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PREDICTORS OF WEIGHT LOSS IN PATIENTS WITH CHRONIC HEART FAILURE AND REDUSED LEFT VENTRICULAR EJECTION FRACTION

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Abstract

Chronic heart failure (CHF) is a heterogeneous syndrome with a poor prognosis.

Aim of the work – to define predictors of body weight (BW) loss in patients with CHF and a reduced left ventricular ejection fraction (LVEF).

Materials and methods. 120 patients with stable CHF and LVEF ≤35 %, II–IV NYHA class were examined. Patients were divided into two groups according to the value of BW loss for 6 months: the first group - loss of BW <6 %, the second – ≥6 %.

Results. Out of the 120 patients who were studied, a BW loss of ≥6 % occurred in 59 (49.2 %) patients. According to the results of binary logistic regression, predictors of BW loss of ≥6 % in patients with CHF and LVEF≤35 % were: age, coronary heart disease, anaemia, and the number of hospitalizations over the last year. People with poorer quality of life, bigger number of points on the Beck depression scale and DEFS, with lower levels of physical activity and worse endothelium-dependent vasodilator response; higher sizes of the right atrium, right ventricle, and pulmonary artery systolic pressure, E/E'. Higher levels of C-reactive protein (CRP), uric acid are associated with a risk of losing BW≥6 %.

Conclusions. Weight loss ≥6 % is observed in 49.2 % of patients with CHF and LVEF≤35 %. According to multivariate analysis, independent predictors of BW loss of ≥6 % in patients with CHF and LVEF≤35 % are age, CRP level, III-IV NYHA class, lower cholesterol levels, as well as lower rates of flow-dependent vasodilator response and hip circumference.

Keywords: chronic heart failure, weight loss, cardiac cachexia, predictors.

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1. Introduction

Chronic heart failure (CHF) is a heterogeneous syndrome with a poor prognosis, independent predictors of which are neurohormonal activity disorders [1] and the development of cachexia. Cardiac cachexia is known to be a factor in reducing survival in CHF, regardless of other important variables, such as age, functional class, ejection fraction and physical capabilities [2, 3]. According to statistical estimates, the prevalence of unintentional weight loss, defined as a loss of 6 % or more of body weight over 6 months, ranges from 12 to 16 % in stable outpatients with heart failure (HF) [4], up to 50 % in patients with severe CH [5]. It was found that the mortality rate of patients with heart failure and progressive weight loss (BW) can reach 50 % within 18 months [6]. The pathophysiological mechanisms underlying the loss of BW in patients with CHF are not entirely understandable; a number of factors have been identified that lead to an imbalance of catabolic and anabolic processes [7]. On the other hand, the clinical characteristics of patients with CHF associated with their loss of BW have been little studied.

Aim of the research – to define the predictors of BW loss in patients with CHF and reduced left ventricular ejection fraction (LVEF).

2. Materials and methods

120 hemodynamically stable patients with CHF (97 men, 23 women), 18–75 years old (mean age 61±0.86) II–IV NYHA class, with left ventricular ejection fraction (LVEF)≤35 % were exam-

ined and observed on the basis of the heart failure department of the State Institution "National Scientific Center "Institute of Cardiology named after Acad. N. D. Strazhesko" from 2014 to 2019.

Inclusion criteria. The study included patients aged 18–75 years with clinical signs of CHF, II–IV NYHA class, LVEF≤35 %, the etiological factor of CHF of which was coronary heart disease (CHD), including in combination with arterial hypertension (AH) or dilated cardiomyopathy (DCMP).

Exclusion criteria. The study did not include patients over the age of 75, with acquired and congenital heart defects, gastrointestinal diseases in the acute stage, which limit the ability to eat or are characterized by malabsorption syndrome, hypo- and hyperthyroidism, myocardial infarction, cerebral stroke, or thromboembolism of pulmonary artery branches up to 3 months old, inflammatory and restrictive heart disease, insulin-dependent diabetes mellitus, chronic pulmonary heart disease, chronic renal failure stage V, terminal stage of liver failure, bronchial asthma or chronic obstructive pulmonary disease of the III–IV stages, oncological and infectious diseases.

The members of the Ethics Commission (extract from protocol No. 2 dated January 20, 2014) at the State Institution "National Scientific Center "Institute of Cardiology named after academician N.D. Strazhesko" of the National Academy of Medical Sciences of Ukraine decided that this study would not contradict the main provisions of the GCP, Convention Council of Europe on human rights and biomedicine, the Helsinki Declaration of the World Medical Association on ethical principles for the conduct of scientific medical research with the participation of man and the Law of Ukraine "On Medicines". All patients signed an informed consent to participate in a clinical trial.

The diagnosis of the underlying disease was determined on the basis of a general clinical examination, special instrumental and laboratory methods. CHF was diagnosed according to the recommendations for the diagnosis and treatment of heart failure of the European Society of Cardiology and the relevant recommendations of the Association of Cardiology of Ukraine [8, 9]. Patients were included in the study in the phase of clinical compensation.

The compulsory examination protocol included electrocardiography, standard echocardiography [10], routine laboratory tests (general clinical and biochemical), glomerular filtration rate using the EPI formula [11], an assessment of life quality using the Minnesota Living with Heart Failure Questionnaire (MLHFQ), an assessment of physical activity level using the Duke University questionnaire [12; 13], an assessment of psychological status on the Beck Depression scale [14], and an assessment of fatigue after physical exertion on the DEFS (Dutch Exertion Fatigue Scale) scale [15]. All patients underwent a general clinical physical examination, measuring the thickness of the skin-fat fold (SFF) at four points using a caliper, measuring the circumference of the shoulder of an unstrained and tense arm, waist and hip. Evaluation of the vasodilating function of the endothelium (flow-mediated vasodilation – FMV) was carried out using the ultrasonographic method using a sample with reactive hyperaemia [16]. The functional capabilities of the patients were investigated using a standard test with 6-minute walking and a standardized test with extension of the lower extremity, the results of which evaluated the exposure of the quadriceps femoris. The criterion for the patients distribution into groups was the value of weight loss for the last 6 months that preceded the patient inclusion in the study, ≥6 % according to the European recommendations for the diagnosis and treatment of CHF [8]. Information on the dynamics of body weight for the specified period was obtained from medical history data and data from medical records of patients.

Statistical processing of information was carried out using Microsoft Exel programs, IBM SPSS Statistics (version 23.0). At the first stage, BW loss factors were tested using binary logistic regression (univariate analysis); uncorrelated odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated. At the next stage of our work, statistically significant factors of BW loss ($p<0.05$) were consistently included in the multivariate logistic regression model. In a multidimensional model, factors were excluded when pair correlation gave a high linear correlation coefficient ($r>0.7$). The quality of multivariate logistic regression was determined based on a likelihood comparison test. (Likelihood Ratio Test).

3. Results

Out of the 120 patients who were studied, a BW loss of ≥6 % occurred in 59 (49.2 %) patients. Their clinical characteristics are presented in **Table 1**.

Table 1

Clinical characteristics and pharmacotherapy structure of patients with CHF and reduced LVEF

Parameter	n	%
Number of patients	120	100
Men	97	80.8
CHD	104	86.7
DCMP	15	12.5
Hypertension	102	85.0
MI	49	40.8
AF	69	57.5
II NYHA class	46	38.3
III–IV NYHA class	74	61.7
Anaemia	23	19.2
Diabetes mellitus	34	28.3
ACE inhibitors	70	58.3
BB	94	78.3
MRA	82	68.3

Note: CHD – coronary heart disease; DCMP – dilated cardiomyopathy; MI – myocardial infarction; AF – atrial fibrillation; ACE inhibitors – angiotensin-converting enzyme inhibitors; BB – beta-blockers; MRA - mineralocorticoid receptors antagonists

According to the results of binary logistic regression, the predictors of BW loss of ≥6 % in patients with CHF and LVEF≤35 % were: age, coronary heart disease, the presence of concomitant anaemia, and the number of hospitalizations over the last year. Significantly higher risk of BW loss was for people with poorer quality of life and a high NYHA class, a large number of points on the Beck depression scale and the fatigue scale after physical exertion. At the same time, patients with a higher level of physical activity and a better endothelium-dependent vasodilator response had a lower risk of progressive loss of BW (**Table 2**).

Table 2

Predictors of BW loss ≥6 % over the last 6 months in patients with CHF and reduced LVEF according to binary logistic regression analysis (clinical and demographic parameters)

Indicator	B (SE)	Odds ratio	95 % confidence interval		P
			Lower	Upper	
1	2	3	4	5	6
Age	0.05 (0.02)	1.05	1.01	1.10	**0.018**
Men	0.50 (0.47)	1.65	0.66	4.31	0.287
Anaemia	1.05 (0.5)	2.87	1.12	8.05	**0.034**
Diabetes mellitus	0.54 (0.41)	1.72	0.77	3.85	0.185
CHD	1.21 (0.61)	3.37	1.09	12.67	**0.046**
CHD combined with hypertension	0.97 (0.53)	2.63	0.94	7.40	0.067
Hypertension	1.07 (0.56)	2.92	0.97	9.67	0.056
DCMP	–1.1 (0.62)	0.331	0.099	1.105	0.072
AF	0.01 (0.37)	1.01	0.49	2.08	0.978
II NYHA class	–3.41 (0.59)	0.03	0.01	0.10	<0.001

Continuation of Table 2

1	2	3	4	5	6
III–IV NYHA class	3.41 (0.59)	30.39	10.62	111.4	**<0.001**
MLHFQ score	0.08 (0.02)	1.08	1.05	1.12	**<0.001**
Duke physical activity index	–0.1 (0.02)	0.91	0.87	0.94	**<0.001**
Beck Depression scale score	0.19 (0.06)	1.20	1.08	1.37	**0.002**
DEFS score	0.09 (0.02)	1.10	1.05	1.15	**<0.001**
FMV	–0.29 (0.08)	0.75	0.63	0.86	**<0.001**
Duration of CHF	–0.003 (0.005)	0.99	0.99	1.05	0.488
Duration of hypertension	0.01 (0.02)	1.01	0.98	1.05	0.484
Duration of CHD	0.001(0.03)	1.01	0.94	1.06	0.997
Number of hospitalizations over the last year	0.47 (0.17)	1.60	1.15	2.30	0.007
Lower limb extension test	–0.03 (0.02)	0.97	0.93	1.01	0.130
6 minute walk test	–0.007 (0.003)	0.99	0.99	1.00	**0.009**

Note: CHD – coronary heart disease AF – atrial fibrillation; FMV – flow-mediated vasodilation; MLHFQ – The Minnesota Living with Heart Failure Questionnaire; DEFS – Dutch Exertion Fatigue Scale

As for the anthropometric parameters, the risk of losing BW ≥ 6 % is lower at higher bicep circumferences of the tight and loose arm, waist and hip, SFF thicknesses above the biceps, triceps, under the shoulder blade and in the inguinal region (**Table 3**).

Table 3

Predictors of BW loss ≥6 % over the last 6 months in patients with CHF and reduced LVEF according to binary logistic regression analysis (anthropometric parameters)

Indicator	B (SE)	Odds ratio	95 % confidence interval		P
			Lower	Upper	
The circumference of the biceps of relaxed arm	–0.31 (0.07)	0.74	0.64	0.83	**<0.001**
The circumference of the biceps of tight arm	–0.31 (0.06)	0.74	0.64	0.83	**<0.001**
Waist circumference	–0.06 (0.02)	0.94	0.91	0.97	**<0.001**
Hip circumference	–0.24 (0.05)	0.79	0.71	0.86	**<0.001**
SFF thickness over biceps	–0.16 (0.05)	0.85	0.77	0.93	**0.001**
SFF thickness over triceps	–0.13 (0.03)	0.88	0.83	0.93	**<0.001**
SFF thickness under the shoulder blade	–0.09 (0.02)	0.92	0.88	0.96	**<0.001**
SFF thickness in the inguinal region	–0.05 (0.02)	0.95	0.91	0.98	**0.001**

Note: SFF – skin-fat fold

An analysis of laboratory parameters revealed that higher levels of C-reactive protein (CRP), uric acid, as well as aspartate aminotransferase (AST) in our patients were significantly associated with a risk of losing BW≥6 % among them. It was also found that higher levels of haemoglobin, hematocrit, cholesterol, triglycerides and albumin were associated with a lower risk of BW loss among studied patients **(Table 4)**.

When studying hemodynamic and echocardiographic parameters, it was determined that the risk of losing BW≥6 % in our patients increases with an increase in the size of the right atrium, right ventricle, pulmonary artery systolic pressure, and E/E'. At the same time, higher rates of diastolic blood pressure, TAPSE, and the ratio of TAPSE to pulmonary arterial systolic pressure (PASP) are associated with a reduced risk of loss of BW≥6 % (**Table 5**).

Table 4

Predictors of BW loss of ≥6 % over the last 6 months in patients with CHF and reduced LVEF according to binary logistic regression analysis (laboratory parameters)

Indicator	B (SE)	Odds ratio	95 % confidence interval		P
			Lower	Upper	
Haemoglobin	–0.02 (0.01)	0.98	0.96	0.99	**0.023**
Hematocrit	–7.2 (3.05)	0.001	0.001	0.30	**0.019**
Lymphocytes, %	–0.02 (0.02)	0.98	0.94	1.02	0.291
Potassium	0.95 (0.56)	2.59	0.90	8.14	0.087
Uric acid	0.004(0.001)	1.004	1.001	1.007	**0.006**
Bilirubin	0.02 (0.01)	1.02	0.99	1.05	0.199
AST	0.05 (0.02)	1.05	1.02	1.10	**0.010**
Cholesterol	–0.8 (0.22)	0.45	0.28	0.68	**<0.001**
Triglycerides	–1.27 (0.46)	0.28	0.11	0.65	**0.006**
Albumin	–0.18 (0.05)	0.84	0.75	0.92	**0.001**
CRP	0.42 (0.08)	1.51	1.30	1.80	**<0.001**

Note: AST – aspartate aminotransferase; CRP – C-reactive protein

Table 5

Predictors of BW loss ≥6 % over the past 6 months in patients with CHF and reduced LVEF according to binary logistic regression analysis (hemodynamic and echocardiographic parameters)

Indicator	B (SE)	Odds ratio	95 % confidence interval		P
			Lower	Upper	
SBP	–0.01(0.02)	0.99	0.96	1.02	0.598
DBP	–0.06 (0.03)	0.94	0.89	0.99	**0.015**
HR	–0.01(0.01)	0.99	0.97	1.01	0.338
LVEF	–0.04 (0.03)	0.963	0.910	1.019	0.189
LA	0.43 (0.3)	1.54	0.85	2.79	0.158
LA volume	0.002(0.004)	1.002	0.99	1.01	0.554
LV EDVI	0.008 (0.01)	1.01	0.99	1.02	0.196
LV ESVI	–0.001(0.003)	0.99	0.99	1.01	0.625
LV MMI	–0.001 (0.005)	0.99	0.99	1.01	0.912
RA	0.5 (0.23)	1.65	1.10	2.66	**0.026**
RV	0.75 (0.27)	2.12	1.29	3.68	**0.005**
E/E’	0.11 (0.03)	1.12	1.05	1.20	**0.001**
TAPSE	–0.19 (0.06)	0.82	0.72	0.92	**0.001**
TAPSE/PASP	–7.39 (2.31)	0.001	0.001	0.04	**0.001**
PASP	0.08 (0.02)	1.08	1.04	1.13	**<0.001**

Note: SBP – systolic blood pressure; DBP – diastolic blood pressure; HR- heart rate; LVEF – left ventricular ejection fraction; LA – left atrium; LV EDVI – left ventricular end diastolic volume index; LV ESVI – left ventricular end-systolic volume index; LV MMI – left ventricular myocardial mass index; RA – right atrium; RV – right ventricle; E/E’ – the ratio of the maximum rate of early filling of the ventricle to the peak velocity of the lateral part of the mitral ring in the same phase of the cardiac cycle; TAPSE – tricuspid annular plane systolic excursion; PASP – pulmonary arterial systolic pressure

In the multivariate regression model, the independent predictors of BW loss ≥6 % in our patients were age, CRP level and III-IV NYHA class. However, higher cholesterol, a flow-dependent vasodilator response, and a hip circumference were associated with a lower risk of BW loss (**Table 6**).

Table 6

Independent predictors of BW loss ≥6 % over the past 6 months in patients with CHF and reduced LVEF according to multivariate analysis

Indicator	B (SE)	Odds ratio	95 % confidence interval		P
			Lower	Upper	
Age	0.11 (0.05)	1.11	1.01	1.25	**0.045**
III–IV NYHA class	4.35 (1.27)	77.15	8.94	1377.73	**0.001**
CRP	0.62 (0.18)	1.85	1.38	2.83	**0.001**
FMV %	–0.45 (0.2)	0.64	0.40	0.90	**0.023**
Cholesterol	–0.88 (0.40)	0.42	0.17	0.85	**0.028**
Hip circumference	–0.26 (0.09)	0.77	0.62	0.90	0.004

4. Discussion

The study allowed us to confirm data on the association of BW loss in patients with CHF and reduced LVEF with age. It is known that muscle loss increases with age, as protein synthesis levels in the body decrease, which is a consequence of the ongoing decline in testosterone and other anabolic hormones [17, 18]. The loss of BW accompanied CHF is observed much more often in patients III–IV NYHA class and is associated with their worst functionality according to the 6-minute walk test and the assessment of the physical activity index using the DASI questionnaire, which is reflected in the worse quality of life of such patients.

The relationship we found between the loss of BW in patients with CHF and impaired function of the right heart is confirmed in previous studies [19, 20]. According to the data of M. Valentova and colleagues, right ventricular failure is hemodynamically associated with venous hyperaemia of the gastrointestinal tract. Edema of the intestinal mucosa is associated with abdominal discomfort, loss of appetite and postprandial satiety, which entails a deterioration in food intake and a progression in loss of BW [21]. The second mechanism, according to which venous congestion in the intestinal wall can contribute to the development of cardiac cachexia, lies through the production of pro-inflammatory cytokines induced by lipopolysaccharides [22]. Researchers suggest that chronic intestinal hyperaemia, which is determined by cardiac cachexia, contributes to the constant translocation of lipopolysaccharides into the bloodstream even in stable patients. Detection of higher serum CRP levels in patients with cachexia that correlated with a larger intestinal wall thickness confirms this point of view [21] .

The role of main pathology (CHD) as a predictor of BW loss in patients with CHF can be considered from the point of view of the generality of their pathophysiological mechanisms, in particular, pro-inflammatory activation. It is known that inflammation plays an important role in the progression of atherosclerosis; highly sensitive C-reactive protein is an important risk factor for systemic atherosclerosis and is associated with its clinical complications [23, 24]. In the pathogenesis of heart failure, activation of the immune system also plays a significant role [25], plasma CRP level is increased in patients with heart failure. The results of the study show that patients with higher CRP show signs of more severe heart failure [26]. There is also evidence that an elevated CRP level independently predicts unintended weight loss [5]. CRP is a product of inflammation, the synthesis of which is stimulated by the liver in cytokines in response to an inflammatory stimulus [27]. Interleukin-6 is the main determinant of CRP production [28] in the liver and is produced in monocytes, macrophages, endothelial cells, vascular smooth muscle cells, fibroblasts and heart myocytes under hypoxic stress [29]. Left ventricular dysfunction, liver or kidney damage caused by low cardiac output, hypoperfusion, hypoxia and venous congestion can stimulate the production of interleukin-6 and, therefore, CRP production [30]. It is also possible that the relationship between plasma CRP and heart failure is causal. CRP can activate the complement system and stimulate the production of cytokines [27], thus causing myocyte apoptosis and contributing to remodeling and left ventricular dysfunction. It was shown that CRP inhibits the production of nitric oxide [31] and has a direct pro-inflammatory effect on human endothelial cells [32], disrupting their function, which is reflected in our results regarding flow-dependent vasodilation. The pathophysiological

mechanism of endothelial dysfunction (ED) in CHF is associated with a state of increased oxidative stress in this patient population through a variety of mechanisms – reduced nitric oxide (NO) synthesis with the possible involvement of genetic polymorphism of endothelial nitric oxide synthase (eNOS) [33], oxidative NO inactivation [34], increased activity of endothelium-bound xanthine oxidase and decreased activity of extracellular superoxide dismutase [35]. Discussing the fact of the conjugation of the cachectic process and ED, the latter can be interpreted from at least two points of view. On the one hand, ED, reducing the ability to regulate peripheral blood flow in skeletal muscles, contributes to the deterioration of perfusion, and, consequently, the conditions for the loss of their mass [36]. On the other hand, there is no reason not to evaluate ED in such patients as a concomitant factor (the "role of a witness"), since along with a decrease in body weight with the clinical progression of CHF, the state of endothelial function worsens to the same extent [37].

The role of cholesterol as a factor associated with the preservation of BW in CHF can be associated with the ability to reduce the biological activity of lipopolysaccharides and, consequently, the level of inflammatory cytokines [38]. This may partially explain why low serum cholesterol is an independent prognostic factor for poor outcome in CHF [39].

Study limitations. A limitation of the study is the fact that the data on changes in body weight of some patients were based on their statements due to the lack of measured values in medical records, which could potentially be a source of inaccuracies. The lack of information in medical data cannot be completely avoided in real clinical practice. However, it was shown that the anthropometric data provided personally by the patient are suitable for use in clinical trials, since the differences are considered small compared with the measured values [40].

5. Conclusions

1. Weight loss ≥6 % is observed in 49.2 % of patients with CHF and LVEF≤35 %. In addition to the older age, its clinical predictors are NYHA class, coronary artery disease, and anaemia.

2. According to a univariate regression analysis, patients with a worse score for quality of life, a large number of points on the Beck depression scale and the fatigue scale after physical exertion have a higher risk of losing BW≥6 %. The risk of developing progressive BW loss is also associated with poorer functional status of patients (6-minute walk test, DASI index) and endothelium-dependent vasodilator response; this risk is reduced at higher values of the bicep circumference of a tight and relaxed arm, waist and hip, SFF thicknesses above the biceps, triceps, under the shoulder blade and in the inguinal region.

3. The risk of losing BW is associated with higher levels of CRP, uric acid, as well as aspartate aminotransferase (AST), and decreased levels of haemoglobin, hematocrit, cholesterol, triglycerides, and albumin. The risk of losing BW≥6 % in our patients increases with an increase in the size of the right atrium, right ventricle, systolic pressure in the pulmonary artery, and E/E'. Higher diastolic blood pressure, TAPSE, and TAPSE to pulmonary systolic pressure ratios are associated with a lower risk of BW loss ≥6 %.

4. According to multivariate analysis, independent predictors of BW loss of ≥6 % in patients with CHF and LVEF≤35 % are age, CRP, III-IV NYHA class, lower cholesterol levels, as well as lower rates of flow-dependent vasodilator response and hip circumference.

Conflict of interest

No conflict of interest.

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EXPERIMENTAL STUDY OF ANTIMICROBIAL PROPERTIES AND ACUTE TOXICITY OF THE PEO-BASED COMBINED SUPPOSITORIES

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Abstract

Aim. The research of antimicrobial and toxicological parameters of a promising pharmaceutical composition with indole-3-carbinol and meloxicam in the form of rectal suppositories.

Materials and methods. The research of antimicrobial activity was carried out in vitro by diffusion in nutrient agar in the modification of "holes" on the reference strains of common pathogens Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Candida albicans.

Adult rats were used for the study of acute toxicity. Suppository mass were administrated in the largest possible volume rectally or orally. The animals were periodically monitored according to the experimental plan – the assessment of physiological parameters before administration and after 6, 12, 18, 24 hours, at 3, 7 and 14 days. The animals were removed from experiment and necropsy provided after 1, 3, 7, and 14 days.

Results. The sizes of zones of inhibition of the microorganisms growth were most significant (from 19.27±0.61 mm of E. coli to 40.80±0.42 mm of S. aureus) near sample of the combined composition suppository compared with other combination of active substances and excipients.

During the observation of animals for 14 days and the study of internal organs after autopsy, deviations in physiological (weight, temperature, activity, respiratory rate) and macroscopic morphological indicators of animals from reference values were not detected.

Conclusion. According to the results of microbiological studies, a moderate antimicrobial effect of suppositories of combined composition in relation to all the studied pathogens was revealed. The absence of manifestations of acute toxicity allows us to conclude that the pharmaceutical composition can be classified as practically non-toxic substances.

The obtained results allow us to recommend a pharmaceutical composition with indole-3-carbinol and meloxicam on a polyethylene oxide basis in the form of suppositories for further preclinical studies of specific pharmacological effects as a prostate protective agent.

Keywords: indole-3-carbinol, meloxicam, antimicrobial activity, acute toxicity.

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1. Introduction

The metabolite of plant glucosinolates indole-3-carbinol (I3C) is a promising treatment for prostate diseases [1]. The effectiveness of I3C as a component of complex therapy of hormone-dependent diseases of benign prostatic hyperplasia (BPH), prostate carcinoma, uterine fibroids, fibrocystic mastopathy is confirmed. Experimentally demonstrated high antitumor activity of I3C and its metabolite DIM with respect to prostate cancer [2], the ability to induce cell apoptosis, inhibit the growth of androgen-dependent and androgen-independent cell cultures of this gland [3]. As well as the ability to block the proliferation of signals (in particular, the activation of nuclear transcription factor NF-kB and protein kinase Akt [4]) in prostate cells by inhibiting the expression of androgen receptors, which play an important role in the pathogenesis of BPH [5]. I3C exerts a modulating effect on estrogen receptors (ER) and the number of androgen receptors (AR) [6] Based on its pharmacodynamic characteristics, it can be assumed that I3C may have a complex effect on the mechanisms of BPH and CP.

Useful for the treatment of prostatitis is the antimicrobial activity of I3C, as there are reports that the substance exhibits broad-spectrum antibacterial activity, in particular, in Candida albicans, I3C stops the cell cycle in phase G(2)/M, thus destroying the structure of the cell membrane of *C. albicans* [7].

Diseases of the gland are also characterized by an increase in the activity of cytokines, growth factors, cyclooxygenase (COX) type 1 and 2, inducers of angiogenic, anti-apoptotic and inflammatory processes. It is believed that the inhibition of inflammation may reduce the risk of prostate disease. It is therefore important that I3C exhibits antimicrobial, anti-inflammatory, antioxidant properties.

Non-steroidal anti-inflammatory drugs (NSAIDs) have a more specific anti-inflammatory effect. They are widely used in the world because of their antinociceptive, anti-inflammatory effects, particularly in cancer and other prostate diseases, although there is still no consensus on the usefulness of NSAIDs in prostate diseases, but they believe this to be the case for treating BPH and prostate cancer [8]. With the use of NSAIDs, COX-1 and COX-2 inhibitors reduce the symptoms of inflammation and improve the quality of life of patients with chronic prostatitis [9], reduce the risk of BPH development and progression [10]. In clinical studies, it has been shown that, with the use of NSAIDs, a greater reduction in the average international prostate symptom score and faster relief of BPH symptoms have been observed [11], thought to be due to a decrease in viability and inhibition of BPH cell line proliferation [12].

A combination of I3C and a non-steroidal anti-inflammatory drug is considered to be appropriate to increase the efficacy of the drug and expand the range of pharmacological effects and indications for use. Meloxicam is a selective cyclooxygenase type 2 inhibitor with pronounced anti-inflammatory properties due to inhibition of arachidonic acid metabolism. He is thought to be at lower risk of damage to the gastric mucosa [13], although there is a risk of cardiovascular events, especially with prolonged treatment [14].

That is why, when designing new highly effective drugs, the dosage form is important, which provides optimal therapeutic effect with minimal side effects. The use of rectal suppositories as the optimal dosage form is relevant for the problem of prostate treatment [15]. They have several advantages over other dosage forms: independence of the completeness of absorption from the functional state of the gastrointestinal tract; the maximum part of the dose of the drug bypasses the processes of presystemic metabolism and invariably enters the prostate; high absorption rate (comparable to parenteral routes of administration), in most cases no allergic effect on the body; independence of the input path from the state of other systems; ease of use; metering accuracy. This is confirmed by studies of local reaction, safety and efficacy of meloxicam suppositories (15 mg) over 3 weeks, which showed no significant adverse events, and approximately 90 % of people rated meloxicam response and treatment efficacy as good or very good [16].

During the research, the authors proposed the optimal composition of a promising pharmaceutical composition for the treatment of prostate diseases in the form of rectal suppositories with I3C and meloxicam. To compare the efficacy of the release of the active substances from the two variants of the suppository base (solid fat and polyethylene oxide (PEO)), a screening study was performed on a

model of turpentine prostatitis [17]. The availability of the active components was determined by the degree of inhibition of inflammation and the effect on the hormonal background of the animals. Because PEO-based suppositories had a more pronounced effect on the course of the inflammatory process and reduced serum testosterone content, they were selected as a promising drug for further in-depth pharmacological research including the determination of acute toxicity and antimicrobial activity.

Aim of the research. Microbiological and toxicological study of a new preparation in the form of rectal polyethylene oxide-based suppositories (SCS-PEO) containing indole-3-carbinol and meloxicam.

2. Materials and methods

In the study of the antimicrobial properties of SCS-PEO, as the substances were used meloxicam (BoehringerIngelheimGmbH, Germany) and I3C (Sigma-Aldrich Co., USA), as well as samples of some variants of suppositories of the following composition:

– Sample 1 – PEO-base: a mixture of PEO-1500 and PEO-400 (95:5); Montanox 80 (Polysorbate-80);

– Sample 2 – PEO basis; Montanox 80; indole-3-carbinol (0.2 g/suppository);

– Sample 3 – PEO basis; Montanox 80; meloxicam (0.0075 g/suppository);

– Sample 4 – SCS-PEO;

– Sample 5 – PEO basis; indole-3-carbinol (0.2 g/suppository);

– Sample 6 – PEO basis; indole-3-carbinol (0.2 g/suppository); meloxicam (0.0075 g/1 suppository).

Microbiological study was performed on clinically significant pathogens of inflammatory diseases of the genitourinary system (including prostatitis). The reference strains of bacteria and fungi were taken from the Ukrainian Collection of Microorganisms of the Institute of Microbiology and Virology named after I. V. Zabolotnyi (Kyiv, Ukraine): *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231).

The bacterial test strains were cultured at about 35 °C for 18 to 24 hours. on soybean casein agar. The studied C. albicans microorganism was grown on Saburo dextrose agar at a temperature of about 35 °C for 24 to 48 hours.

Before starting the experiment, the purity of each culture and its typical properties were checked for morphological, cultural and staining (for bacteria) characteristics.

A standard pharmacopoeial turbidity sample (PSS SPU) of 10 IU was used to prepare a microbial suspension of standard concentration [19–21].

Studies on the specific antimicrobial action of the drug were performed in vitro by diffusion into nutrient agar with the modification of the wells in accordance with the recommendations [22–25].

Acute toxicity studies were performed with single administration of the maximum possible amount of the drug, since known maximum toxic doses for the active substances could not be achieved.

All experiments were carried out in accordance with the Law of Ukraine "On the Protection of Animals against Cruelty", which was agreed with the European Convention on the Protection of Vertebrate Animals of 1986 [26, 27]. Experiments were approved by the Bioethics Commission of the National University of Pharmacy (excerpt from protocol No. 3 of 20.04.2016).

Acute toxicity studies were conducted on 32 adult male rats randomized to 2 subgroups for oral and rectal administration of the study agent. Rats from subgroup 1 were injected intragastrically with a single dose of 4.5 g of SCS-PEO suppository, which corresponded to the administration of 10.75 mg meloxicam and 300 mg I3C per animal. Rats of subgroup 2, using a soft probe, performed rectal administration of the drug at a dose of 1 g, which corresponded to the administration of 2.5 mg meloxicam and 67.7 mg I3C per animal [18].

Over the next 24 hours, the animals were continuously monitored for physiological parameters. From 1 to 14 days we carried out daily assessment of the animals. The animals were removed according to the scheme – 4 animals from both subgroups after 1, 3, 7 and 14 days after the introduction of the studied SCS-PEO by rapid decapitation.

Physiological (measurement of basal body temperature, respiratory rate, monitoring of activity, feed and water consumption) and morphological parameters (body and organ weight, macroscopic morphological evaluation of internal organs) were used to evaluate toxicodynamics [28].

Statistical processing of the results was carried out using software using variation statistics methods using parametric and non-parametric methods of analysis (Student and Mann-Whitney criteria) and presented as comparative tables with the results of different groups as arithmetic mean and its error (M±m) or Median and 25 and 75 percentile (Me, LQ; UQ). Differences were considered statistically significant at $p<0.05$ [29].

3. Results

The results of a comparative study of the antimicrobial activity of suppository samples by agar diffusion (**Table 1**) against reference strains of bacteria and fungi showed that Sample 1, which includes Montanox 80 and PEO-base, has no antimicrobial effect against *B. subtilis* (ATCC 6633), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231). Small areas of inhibition of growth of *E. coli* (ATCC 8739) with a diameter of 13.27±0.36 mm were observed, indicating little antimicrobial activity against this bacterium.

Despite the absence of a well-described mechanism of antimicrobial activity, suppositories of I3C (Sample 2) showed antimicrobial action against all tested microorganisms used as reference. The most pronounced activity was observed for *S. aureus* – the area of inhibition of growth of *S. aureus* (ATCC 6538) was 38.60±0.99 mm, i. e., indicates a high level of antibacterial activity. High antibacterial activity was also observed in relation to gram-positive *B. subtilis* (ATCC 6633) – the diameter of the growth inhibition zone was 27.83±0.77 mm. Gram-negative bacteria showed moderate sensitivity to Sample 2, the diameter of the zone of inhibition of growth of *E. coli* and purulent sticks was less than 20 mm (**Table 1**). Concerning the strain of the yeast fungus *C. albicans* (ATCC 10231), Sample 2 showed a moderate antifungal effect – the diameter of the growth inhibition zone was 22.43±0.33 mm.

The severity of antibacterial and antifungal action of suppositories with I3C was not significantly dependent on the presence (Sample 5) or absence (Sample 2) of Montanox 80. The diameter of the growth inhibition zones of each of the studied organisms *B. subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538), and *C. albicans* (ATCC 10231) for Sample 5 did not differ significantly from the values obtained for Sample 2 (**Table 1**).

Suppositories with meloxicam (Sample 3) did not show antimicrobial activity against the microorganisms *B. subtilis* (ATCC 6633), *P. aeruginosa* (ATCC 9027), *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231) – no growth inhibition zones were present. About *E. coli* (ATSC 8739) had poorly expressed activity – the diameter of the growth inhibition zone was significantly smaller than the diameter of the growth zones of *E. coli* for Sample 1.

Table 1

Antimicrobial activity of tested samples of suppositories

The studied microorganisms	Growth inhibition zone diameter, mm (M±m, n=6)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
B. subtilis (ATCC 6633)	No zone	27.83±0.77	No zone	29.96±0.69	25.38±1.20	29.22±0.51[c)]
E. coli (ATCC 8739)	13.27±0.36	18.52±0.26	11.68±0.39[a)]	19.27±0.61	18.50±0.21	18.38±0.33
P. aeruginosa (ATCC 9027)	No zone	16.35±0.43	No zone	17.75±0.34 [b)]	16.87±0.45	17.65±•.31
S. aureus (ATCC 6538)	No zone	38.60±0.99	No zone	40.80±0.42	37.43±0.78	39.53±0.53
C. albicans (ATCC 10231)	No zone	22.43±0.33	No zone	20.27±0.48 [b)]	22.43±0.57	19.95±0.26[c)]

Note: [a)] – a significant difference ($p<0.05$) compared to the results of Sample 1; [b)] – a significant difference ($p<0.05$) compared to the results of Sample 2; [c)] – a significant difference ($p<0.05$) compared to the results of Sample 5

The antimicrobial activity of samples of suppositories containing I3C and meloxicam did not differ significantly from the activity of suppositories containing only I3C. It should be noted that there was a certain tendency to increase the degree of antibacterial activity in the presence of meloxicam in suppositories, but the difference in the diameters of growth inhibition zones was only significant in two cases – for Samples 2 and 4 for *P. aeruginosa* (ATCC 9027) and for Samples 5 and 6 regarding *B. subtilis* (ATCC 6633). In contrast, the antifungal activity of suppositories containing I3C and meloxicam against *C. albicans* (ATCC 10231) was significantly lower than that of suppositories containing I3C only.

After a single intragastric or rectal administration of the suppository mass of SCS-PEO, the animals remained alive on the first day and had normal appearance and behavior. In the future, at all times of observation, mobility, condition of the hair, open areas of the skin, eating behavior, excretion did not differ from the usual for laboratory rats. There were no unusual movements, tremors, and more. Body weight did not change statistically significantly over 14 days.

The results of the study of toxicodynamics by physiological (basal temperature, respiratory rate, observation of activity, feed and water consumption) and morphological (mass and macroscopic morphological evaluation of internal organs, determination of body weight and its changes) indicators in rats, which were once administered with suppository SCS-PEO shown in **Fig. 1**. The data obtained during the first observation day every 6 h and in the following observation periods are presented: 3, 7 and 14 days.

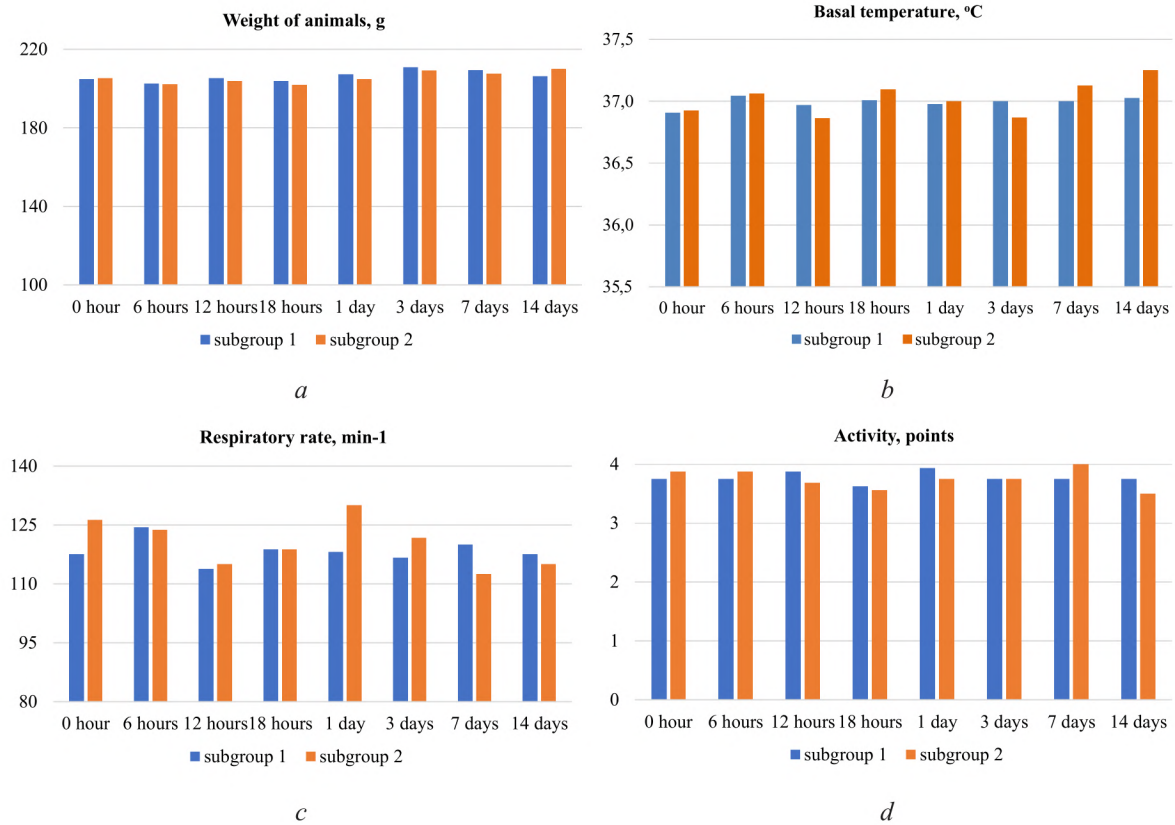

Fig. 1. Physiological indices of rats receiving suppository mass of SCS-PEO: subgroup 1 – oral administration; subgroup 2 – rectal administration. For 0, 6, 12, 18, 24 hours: n=16; 3 days: n=12; 7 days: n=8; n=4; *a* – animal body weight; *b* – basal temperature; *c* – respiratory rate; *d* – activity

At the necropsy of animals at day 14, it was noted that the hair of the animals was soft and shiny, the natural ostium (eyes, mouth, ears, anus and penis) were dry, without secretions, the mucous membranes were shiny, pale pink.

The location, size, density, and colour of the thymus, heart, lungs, liver, spleen, kidneys, prostate, testes, and vesicles did not differ from these control animals. As shown in **Table 2**, in all observation periods the mass coefficients of the organs have no significant differences with the reference indicators and in rats of both groups (oral and rectal administration) remained within the reference age norm [30].

Macroscopic morphological examination revealed that 25 % of subgroups 1 (oral administration) rats withdrawn from the experiment at 24 h and 3 days showed gastric mucosa. This may be related to the effect of meloxicam, since inhibition of cyclooxygenase activity may slightly reduce the antacid protection of the gastric mucosa [31].

It should be noted that the calculated therapeutic dose of the SCS-PEO suppository for meloxicam is 0.45 mg/kg, which, respectively, for rats weighing 200–220 g is a dose of 0.09–0.10 mg/kg. In our study, rats in subgroup 1 received oral meloxicam at a dose of 10.45 mg. Considering that the therapeutic dosage of the study drug was exceeded more than 100 times, the mucous membrane hyperaemia can be considered as an expected undesirable manifestation.

Table 2

Mass ratios of organs of rats receiving suppository mass of SCS-PEO orally (subgroup 1) or rectally (subgroup 2) ($1 \cdot 10^{-3}$, n=4, Me, LQ; UQ)

Reference rate [12]	24 hours	3 days	7 days	14 days
1	2	3	4	5
		Subgroup 1 (oral administration)		
Heart 4.2 (3.8; 4.6)	4.44 (4.37; 4.48)	4.32 (4.29; 4.33)	4.28 (4.26; 4.31)	4.43 (4.41; 4.47)
		Subgroup 2 (rectal administration)		
	4.35 (4.23; 4.46)	4.46 (4.38; 4.50)	4.25 (4.24; 4.26)	4.49 (4.42; 4.53)
		Subgroup 1 (oral administration)		
Lungs 17.6 (15.6; 18.6)	16.50 (16.27; 16.62)	16.71 (16.31; 16.97)	16.34 (15.91; 16.81)	16.30 (16.11; 16.65)
		Subgroup 2 (rectal administration)		
	16.85 (16.47; 17.15)	16.96 (16.52; 17.22)	16.69 (16.28; 17.07)	16.50 (16.19; 16.77)
		Subgroup 1 (oral administration)		
Thymus 2.2 (2.1; 2.3)	2.17 (2.14; 2.19)	2.24 (2.19; 2.24)	2.20 (2.17; 2.22)	2.18 (2.13; 2.19)
		Subgroup 2 (rectal administration)		
	2.11 (2.08; 2.15)	2.21 (2.13; 2.26)	2.16 (2.11; 2.21)	2.13 (2.11; 2.13)
		Subgroup 2 (rectal administration)		
Liver 51.80 (50.4; 53.2)	51.49 (50.93; 52.03)	51.67 (50.56; 52.44)	52.08 (51.67; 52.43)	51.97 (51.16; 52.53)
		Subgroup 2 (rectal administration)		
	52.42 (51.17; 53.66)	51.76 (51.20; 52.52)	50.53 (50.26; 51.50)	51.17 (51.05; 51.34)
		Subgroup 2 (rectal administration)		
Spleen 6.2 (5.8; 6.6)	6.23 (6.17; 6.28)	5.94 (5.92; 6.05)	6.08 (5.94; 6.20)	6.15 (6.06; 6.22)
		Subgroup 2 (rectal administration)		
	6.00 (5.95; 6.06)	6.07 (5.99; 6.11)	6.06 (6.01; 6.10)	6.32 (6.19; 6.35)
		Subgroup 1 (oral administration)		
Kidneys 16.0 (15.6; 16.4)	15.89 (15.62; 16.15)	15.87 (15.57; 16.29)	16.07 (15.91; 16.16)	16.07 (15.71; 16.36)
		Subgroup 2 (rectal administration)		
	16.58 (16.14; 16.79)	16.65 (16.44; 16.71)	15.64 (15.63; 15.71)	16.18 (16.02; 16.24)

Continuation of Table 2

1	2	3	4	5
		Subgroup 1 (oral administration)		
Prostate 3.2 (3.0; 3.4)	3.15 (3.14; 3.18)	3.18 (3.07; 3.27)	3.21 (3.12; 3.26)	3.09 (3.07; 3.11)
		Subgroup 2 (rectal administration)		
	3.19 (3.09; 3.27)	3.18 (3.13; 3.22)	3.11 (3.10; 3.14)	3.18 (3.12; 3.23)
		Subgroup 1 (oral administration)		
Seminal vesicles 5.1 (5.0; 5.3)	5.05 (4.99; 5.10)	5.23 (5.06; 5.33)	5.21 (5.14; 5.23)	5.23 (5.11; 5.28)
		Subgroup 2 (rectal administration)		
	5.07 (4.99; 5.18)	5.25 (5.15; 5.29)	5.10 (5.05; 5.13)	5.15 (5.04; 5.22)
		Subgroup 1 (oral administration)		
Testicular 13.5 (13.0; 14.0)	13.70 (13.50; 13.81)	13.11 (13.06; 13.21)	13.53 (13.42; 13.60)	13.79 (13.66; 13.92)
		Subgroup 2 (rectal administration)		
	13.57 (13.14; 13.94)	13.47 (13.32; 13.55)	13.23 (13.04; 13.60)	13.27 (13.18; 13.34)

Thus, SCS-PEO suppositories, which on the model of turpentine prostatitis showed a distinct prostate protective effect, showed antimicrobial properties against clinically significant agents of inflammation. In terms of toxicodynamics suppositories of the proposed composition can be considered a virtually non-toxic composition, which justifies the feasibility of further study of SCS-PEO as a promising prostate protector.

4. Discussion of the results

The absence of statistically significant differences of the studied indicators from the reference ones indicates that the degree of toxicodynamic effect of the combination of active components [32, 33] and the excipients [34] of SCS-PEO is consistent with the data reported in the literature. The results of the study on the parameters of acute toxicity indicate that SCS-PEO may be recommended for further preclinical study of specific prostate protective action, as significant toxic effects with single administration at doses exceeding the proposed therapeutic 3-4 times, were not detected. The proposed pharmaceutical composition based on polyethylene oxide based on the parameters of acute toxicity can be attributed to almost non-toxic substances.

Study limitations. The presence of antimicrobial activity in SCS-PEO suggests that there is both an additional therapeutic benefit (inhibitory effect on infectious prostatitis pathogens), and be a potential source of side effects (development of intestinal dysbiosis). The presence of meloxicam in suppositories does not significantly affect the antibacterial effect, but significantly reduces the antifungal activity of the pharmaceutical composition.

Prospects for further research. Given the potential risks of prolonged use, there is a need to consider further studies of SCS-PEO with the long-term introduction of cumulative toxic properties and adverse effects on the intestinal biotope.

5. Conclusions

The results show that suppositories with indole-3-carbinol and meloxicam meet the expected toxicity parameters.

SCS-PEO suppositories exhibit a pronounced antibacterial effect against gram-positive and gram-negative bacteria and antifungal action. The revealed features of antimicrobial action make it possible to recommend the use of SCS-PEO for the treatment of not only aseptic but also bacterial (infectious) forms of prostatitis.

Suppositories of the combination composition with indole-3-carbinol and meloxicam on a polyethylene oxide basis are a relatively safe preparation and may be recommended for further study of the specific pharmacological action.

Conflict of interests

No conflict of interest.

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THE INFLUENCE OF VAGINAL SUPPOSITORIES "MELANIZOL" ON THE SYSTEM OF LPO-AOS IN CONDITION OF MODEL OF A NONSPECIFIC VAGINITIS IN RATS

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Abstract

It is known that almost any pathology is accompanied by an imbalance of the lipid peroxidation (LPO) and the antioxidant system (AOS), and diseases of the female reproductive system are no exception. Thus, modern therapies of nonspecific vaginitis (NV) should include drugs with an antioxidant effect in the complex treatment, because the role of the LPO-AOS system in the mechanism for the development of NV is great. This problem can be solved by new complex drug with plant material with a wide spectrum of action.

Aim. The aim of our research was to study the effect of new vaginal suppositories "Melanizol", containing metronidazole and tea tree oil on the system of LPO-AOS on a model of nonspecific vaginitis in rats.

Materials and methods. The study object was the new vaginal suppositories "Melanizol". As reference drugs there were taken suppositories "Gravagin" and "Hippophaes oleum suppositories". The study has been carried out on the model of experimental nonspecific (irritative) vaginitis caused in rats by the mixture of turpentine oil and dimethyl sulfoxide. Blood serum and vaginal tissue were used as the material for the study. Thiobarbituric acid-active products (TBA-AP) were determined as the component of a lipid peroxidation. The catalase (CAT), the superoxide dismutase (SOD) and the reduced glutathione (G-SH) were determined as the component of an antioxidant system.

Results and discussion. On the background of experimental vaginitis in serum and in vaginal tissue homogenate in rats, significant changes in indicators of the LPO-AOS were noted. Change of these indicators show a systemic response of the body to pathological changes in the vagina, which is manifested in the depletion of AOS and activation of the LPO. The vaginal suppositories "Melanizol" in the study on the model of nonspecific (irritative) vaginitis in rats showed an antioxidant effect. Suppositories "Melanizol" significantly reduce the level of TBA-AP, interfering with lipid peroxidation processes, and restore the level of G-SH, as well as the activity of SOD and catalase in the blood and in the vaginal homogenate, helping to protect the cell membranes. Suppositories "Melanizol" significantly exceed the reference suppositories "Gravagin" and are not inferior to the reference drug "Hippophaes oleum suppositories" in activity to reduce the level of TBA-AP, and to restore the level of G-SH, as well as restoration the activity of SOD and CAT. Antioxidant activity of vaginal suppositories "Melanizol" can be explained by the presence of tea tree oil and metronidazole in their composition, which is confirmed by literature data.

Conclusions. The conducted researches have shown that the new vaginal suppositories "Melanizol" demonstrated antioxidation activity. Results of this experiment allow us to recommend them for the further studies as medication for the treatment of nonspecific vaginitis.

Keywords: vaginal pessaries, vaginitis, metronidazole, tea tree oil, antioxidant activity, lipid peroxidation, rats.

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1. Introduction

In recent years the study of the role of lipid peroxidation (LPO) and the antioxidant system (AOS) in the pathogenesis of many diseases has been of interest to researchers. It is known that almost any pathology is accompanied by an imbalance of the LPO-AOS system, and diseases of the female reproductive system are no exception [1–3]. Also a change in the potential of LPO-AOS in nonspecific vaginitis (NV) has been noted [3]. This is because NV is accompanied by an

increase in the number of conditionally pathogenic microflora, which provokes the activation of polymorphonuclear leukocytes, as the first line of innate protection and the effector of adaptive immunity. Their release activates excessive inflammation and oxidative stress in the vagina, which leads to the production of reactive oxygen species (ROS) [4]. The formation of ROS activates the processes of LPO are a factors that damage a lot of cellular components (membrane lipids, enzymes, DNA, etc.) [5], leading to cell death.

A change in the level of thiobarbituric acid-active products (TBA-AP) is the result of the discrepancy between the capabilities of antioxidant enzymes and the rate of free radical oxidation [5], which also reflect the degree of damage of cell membranes under the action of ROS [6].

The state of AOS on the contrary displays the protective capabilities of the body and cell membranes from damage by free radicals [5].

Glutathione is one of the most studied antioxidants. It is an intracellular antioxidant with a powerful detoxifying effect. The glutathione system includes glutathione and three enzymes (glutathione peroxidase, glutathione transferase and glutathione reductase) and is the only one system in the body that participates in three of four lines of defense. The presence of a sufficient concentration of reduced glutathione is a critical factor in the survival of cells in oxidative stress [7].

Also, the functions of the antioxidant defense system are realized by enzymes superoxide dismutase (SOD) and catalase (CAT). Many inflammatory diseases are accompanied by changes in the activity of SOD and CAT in the blood [8]. Superoxide dismutase catalyzes the dismutation (or partitioning) of the superoxide radical – a product of one-electron reduction of molecular oxygen (to oxygen and hydrogen peroxide), which is formed in almost all body cells, that contact with oxygen. Superoxide radical plays a leading role in the processes of ROS toxicity [8]. Catalase removes toxic hydrogen peroxide (H_2O_2), catalyzing its decomposition to O_2 and H_2O and prevents its accumulation. This highly active enzyme does not require energy to activate [3, 9]. And its high activity is crucial to ensure the function of SOD [3].

Thus, modern therapies of NV should include drugs with an antioxidant effect in the complex treatment, because the role of the LPO-AOS system in the mechanism for the development of NV is great.

In most cases, along with the rest, local treatment of NV is effective. It creates a higher local concentration of drugs in vagina and decreases drug interactions and side effects. In addition, the resistance of microorganisms to antibiotics and the difficulties resulting from frequent relapses create the need for more effective new drugs with herbal material with a lot of range of effects [10].

The aim of our research was to study the effect of new vaginal suppositories “Melanizol”, containing metronidazole and tea tree oil [11], developed at the Department of TL NUPh under the guidance of prof. T. G. Yarnykh on the LPO-AOS system on a model of nonspecific (irritative) vaginitis in rats.

2. Materials and methods

As studied drugs there were taken: suppositories “Melanizol” (1 pessary contains 250 mg of metronidazole, 100 mg of tea tree oil, the mixture of PEO-1500 and PEO-400 (9:1) As reference drugs there were taken: suppositories “Gravagin” (nonproprietary name, production of “Sperco Ukraine”, Ukraine; 1 suppository contains 500 mg of metronidazole), similar in composition to “Melanizol” and recommended in protocols for the treatment of gynecological diseases; “Hippophaes oleum suppositories” (nonproprietary name, production of “Nizhpharm”, Russia; 1 suppository contains 500 mg of oleum Hippophaes), to have antioxidant properties and are used in gynecological practice for the complex treatment of inflammatory processes of the vagina and cervix, as well as by erosion of the cervix.

The study was conducted on 36 non-linear white laboratory female rats weighing 190±20 g. The animals were kept on a standard diet of the vivarium. Care of them (including euthanasia) during the experiment was carried out according to the requirements of documents, which regulate organization of the work with experimental animals. The principles of the “European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes” were followed (Strasbourg, 18.3.1986) [12], adopted by The I National Congress on Bioethics (Kyiv, 2000),

which is coordinated with the "European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes", Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, Order of the Ministry of Health of Ukraine from February 13, 2006 No. 66, Law of Ukraine "On protection of animals from cruelty" (No. 3447-IV 21.02.2006). After completion of the experiment, the rats were taken out from the experiment in accordance with the ethical principles of animal testing.

Before the beginning of the experiment, the estrous cycle was examined in female rats and the animals in proestrus-estrus phases were selected, in order to minimize the differences in the assessment of the functional state of the vaginal mucosa when comparing the obtained results.

Nonspecific (irritative) vaginitis was modeled by a single intravaginal application of a mixture of irritants [13]: presented by the mixture of turpentine oil (JSC "Yantar", Ukraine) with dimethyl sulfoxide (Dimexid, "Arterium", JSC "Halychpharm", Ukraine) in the ratio of 1:1 at the dose of 0.5 ml/100 g of the body weight of the animal. The animals were divided into 6 groups of 6 animals in every group each as follows: 1st group – intact animals (IC, intact control, no pathology), 2 group – control pathology (CP, positive control), 3 group – animals treated with vaginal suppositories "Melanizol", group 4 – animals treated with the reference vaginal suppositories "Gravagin"; group 5 – animals treated with the reference drug "Hippophaes oleum suppositories"; group 6 – animals, on which the pessary base in an equivalent amount was applied (placebo; negative control; the mixture of PEO-1500 and PEO-400 (9:1). Treatment began 24 hours after the application of the irritant. The studied drugs were being injected into the vagina during 7 days, once a day. The studied pessaries and reference drugs were injected vaginally once a day at doses calculated using the specific sustainability factors by Y. R. Rybolovliev, applied in the experimental pharmacology [14].

After finishing experiment, the animals were taken out from the experiment by the method of euthanasia. Blood serum and vaginal tissue were used as the material for the study. The determination of the components of LPO-AOS activity was performed on a spectrophotometer "SPEKOL 1500" (Germany). TBA-AP, CAT and SOD were determined in the serum [15]. CAT, TBA-AP and reduced glutathione (G-SH) were determined in the vaginal tissue [15].

Changes in these indicators allow us to assess the severity of the inflammatory process, in which a disbalance between the processes of LPO and the protective systems of the body is present. The content of TBA-AP – product reactive with thiobarbituric acid, most often determine as an indicator of intensity POL. Under their influence occurs damage of cell membrane. There is one of the leading factors in the development of the inflammatory process [6]. In turn, the state of the antioxidant system was assessed by the level of G-SH and the activity of intracellular antioxidant enzymes CAT and SOD [16].

Statistical treatment of the obtained results was carried out with the program "Statistica 6.0" (Free Trial Version https://www.tibco.com/), using the Newman-Keuls criterion, the data were considered reliable when the difference between the mean values $p<0.05$.

3. Research results

Data on the effect of new vaginal suppositories "Melanizol" on the status of indicators of the LPO-AOS system in blood serum and vaginal tissue of rats on the background of experimental nonspecific (irritative) vaginitis are presented in **Fig. 1, 2**.

An imbalance in the LPO-AOC system in the vaginal tissue homogenate of rats was observed on the background of nonspecific (irritative) vaginitis (**Fig. 1**). The level of catalase was significantly reduced by almost 2 times the level of G-SH significantly reduced by 1.4 times, and the level of TBA-AP significantly increased by 1.5 times. It indicates the depletion of AOS and the activation of LPO in vaginal tissues and is accompanied by inhibition of oxygen-dependent processes.

The restoration of antioxidant protection was observed under the influence of the investigated suppositories "Melanizol" in the vaginal tissue, which is manifested by a significant increase in the level of catalase by 1.7 times and an increase in the level of G-SH by 1.3 times in comparison with animals of the CP group. A significant decrease in the level of TBA-AP was observed by

1.8 times in the vaginal tissue under the influence of the tested suppositories "Melanizol" in comparison with a group of animals of the CP.

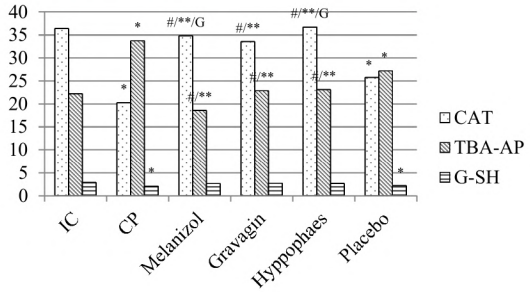

Fig. 1. The level of catalase (μmol/minute·g), TBA-AP (μmol/g) and G-SH (μmol/g) in the vaginal homogenate of rats on the background of nonspecific (irritative) vaginitis: * – significant in relation to intact control, IC ($p<0.05$); ** – significant in relation to placebo ($p<0.05$); # – significant in relation to the control pathology, CP ($p<0.05$); G – significant in relation to Gravagin suppositories ($p<0.05$)

On the background of nonspecific vaginitis, the suppositories "Melanizol" were not inferior to the reference drug "Hippophaes oleum suppositories" and significantly exceeded the reference suppositories "Gravagin" in terms of their effectiveness to restore catalase activity. In the ability to reduce the TBA-AP activity and to restore the G-SH level in the vaginal tissue homogenate, the tested suppositories "Melanizol" are not inferior to the reference drugs "Gravagin" and "Hippophaes oleum suppositories".

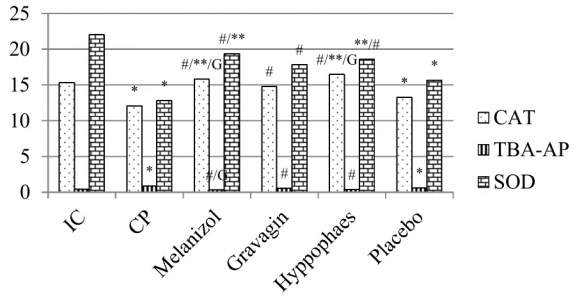

Fig. 2. The level of CAT, μmol/(minute·l), TBA-AP, μmol/g and SOD, conventional units (cu), used in the serum of rats on the background of nonspecific (irritative) vaginitis: * – significant in relation to intact control, IC ($p<0.05$); ** – significant in relation to placebo ($p<0.05$); # – significant in relation to control pathology, CP ($p<0.05$); G – significantly in relation to Gravagin suppositories ($p<0.05$)

On the background of experimental nonspecific (irritative) vaginitis in serum (**Fig. 2**), significant changes in indicators of the system LPO-AOS characteristics were noted. There are significant decrease in CAT activity by 1.3 times, significant decrease in SOD activity by 1.7 times and significant increase in the level of TBA-AP by 2.25 times in comparison with the IC group.

Recovery of antioxidant protection to the level of the values of the animals of the IC group was observed under the influence of the investigated suppositories "Melanizol" in the serum of rats with nonspecific (irritative) vaginitis. Its manifested in a significant increase in the level of CAT activity by 1.3 times and significant increase SOD activity by 1.6 times in comparison with animals of CP group. As well as, the level of TBA-AP was significantly reduced by 2.8 times as compared with animals of CP group. The data indicate a restorative effect of the studied suppositories "Melanizol" on indicators of LPO-AOS.

As a result of the experiment positive effect of suppositories "Melanizol" was noted. During the treatment with the studied drug and reference drugs, the suppositories "Melanizol" signifi-

cantly exceeded the reference suppositories "Gravagin" in their activity to reduce TBA-AP and to increase the activity of CAT and SOD in the blood serum of rats on the background of experimental vaginitis. The suppositories "Melanizol" were not inferior to the reference drug "Hippophaes oleum suppositories" in their restoration effects on the POL-AOS system.

In animals treated with placebo, no positive effect on investigated indexes of LPO-AOS was observed both in blood serum and in vaginal tissue homogenate.

5. Discussion

On the background of experimental vaginitis in serum and in vaginal tissue homogenate in rats, significant changes in indicators of the LPO-AOS were noted. There are decreasing of CAT activity, decreasing of SOD activity, increasing of the level of TBA-AP in the blood serum and decreasing of CAT activity, decreasing of the level of G-SH, increasing of the level of TBA-AP in the vaginal tissue homogenate. Changes of these indicators characterize of the inflammatory process [16]. Thus, changes of these indicators show a systemic response of the body to pathological changes in the vagina, which is manifested in the depletion of AOS and activation of the LPO.

Antioxidant activity of vaginal suppositories "Melanizol" can be explained by the presence of tea tree oil in their composition, which is confirmed by literature data [17]. The antiradical activity of herbal drugs is explained by presence of phenolic compounds, monoterpene alcohols, ketones, aldehydes, carbohydrates, etc., in their composition [18]. Literature data shows that tea tree oil has a strong ability to remove free radicals and to inhibit lipid peroxidation. This is explained by the activity of the components of the oil, in particular phenols, which are able to inhibit or reduce the rate of aerobic oxidation of organic substances [17]. Data in the literature indicates that monoterpene hydrocarbons (chemical *components* contained within tea tree oil composition), such as terpinen-4-ol, γ-terpinene and α-terpinene have in the structure activated methylene groups and are more active antioxidant compounds than sesquiterpenes [19].

Also, literature data show that "Metrogil" gel (contains 1 % metronidazole) for external use has antioxidant properties. It can reduce the production of ROS by neutrophilic leukocytes, such as hydroxyl radicals and hydrogen peroxide, that are oxidants [20].

Thus, new vaginal suppositories "Melanizol" have antioxidant properties, because metronidazole and tea tree oil are present in their composition [17-20]. This activity is the ability to restore pathologically reduced levels of G-SH, to restore the activity of intracellular antioxidant enzymes (superoxide dismutase, catalase) and to reduce pathological level of TBA-AP in the vaginal homogenate and in the blood serum of rats with nonspecific vaginitis modeled by an intravaginal application of the mixture of turpentine oil with dimethyl sulfoxide.

This indicates a positive effect of the tested suppositories on the state of the prooxidant-antioxidant system locally and systemically. It is positive in the treatment of nonspecific vaginitis and will prevent the development of systemic complications [21].

Limitations of the study. Obtained results confirm the important role of LPO-AOS in the development of nonspecific vaginitis. New vaginal suppositories "Melanizol", containing metronidazole and tea tree oil, can restore indexes of LPO-AOS (CAT, TBA-AP, G-SH, G-SH) in the blood and in the vaginal homogenate in condition of model of experimental nonspecific vaginitis in rats. That allow us to recommend them for the further studies as medication for the treatment of nonspecific vaginitis. But this mechanism requires further detailed study.

6. Conclusions

In summary, studies have shown that the following conclusions can be drawn:

1. It has been experimentally proved that the new vaginal suppositories "Melanizol" in the study on the model of nonspecific (irritative) vaginitis in rats showed an antioxidant effect. Suppositories "Melanizol" significantly reduce the level of thiobarbituric acid-active products, interfering with lipid peroxidation processes, and restore the level of reduced glutathione, as well as the activity of superoxide dismutase and catalase in the blood and in the vaginal homogenate, helping to protect the cell membranes.

2. Vaginal suppositories "Melanizol" significantly exceed the reference suppositories "Gravagin" and are not inferior to the reference drug "Hippophaes oleum Suppositories" in activity to reduce the level of thiobarbituric acid-active products, and to restore the level of reduced glutathione, as well as restoration the activity of superoxide dismutase and catalase.

3. The preliminary studies proved anti-inflammatory [22], antibacterial [23] properties of the new vaginal suppositories "Melanizol", and the antioxidant activity proved by us in this experiment allow to recommend them for the further studies as drug for the treatment of non-specific vaginitis.

Conflicts of interest

No conflict of interest.

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METHODOLOGY FOR ASSESSING THE LOGISTICS POTENTIAL OF THE FOREIGN ECONOMIC ACTIVITY OF A PHARMACEUTICAL COMPANY

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Abstract

The aim of the article is to determine the essence of the logistics potential of a foreign trade activity of a pharmaceutical enterprise and justify the methods for determining it.

The materials used in the study include statistical data of the investigated pharmaceutical enterprises, namely JSC FF "Darnitsa", PJSC NPC "Borschagovsky Chemical and Pharmaceutical Plant", PJSC "Pharmak", LLC "FC Zdorovia" and JSC "Lekhim-Kharkiv". The study used methods of analysis and synthesis, generalization, content analysis, questionnaires and methods for assessing potential. The questionnaire was used to select indicators that should be part of the logistics potential of a foreign trade activity of a pharmaceutical enterprise.

The experts were 100 leading specialists of pharmaceutical companies. They are all involved in foreign economic activity. Experts are gender–divided into women (73 %), men (27 %); by age: up to 25 years – 8 %, 25–35 years – 14 %, 35–45 years – 27 %, 45–55 years – 36 %, over 55 years – 15 %; by experience: up to 5 years – 11 %, 5–10 years – 15 %, 10–20 years – 32 %, 20–30 years – 36 %, over 30 years – 6 %. The experts' conclusions are valid, the coefficient of concordance is 0.86, and the Pearson test exceeds the table value.

The essence of the definition of "potential of logistics of pharmaceutical enterprise's foreign trade activity" is investigated. The types of logistics potential of foreign economic activity and indicators that are appropriate to use for determining the level of development of the logistic potential in foreign economic activity are offered.

The potential of logistics of foreign trade activities of pharmaceutical enterprises consists of the potentials of logistics in the field of export and import. The system of indicators for measuring the logistics potential of a foreign trade activity of a pharmaceutical enterprise contains indicators selected through content analysis and questionnaires.

The method of estimation of logistics potential in foreign economic activity of pharmaceutical enterprise is offered.

Keywords: potential, logistics, foreign economic activity, pharmaceutical enterprise.

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1. Introduction

In the current dynamic conditions, among the main causes of exacerbation of problems with the sale of pharmaceutical products, reducing the cost of production and marketing of drugs and increase its efficiency, scientists highlight the restructuring of the world economy, the intense development of the pharmaceutical market, the growing role of economic unions (alliances) and the activation of economic ties between pharmaceutical companies, suppliers of active pharmaceutical ingredients, basic and auxiliary materials, distribution oramy, active dissemination of new information and communication technology and logistics, change directions and volumes of international cargo and complexity of production processes, enterprises themselves inside and outside, that is, foreign economic activity.

Correctly built foreign economic activity of the enterprise due to the formation and support of business relations at the international level will bring him additional economic benefits. But it must be taken into account that the enterprise should carry out this activity only in those countries and with that partner that are the most profitable for a specific period of time.

Domestic pharmaceutical enterprises are distinguished by their unique geographical location at the crossroads of trade routes, therefore they can receive significantly more benefits from globalization and international cooperation. This predetermines the need to develop effective approaches to managing the processes of purchasing, transportation, storage and marketing of products, actualizes the need to use a scientific approach to improving the economic and organizational mechanism for the development of logistics processes in managing the movement of material, financial and information flows.

Theorists and practitioners [1, 2] have already repeatedly proved that today it is logistics that provides the appropriate level of enterprise potential, together with natural conditions, resources, means and values, provides opportunities for more complete use of resources and, consequently, economic development for gaining competitive advantages in the market environment. So, it is quite clear that logistics from fundamental science is gradually turning into the practical dominant of the pharmaceutical enterprise, and the effective use of logistics tools can reduce costs, improve productivity and improve customer service. Using logistics approaches allows enterprises to carry out productive activities, and increases the role of the logistics component in the process of rational capacity building.

The aim of the article is to determine the essence of the logistics potential of the foreign economic activity of a pharmaceutical company and the rationale for its determination.

2. Materials and methods

The materials used in the study include statistical data of the investigated pharmaceutical enterprises, namely JSC FF "Darnitsa", PJSC NPC "Borschagovsky Chemical and Pharmaceutical Plant", PJSC "Pharmak", LLC "FC Zdorovia" and JSC "Lekhim-Kharkiv".

The study used methods of analysis and synthesis, generalization, content analysis, questionnaires and methods for assessing potential.

The experts were 100 leading specialists of pharmaceutical companies involved in foreign economic activity, among which women – 73 %, men – 27 %. According to age, the experts were divided as follows: up to 25 years old – 8 %, 25–35 years old – 14 %, 35–45 years old – 27 %, 45–55 years old – 36 %, over 55 years old – 15 %. According to the experience, experts were divided – up to 5 years – 11 %, 5–10 years – 15 %, 10–20 years – 32 %, 20–30 years – 36 %, over 30 years – 6 %. Expert conclusions are justified, the concordance coefficient is 0.86, the Pearson criterion exceeds the table value.

The questionnaire was used to select indicators that should be part of the logistics potential of a foreign trade activity of a pharmaceutical enterprise. Score was scored on a ten-point scale ranging from the lowest (1 point) to the highest (10 points). Indicators with a weighted average of the expert evaluation score less than 3 were not used in the subsequent calculations.

Experimental procedures.

The study was conducted in five stages. At the first stage, based on the study of the opinions of scientists and the specifics of the pharmaceutical sector of the healthcare industry and the activity of pharmaceutical enterprises, the definition of "logistics potential of foreign economic activity of the pharmaceutical enterprise" was defined. The second stage is devoted to the study of the logistics potential composition of the foreign economic activity of a pharmaceutical company, which was carried out on the basis of content analysis and questionnaires. The third stage is an assessment of the logistics potential of the foreign economic activity of a pharmaceutical company. The fourth stage is devoted to determining the factors influencing it. In the fifth stage, the development of an optimal strategy for developing the logistics potential of foreign economic activity of the pharmaceutical enterprise was done.

3. Results

The analysis of publications on this range of issues indicates that there is little scientific work in the field. A number of issues remain under-researched, such as: theoretical substantiation of the essence of the logistics potential of a pharmaceutical enterprise (PhE), especially those that are subjects of foreign economic activity [3, 4]; lack of a unified systematic approach to the mechanism of its formation [5], operation and development, lack of applied development of organizational measures to optimize the management of logistics potential [6, 7].

In foreign publications, the potential is mostly regarded as a resource, but, for the most part, relative to a country or region (military, industrial, natural) [8, 9], only some [10, 11] understand the degree of power (hidden opportunities) in any respect. Domestic economic literature [12, 13], on the contrary, is more inclined to understand it as opportunities, available forces, reserves and means that can be used to achieve a specific goal of the enterprise [14, 15]. However, some scholars [16, 17] argue that the potential is more properly called the set necessary for the functioning and development of a system of different types of resources. Therefore, despite the diversity of views on the essence of the concept and based on the general features of each interpretation, we can agree that the potential, in any case, means the presence of someone (person, staff, enterprise, society) hidden to activities in certain areas that have not yet emerged [17, 18].

Home authors, considering the functional features and structure of logistic processes by subject characteristics (personnel logistics, logistics of materials and finished goods, energy and information flows), determine the logistical potential of the enterprise as the maximum productivity (functional capacity) of systemically integrated subdivisions, all subdivisions, in space and time movement of personnel, materials and finished products, energy and information flows [19, 20].

The place of the FEA logistics potential in the structure of the PhE logistics potential in a formalized form is reflected as follows:

$$\begin{cases} P_{lFEA} \equiv f\{P_{l-exp}; P_{l-imp}\}; \\ P_{lFEA} \equiv f\{A_{prod}; B_{warehouse}; C_{tr}; D_{purch}; l_{pal}; F_{inf}; H_{pers}; K_{serv}\}; \\ P_{lFEA} \supset P_{l-exp} \wedge P_{LFEA} \bigcap P_{l-exp} \because P_{l-imp} \supset A_B \wedge P_{l-imp} \neq A_B; \\ P_{l-imp} \supset B_{warehouse} \wedge P_{l-imp} \neq B_{warehouse}; \end{cases}$$

P_{lFEA} – many components of the logistics potential of foreign economic activity of a pharmaceutical company; $P_{l\text{-}exp}$ and $P_{l\text{-}imp}$ – a set of components of the logistics activity potential in the pharmaceutical export import sphere respectively; A_{prod}, $B_{warehouse}$, C_{tr}, D_{purch}, I_{sal}, F_{inf}, H_{pers}, K_{serv} – set of elements in the structure of the potential of logistics activity in the field of export and import of the pharmaceutical enterprise, respectively, which is represented by the potential of production, warehouse, transport, purchasing, sales, information, personnel and logistics services.

Foreign Trade Logistics Potential is composed of export and import logistics. The system of indicators for measuring the logistics potential of the FEA of a pharmaceutical enterprise is shown in **Fig. 1**. The scorecard includes indicators selected through content analysis and questionnaire surveys.

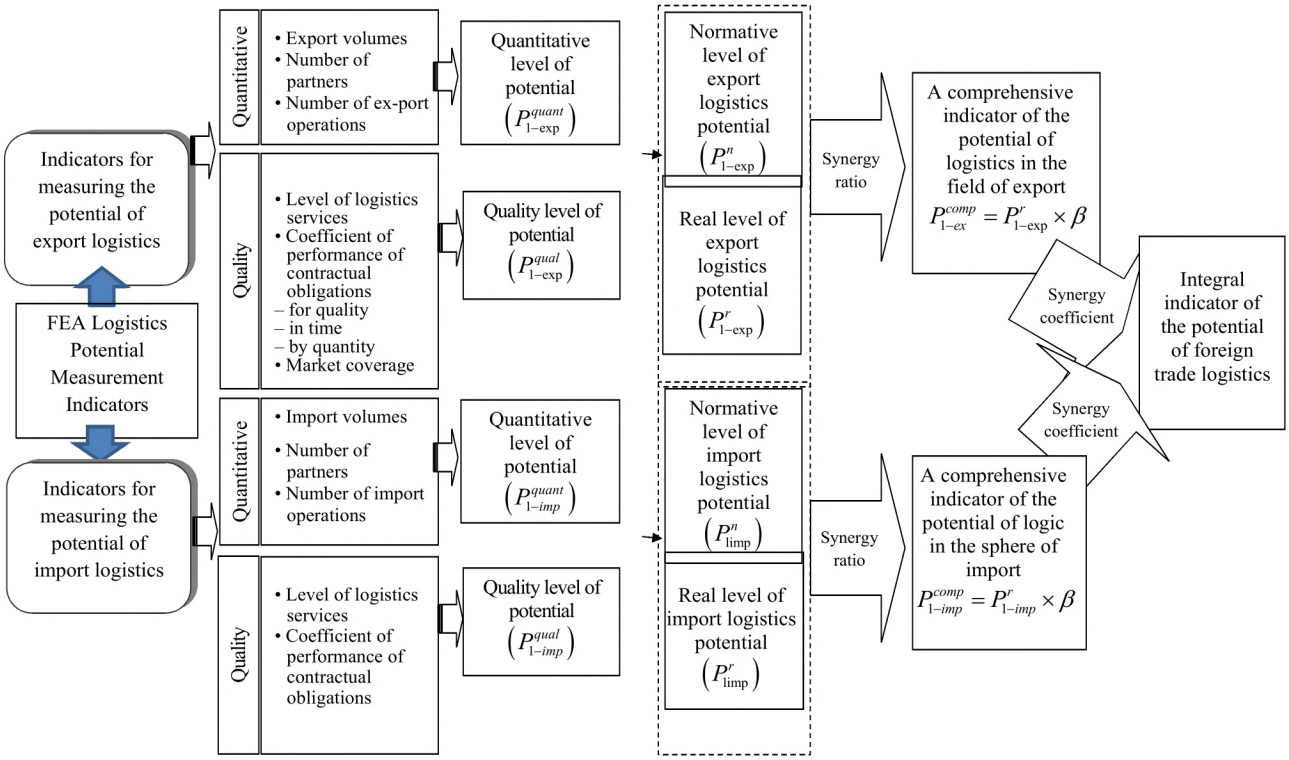

Fig. 1. System of indicators of measuring the potential of logistics of pharmaceutical enterprise FEA

Based on the calculated synergy coefficient, the enterprise monitors potential development in order to quickly respond to negative trends. The value of the synergy coefficient is in the range from $0<\beta<1$. If the estimated $\beta>1$, then the enterprise for a certain time has become more efficient in managing its potential. If $0.8<\beta<1$, then it is necessary to take measures to improve logistics. If $0.79<\beta$, then it is necessary to take strict measures for the efficient functioning of logistics and the enterprise as a whole.

The norm is the best level of PhE logistics potential for a certain period of time.

The dynamics of the logistics indicators of foreign economic activity of the studied PhE are shown in **Fig. 2**.

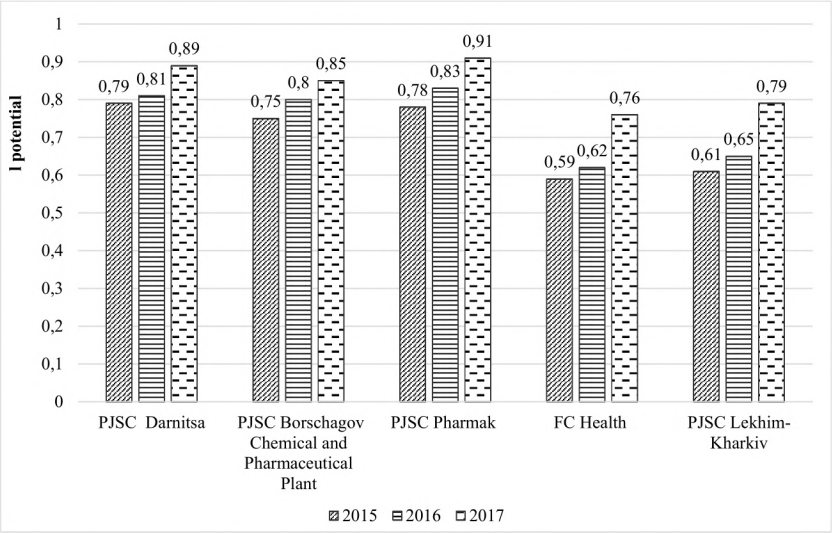

Fig. 2. Dynamics of integral index of logistics of FEA of the investigated PhEs

4. Discussion

Taking into account the theoretical positions and views of scientists, we consider it appropriate to use the definition of the logistics potential of a pharmaceutical enterprise subject to foreign economic activity, according to which it is a dynamically balanced system of resources and competencies, the effective use of which is based on the implementation of the optimization and integration properties of logistics (coordination of activities of functional units in organizing the movement of material, financial, information on resources per hundred actions of the logistic chain), determines the possibility of effective activity of the enterprise in the foreign market to ensure its development.

The proposed approach differs from the others [4, 20] by the complexity of the approach to the spheres of logistic activity of the enterprise, secondly, in the works [9] only quantitative indicators are taken into account.

As noted above, to date, insufficient attention has been paid to the problem under study. The vast majority of scientists investigate the level of logistic potential of the pharmaceutical enterprise as a whole, others have studied the potential of foreign economic activity of enterprises without taking into account the specificity of the pharmaceutical enterprise, its social orientation and the features of storage and transportation of finished medicines.

The proposed methodology differs from the existing ones and the set of indicators used to assess the level of potential. The obtained results allow us to conclude that the logistics of foreign economic activity of the studied domestic PhEs is quite different, since the value of integral indicators ranges from 0.59 to 0.91.

Scale of assessment of the status of FEA logistics by the magnitude of the integral indicator has the following ranges: from 0 to 0.25 – unsatisfactory level of logistics of FEA; from 0.26 to 0.50 – average level of foreign trade logistics*)*; from 0.51 to 0.75 – the level of foreign trade logistics is above average; from 0.76 to 1 – high level of FEA logistics.

Based on the calculations, the state logistics FEA any of the studied pharmaceutical enterprises can be described as high. Positive trend has all investigated pharmaceutical enterprises, rather high indicators are characteristic for PJSC “Pharmak”, PJSC “Darnitsa” and PJSC NPC “Borschagovsky HFZ”.

Therefore, we can conclude that the need to take measures to improve the level of logistics FEA at domestic pharmaceutical enterprises logistic potential, like all types of potentials inherent in AF, is formed under the influence of a large number of factors that exert both positive and negative effects, as well as constantly changing, which include not only the internal environment (the size of the pharmaceutical enterprises, the range of drugs manufactured enterprises, infrastructure

development, etc.) and macroenvironment (demand, entry into the pharmaceutical market, competitors, etc.).

5. Conclusions

1. The definition of "logistics potential of foreign trade activity of pharmaceutical enterprise" is defined, under which it is expedient to understand a dynamically balanced system of resources and competencies, the effective use of which is based on the realization of optimization and integration properties of logistics (coordination of activity of functional units during the organization of movement of material, financial resources, by stages of the logistics chain), determines the possibility of effective activity of the enterprise in the foreign market to ensure its development.

2. A system of indicators has been developed to determine the level of development of logistics potential of foreign economic activity of a pharmaceutical enterprise. Thus, the logistics potential of a foreign trade activity of a pharmaceutical enterprise consists of the logistics potential in the sphere of export and the potential of logistics in the sphere of import. Quantitative indicators for measuring the potential of logistics in the export field include export volumes, number of partners and number of export operations; quality indicators include the level of logistical services, the rate of fulfilment of contractual obligations (in terms of quality, time, quantity) and the breadth of coverage of the markets for pharmaceutical products. Quantitative indicators for measuring the potential of import logistics include import volumes, number of partners, and number of export operations; to quality indicators – the level of logistic service, the rate of fulfilment of contractual obligations (in quality, time, quantity).

3. A system of indicators has been developed to determine the proposed methodology for evaluating the logistics potential of a foreign trade activity of a pharmaceutical enterprise, which takes into account the normative and real level of its development and the synergy coefficient.

4. The scale of estimation of the state of logistics of FEA by the magnitude of the integral index is offered, which allows to estimate the level of development and to develop directions of improvement on its basis.

Conflict of interest

No conflict of interest.

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ANALYSIS OF THE CREATION OF A MODERN PHARMACEUTICAL SUPPORT SYSTEM IN UKRAINE IN A RETROSPECTIVE DEVELOPMENT OF THE STATE AND CIVIL SOCIETY RELATIONS

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Abstract

The effectiveness of the system of pharmaceutical supply to the population depends on the proper interaction of the state and market mechanisms for regulating the interests of all subjects of the pharmaceutical market for the implementation of the social policy of the state regarding the availability of quality and effective medicines for the population. In the context of healthcare reform, the issues of assessing the main stages of the development of the pharmaceutical supply system of the population become especially relevant.

Aim. Determination of key, from organizational, economic and social points of view, stages of formation of the domestic system of pharmaceutical supply of the population, analysis of the main tendencies of its development in retrospect years.

Materials and methods. The materials of the research were selected by the regulatory acts, the data of the specialized literature, certain aspects of activity of subjects of the pharmaceutical market, etc. Methods such as historical, informational-analytical, analytical-comparative, systemic, logical, hypothetical-deductive and generalization were used.

Results. Based on the conducted research, five stages of the formation of the pharmaceutical supply system have been identified and outlined, which differ fundamentally across the range of criteria for analyzing effectiveness in building relationships between the state, civil society and the professional community. These stages were elaborated in accordance with the results of the analysis of the nature of relations between the authorities and society under the conditions of gradual transition from paternalistic to the elements of the patient-oriented model of medical and pharmaceutical services to the population:

1) 1990–1993;
2) 1994–2000;
3) 2001–2007;
4) 2008–2013;
5) from 2014 to the present.

It has been proved that the stages of development of the domestic pharmaceutical supply system depend to a large extent on the nature of the influence of external environmental factors: changes of political elites in the country, global and internal financial crises, changes of priorities of the state development, breaking of social consciousness of the population against the background of political crisis, etc. It should be noted that the most important in terms of positive, first of all socio-economic, development characteristics is the fourth stage of formation (2008-2013) of the domestic system of pharmaceutical supply of the population. The last period (since 2014) is characterized by the existence of a crisis of relations between all subjects in the system of pharmaceutical provision of the population against the background of systemic transformations in the state, strengthening of the role of public professional associations and critical raising of public expectations from the state regarding the organization of providing affordable medical care to the population. and pharmaceutical assistance in the face of mass impoverishment.

Conclusion. The basic stages of the formation of the domestic pharmaceutical supply system are defined and their characteristics are envisaged, which allow to form in the future fundamentally new approaches to building rational, economically speaking, and simultaneously socially responsible relations between different subjects in the pharmaceutical market.

Keywords: pharmaceutical supply system, health care, state regulation of pharmaceutical activity, pharmacy establishments, civil society.

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1. Introduction

The peculiarity of the functioning of the pharmaceutical supply system in any country is due to several factors, among which the following deserve attention. First, the regulated circulation of medicines (drugs) in the pharmaceutical market (FM) emerges as an important socially important mechanism, the effective functioning of which enables the exercise of the constitutional right of citizens to preserve the fundamental value in society, namely health and human life. Second, FM is a consumer market sector in which market relations between different entities operate. Considering the aforementioned, the effective functioning of the system of pharmaceutical supply to the population depends on the proper interaction of the state and market mechanisms of regulating the interests of all subjects of the FM for the implementation of the social policy of the state regarding the availability of quality and effective drugs for the population. Thus, the effective functioning of the FM must ensure that the social and economic interests of all its subjects are balanced. The nature of the interaction between the categories of public health and the indicators of the level of solvency of citizens depends on the general structure of expenditures on pharmaceutical supply of the population, which, in the final analysis, supply and demand in the market of medicines, the corresponding structure of FM, sales volumes, assortment of goods, etc.

Despite the market nature of the functioning of various economic entities, the FM is increasingly focusing on increasing the social responsibility of the pharmaceutical business to society, above all, providing the population with quality, effective and affordable drugs. A special place in building this type of relationship in the pharmaceutical supply system belongs to public professional organizations and unions. The most effective functioning of the pharmaceutical community and professional self-government, which is based on public organizations, associations, unions, in cooperation with state structures, should ensure the formation of socially responsible relations in the modern FM in Ukraine. Currently, the country's health care system is at a turning point. The first stages of public relations pharmaceutical reform, which began in 2017, demonstrated the depth of those socio-economic problems that have plagued the population for many years. Given the high level of public expectations for improving the availability of medical and pharmaceutical care, the question of changing the formats of relationships between entities in the FR and in the health care system is emerging as socially significant. Against this background, the analysis of the stages of formation of the domestic pharmaceutical supply system in order to identify the directions of its effective development is relevant and of social importance.

Problems of formation of the system of pharmaceutical supply of the population in terms of relations between the state and civil society have been repeatedly researched by scientists. Thus, the priorities for reforming the system of state and social regulation in pharmacy were studied by Nemchenko A. S., Susharina I. V., Khomenko V. M. [1]; questions of introduction of concept of social responsibility in practical pharmacy, approaches to development of social responsibility during professional training – Gromovik B. P., Tkachenko N. O. [2]; the main stages of the formation of social pharmacy in the world and in Ukraine since the 50's of the twentieth century – Kotvitska A. A., Kubareva I. V. [3]; expediency of participation of public pharmaceutical organizations in standardization of processes of pharmaceutical supply of population – Unguryan L. M. [4]; questions of social responsibility of pharmaceutical business and directions of its regulation in accordance with international standards – Bratishko Yu. S. [5]. Also, the work of foreign authors has explored the issue of strengthening the role of pharmaceutical workers and public health services, which requires changes in the behavior of both professionals and society – Eades C. E., Ferguson J. S., O'Carroll R. E. [6]; pharmacists' experience in healthcare teams, providing patient care systems – Bush P. W., Daniels R. [7]; pharmacists' involvement in optimal drug use, pharmacotherapy management, which reduces overall health care costs – Blouin R. A., Adams M. L. [8].

Aim – identification of key, from organizational, economic and social points of view, stages of formation of the domestic system of pharmaceutical supply of the population, analysis of the main tendencies of its development in retrospect years.

2. Materials and methods

The subject of the research was the historical processes of development of the national health care system and pharmaceutical supply of the population in the context of state formation and formation of civil society, and the objects – mechanisms of state regulation of FM of Ukraine and market development of relations between different entities in the process of providing the population with medical and pharmaceutical assistance, basic approaches in the organization of pharmaceutical activity at different stages of formation of the national health care system. The materials of the study selected regulatory legal acts (RLAs) regulating the organization of provision of medical and pharmaceutical services to the population, data of special literature, which outlines the main directions of development of the pharmaceutical supply system of the population, certain aspects of the activity of subjects of FM and indicators of its development in the dynamics years (volume of retail sales and hospital supplies of drugs in monetary and physical terms, level of provision by pharmacy establishments). In theoretical studies, we have used such methods as historical (study of stages of development in chronological sequence), information-analytical (detection of common and different between different stages of development), analytical-comparative (search for properties of certain objects in comparison with others), systemic (establishing links between the elements of the system under study), logical (establishing logical patterns of development), hypothetical-deductive (hypothesis and deduction by deduction) and generalization (results of the study are summarized).

The system of pharmaceutical supply to the population in the classical definition appears as an integral structure, characterized not only by the complexity of the construction, but also by close links with the environment [1, 9]. Therefore, a retrospective analysis of the development of the said macrostructure of the country was considered by us, above all, in the context of the formation of the Ukrainian state (declaration of independence of Ukraine in 1990), the development of civil society and changes in the worldviews of people regarding the attitude to "health" as the highest value in the humanistic oriented states.

3. Results of the research

Summarizing the results of the theoretical studies, we have identified the following stages of development of the system of pharmaceutical supply of the population, outlined the main trends and problems. So, in the early 1990s, when Ukraine gained independence, the issue of providing drugs to the population was acute. From the planned economy under the Law "On Enterprises in Ukraine" of 26.03.1991, the country embarked on the path of its liberalization and introduction of market relations in almost all sectors of the economy. In 1992, the decline in our own production of drugs was maximum, during this period, Ukraine received $ 280 million worth of drugs at wholesale prices, but it was three times less than during the Soviet Union (USSR) period. However, as of January 1, 1992, there were 6,413 pharmacies operating in the system of the Ministry of Health of Ukraine, most of them located in cities and towns. The population served by one pharmacy in average was 7.9 thousand people, which was significantly higher than in Europe [10].

The need for market transformation for all parts of the macroeconomic complex of the state, including FM, became relevant, given the regulatory influence of market relations on establishing the necessary proportions between the volume of needs of society in certain goods and their production. Under the conditions of transition to a market economy in accordance with the Laws of Ukraine "On privatization of state property", "On privatization of small state-owned enterprises (small privatization)", adopted in 1992, processes of privatization of pharmaceutical enterprises, demonopolization, creation of alternative structures were observed, including non-state ownership, even in socially-oriented industries. The spontaneous, irrational opening of pharmacies has begun with the simultaneous catastrophic reduction of their number in rural areas due to the unprofitable situation for owners, the creation of associations and pharmacy chains: "Arnica", "Falby", "Med-Service", "Zi" and others. (1993–1995). There are also the first powerful distribution companies – "Alba" (1993), "BaDM" (1994), "Optima Farm" (1994), "BBC-LTD" (1994), "Venta LTD" (1995).

According to the theory of market relations, social protection of the person in the initial stages of the formation of relations between capital and labor resources is not a dominant in society.

Thus, it was during this period that the centralized system of providing the population with drugs, which had existed in the former USSR for several decades, was completely destroyed in Ukraine. The state has withdrawn from the fulfilment of the financial obligations declared in the Constitution of Ukraine regarding the protection of public health. It is at this time that the shadow market, the production and circulation of counterfeit drugs, develops, and aggressively advertises biologically active additives of unknown production. Domestic pharmaceutical manufacturers are on the verge of bankruptcy. This stage of market relations is characterized by low level of development of pharmaceutical industry enterprises, low physical and socio-economic availability of drugs for the population, as well as inefficiency of functioning of state mechanisms of regulation of quality of medicines at all stages of their promotion through the distribution network, from import into the country to sale to the end consumer pharmacies. Analyzing the data of the specialized literature, it can be argued that the government at that time did not realize that one of the guarantors of Ukraine's sovereignty was the effective pharmaceutical supply of the population [10, 11]. However, despite the systemic crisis in the state, in 1992 the "Comprehensive Program for the Development of the Medical, Veterinary and Microbiological Industry, Improving the Supply of the Population and Livestock with Medicines, Medical and Veterinary Equipment for 1992–1996" was developed and adopted for construction of new and reconstruction of existing enterprises, expansion of the range of production of drugs and medical devices. Positive during this period (1992) was also the establishment of important institutions, which were subordinate to the Ministry of Health (MOH) of Ukraine and had control functions in the sphere of drug trafficking, in particular, the State Inspectorate for Quality Control of Drugs, the Pharmacological Committee, Pharmacopoeial Committee, the successors of which still play an important role in the regulation of pharmaceutical activity in Ukraine. In 1993, significant changes occurred in the structure of pharmacy management. Thus, the State Committee of Ukraine for Medical and Microbiological Industry (SCUMMI) and the Ukrainian Association "Ukrpharmacy" were created. Already from this period, it can be argued about the gradual revival of the national health care system and pharmaceutical supply of the population as an important component of the country's macroeconomic complex, the effective functioning of which creates favourable conditions for social stability and well-being of the population. Thus, **the first stage of development** lasted **from 1990 to 1993**.

The second stage of development begins **in 1994**, when the Ukrainian authorities signed the Partnership and Cooperation Agreement between Ukraine and the European Community and their Member States and declared their desire for European values and standards, but the processes of divestiture of pharmacies did not contribute to the stated purpose. According to experts, at that time, there were no private drugstore chains in any European Union (EU) country that actually forced the society into its own understanding of the drug supply process in accordance with its commercial interests. It was at the second stage of the development of the domestic pharmaceutical supply system that an unmistakably significant event took place, namely in 1996 the Law of Ukraine "On Medicines" was voted, according to which it was envisaged to regulate by-laws all issues related to drug trafficking in Ukraine. During this period, qualitative structural changes took place on the domestic FM. Thus, in the use of drugs, the share of domestic drugs began to increase gradually, but for the vast majority of the population, medicines remained almost inaccessible. Therefore, at the state level, the question arises of the development and implementation of effective mechanisms to ensure the physical and socio-economic availability of medicines for the general population. At that time, part of the physical shortage of drugs was satisfied with the efforts of domestic manufacturers, although the level of socio-economic availability of drugs remained very low. Thus, gradually FM drugs began to be saturated, in the vast majority due to drugs from domestic production and generics of Indian and Eastern European production. By the end of 1997, drugs were manufactured in Ukraine 60 % more than in 1992. In 1998, drugs were imported into Ukraine from almost 50 countries [10]. The logical consequence of the development of the domestic system of pharmaceutical supply to the population and the gradual transition to the implementation of European norms and requirements in the pharmaceutical sector of the economy was the large-scale development and implementation of a series of steps, regulating drug trafficking in Ukraine.

At this stage, the idea of reforming the pharmaceutical health care industry was to create a system of providing the population with affordable and quality drugs, as recommended by the World Health Organization (WHO) "Focus on the Patient" (1998) [4]. This was an important qualitative breakthrough in the state's vision for the development of the national health care system.

In the context of the economic crisis of September 1998, the fall of the national currency, the growth of competition in the pharmaceutical sector, the decrease in the level of solvency of the population, in Ukraine there was a tendency to increase the level of wholesale and retail prices for drugs. However, due to the lack of a consistent national drug policy under the slogan of reforming the pharmaceutical sector within the Ministry of Health of Ukraine, organizational structures were created, with provisions for activities declaring comprehensive functions that could not be put into practice in Ukrainian realities (1999-2000 – National agency on quality and safety control of food, medicines and medical products). This structure was to combine: production control (until now State Committee on Biomedicine Industry), control in the field of drug development, registration and use (Pharmacological Committee and Committee on Immunobiological Preparations), control in the field of implementation (State Inspectorate for Quality Control of drugs). That was an attempt to build a centralized model for regulating drug trafficking in Ukraine. However, this state structure did not exist for 2 years. By decree of the President of Ukraine dated July 24, 2000, No. 918/2000, the activity of the said body was cancelled. Following the elimination of the above-mentioned National Agency in 2000, a State Department for Quality Control, Safety and Production of Drugs and Medical Devices was formed within the Ministry of Health, despite its functioning in the country of the State Inspectorate for Quality Control of Drugs. Thus, at this stage, a foundation was laid for the operation of an expanded (multiple) model of drug trafficking on FM.

The third stage (2001–2007) of the development of the pharmaceutical supply system begins in 2001, when the government took steps to ensure the physical and socio-economic availability of drugs to the public. First, the Verkhovna Rada of Ukraine adopted Resolution No. 2564-III "On Information of the Cabinet of Ministers of Ukraine on Implementation of the Policy of State Regulation of Drugs and Medical Devices", which defined a program of measures to increase the availability of drugs and medical devices for the population, rational use of funds for the purchase of drugs, the development of a national list of essential (vital) drugs and medical products, and the introduction of a formulated system of medical supply for the population. At the end of 2001, a number of important RLAs were adopted that regulate pharmaceutical activity in the country as a whole and the circulation of drugs in particular:

– Resolution of the Cabinet of Ministers of Ukraine (CMU) No. 1482 "On Approving the National List of Essential (Essential) Drugs and Medical Devices";

– Order of the Ministry of Health of Ukraine No. 479 "On Amendments to the List of Medicines of Domestic and Foreign Production that may be Purchased by Health and Medical Institutions Fully or Partially Funded from the State and Local Budgets";

– Order of the Ministry of Health of Ukraine and the Ministry of Economy and European Integration of Ukraine No. 480/294 "On Approval of the List of Domestic and Imported Medicines and Medical Devices, Prices for which are Subject to State Regulation".

With the increase of financial stability and growth of the country's gross domestic product, the volume of domestic FM significantly increased during this period. Thus, as of January 1, 2003, 6620 registered drugs were registered in Ukraine, covering almost all pharmacotherapeutic groups, of which 40 % were of domestic production. A large-scale process of step-by-step implementation of GMP requirements at such powerful enterprises as "Styrol", "Darnitsa", "Farmak", "Borschagovsky HFZ", "Indar" and others has begun to increase the quality requirements of drugs. On the whole, the macroeconomic situation in the Ukrainian FM improved significantly during this period, with an average annual growth rate of 21 %. Due to the increase in real incomes, the consumption of drugs increases – from $ 23 to $ 48 per person. However, these indicators are much lower than the existing indicators in the EU countries at the time (Czech Republic – $ 331, Slovakia – $ 254, Poland – $ 154).

An important step towards improving physical and socio-economic accessibility was the approval in 2006 of CMU Resolution No. 400 of the "Second Edition of the National List of Ba-

sic Drugs and Medical Devices", which already contained 783 international non-proprietary or accepted names of active substances [12, 13]. Adopted at the end of 2007, "The Concept for the Development of the Pharmaceutical Sector of Healthcare in Ukraine" defined the task of developing a national policy for setting social priorities in providing the population with drugs, as well as implementing international standards (production, clinical, laboratory, distribution, pharmacy and other good practices) as the most important components in shaping a modern model of regulation of drug trafficking on FM.

With the onset of the global financial and economic crisis (2008), **the fourth stage (2008–2013)** of the development of the pharmaceutical supply system for the population began, which Ukraine has done quite successfully, building a strong structure within the country's macroeconomic complex. It should be noted that the beginning of this stage of development of the pharmaceutical supply system was characterized by the emergence of negative trends in FM, primarily due to its import dependence. Against the background of the gradual rise in drug prices, the Government of Ukraine has made it possible to remedy the situation by introducing a number of measures aimed at curbing purchase, wholesale, and retail prices. The process of improving the functioning of the state system of quality control of medicines also continued. In the event of worsening of the crisis phenomena on FM, the signing of the Memorandum on joining, accepting, amending and reviewing complaints within the framework of Rules for proper promotion by pharmaceutical companies to drug professionals by the representatives of 35 foreign and domestic pharmaceutical companies of the Ministry of Health of Ukraine looks positive in the summer of 2008. From this period it can be argued that Ukraine has laid the foundations for the implementation of important components in building a socially oriented model of the pharmaceutical business [14, 15]. Another important document that outlined the scale and irreversibility of changes in this direction was the Code of Ethics for Pharmaceutical Workers of Ukraine, which was adopted in September 2010 at the VII National Congress of Pharmacists of Ukraine [16]. This document for the first time in the history of domestic pharmacy declared a professionally and socially oriented model of behavior of pharmacy professionals in society in accordance with the modern paradigm of transformation of pharmaceutical assistance and services in the direction of increasing the social responsibility of pharmacies.

Consistency in the implementation of state policy in the system of pharmaceutical supply to the population was demonstrated by the fact that in 2010 by the order of the Ministry of Health of Ukraine No. 769, the "Concept of development of the pharmaceutical sector of health care of Ukraine for 2011–2020" was adopted, which was developed on behalf of the CMU in order to ensure the availability of high quality drugs, medical devices and medical equipment for the population [17]. improving the physical and socio-economic availability of drugs with proven efficacy, safety and quality through the use of drugs in accordance with drug forms, protocols and treatment standards, the introduction of state pricing regulations, and international standards for quality assurance of products and medical and pharmaceutical services. It should be noted that despite the import dependence of domestic FM and the consequences of the financial crisis in 2010–2012 in Ukraine, the physical availability of drugs has gradually increased. From a socio-economic point of view, the fact is that the domestic industry is showing a steady tendency for overproduction, which contributed to the gradual development of drug exports, especially to the countries of the former USSR. In order to solve the problem of low socio-economic availability of drugs, one of the perspective directions for the development of the system of pharmaceutical supply to the population is the introduction by the Government of the country of introduction of mechanisms for reimbursement of expenditures for drugs, a formulary system at all levels of provision of medical and pharmaceutical assistance and a model of compulsory state medical insurance [18].

Due to the transition of many quantitative positive changes to qualitative ones in 2011, Ukraine, represented by the State Drugs Service of Ukraine, became a full member of the International Pharmaceutical Inspection Co-operation Scheme (PIC/S). It should be noted that today from the list of countries of the former USSR, except for the Baltic states, which are members of the European Union, only Ukraine is part of the mentioned influential international organization.

For the first time on domestic FM, in order to increase the economic availability of drugs, in April 2012 the Cabinet of Ministers adopted Resolution No. 340 "On the implementation of a pilot project on the introduction of state regulation of the prices of drugs for the treatment of persons with hypertension", which introduced the state regulation of the prices for drugs for that group. patients by setting the limit of wholesale prices for such drugs and working out a mechanism for partial reimbursement of their value. The implementation of this document was aimed at increasing the availability of drugs for low-income and socially unprotected patients with hypertension.

As a result of the Ukrainian government's earlier stated intention to implement a set of international good practice standards, primarily GMP, the number of licensees decreased from 151 to 111, respectively, in the period 2009-2013. At the same time, against the background of a decrease in the number of domestic producers, the volume of industrial production of products in monetary terms increased by almost 117 % and exports – by 118 %. It should be noted that the share (%) of drugs produced by domestic production in total volume on domestic FM increased from 25.3 % in 2009 to 35.5 % in 2013 [19, 20]. Systematizing the results of the study, it can be argued that 2013 is one of the most successful periods in the development of Ukraine's pharmaceutical sector.

With the advent of the political, financial and socio-economic crisis in 2014, **the fifth stage** of the development of the pharmaceutical supply system for the population, which has been significantly influenced by external factors, begins. In 2014, the country experienced dramatic changes in virtually all areas of public relations. The systemic crisis in the country, the loss of territories due to the start of hostilities in the east of the country and the annexation of the Autonomous Republic of Crimea have led to significant structural changes in the system of pharmaceutical supply to the population. In 2014, as a result of fundamental changes in the exchange rate policy of the National Bank of Ukraine and as a result of the uncontrolled devaluation of the hryvnia (from February to August, more than 70 %), there was a significant decrease in the consumption of drugs and medical devices. This period was characterized by a decrease in sales volumes, both in US dollars and in kind. In 2015, the lowest FM drug volume was recorded in US dollars (US $ 2.28 billion) (**Fig. 1**). In terms of in-kind drug sales, there was also a decline from 1.4 billion packs in 2012 to a minimum in 2015 (1.1 billion packs) and a gradual increase further to 1.3 in 2018 [21].

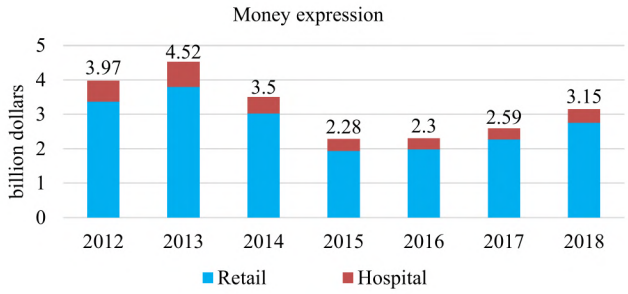

Fig. 1. Dynamics of retail sales and hospital supply of drugs in monetary terms, indicating the share of each of the segments

The increase (38.0 %) of hryvnia sales, which was observed in 2015, was due to the devaluation of the national currency. Amid the financial crisis, weakening of the executive branch, lack of a systematic approach to managing the industry, there was a sharp rise in drug prices and a sharp decline in drug consumption. It became very difficult to control the situation on FM, so the government introduced mechanisms of direct government regulation over a wide range of pharmaceutical activities, restrictions, differentiation of retail allowances, the declaration of wholesale and selling prices for drugs purchased for budgetary funds, the introduction of a 7 % value added tax on drugs (April 2014), changing the rules for centralized drug procurement [22]. Under these conditions, the need for deregulation of the FM retail chain has become increasingly discussed in the pharmaceutical community.

It is well known that increasing regulatory pressure on FM subjects always automatically entails additional costs for businesses, with the direct consequence of reducing the socio-eco-

nomic and further physical availability of drugs [23–25]. Significant structural shifts in drug use have occurred during the fifth period of the pharmaceutical supply system's development. Thus, with the gradual stabilization of the economic situation (2015–2016) in the country, Ukrainian consumers have increasingly preferred foreign-made drugs. An important event in the development of the national system of pharmaceutical supply to the population was the implementation of the government program "Available medicines" for persons suffering from cardiovascular diseases, type II diabetes, bronchial asthma since April 2017. The Affordable Care program was implemented against the background of reforming the entire healthcare system and implementing e-Health.

An important place in the development of the system of pharmaceutical supply to the population in the direction of implementation of modern norms and requirements was taken by the Cabinet of Ministers of Ukraine №1022 of December 5, 2018 "State strategy for the implementation of the state policy of providing the population with medicines for the period up to 2025" [17]. The document outlined policy commitments to ensure the availability and rational use of effective and safe drugs. First of all, it concerned the socio-economic availability of drugs, as physical availability was provided by a sufficient number of pharmacies in Ukraine. For example, as of January 1, 2019, 1 pharmacy serves an average of 2,044 citizens (**Fig. 2**) [21].

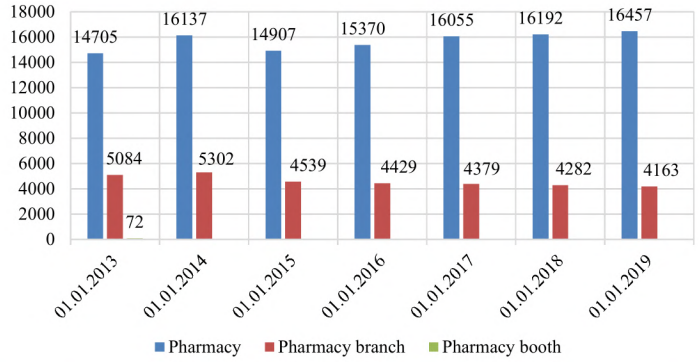

Fig. 2. Dynamics of the number of different types of shopping unit on FM Ukraine

Systematizing the assets of the fifth stage of development of the domestic system of pharmaceutical supply to the population, it is necessary to determine the following characteristics:

– introduction of e-Health elements in the organization of providing medical and pharmaceutical assistance to patients, in particular servicing the population by electronic prescriptions at the primary health care unit;

– the adoption in 2017 of a new National List of Essential Drugs (427 INN), which occupies a dominant position in the FM regulatory space and complies with WHO requirements (20thWHOEML (March 2017);

– the operation of the Government's Affordable Care Program;

– changes in the order of centralized procurement of drugs used to resolve socially significant issues through "ProZorro" and international agencies;

– consolidating the efforts of the professional community to enhance the influence of professional associations on the processes of organizing the provision of pharmaceutical assistance to the population and regulating pharmaceutical activity in the country;

– increasing the level of social responsibility of the pharmaceutical business and the formation of an updated model of capital-society relations on FM;

– implementation of European rules on the registration of drugs (simplified registration and mutual recognition procedure).

Thus, it can be argued that, despite the complexity of development, the domestic pharmaceutical supply system has shown, from one point of view, a significant dependence on external environmental factors, and on the other, significant development potential and the ability to respond quickly to numerous changes in factors of influence.

4. Discussion of the results

On the basis of generalization of the processed material, we have identified the main five stages of the formation of the system of pharmaceutical supply of the population in terms of the formation of relationships between authorities and society from paternalistic to the emergence of elements of the patient-oriented model:

– 1990–1993 – chaotic development of FM and gradual emergence of market relations in the organization of provision of pharmaceutical services to the population, low level of efficiency of state regulation of pharmaceutical activity in its various aspects and directions;

– 1994–2000 – laying the legal, organizational, economic and administrative bases for the construction of a European-oriented model of pharmaceutical activity, such as an expanded (multiple) model of regulation of drug trafficking on FM;

– 2001–2007 – further development of a European-oriented model of pharmaceutical activity (gradual implementation of a set of good practices, first of all production) and an expanded (multiple) model of regulation of drug trafficking on FM against the background of gradual strengthening of state regulation of pharmaceutical activity at all stages of the promotion of drugs network;

– 2008–2013 – the emergence and gradual development of socially oriented models of relations between different subjects of pharmaceutical relations, subject to the declaration by the state of European standards and requirements for the organization of accessible and effective medical and pharmaceutical care in accordance with the requirements of the WHO National Medicinal Policy;

– from 2014 to the present – crisis of relations between all subjects of relations in the system of pharmaceutical supply of the population against the background of systemic transformations in the state, strengthening of the role of public professional associations and critical raising of public expectations from the state regarding the organization of providing affordable medical care to the population and pharmaceutical assistance in the face of mass impoverishment.

The identified main stages of the formation of the pharmaceutical supply system in Ukraine and their characteristics allow to form in the future fundamentally new approaches to building rational, economically speaking, and at the same time socially responsible relations between different subjects on FM.

5. Conclusions

1. Based on our research, we have identified and outlined five stages in the development of the pharmaceutical supply system, which differ fundamentally across the spectrum of performance analysis criteria in building relationships between the state, civil society and the professional community.

2. It is proved that the stages of development of the domestic pharmaceutical supply system depend to a large extent on the nature of influence of external environmental factors: changes of political elites in the country, global and internal financial crises, changes of priorities of the state development, breaking of social consciousness of the population against the background of political crisis, etc.

3. It should be noted that the most important of the positive, first of all socio-economic, characteristics of development is the fourth stage of formation (2008–2013) of the domestic system of pharmaceutical supply of the population.

4. A promising direction for further research will be the development of theoretical and applied approaches to improving socially-oriented relations between different subjects of FM on the path from a paternalistic to a patient-oriented model of development of national health care in general and the system of pharmaceutical supply to the population in particular.

Conflict of interest

No conflict of interest.

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TOPICALITY OF IMPLEMENTATION OF MOBILE MEDICAL APPLICATIONS WITHIN THE FRAMEWORK OF ELECTRONIC HEALTH IN UKRAINE: THE EXPERIENCE OF LEADING COUNTRIES OF THE WORLD

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Abstract

Today, in the field of healthcare of the leading countries of the world there is a technological breakthrough due to the use of modern electronic systems. Thanks to the use of medical electronic technologies, it is possible to improve the quality of medical and pharmaceutical care for all segments of the population. The eHealth system provides opportunities for direct participation of patients in their health, which involves organization of a healthy lifestyle and disease prevention.

The aim of the study was to analyze the experience of implementation of mobile medical applications and electronic medical systems within eHealth in the healthcare systems of the leading countries of the world and Ukraine.

Materials and Methods. Ministry of Health of Ukraine, FDA, WHO, NHS, ABI Research and other open information databases and statistical agencies were used in the work. The analytical, comparative and generalization of scientific information methods were chosen for the research.

Results and Discussion. Modern reformation and further development of the healthcare system of Ukraine provides for the introduction of not only government programs of reimbursement, but also the introduction of electronic information systems, mobile applications for medical, pharmaceutical workers and patients at all stages of medical and pharmaceutical care. The integrated implementation of eHealth determines availability of the necessary healthcare management information system, which should have the following components: registers of medical institutions, services, doctors, patients, diagnosis coding system and electronic medical documentation (electronic medical records and prescriptions). Currently, 1472 medical institutions, 24506 doctors and 21068249 patients have joined eHealth in Ukraine.

Conclusions. It has been determined that information electronic technologies allow not only to reduce government spending on the healthcare, but also to achieve a reduction in the number of errors in prescriptions, visits to doctors without the necessary needs and decrease the total number of deaths. The introduction of electronic technologies, in particular mobile applications for the national healthcare system in Ukraine should be controlled by state authorities and independent international organizations on the control and development of information electronic systems.

Keywords: information technology, healthcare, electronic system.

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1. Introduction

The current reform of the national healthcare system (NHS) is aimed at Ukraine's aspiration to join the European community. One of the main features of each developed European country is

the appropriate level of NHS, which key features are the high level of medical and pharmaceutical services. It is known that modern trends in the development of NHS in the leading countries of the world are electronic technologies – mobile and electronic medical systems, especially electronic health system (eHealth), which rapid spread and use open up new and innovative means for improving the provision of medical and pharmaceutical care. In addition, thanks to modern electronic technologies, in particular online training, it is possible to have the increased access of the population in middle-and low-income countries to first aid training [1].

2. Aim of the research

According to the forecasts of the RAND Institute the large-scale distribution of eHealth will determine reduction of the healthcare costs by 142–371 billion USD each year [2]. Therefore, the **aim** of our study was to analyze the experience of implementation of mobile medical applications and electronic medical systems within eHealth in the healthcare systems of the leading countries of the world and Ukraine.

3. Materials and methods

Materials of the Ministry of Health of Ukraine, FDA, WHO, NHS, ABI Research and other open information databases and statistical agencies were used in the work [3–6]. The analytical, comparative and generalization of scientific information methods were chosen for the research.

4. Result

Low solvency of citizens in low- and middle-income countries leads to low availability of treatment of the most common diseases and deterioration of the epidemiological situation in these countries. Ukraine is not an exception; its current state of the healthcare development does not meet the needs of the majority of citizens. In this regard, NHS of Ukraine has taken a tack of development for the introduction of similar systems in the leading countries of the world, which governments are actively implementing the national programs to improve medical and pharmaceutical care and increase the availability of drugs for all segments of the population. For example, the "Available medicines" program was introduced by the Cabinet of Ministers of Ukraine in 2017, and with the support of Management Sciences for Health (MSH) from 1.09.2017 the country launched the project "Safe, available and effective medicines for the Ukrainians" (SAFEMed) [7, 8]. The aim of this project is to improve and optimize the functioning of the financing mechanisms of NHS. At the same time, the introduction of eHealth into NHS of Ukraine is hampered by limited budget funding and the absence of the rational development concept. In addition, the issue of the introduction of electronic information technologies in the healthcare, in particular mobile applications for use by medical and pharmaceutical specialists, as well as patients, remains unsolved, and taking into account the foreign experience such mobile applications have a huge demand [1]. It should be noted that eHealth involves the use of mobile devices in the process of collecting medical information about patients and transferring it to doctors.

In connection with the above, the study of the foreign experience in application of separate elements of eHealth on the example of some developed countries is rather relevant for NHS of Ukraine.

According to the estimates of the Commonwealth of Nations NHS fund, the United Kingdom now has the most effective NHS due to large-scale and effective use of information technologies in this area. Under the UK law, general practitioners (family doctors) are allowed to send information messages regarding drug prescriptions from the hospital post office to the pharmacy. This has led to the fact that the process of receipt of an electronic prescription in pharmacies has become more effective and convenient for both patients and pharmacists. In addition, the results of studies by experts of Commonwealth of Nations showed that in the UK, USA and Switzerland the number of errors in electronic prescriptions decreased by 60 %. At the same time, the introduction of information mobile systems in these countries reduced the overall mortality rate by 45 %, from emergency events by 20 %, the number of visits to medical professionals by subject-related reasons decreased by 14 %, tariff costs decreased by 8 %, more than 50 % of the population began to use Internet resources to communicate with the doctor [9, 10].

As it was mentioned, recently mobile applications in medicine, involving the use of mobile devices in the process of collecting medical information about patients and transferring it to doctors, as well as monitoring (in real time) the vital functions of the patient's body and direct remote aid through special mobile devices, are in great demand.

According to the data of ABI Research analytical company, it has been found that by 2019 the number of users of medical mobile applications will increase to 500 million people. Moreover, the volume of the market of mobile applications in medicine in 2017 increased almost 11 times compared to 2013. It should be noted that the first place by the number of downloads are applications with reference books of medicines (23.7 %), the second place is occupied by applications with physical exercises (20.2 %), the third one – applications that help to track the health status (12.9 %), and the fourth place – applications for disease prevention (10.1 %) [9]. According to the WHO data various types of services are implemented within the framework of mobile medicine, the most common of them are shown in **Fig. 1**.

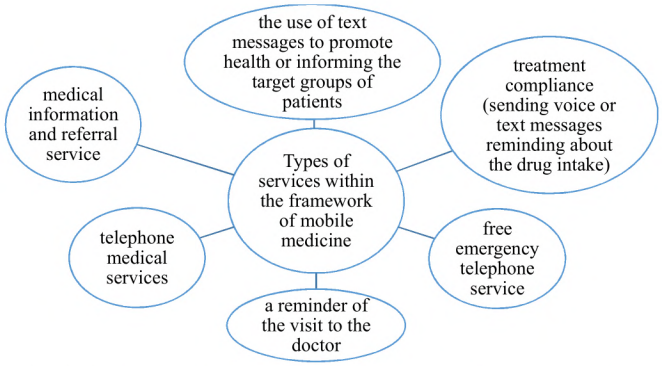

Fig. 1. The most common types of services within the framework of mobile medicine (the WHO data)

Therefore, mobile medicine works to raise awareness of the population, for example concerning topical issues of healthcare. It should be noted that according to the KPCB forecasts the world market of mobile medical applications would reach 102.43 billion USD by 2022. Today, the largest market of medical mobile applications is the United States. Potential markets of mobile applications in healthcare are projected to grow at maximum speed over the next five years. This concerns the Asia-Pacific region, Latin America and Europe [11–15].

The characteristics of the ranking of the top 15 medical mobile applications in popularity having a direct connection with the existing NHS are presented in **Table 1**. According to this **Table 1**, it has been found that 73 % of mobile applications are partially paid and 13 % are fully paid and free, respectively. Conventionally, all applications that are listed in the **Table 1** can be divided into applications for doctors; patient applications; doctor and patient collaboration applications. A brief description of the applications is given below.

Applications such as Medscape, Epocrates, Read by QxMD, UpToDate, Isabel, Dyna Med Mobile are similar to each other and represent an encyclopedia on medical information, they have the same interface and principle of operation [16–18]. These applications contain information about various diseases and their treatment methods [19–21].

NEJM This Week and **Fig. 1** Medical Images mobile applications are with the same direction of work – they help doctors exchange medical images of the patient for consultation or diagnosis while maintaining confidentiality [22, 23].

Applications Virtual Practice for Doctors, My Chart, and iPharmacy designed specifically for doctor and patient collaboration. They could be used in case you do not get to the doctor or do not receive the necessary clarifications regarding medication [24, 25]. The uniqueness of the doctor-patient applications creates conditions remotely and always in touch, as well as more detailed personal consultation with the patient [26].

It is worth noting that such mobile applications as Cardio Smart, Calculate by QxMD, which are created specifically for the doctor to work with patients suffering from cardiovascular diseases. These diseases occupy a leading place in the world in the number of prevalence among the working-age population. Therefore, there is no doubt about the relevance of creating applications for this pathology. Applications 1 help both doctors and patients to understand what happens to the vessels and the heart in various types of pathologies of the cardiovascular system [27, 28].

The mobile app Doximity deserves special attention. This application is one of the largest networks of interconnected doctors of various specialties in the United States. This is very convenient as you can quickly respond to questions from colleagues on the formulation of a diagnosis or to have the opinion of several experts [29].

Goo Patient created for patients who monitor their health. Thanks to the work with the application, it becomes possible to upload your analyzes and have them at hand always in dynamics [30].

Thus, the positive experience of the implementation of mobile applications within eHealth in the leading countries of the world provides NHS with tools for the effective and rational use of medical and pharmaceutical resources of the sector and the high-quality medical and pharmaceutical care to patients.

Now there is also a tendency to the introduction of information electronic systems in Ukraine. Thus, since 2008 on the initiative of the Ministry of Health of Ukraine there was an attempt to introduce an electronic medical passport of the patient and the all-Ukrainian electronic register of patients, but only in 2018, as part of the reform of NHS, the eHealth project was launched, the total cost of its development amounted to 400 thousand USD. The aim of eHealth was to optimize provision of medical care to patients and improve the work of doctors with the involvement of electronic healthcare, as well as monitoring the rationality of the use of budgetary funds [31–33].

It should be noted that the effectiveness of eHealth implementation requires availability of the appropriate healthcare management information system, which has the following components: registers of medical institutions, services, doctors, patients, diagnosis coding system and electronic medical documentation (electronic medical records), in particular electronic prescriptions.

Today one needs to choose one of 14 available medical information systems to work with eHealth: Helsi, MIC EMCiMED, Doctor Elex, MEDSTAR, MEDICS, Polyclinic without queues, MedAir, MedCard Plus, Askep.net, Health24, nHealth, UASMART, Medinfoservice, MIS "Kashtan". Currently, in Ukraine 1472 medical institutions, 24506 doctors and 21068249 patients joined eHealth. The largest number of institutions participating in the project was in the Lviv region – 140 institutions (9.51 % of the total number of participating institutions), the Dnipropetrovsk region was in the second position– 94 (6.38 %) in the third position was Kyiv – 90 (6.11 %) and the fourth was the Kharkiv region – 87 (5.91 %) institutions, respectively. At the same time, the smallest number of participating institutions was in the Luhansk region– 19 (1.29 %).

The largest number of doctors who took part in the project was observed in the Dnipropetrovsk region – 1924 (7.85 % of the total number of participating doctors), in the Kharkiv region – 1771 (7.22 %), in Kyiv – 1731 (7.06 %), and in the fourth position there was the Lviv region – 1668 (6.80 %) doctors, respectively. By the number of patients who signed a contract (declaration) with the hospital and the family doctor the leader was the Dnipropetrovsk region – 1779743 (8.44 % of the total number of declarations), the Kharkiv region was in the second place – 1602772 (7.60 %) the Lviv region was third – 1459589 (6.92 %) and in the fourth position was Kyiv – 1284785 (6.09 %) declarations, respectively.

It should be noted, that most healthcare institutions are not in a hurry with the introduction of medical information systems required for effective participation in the abovementioned project. It may be connected with the unwillingness of health professionals to use electronic information systems due to the age of specialists, their inability or unwillingness to master the tools of modern information globalization.

Therefore, currently eHealth is the initial link in creating the national information electronic system of the healthcare. The next step in the reform of NHS should be distribution of mobile medical applications that will interact with the electronic information system. The characteristics of the basic principles of mobile medical applications are presented in **Fig. 2**.

Table 1

The top 15 mobile medical applications for doctors, pharmacists and patients

Name of a medical mobile application	Characteristics
Medscape	Multi-purpose medical reference book comprising original articles on various subjects, news from the world of medicine, more than 7 thousand patient information leaflets, tests for the drug compatibility, etc. It is one of the most informative resources for both physicians and consumers (free)
Epocrates	It is used mainly by doctors to obtain information about drugs and their interaction (part of the content is free, additional information is paid)
Read by QxMD	It allows you to read and download research results, journals and articles from a variety of sources (open access journals, Pubmed and documents from relevant institutions). "Read" is a free application, but some journals and Pubmed may require a subscription
UpToDate	Medical reference book (the program is free, but access to information provides for a subscription to the database – 499 USD for a year)
Doximity	The largest social network for doctors in the United States (about 40 % of medical professionals are users of this program). With a mobile phone and web platform, doctors can use the Doximity functionality to securely share Health Insurance Portability and Accountability Act data. Downloading the application is free
Isabel	The Isabel database has more than 6 thousand reports about diseases and symptoms, and it gives the possibility to specify results by age, sex of the patient, etc. To use the application the online access is required (it is free, but to use all of the functions you need to purchase a paid subscription)
Calculate by QxMD	Application for doctors practicing in cardiology, oncology and obstetrics. "Calculate" turns authoritative scientific medical research into practical tools for diagnosis, selection of doses of drugs, management of treatment, etc.
Virtual Practice for Doctors	It allows the doctor to be in contact with the patient outside work (remote control of the patient and the ability to answer text questions)
NEJM This Week	It has access to the latest articles, allows you to view images of various medical pathologies, listen and see audio and video reviews of articles
Figure 1 Medical Images	It allows to view and share medical images with other professionals. The application ensures the privacy of the patient by using an automatic blocking of the person and eliminating the identifying information
My Chart	It is designed for individual use by patients. MyChart creates a direct communication between patients and healthcare professionals
Dyna Med Mobile	It includes medical resources, information about places of providing medical care, reviews of more than 3,400 various topics (it costs 395 USD a year for a medical practitioner)
iPharmacy	It is a tool for patients and doctors that helps to find the prescribed drugs at the lowest price and has an electronic discount card
Goo Patient	It allows to create the own electronic medical card, store the results of examinations (MRI, ultrasound), copies of insurance, prescriptions, etc. The paid version allows you to register an account for several family members
Cardio Smart	It was created to discuss cardiovascular disease with patients. The doctor will be able to demonstrate to the patient exactly what happens to the heart muscle during a myocardial infarction or describe the process of coronary angiography, etc.

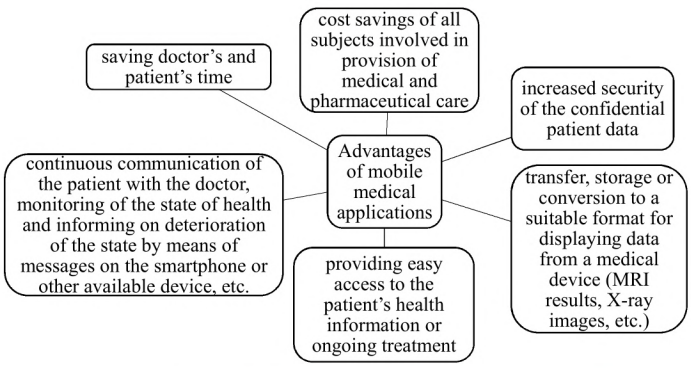

Fig. 2. The characteristics of the basic principles of mobile medical applications

5. Discussion

When developing and introducing mobile medical applications in NHS, the Guidelines to mobile medical applications for professionals in the field of industry and food and medicines released by the FDA (USA) should be taken into account. This document explains that the agency monitors mobile medical applications that pose a greater risk to patients if they do not work properly, as well as applications that cause smartphones or other mobile platforms to affect the functionality or performance of traditional medical devices.

Currently, this Guidelines is a basic tool that developers can use to determine the extent to which FDA will regulate the application. The Guidelines defines a "mobile medical application" to refer to a mobile application or is intended to be used as an accessory to a regulated medical device [34–36].

Today there are 286 applications approved by FDA [37–39]. However, it should be noted that medical devices, including mobile applications, are classified into three classes depending on their risk profile:

– class I (overall control, low risk);
– class II (special and general control);
– class III (pre-approval, high risk).

Therefore, the introduction of mobile applications for NHS in Ukraine should be controlled by state authorities and independent international organizations dealing with the control and development of information electronic systems.

It should be noted some aspects, both positive and negative, from the introduction of mobile applications that affect the efficiency of the medical and pharmaceutical care to patients. The paper Ross, J et al., provides an overview of the factors that influence the implementation of e-health. According to the review, it was found that e-health is certainly progress and an impetus in the development of the entire patient care system, but there are also negative consequences of such an introduction that should also be addressed [41]. The work by Ludwick D. reviewed the results of the introduction of electronic medical records of patients in the health care system. It was revealed that, by the example of implementation in 7 countries, there is a problem of responsibility for the personal data of patients and, in general, the problem of confidentiality of all data existing in hospitals [42]. For example, in the study, the authors Liang X. et al., evaluated the impact of mobile phone intervention on the glycemic control in diabetes. It was found that intervention with a mobile phone led to a statistically significant improvement in glycemic control in patients with type 2 diabetes [43].

The author Boulos M. N. K. et al., presents an analysis of the impact of mobile phones and diligence on the development of health care and society as a whole. It has been found that smartphones are useful for medicine and health-related professions, because they are mobile, portable, easy to use and can be used on the road [44]. In the study "The impact of mobile handheld technology on hospital physicians" work practices and patient care: a systematic review, the authors concluded that the use of mobile applications demonstrates advantages in conditions where time is a critical factor, and quick response is decisive [45]. The author C. Free et al., found that the impact of mobile applications on the provision of medical and pharmaceutical care has shown positive results. This is due to the simplicity and efficiency of working with mobile applications [46].

According to the introduction of mobile health in all countries, should be carried out with the help of specialists. To this end, the mHealth Knowledge platform was created that connects healthcare professionals around the world – with people, products and ideas that are needed for effective work [47, 48]. To keep track of new technologies in the field of mobile healthcare, the WHO released a collection of Compendium of new and emerging technologies aimed at solving global healthcare problems. This compilation was created as a neutral platform for technologies that can be used in conditions of limited resources. To this end, research2guidance has launched the digital health care market research program mHealth App Developer Economics since 2010 [49, 50].

Summing up at the same time, it is necessary to remember and take into account the relevant risks that may arise in the process of using mobile applications or electronic information systems, for example, incorrect interpretation of their data by patients, incorrect diagnosis and further drug

use. Those inadequate self-medication or risks associated with the leakage of confidential patient data, etc. [51, 52].

6. Conclusion

The results of the study suggest that the introduction of mobile medicine in NHS will not only expand the access to medical and pharmaceutical care and health information, but will also strengthen the ability to diagnose, monitor and track diseases in a timely manner. Moreover, mobile applications can provide a lot of useful information to patients, helping them to access medical and pharmaceutical knowledge.

Distribution of electronic information systems in the healthcare and mobile applications allow not only to reduce government spending on the healthcare, but also to decrease the overall mortality rate of the population, reduce visits to doctors without the necessary needs and errors in prescriptions. At the same time, the introduction of medical mobile applications should be in accordance with international guidelines, in particular FDA, and the relevant state control.

Conflict of interest

No conflict of interest.

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PROSPECTIVE BIOLOGICAL ACTIVE COMPOUNDS AMONG 7-SUBSTITUTED OF 3-BENZYL-8-PROPYLXANTHINES FOR TREATMENT OF METABOLIC SYNDROME PATHOLOGIES

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Abstract

Aim – the search for biologically active compounds with diuretic and hypoglycemic action among 7-substituted of 3-benzyl-8-propylxanthines, which can be used for the treatment of pathologies of "metabolic syndrome".

Materials and methods. 30 new chemical compounds among derivatives of 7-substituted of 3-benzyl-8-propylxanthines by PASS prediction filter were chosen for diuretic and hypoglycemic activity researches. Diuretic, hypoglycemic activity and for the most active compounds – the acute toxicity were studied in vivo. The molecular docking, which is based on EADock DSS mechanism of the modulation displayed the interaction between some of functional groups inside discovered xanthine ligands and known receptors and enzymes presented in Ligand-protein Database of Swiss Institute of Bioinformatics.

Results. It has been shown that synthesized compounds displayed strong diuretic and medium hypoglycemic activities. The molecular docking modulation (SwissDock) of interaction of xanthine derivatives hits with proposed receptors and enzymes revealed prospective of using 7-substituted of 3-benzyl-8-propylxanthines as potential drugs for treatment of metabolic syndrome pathologies.

Conclusion. The diuretic activity of the new 23 compounds of 7-substituted of 3-benzyl-8-propylxanthines and hypoglycemic activity of the new 7 derivatives of 7-substituted of 3-benzyl-8-propylxanthines was studied. Results of performed investigation illustrate that 7-substituted of 3-benzyl-8-propylxanthines demonstrate biological activity comparable to standard drugs. We also proposed probable molecular targets for the most active compounds by molecular docking method. It was shown that derivatives of 7-substituted of 3-benzyl-8-propylxanthines can be used for metabolic syndrome disorders prevention.

Keywords: metabolic syndrome, xanthines, diuretic activity, hypoglycaemic activity.

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1. Introduction

The prevalence of the metabolic syndrome (MS) is the actual problem among adult population of the developed countries [1]. In general MS is associated with increasing of everyday calories intake, sedentary lifestyle, which is based on the improvement access to quality food and the progress of the technology in which human is engaged on intelligent levels of management [2].

Unfortunately, according to the WHO data [3] of post-industrial countries the frequency of MS is 10–20 % among 30 years and older population, in the USA – 34 % (44 % for those who are

older 50 years). In addition, survey of American Diabetes Association shows that current syndrome demonstrates impetuous growth of appearance between teenagers and young people [4].

Nowadays, MS includes so-called "deadly" quartet – arterial hypertension, diabetes mellitus type 2, dyslipidemia and alimentary obesity [5].

An unpleasant feature of the combination of these diseases is that each of them get worse the progression of the other, that is, a vicious circle is formed.

Therefore, the search for drugs that would affect individual links of MS, improving carbohydrate and lipid metabolism, reducing arterial pressure can be useful in treating this "disease of civilization".

The attention of scientists who are searching for biologically active substances, is attracted by the natural nitrogen-containing heterocyclic systems [6], and the xanthine derivatives [7, 8] occupy a special place in this, among which, as well as several effective non-toxic drugs.

Aim of the research. Considering the above objective, the purposeful search for biologically active compounds among 7-substituted of 3-benzyl-8-propylxanthines became an aim of our work.

2. Materials and methods

Derivatives of 7-substituted of 3-benzyl-8-propylxanthines were previously synthesized by our scientific team [9, 10].

The research was coordinated with the internal commission of Zaporizhzhia State Medical University on bioethics (ZSMU Act No. 1 of 10.01.2019).

Experiments with animals were performed in the Scientific medical-laboratory center of the Zaporizhzhia State Medical University, certified by the SEC of the Ministry of Health of Ukraine (certificate No. 039/14). During the experiment, the animals were kept in the vivarium at a temperature of 20–22 °C and 50 % humidity in a well-ventilated room, under the conditions of natural dark/light cycle with free access to food and water.

All manipulations with animals were performed out in accordance with the requirements of GLP, the recommendations of "European Union Directive 2010/63 / EU on the protection of animals used for scientific purposes" [11].

Animals were achieved from nursery of State Institute of Pharmacology and Toxicology of National Medical Academy of Ukraine.

The study of *diuretic activity* was performed on nonlinear rats weighing 135-288 g using the Berkhin method [12]. For the observation of diuretic activity was used 7 rats in each group. In the study of water diuresis, rats were kept on a constant diet with free access to water. To the water load animals were kept for two hours without food and water. Subsequently, the animals were injected into the stomach with the help of a probe of the test substance in the form of aqueous suspension at the same time as an aqueous loading in the amount of 3 ml per 100 g body weight of the animal. Urine was collected every hour for 4 hours. Hydrochlorothiazide was used as reference drug.

Hypoglycemic activity (in vivo) was modelled by an oral glucose tolerance test [13], which was simulated by a load of animals at a dose of 2 g/kg body weight. The study used nonlinear white rats weighing 128–163 g, divided into 10 groups of 7 rats in each: 1 – intact; 2 – control of glycaemia without treatment; 3–9 – with the use of the studied xanthine derivatives; 10 – with glibenclamide therapy (5 mg/kg).The blood sample was taken from the femoral vein in 30 minutes. The glucose level was measured by the glucose oxidase method.

Molecular docking experiments were performed at the SwissDock web server (http://www.swissdock.ch, this server is free for academic use) [14, 15].

The results of the research were processed by modern methods of the analysis on personal computer with the use of a statistical package Statistica® for Windows 6.0 license program (StatSoft Inc., License No. AXXR712 D833214FAN5). The normality of distribution of quantitative signs was analyzed by Shapiro–Wilk's test. The parameters had normal distribution. The comparison of indicators in groups was carried out with the use of Student's test. The difference on $p<0.05$ was considered statistically significant. All tests were bilateral. For definition of reliable distinctions between quality indicators the non-parametric statistical criteria were used (the analysis of tables – criterion χ^2).

4. Results

For the enzyme DPP-4 [16], we have calculated the interaction with phenylthiosemicarbazone of 3-benzyl-8-propylxanthynyl-7 acetic acid. The classic inhibition of this enzyme is the covalent interaction with serine 630 (the major nucleophile involved in the hydrolase reaction). Molecular docking displayed possible interactions to form a hydrogen bond with threonine 304 and hydrophobic interaction with phenylalanine 208, which prevents suitable substrate to the active site nucleophile.

Molecular docking revealed the formation of three hydrophobic bindings between phenylthiosemicarbazone of 3-benzyl-8-propylxanthynyl-7 acetic acid and glycine 47, isoleucine 46 and 218, which correlates with the inhibition data of enzyme 11β-hydroxysteroid dehydrogenase type 1 (HSD 11B1) [17].

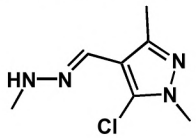
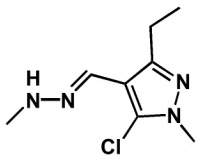
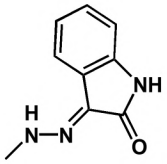
Also, by molecular docking [18], we have found possible agonistic activity through forming hydrogen bonds between phenylthiosemicarbazone of 3-benzyl-8-propylxanthynyl-7 acetic acid and the receptor peroxisomal proliferative activating receptor gamma (γ-PPAR).

We achieved the result of molecular docking, which indicate the presence of interactions between hydrazide 3-benzyl-8-propylxanthinyl-7 acetic acid and phenylalanine 1276, which blocks interaction of A1-receptor [19] with targeted mediator – adenosine.

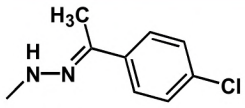
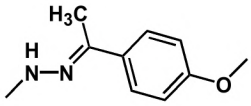
Results of diuretic and hypoglycemic activities is presented in **Tables 1, 2**.

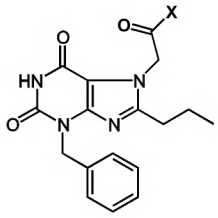
Table 1

Diuretic activity of derivatives of 3-benzyl-8-propylxanthinyl-7 acetic acid

Compound	X*	120 min, Δ %	240 min, Δ %
1	2	3	4
Hydrochlorothiazide		78.79	73.10
E-71	–NH–N=CH–C_6H_5	14.42	42.62
E-104	–NH–N=CH–p–C_6H_4Br	70.19	82.51
E-79	–NH–N=CH–p–C_6H_4F	95.19	81.42
E٦٣-	–NH–N=CH–p–C_6H_4–$N(CH_3)_2$	–20.19	–24.04
E-78	–NH–N=CH–p–$C_6H_4NO_2$	–9.62	13.66
E-121	–NH–N=CH–m–$C_6H_4CH_3$	2.97	132.57
E-77	–NH–N=CH–m–$C_6H_4NO_2$	29.70	26.29
E-122	–NH–N=CH–o–C_6H_4–OEt	3.96	–5.14
E-125	–NH–N=CH–o–$C_6H_4NO_2$	60.40	95.43
E-127	–NH–N=CH–CH=CH–CH_3	37.62	27.43
E-126	–NH–N=CH–CH=CH–C_6H_5	–14.85	13.14
E-129		81.40	67.20
E-130		2.33	32.80
E-91		66.28	34.95

Continuation of Table 1

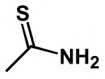
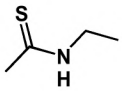
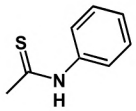
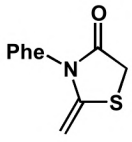
1	2	3	4
E-153		86.05	6.45
E-154		67.44	39.78
E-61	–NH–NH_2	88.46	144.26
E-166	–O–n–Hex	62.63	68.28
E-167	–O–n–Hep	5.05	121.38
E-147	–p–C_6H_4–OCH_3	39.39	133.10
E-174	–O–n–But	62.63	108.97
E-58	–O–n–Pr	53.54	114.48
E-62	–C_6H_5	8.08	66.21



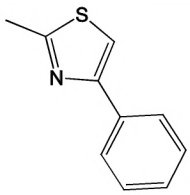
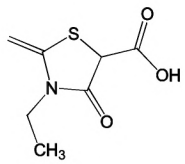
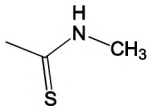
Note: * –

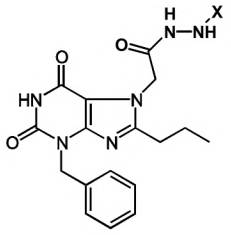
Table 2

Hypoglycemic activity of derivatives of hydrazide of 3-benzyl-8-propylxanthinyl-7 acetic acid

Compound	X*	Glucose level, %
1	2	3
Control		100
Intact		–35.76
Glibenclamide		–45.66
E-168		–0.91
E-165		–1.24
E-64		–50.29
E-135		–11.23

Continuation of Table 2

1	2	4
E-178		2.31
E-179		6.03
E-180		0.33

Note: * – 

As it shown in the **Table 1**, most of the tested compounds demonstrated diuretic activity. Exception – compound E-63 with antidiuretic activity in period 2 and 4 hours, compounds E-78 and E-126 with antidiuretic manifestation in short period (2 h) and weak diuretic activity after 4 h. The most active compound – E-61 (hydrazide of 3-benzyl-8-propylxanthinyl-7 acetic acid) displayed activity in 1.97 time bigger than reference drug hydrochlorothiazide (144.26 % in comparison with 73.10 %).

Table 2 data illustrate that four of investigated compounds have hypoglycemic activity, but only E-64 exceeds the value of reference drug – glibenclamide (decreasing of glucose level on 50.29 %).

5. Discussion

The most promising mechanisms of the hypoglycemic activity is the increasing of insulin secretion. It is known that inhibition of the enzyme dipeptidyl peptidase 4 (DPP 4) [20] leads to the accumulation of incretins – Glucagon-like peptide-1 (GLP-1) [21] and glucose-dependent insulinotropic peptide (GIP) [22], that stimulate insulin release and inhibit glucagon secretion. In difference of the most commonly used drugs, such as insulin and sulfonylurea, GLP-1 therapy is associated with both weight loss and is accompanied by a lower risk of pathological hypoglycaemia, important manifestations for patients with type 2 diabetes mellitus.

In addition, it is known that cortisol is a powerful contrinsular hormone [23]. Accumulation of this glucocorticoid leads to an increase in gluconeogenesis, suppression of glucose transportation to cells, desensitization of insulin receptors, the stimulation of anabolic pathways for the deposition of energy macromolecules and increased blood pressure. In the biosynthesis of this corticosteroid hormone the crucial role is played by the enzyme – 11β-hydroxysteroid dehydrogenase type 1 (HSD 11B1), inhibition of which can improve the stresses caused by this hormone.

Peroxisomal proliferative activating receptor gamma (γ-PPAR) [24] is a transcriptional factor that belongs to the superfamily of nuclear receptors, is involved in the absorption and storage of

fatty acids, glucose homeostasis, and has an anti-inflammatory effect. Synthetic g-PPAR agonists can be widely used in the treatment of dyslipidemia, hyperglycemia and their combination.

According to numerous investigations [25], diuretic drugs play an important role for the treatment of arterial hypertension. Many natural and synthetic xanthines are characterized by diuretic action, which, corresponding to the literature, is realized, due to the blocking of A1 adenosine receptors [26].

Up-to-date commonly used in silico methods, regrettably, have certain restrictions due imperfect prediction algorithms. Despite to some raw estimation of calculated results, presented investigations may been used as a possible way for the future researchesfor the prediction of the biological activity among substituted xanthines.

6. Conclusion

The diuretic activity of the new 23 compounds of 7-substituted of 3-benzyl-8-propylxanthines and hypoglycemic activity of the new 7 derivatives of 7-substituted of 3-benzyl-8-propylxanthines was studied. Results of performed investigation illustrate that 7-substituted of 3-benzyl-8-propylxanthines demonstrate biological activity comparable to standard drugs. We also proposed probable molecular targets for the most active compounds by molecular docking method. It was shown that derivatives of 7-substituted of 3-benzyl-8-propylxanthines can be used for metabolic syndrome disorders prevention.

Conflict of interest

No conflict of interest.

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