ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

The Investigation of the Stability of the Granules Based on Arginine and Tincture of Ginseng

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Abstract

The article presents the stability studies of the granules based on arginine and tincture of ginseng with neuroprotective (nootropic) action for the treatment of various disorders of the functional activity of the brain. For determining stability and shelf life of the developed medicine, several series of granules were stored at a temperature 15-25 °C in a dark, light protected place. Based on the research, it can be concluded that within 24 months from the date of manufacture, granules remain stable in all respects that have been studied.

Keywords: *Arginine, Tincture of ginseng, Granules, Stability, Investigation.*

Introduction

A sharp increase in the number of patients with established impairments in the functional activity of the brain (cognitive disorders of varying severity) contributes to an increase in demand for medicines of the nootropic group. A significant increase in the frequency of neurological and psychiatric pathology, including dementia and encephalopathy of various genesis, especially in old age, also necessitates the conduct of scientific research in the field of creating new medicines of neuroprotective (nootropic) action [1, 2, 3]. The modern assortment of medicines of the group N06BX "Other psycho-stimulating and nootropic medicines" makes more than 80 trade names (Fig. 1). Most often in the treatment of violations of the functional activity of the brain, medicines based on piracetam are used (28%). A significant part of the pharmaceutical market also belongs to herbal medicines based on the extract of leaves of Ginkgo Biloba (24 %). Citicoline and medicines based on it occupy 16 % of the market segment of neuroprotectors, phenibut and vinpocetine-12 % each, other medicines account for less than 5 % [4, 5].

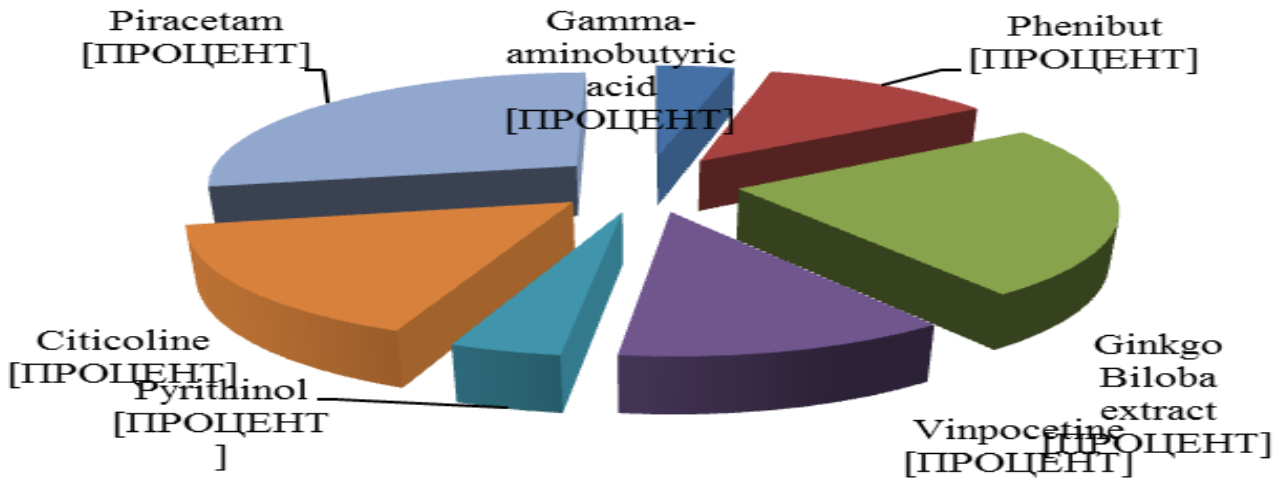

Fig. 1: The percentage of neuroprotectors relative to the share of the pharmaceutical market, depending on the main active ingredients

A modern range of medicines of nootropic action is formed by such manufacturing countries as Austria, Latvia, Italy, Poland, Hungary, India, Bulgaria, Slovenia, Spain, Germany and France (Fig. 2).

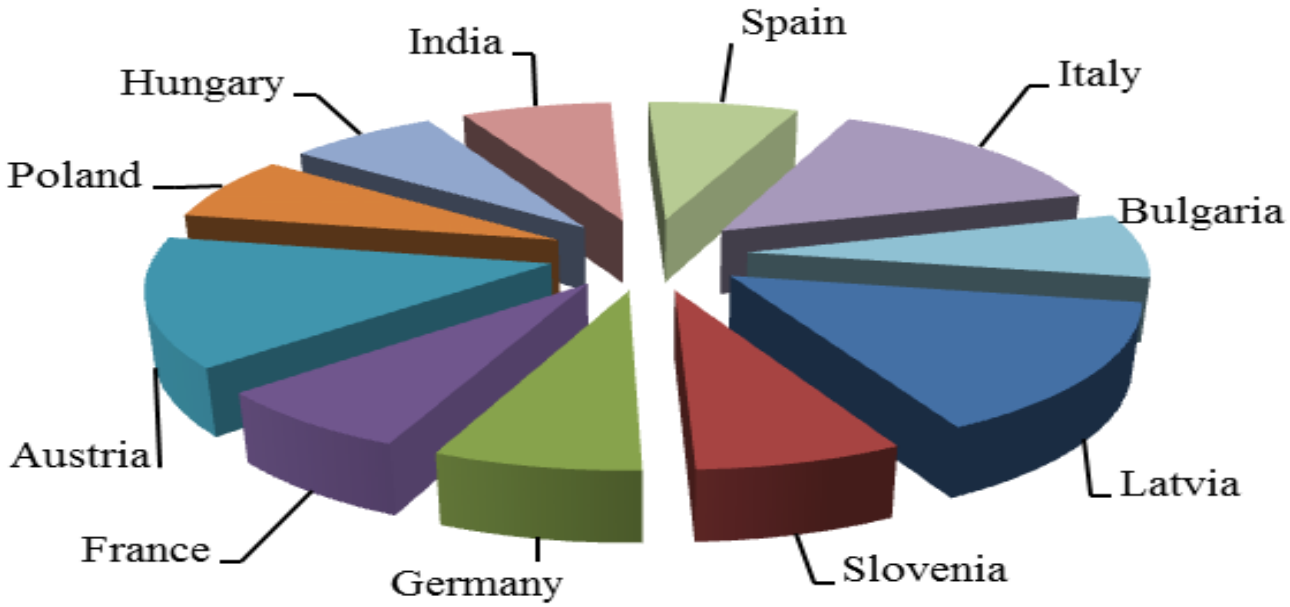

Fig.2: Proportion of countries producing nootropic (neuroprotective) medicines in the pharmaceutical market

A more detailed study of the assortment of neuroprotectors (nootropics) in the European pharmaceutical market showed the absence in this pharmacological group of medicines containing the amino acid arginine. However, recently, this substance is widely used for the prevention and treatment of a number of pathologies, including those associated with impaired cerebral circulation.

Arginine has a fairly wide range of pharmacological effects: it is involved in the regulation of the tone of smooth muscles, permeability and microcirculation of blood vessels, blood pressure; improves peripheral and central circulation; reduces hypoxia of the myocardium, brain, limbs and other organs; makes beneficial effect on the functional activity of the brain with age-related degenerative pathologies and cognitive impairment; reduces the progression of Alzheimer's disease; stimulates memory and intellectual abilities [6, 7, 8].

Thus, it is actual to create a new medicine based on the amino acid arginine with neuroprotective (nootropic) action. To date, at the National University of Pharmacy (Kharkiv, Ukraine) at the Pharmaceutical Technology of Drugs Department granules for the preparation of a solution for oral administration based on arginine and tincture of ginseng were developed [9, 10, 11, 12, 13].It is known that the preparation and especially the storage of medicines are often accompanied by a change in their properties with different degrees of manifestation. This leads to a decrease in the content of medicinal substances or to a decrease in their pharmacological activity, the appearance of new impurities, changes in the properties of dosage forms, etc. Such phenomena significantly affect the shelf life of medicines, which can vary widely: from several hours to several years. The **objective** of this work is to study the stability of developed by us granules based on arginine and tincture of ginseng with neuroprotective (nootropic) action for the treatment of various disorders of the functional activity of the brain.

Materials and Methods

As objects of research were selected granules based on arginine and tincture of ginseng, developed at the Pharmaceutical Technology of Drugs Department of the National University of Pharmacy (Kharkiv, Ukraine).The preparation of the granules was carried out by the conventional wet granulation method.

Auxiliary substances, namely granulating (binding) liquids, which determine the quality of the granulated material and the granules themselves (strength, disintegration, dissolution), play an important role in granulation.

The requirement for the granulated mass is its plasticity and the absence of sticking properties.

The resulting granules for determination of the conditions of their storage and shelf life, at which no change in their physical, chemical and technological properties did not observed, were analyzed and standardized. The fractional composition was determined by means of a vibration shaker BA 200N (CISA, Spain). The flow ability of the granules, as well as the angle of repose, were studied on an automated powder testing facility PTG-S4 (Pharma Test, Germany), according to the pharmacopoeia techniques EP <2.9.36>, USP <1174> and ISO 4324.

To determine the bulk volume and the bulk volume after exhaustion, a PT-TD200 semi-automatic tester was used (Pharma Test, Germany) according to the procedures described in USP <616 App.1-3> and EP <2.9.15>. The disintegration test for the granules was carried out on the identifier of the disintegration process of DT-1000 (Lab India, India) ("swinging basket", medium-purified water, medium's volume-1000 ml, temperature-(37.0±2.0)°C, speed of movement-(30 ± 2) cycles/min.

The dissolution of the granules was studied on a Type II ("Blade Mixer") DS-8000 (Lab India, India). The determination was carried out at the temperature (37/0 ± 1.0) °C for up to 8 hours. As the dissolution mediums used purified water, 0.1 M hydrochloric acid (pH 1.2) with a volume of 900 ml, with a stirring speed of 50 rpm. The abrasion resistance of the granules was determined using a drum tester of friability and abrasion AE 1 (Lab line, Austria). The moisture content of the granules was studied with the Moisture Analyzer OHAUS MB-35 (Switzerland). The degree of release of active substances was determined using traditional methods of analysis.

As a method of identification of the amino acid arginine in the granules, we selected a highly selective analytical method that allows us to identify the amino acid composition of multicomponent mixtures-high-performance liquid chromatography (HPLC), which is characterized by high rate of results, convenience, availability, high selectivity, accuracy and sensitivity in research. As a comparison object, the certified L-Arginine substance (SYNNEX Pharma Technologies Co., Ltd, China) was used in the composition of granules for the qualitative and quantitative identification of the amino acid arginine. The studies were carried out on a high-performance liquid chromatograph "Geilston" (France), followed by computer processing of the results of the study using the program "Multi Chrome" for "Windows". For the study, a chromatographic column measuring 150 × 2.0 mm, filled with a Reprosil-Pur C18-AQ sorbent with a particle size of three µm was chosen.

For work used measuring tableware of class A. A phosphate buffer solution of 0.1 M sodium phosphate was used as a mobile phase and the pH was adjusted to 2.5 with phosphoric acid and acetonitrile. The mobile phase "A" is a mixture of a phosphate buffer solution (pH 2.5) and acetonitrile in a 90:10 ratio, the mobile phase "B" is the indicated mixture at a ratio of 15:85.

Results and Discussion

In the course of studying the technological characteristics of the active substance arginine, it was found that the flow ability of this component has an insufficient index. Therefore, in order to increase the flow ability of the granulation mass, we considered the possibility of adding auxiliary components, namely, potato starch, lactose monohydrate, magnesium oxide and glucose, using their mixtures with arginine in various ratios (Fig. 3.). Based on the data obtained experimentally in the study of the flowability of the mixture of arginine with the selected auxiliary components (Fig. 3), the addition of lactose monohydrate (Flow Lac 100, MEGGLE GMBH, Germany) in a 1:1 ratio significantly improved the flow ability of the granulation mass.

Flow Lac 100 is today a promising antifriction substance in the manufacture of solid medicines. Due to the spherical shape of the particles, the lactose is well distributed between the particles of substances in the mixture, improves flow and compacts the powder. In addition, thanks to the choice of lactose as a filler in the manufacture of granules, along with the improvement of technological properties, we were able to provide a pleasant (sweetish) taste of the solution for oral administration, obtained by dissolving granules.

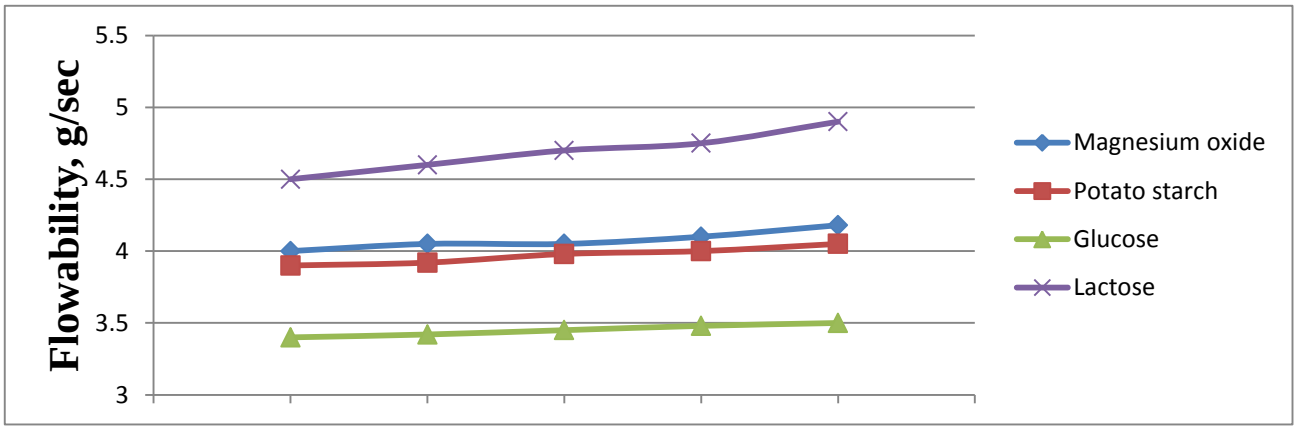

Fig. 3: The dependence of flowability of the granulation mass on the content of auxiliary substances

The introduction of the second active ingredient (tincture of ginseng) into the granules was decided by using this component as a granulating liquid, which in turn made it possible to significantly reduce the laboriousness of the technological process.

While studying the technological properties of the obtained granules, a significant improvement in flow ability was observed, however, high vibrations were observed in the study of the fractional composition, the heterogeneity of the shape and size of the granules. In order to eliminate the established shortcomings of the obtained dosage form, it became necessary to use binder substances as a granulating liquid.

During the experiment we used aqueous solutions of starch (5 %), methylcellulose (3 %), aqueous and alcohol solutions of polyvinylpyrrolidone (5 %) together with tincture of ginseng root. It was experimentally established that a 5 % solution of polyvinylpyrrolidone (solvent - ethanol 70% and tincture of ginseng root in the ratio 1:9) should be used as a granulating liquid. This technological process allowed to avoid excessive moistening of the granulation mass, as well as to obtain granules whose technological parameters corresponded to the established norms.

The granulate was dried in a thin layer at a temperature of 50-60 °C, then the fractional composition of the granules was determined (separating the smaller and larger granules) and packed in hermetically sealed containers of dark glass. The technological properties of the substance L-Arginine, the granulation mass, as well as two samples of granules obtained using a different humectant are shown in Table 1.

According to the data presented in Table 1, the addition of lactose monohydrate modified (Flow Lac 100) as an auxiliary substance in the granulation mass allowed not only to increase the flow ability, but also to reduce the moisture content of the granulation mass, due to low hygroscopicity.

Granules obtained by moistening the mass for granulation with tincture of ginseng are distinguished by a significant improvement in flowability; however, significant fluctuations in the bulk volume and bulk volume after shrinkage indicate the heterogeneity of the shape and size of the granules, which was also confirmed by studying the fractional composition of the granules and determining their organoleptic properties.

Granules based on L-Arginine and tincture of ginseng, obtained by moistening with 5 % alcohol solution of polyvinylpyrrolidone, show the best technological characteristics, namely: flow ability sufficient for automated dosing, which in turn is confirmed by the value of the angle of repose; homogeneity of the shape and size of the granules, established by studying the fractional composition, organoleptic control and reducing the volume and bulk volume fluctuations after shrinkage, in comparison with granules moistened with ginseng tincture.

In addition, a low moisture content of granules helps to increase the stability of their technological properties during storage. Further, several series of granules based on arginine and tincture of ginseng were stored at a temperature of + 15 °C to + 25 °C in a dark, light protected place. At intervals of 6 months, the granules were subjected to a subsequent study according to the above-

mentioned study indices and the procedures given.

The data obtained are presented in Table 2.

Table 1: Properties of L-Arginine, mass for granulation and two compositions of granules differing in the composition of the humectant

No.	Physical, chemical and technological properties	L-Arginine	Mass for granulation (L-Arginine and lactose, 1:1)	Granules (humectant – tincture of ginseng)	Granules (humectant – 5 % alcohol solution of polyvinylpyrrolidone)
1.	Flow ability, g/sec	3.32 ± 0.06	4.72 ± 0.09	8.31 ± 0.11	12.1±0.08
2.	Angle of repose, degrees	32.1 ± 0.5	30.5 ± 0.4	29.4 ± 0.6	27.3 ± 0.3
3.	Bulk volume, (V0), ml	177.09 ± 4.34	178.10 ± 1.79	266.82 ± 1.06	268.56 ± 1.72
4.	Bulk volume after shrinkage, (V10), ml	157.85 ± 4.68	156.42 ± 0.95	254.66 ± 1.46	256.82 ± 2.64
5.	Bulk volume after shrinkage, (V500), ml	136.72 ± 2.54	136.22 ± 1.04	215.88 ± 2.13	216.46 ± 2.08
6.	Bulk volume after shrinkage, (V1250), ml	132.66 ± 2.82	132.34 ± 1.43	186.10 ±1.39	207.08 ± 2.01
7.	Ability to shrink, (V10 - V500), ml	21.13 ± 4.21	20.20 ± 1.42	39.38 ± 2.57	40.36 ± 2.87
8.	Bulk density, (m / V0), g/ml	0.566 ± 0.014	0.562 ± 0.006	0.374 ± 0.007	0.370 ± 0.009
9.	Bulk density after shrinkage (m / V1250), g/ml	0.754 ± 0.014	0.758 ± 0.010	0.538 ± 0.007	0.480 ± 0.011
10.	Moisture contents, %	13.81 ± 0.12	10.2	9.7	8.08

Table 2: Study of stability of granules based on arginine and tincture of ginseng

Series No.	Duration of storage (months)	Appearance (description)	Fractional composition of granules, %			Flow ability, g / sec	Moisture contents, %	Disintegration time, min.	Mechanical abrasion resistance, %	Release of the substance L-Arginine, %
			less than 0.2 mm	from 0.2 mm to 3.0 mm	more than 3,0 mm					
010516	-	Grains of round, cylindrical forms of cream color	1.5± 0.03	97.3± 0.05	1.2± 0.06	11.8± 0.06	8.40± 0.06	7.9± 0.03	98.6± 0.01	99.20±0.35 99.15±0.28 98.34±0.18 98.76±0.42 98.75±0.37
	6		1.4± 0.02	97.9± 0.02	0.7± 0.01	11.9± 0.08	8.04± 0.05	7.8± 0.04	98.8± 0.03	
	12		1.3± 0.04	97.4± 0.03	1.3± 0.05	12.1± 0.04	8.21± 0.04	8.0± 0.05	99.0± 0.05	
	18		1.4± 0.01	97.5± 0.04	1.1± 0.02	12.0± 0.05	8.16± 0.03	8.2± 0.06	99.1± 0.04	
	24		1.2± 0.03	97.6± 0.06	1.2± 0.07	12.2± 0.09	8.19± 0.07	8.4± 0.04	98.9± 0.05	
020516	-		1.8± 0.07	97.6± 0.04	0.6± 0.03	12.0± 0.05	8.27± 0.02	8.0± 0.07	98.7± 0.07	98.67±0.38 98.85±0.45 98.76±0.58 99.24±0.85 99.07±0.32
	6		1.2± 0.02	97.9± 0.03	0.9± 0.04	11.9± 0.07	8.20± 0.03	8.5± 0.05	99.2± 0.06	
	12		1.0± 0.05	97.8± 0.02	1.2± 0.03	12.3± 0.06	8.11± 0.05	8.2± 0.06	99.0± 0.07	
	18		1.2± 0.04	97.7± 0.06	1.1± 0.04	11.8± 0.08	8.07± 0.04	7.8± 0.08	99.1± 0.03	
	24		1.4± 0.01	97.5± 0.04	1.1± 0.02	12.2± 0.04	8.42± 0.07	7.7± 0.03	99.0± 0.04	

030516	-		1.1± 0.04	98.2± 0.05	0.7± 0.02	12.5± 0.07	8.15± 0.08	8.1± 0.03	99.2± 0.05	99.07±0.26
	6		1.6± 0.01	98.0± 0.03	0.4± 0.03	12.4± 0.06	8.07± 0.05	7.7± 0.04	99.3± 0.02	99.12±0.53
	12		1.0± 0.01	98.4± 0.01	0.6± 0.01	12.0± 0.03	8.01± 0.03	7.9± 0.07	98.7± 0.07	99.10±0.47
	18		1.2± 0.05	98.0± 0.04	0.8± 0.04	12.2± 0.09	8.33± 0.05	8.6± 0.05	99.4± 0.08	98.95±0.82
	24		1.3± 0.04	98.1± 0.06	0.6± 0.05	12.4± 0.05	8.28±0.06	8.2± 0.04	98.9± 0.07	98.74±0.67
040516	-		1.4± 0.05	98.1± 0.03	0.5± 0.04	12.1± 0.08	8.41± 0.01	8.3± 0.02	98.8± 0.06	98.75±0.11
	6		1.1± 0.04	98.2± 0.05	0.7± 0.02	12.2± 0.06	8.22± 0.04	8.4± 0.06	99.0± 0.05	99.04±0.34
	12		1.1± 0.02	98.3± 0.01	0.6± 0.01	12.0± 0.04	8.08± 0.06	8.0± 0.03	99.2± 0.02	98.84±0.23
	18		1.3± 0.05	98.0± 0.04	0.7± 0.02	12.0± 0.03	8.13± 0.07	7.9± 0.05	98.7± 0.03	99.12±0.56
	24		1.4± 0.03	98.1± 0.05	0.5± 0.06	11.9± 0.07	8.25± 0.05	7.8± 0.06	98.6± 0.06	98.80±0.17
050516	-		1.6± 0.02	98.0± 0.03 98.1±0.06 97.9±0.03 98.0±0.04 98.3±0.05	0.4± 0.01	12.2± 0.04	8.14± 0.02	8.4± 0.03	99.4± 0.02	99.37±0.25
	6		1.4± 0.05		0.5± 0.04	12.1± 0.05	8.17± 0.04	7.7± 0.04	99.0± 0.09	99.23±0.16
	12		1.2± 0.02		0.9± 0.03	11.9± 0.06	8.25± 0.06	7.8± 0.05	99.4± 0.08	99.31±0.17
	18		1.2± 0.05		0.8± 0.04	12.0± 0.07	8.26± 0.07	8.3± 0.07	99.3± 0.04	99.02±0.18
	24		1.0±0.04		0.7±0.02	12.1±0.09	8.39±0.03	8.0±0.06	98.7±0.05	99.15±0.19

Based on the data presented in the table 2, it can be concluded that within 24 months from the date of manufacture, the granules remain stable in all respects that have been studied. Thus, by experimental studies, we have established the shelf life of granules based on L-Arginine and tincture of ginseng, which is 24 months

Conclusions

- The composition and technology of the granules based on the amino acid L-Arginine and the tincture of the ginseng root are developed. The auxiliary substances and the granulation method are selected. The technological properties of granules are studied
- The stability of physical, chemical and technological properties of the granules was studied, the results of which allowed to establish storage conditions and shelf life of the granules.

References

1. Gandzjuk VA (2014) Dynamics of the morbidity and prevalence of circulatory system diseases among the population of Ukraine at the present stage: national and regional aspects. Review of social hygiene and health care organizations of Ukraine, 2 (60): 74-78.
2. Zinchenko OM, Golubchikov MV, Mishchenko TS (2015) The state of the neurological service of Ukraine in 2014: the statistical and analytical directory, Kharkiv,.
3. Zozulya AI, Slabkiy GA (2013) Improvement of the system of rendering medical care to the population with cerebrovascular diseases - a component of the overall health care reform in Ukraine. Collection of scientific papers of the NMAPE staff named after P. L. Shupik., 2: 18-21.
4. Pischikov VA, Yaschenko Yu B, Kondratyuk N Yu (2014) Basic approaches to the prevention of circulatory system diseases. Ukrainian medical review, 6 (104): 45-48.
5. Yavorska VO, Flomin Yu V (2010) Specific Treatment of Ischemic Stroke: Neuroprotection. International Neurological Journal, 6: 36.
6. Mommersteeg P M, Schoemaker R G, Eisel U L, Garrelds IM, Schalkwijk CG, Kop WJ (2015) Nitric oxide dysregulation in patients with heart failure: the association of depressive symptoms with L-arginine, asymmetric dimethylarginine, symmetric

dimethylarginine, and ısoprostane. Psychosom. Med., 77: 292-302.

7. Oliveira J, Debnath M, Etain B, Bennabi M, Hamdani N, Lajnef M, Bengoufa D, Fortier C, Boukouaci W, Bellivier F (2015) Violent suicidal behaviour in bipolar disorder is associated with nitric oxide synthase 3 gene polymorphism. Acta Psychiat. Scand., 132: 218-225.
8. Mercimek-Mahmutoglu S, Cordeiro D, Cruz V, Hyland K, Struys EA, Kyriakopoulou L (2014) Novel therapy for pyridoxine dependent epilepsy due to ALDH7A1 genetic defect: L-arginine supplementation alternative to lysine-restricted diet. Eur. J. Paediatr. Neurol., 1: 14-18.
9. Buntsevich LL, Ageeva NM, Kostyuk MA (2014) Cultivation of ginseng in culture in vitro, development of drinks with the use of its extracts. Fruit growing and viticulture of the South of Russia, 30 (06): 33-44.
10. Choi KT (2008) Botanical characteristics, pharmacologic al effects and medicinal components of Korean Panax ginseng. Acta. Pharmacol. Sin., 29: 1109-1118.
11. Neale C, Camfield D, Reay J, Stough C, Scholey A (2013) Cognitive effects of two nutraceuticals Ginseng and Bacopa benchmarked against modafinil: a review and comparison of effect sizes. Br J. Clin. Pharmacol., 75(3): 728-737.
12. Reay JL, Scholey AB, Kennedy DO (2010) Panax ginseng (G115) improves aspects of working memory performance and subjective ratings of calmness in healthy young adults. Hum. Psychopharmacol., 25: 462-471.
13. Ruban EA, Khokhlova LN, Bobritskaya LA, Spiridonov SV (2016) Current trends in the technology of solid medicines. Kharkov, NUPh,.

