: Publishing house of the USSR Academy of Sciences, 1953, Vol. XIX. – Pp. 694–695.*
4. *Derimedved L.V. Biologically active additives containing medicinal plant raw materials / L.V. Derimedved, I.M. Pertsev, V.N. Kovalev // Pharmaceutist. – 2001. – №3. – Pp. 37–40.*
5. *European verbena (Verbena officinalis L.): review of phytochemical and pharmacological studies / Kulyak O.Yu., Adamov G.V., Radimich A.I., Saibel O.L. // Questions of biological, medical and pharmaceutical chemistry. – 2019. – №11. – Pp. 9–18.*
6. *Rabinovich, A. M., Medicinal herbs and recipes of ancient times. – M.: Rosagropromizdat, 1991. – Pp. 121–122.*
7. *OFS.1.5.1.0002.15 "Herbs".*
8. *OFS.1.5.3.0003.15 "Technique of microscopic and microchemical study of medicinal plant raw materials and herbal medicinal products".*

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ASSESSMENT OF THE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES IN FRESH AND DRIED RAW MATERIALS OF SWEET BASIL (OCIMUM BASILICUM L.)

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In this article the authors conducted a qualitative and quantitative determination of a number of known groups of bilogically active substances, such as essential oils, polyphenols and anthocyanins. Qualitative analysis was performed using characteristic qualitative reactions and thin-layer chromatography. Quantitative evaluation of the groups studied was carried out using well-known Pharmacopoeia methods (essential oils, anthocyanins) and the Follin – Chicalteu method, which is widely used in the food industry (polyphenols). The maximum yield of substances is typical for fresh raw materials of Pepper Basil, in which the content of essential oil was 0.82%, polyphenolic compounds – 6.42%, anthocyanins – 0.560%.

Keywords: basil, essential oil, polyphenolic substances, anthocyanins, antibacterial activity, TLC, spectrophotometry, quality factors

Ocimum basilicum L. (Lamiaceae) is a plant with a long history of use as a food and medicinal plant raw material. The description of the plant is found in the works of Hippocrates, Galen, and Dioscorides.

The ancient Sanskrit medical book of India "Yajur-Veda", written before our era, contains recommendations for the use of basil, the flowers of which are recommended as a diuretic and sedative, the fruit – for gonorrhea, the roots – for the treatment of intestinal disorders [1]. The great doctor and thinker of the East, Avicenna in "Canon of medical science" separately describes various types of basil – fibrous, hairy, mountain, sweet, garden. Basil is very popular in folk medicine in the Balkans. Herbalists since the time of the Ottoman Empire have recommended the juice of fresh basil leaves to be used for purulent inflammation of the middle ear, and externally – for non-healing wounds [2].

Over 800 years ago Ganjevi Nizami, the famous Azerbaijani scientist and writer, in his works, summarized national data on effect of medicinal plants of the Caucasus, among which basil used as a diuretic, anti-inflammatory and febrifuge, occupies an important place, [3].

For many years, basil has been included in the Russian State Pharmacopoeia, is a pharmacopoeial raw material in France, and is included in the Pharmacopee Français. Currently, in Russia, basil is cultivated as a spice and oilseed raw material and is used in the food and cosmetic industry. However, raw materials and essential oil of basil are widely studied by scientists, primarily as an antibacterial agent. Thus, the presence of high antibacterial activity of Ocimum basilicum essential oil against the S. Aureus strain was revealed. This study also proved the presence of a synergistic effect when using antibiotics and basil essential oil together [4,5].

In the studies of scientists from South India, the antitumor activity of the essential oil from Ocimum basilicum Linn was revealed. Methylthiazole-tetrazolium assay was used for in vitro cytotoxicity screening against human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cell line (HEp-2), and mouse embryonic fibroblasts NIH 3T3. As a result of the study, it was proved that basil oil has a powerful cytotoxicity [6].

An interesting area of research is the study of the ccompositional analysis of basil essential oil, conducted by scientists from different countries with samples of basil introduced in their localities, which revealed the presence of significant differences in the compositional analysis of basil grown in different latitudes [7,8].

It should also be noted that there is a significant chemical variation of basil chemotypes, among which linalool and eugenol varieties are most common [9].

Also in recent years, researchers have been interested in studying the substances of polyphenolic nature and anthocyanin glycosides, the presence of which causes anti-inflammatory and capillary-strengthening activity of ethanol extracts of basil herb [10].

Despite the large number of scientific publications characterizing the chemical composition of various groups of biologically active substances (BAS) of basil herb and features of the pharmacological action of this raw material, it is important to conduct studies aimed at the choice of criteria of quality of fresh and dried basil herbs for following inclusion into the proposed regulations, because today the basil herb is standardized according to the requirements of GOST R 56562–2015 "Fresh basil – greens. Technical specifications", which includes the definition of quality factors such as the appearance of raw materials, color, smell, taste, mass fraction of defected plants, mass fraction of weeds, the presence of agricultural pests, the presence of mineral impurities and which does not allow to assess the content of biologically active substances and regulate the quality of raw materials.

The purpose of the study is to substantiate methods for identifying the main groups of BAS of basil herb, quantify and evaluate the effect of drying on the content of essential oil, polyphenolic substances and anthocyanins in basil herb.

MATERIALS AND METHODS

As the objects of study, we used fresh basil herb sold in food stores in Moscow that meets the requirements of GOST 56562-2015 "Fresh basil – greens", fresh basil herb of the "Pepper" variety, cultivated in pot culture, as well as dried basil raw material sold as spice by KAMIS, "Home kitchen", "MAGIC TREE" companies (packing – 10 g).

The presence of essential oil in the raw material was determined by steam distillation in accordance with the OFS.1.5.3.0010.15 "Determination of the content of essential oil in medicinal plant raw materials and medicinal plant products".

Qualitative analysis of substances of anthocyanin nature was performed by TLC in the system of solvents "n-butanol – glacial acetic

acid – water" (4:1:2) with detection in the visible range of the spectrum by the method described earlier for identification of anthocyanin pigments [11].

Quantitative determination of polyphenolic substances in basil herb was performed by the Folin-Ciocalteu method, which is widely used for evaluating the quality of food raw materials [12].

Quantitative evaluation of anthocyanin compounds was performed by the method of spectrophotometry [11,13], widely used for the analysis of medicinal plant raw materials. About 1 g (exact weight) of crushed basil herb was transferred to a conical flask provided with a 100 ml ground joint, then, 50 ml of 70% ethyl alcohol containing 1% hydrochloric acid was poured, after which the flask was attached to the return condenser and kept in a boiling water bath for half an hour. At the end of the extraction time, the flask with extract was cooled to room temperature, the missing extractant was added and filtered using a paper filter. 1 ml of filtered extract was placed in a 25 ml volumetric flask and brought to the mark with a 1% solution of hydrochloric acid in 70% ethyl alcohol. The optical density was measured at wavelength of 538 nm, using 70% ethyl alcohol containing 1% HCl as the reference solution. The content of the sum of anthocyanins in basil herb as a percentage (X) in terms of cyanidin-3-O-glucoside was calculated using the formula:

$$X = \frac{A \times 25 \times 50}{m \times 1 \times 100},$$

where A is the optical density of the test solution, m is the weight of the raw material, g, 100 is the specific absorption rate of cyanidin-3-O-glucoside at 538 Nm in 70% ethyl alcohol containing 1% hydrochloric acid.

RESULTS AND DISCUSSION

The use of laboratory express methods allowed us to identify the content of essential oil in all the samples studied. The complex of traditional qualitative reactions confirmed the presence of tannins. When carrying out a qualitative reaction with lead (II) acetate in the presence of a formed amorphous precipitate, the solution above the precipitate changed to

Table

RESULTS OF DETERMINING THE MAIN GROUPS OF BAS IN BASIL HERB

Study sample	Content of essential oil, %	Content of the sum of polyphenols, %	Content of the sum of anthocyanins, %
Fresh basil herb sold in food stores in Moscow "Home kitchen"	0,72	6,04	0,480
Fresh basil herb of the "Pepper" variety, cultivated in pot culture	0,82	6,42	0,560
Dried raw basil sold as a spice by KAMIS	0, 43	4,25	0,039
Dried raw basil sold as a spice by "Home kitchen" company	0,39	3,89	0,064
Dried raw basil sold as a spice by "MAGIC TREE" company	0,47	4,76	0,053

a pronounced pink color, which indicates the presence of anthocyanins, identified by TLC. Chromatograms of extracts obtained from basil leaves show pink spots with values of Rf-0.51, Rf-0.46, Rf – 0.36 (dominant spot), Rf – 0.31 and Rf – 0.21. Comparison with the Rf standard identified cyanidin-3-O-glucoside (Rf-0-36) (standard sample of cyanidin-3-O-glucoside of Xian Le Sen Bio-Technology Co).

The results of quantitative determination of essential oil, the total content of substances of polyphenolic nature and anthocyanins are presented in the table.

Taking into account the results obtained, we found that the maximum content of all BAS groups is typical for fresh basil herb raw materials, along with this the pot basil culture of the "Pepper" variety shows the highest content for all the studied groups, which can be explained by the fact that the raw materials for analysis were cut off immediately before the experiment, while fresh basil raw materials sold in food stores were cut off a day and more earlier. Raw basil sold as a spice was dried in the conditions indicated on the package, which suggests the need for further studies aimed at studying the effect of the drying conditions on the yield of biologically active substances. The greatest losses when drying basil are observed among the class of anthocyanins.

As it is known, anthocyanins are representatives of a class of polyphenolic secondary

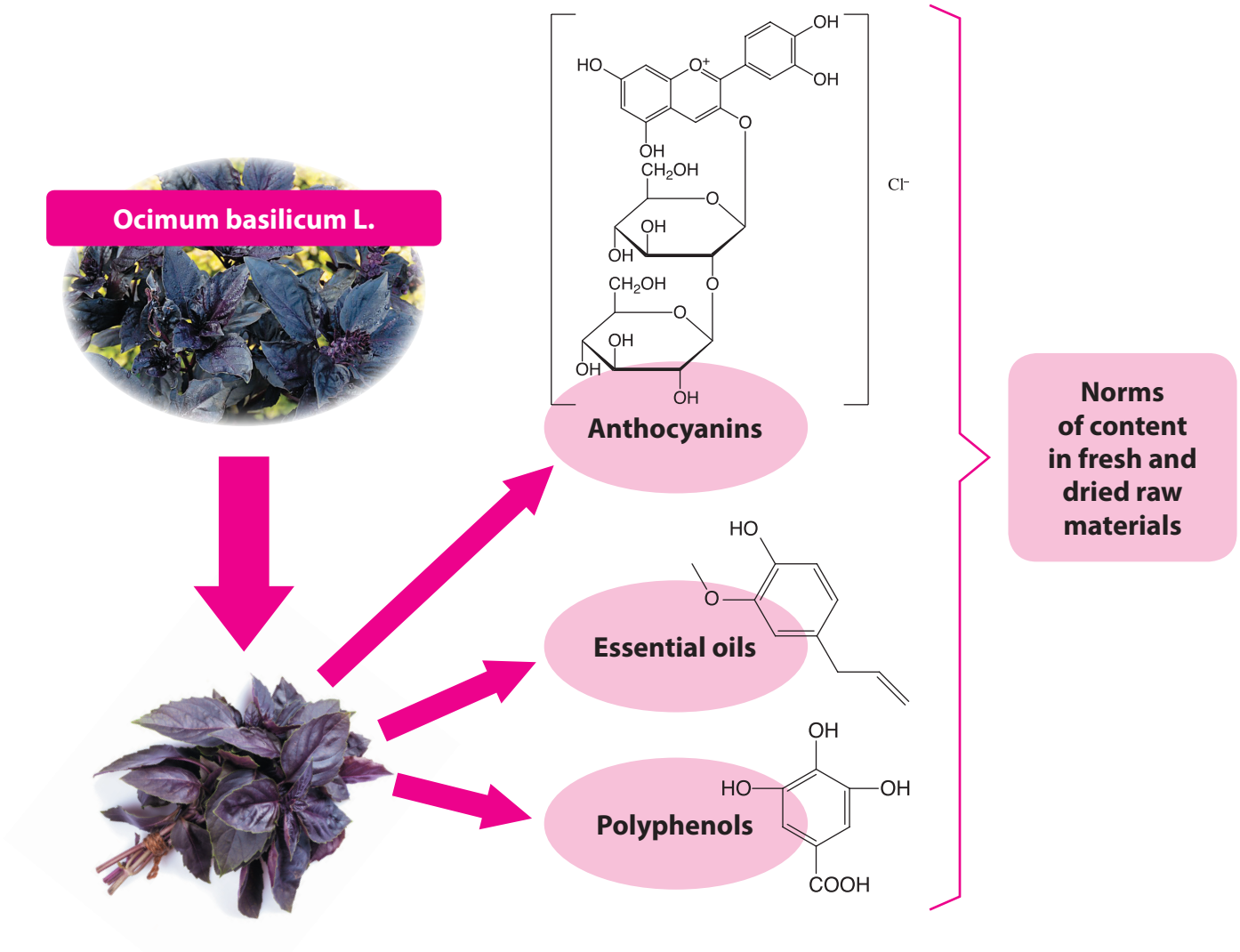

FIG. *Selection of BAS groups for the development of standards for the content of BAS in Ocimum basilicum L. fresh and dried raw materials for inclusion into the developed regulatory documentation*

metabolites-flavonoids, which are widely distributed in the plant world. Under the conditions of modern in vitro and in vivo analysis, the valuable pharmacological properties which are characteristic for anthocyanins, have been identified. The scientific literature often shows the protective function of anthocyanins in the development of oxidative stress, hypoglycemic and hypolipidemic activity, anti-inflammatory effect, the ability to inhibit lipid peroxidation, reducing the permeability and fragility of capillaries.

The need to use fresh basil raw materials as a source of anthocyanins is due to the fact that during the drying of basil leaves, the processes of hydrolysis and polymerization of anthocyanin pigments are carried out, which results in the accumulation of polymeric anthocyanin structures characterized by low bioavailability and absence of pharmacological effects inherent in anthocyanins.

Thus, the quantitative determination of the content of essential oil, polyphenol compounds, and anthocyanins in the raw materials of basil was carried out, on the basis of which it is planned to develop standards for the content of these BASs, according to Fig.

ONCLUSION

During the experiment, the authors determined the content of essential oil, polyphenolic compounds and anthocyanins in fresh and dried basil herb. The identification of these BAS groups was carried out by qualitative reactions and TLC, the Pharmacopoeia method of steam distillation was used for the quantitative determination of essential oil, the polyphenol fraction was determined by the Folin – Chocalteu method, widely used in the food industry, and anthocyanins were determined spectrophotometrically. The maximum yield of substances is typical for fresh raw material of basil of "Pepper" variety in which the content of essential oil was 0.82%, polyphenolic compounds – 6.42%, anthocyanins – 0.560%.

REFERENCES

1. *Chopra R.N., Nayar S.L. Glossary of Indian medicinal plants. – New Delhi, 1956, p. 165–166.*
2. *Staykov V., Ilieva S. Essential-oil and medical culture. – Sofia, 1961, p. 27.*
3. *Efendiev I.K. History of medicine in Azerbaijan. – Baku, 1964, p. 44.*
4. *Javanmardi J., Khalighi A., Kashi A. et al. 2002. Chemical characterization of basil (Ocimum basilicum L.) found in local accessions and used in traditional medicines in Iran // J. Agric. Food Chem., 50: 5878–5883.*
5. *Bassole´ I.H. N., Lamien-Meda A., Bayala B. et al. 2010. Composition and antimicrobial activities of Lippia multiflora moldenke, mentha x piperita l. and Ocimum basilicum L. essential oils and their major monoterpene alcohols alone and in combination // Molecules 15: 7825–7839.*
6. *Poonkodi Kathirve, Subban Ravi. Chemical composition of the essential oil from basil (Ocimum basilicum Linn.) and its in vitro cytotoxicity against HeLa and HEp-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts // Journal Natural Product Research. Volume 26, 2012. – Issue 12. https://doi.org/10.1080/14786419.2010.545357*
7. *Kovalenko N.A., Sunichenko G.N., Sachivko T.V., Bosak V.N. Study of the compositional analysis of Ocimum basilicum L. essential oil from plant raw materials of the Republic of Belarus. The works of BSTU. 2014. №4, Chemistry, technology of organic substances and biotechnology, p. 194–196.*
8. *Christova Yu.P. Study of the composition analysis of essential oils of the representatives of Ocimum basilicum L. genus in conditions of southern coast of Crimea. Collection*

of scientific works of the State Nikitsky Botanical garden. 2011, Pp. 34–37.

9. *João Vito B. Freitas, Elenilson G. Alves Filhob, Lorena Mara A. Silva, Guilherme J. Zocolo, Edy S. de Brito, Nilce V. Gramosa. Chemometric analysis of NMR and GC datasets for chemotype characterization of essential oils from different species of Ocimum // Talanta. Volume 180, 1 April 2018, p. 329–336.*
10. *Pisarev D.I., Sevruk I.A., Malyutina A.Yu., Krikun E.N., Novikova M.Yu. / Study of the composition of anthocyanins of the Ocimum basilicum L. herb within the scientific direction "Pharmaceutical remake". Scientific results of biomedical research. Series "Medicine and pharmacy". 2016, Vol. 2, №2, pp. 101–105.*
11. *Kurkin V.A., Ryazanova T.K. New approaches in the field of standardization of raw materials and preparations of blueberries // Izvestiya of Samara scientific center of the Russian Academy of Sciences, Vol. 13, №1 (8), 2011, pp. 2010–2014.*
12. *Nesterova N.V., Samylina I.A. Study of the qualitative composition and quality factors of leaves of forest and domestic apple trees [Text] // Health and education in the twenty-first century. – 2016. – №5. – Vol. 18. – Pp. 251–258.*
13. *Perova I.B. Study of the content and specific profile of anthocyanins in medicinal plant raw materials. Extended abstract of Cand.Sc. Pharm. dissertation. – Moscow. – 2015, – 24 p.*

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