რეზიუმე

ღვიძლის პორტული ტრაქტის მორფოლოგიური ცვლილებები ექსპერიმენტული ქოლესტაზის პირობებში

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, კლინიკური ანატომიის და ოპერაციული ქირურგიის დეპარტამენტი, საქართველო

ღვიძლში და ნაღვლის სადინრებში ობსტრუქციის დროს განვითარებული მორფოლოგიური ცვლილებების დინამიკა კარგად არის შესწავლილი ექსპერიმენტულ ცხოველებში, თუმცა მონაცემები პორტული ტრაქტის მორფოლოგიაზე მწირია.

კვლევის მიზანს წარმოადგენდა პორტული ტრაქტების სტრუქტურისა და მათი სტრუქტურული ელემენტების ურთიერთდამოკიდებულების შესწავლა ექსპერიმენტული ქოლესტაზის პირობებში.

კვლევა ჩატარდა ვისტარის ჯიშის თეთრ ვირთაგვებზე, რომელთა წონა იყო 200-250 გ. ნაღვლის საერთო სადინრის გადაკვანძვიდან მე-3, მე-6 და მე-12 დღეს ჰემატოქსილინ-ეოზინით, მასონის ტრიქრომის შეღებვის მეთოდით და CK 8 ანტისხეულის მარკერის საშუალებით ჩატარებული იყო ღვიძლის ქსოვილის ჰისტოლოგიური, ჰისტოქიმიური და იმუნოჰისტოქიმიური კვლევა.

ნაღვლის საერთო სადინრის გადაკვანძვა იწვევს სანაღვლე სადინრების სისტემურ რეაქციას და თანმხლები რეაქციების კომპლექსს, რომელიც ჰეტეროგენურია, რაც დამოკიდებულია როგორც თავად სანაღვლე გზების, ასევე პორტული ტრაქტების კალიბრზე. ნაღვლის საერთო სადინრის გადაკვანძვა იწვევს ნაღვლის მსხვილი სადინრების გაფართოებას, მის ზეწოლას კარის ვენებზე, რის შედეგადაც ვითარდება სანათურების დეფორმაცია, პორტული ტრაქტის არქიტექტონიკის ცვლილება და პორტული უბნების გაფართოება, რაც, თავის მხრივ, იწვევს კარის ფიბროზსა და სადინრების რეაქციის ზრდას.

ნაღვლის საერთო სადინრის გადაკვანძვის პირობებში ნაღვლის მცირე სადინრები ნაკლებადაა მიდრეკილი გაფართოებისკენ, მაგრამ აქტიურად ხდება მათი პროლიფერაცია და ფართოდ შეღწევა ღვიძლის წილაკების პარენქიმაში. ამ სადინრების რეაქციის საფუძველზე ვითარდება მზარდი ინტენსივობის ფიბროზი, რომელიც აკავშირებს მომიჯნავე პორტულ ტრაქტებს ერთმანეთთან და აგრეთვე აკავშირებს პორტულ ტრაქტს და ღვიძლის ვენების წვრილი შენაკადების შემაერთებელი ქსოვილის გარსებს. ასეთი ფიბროზის პირობებში ძნელია მცირე კალიბრის ცალკეული პორტული ტრაქტის იდენტიფიცირება და უფრო მეტიც, მათ ელემენტებს შორის ურთიერთობის გარკვევა.

EFFECTS OF IONIZING RADIATION ON COGNITIVE PARAMETERS IN WHITE MICE

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Ionizing radiation has multiple effects on brain functioning, behavior, and cognitive function. These changes are largely dependent on the radiation dose. Studies revealed that ionizing radiation affects the functions of the central nervous system what results in behavior and memory changes; these changes occur as a result of a direct effect of irradiation of the central nervous system and also its indirect effects, resulted of response to irradiation of other organ systems [6].

The central nervous system is considered a radiosensitive system, and the degree of its dysfunction can be evaluated by electrophysiological, biochemical, and behavioral parameters. Impairments of these parameters can be observed after local, also total irradiation of the whole body [14]. Nowadays, there is increasing evidence literature date that the response of the central nervous system to radiation is a continuous and interactive process. Particular attention is paid to apoptotic cell (neuronal) death, as well as, mechanisms of cells' damage and death induced by secondary injury [9].

Recent studies revealed cranial radiation therapy's impact on a wide range of brain functions resulting in cognitive and memory deficiency. Radiation-induced alterations develop with a dose-volume-dependent severity. After irradiation of the brain, detrimental effects develop, acute, early, delayed, and late injuries are observed [11]. High doses of ionizing radiation induce reactive gliosis, white matter necrosis, vascular abnormalities, which are irreversible and result in clinical symptoms [4]. Low doses can also induce a wide array of cognitive dysfunctions without any significant morphological changes [7].

Dysfunction of the central nervous system is manifested after a certain interval of time (months or years) of radiation exposure. Cognitive impairment is revealed in various degrees of learning difficulties, behavior changes, and memory deficits [5].

The presence of cognitive disorders after exposure to high dose irradiation have a connection to the hippocampus glial cells and proliferating progenitor cells in the sub-granular zone of the dentate gyrus. [8]. This region is especially sensitive to ionizing radiation [1]. Doses of ionizing radiation above 1 Gy decreased the number of neural progenitor cells, which results in the arrested cell cycle, induces oxidative stress [3], and cognitive disability, memory, and learning dysfunctions [10, 12, 15].

The aim of the study was to establish the dependence of memory processes and learning ability in gamma-irradiated white mice on the age and period after irradiation.

Material and methods. The experimental protocol was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU).

Animal care and maintenance:

3-month and 1e-year-old male mice (Mus musculus), were obtained from the Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 20^0-220C and relative humidity of 50-70%. Also, a time-controlled system provided 08:00-20:00 h of light and 20:00-08:00 h dark cycles. All mice were given a standard rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes.

Experimental design:

After acclimatization for a week to laboratory conditions, the mice were divided into four different groups. The I control group - of 3-month-old not irradiated mice, II experimental group - 3-months-old irradiated mice, III control group - 1-year-old not irradiated mice, and IV experimental group – 1-year-old irradiated mice. Mice whole-body irradiation with 137Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a "Gamma-capsule-2" (group II and IV).

Spatial learning and formation of memory were estimated in the elevated-type multi-way maze and elevated plus-maze.

The maze consists of 10 platforms (40x10 cm) fixed at a height of 25 cm. The motivation for movement along the maze under test conditions was to go back in the box-nest fixed at the end of the maze.

Experiments were carried out after 48 hours and 30 days of irradiation for seven days (five trials each day). Animals were placed in the start point facing the pathway of the maze. The familiarization session consisted of free exploration of the start and familiar arms for 10 min. On the first day, the experimenter helped the animal to find the optimal way leading to box-nest.

The number of errors (deviations from optimal trajectory) and total time for crossing the maze were calculated. Analysis of the obtained numerical data allowed us to estimate the dynamics of the learning process. Free passing in the labyrinth during 10-15 sec and the achievement of automated behavior was considered as a criterion of the complete learning process.

All experimental areas of labyrinth were wiped with 20% ethanol after each trial. All behavioral experiments were conducted during the light cycle after two hours of acclimation.

Statistical analysis of data was carried out using the "Statistical Package for Social Sciences (SPSS) for Windows (SPSS version 11.0). Data were reported as mean ± SD. A significant level of 0.05 was chosen to assess the statistical significance.

Results and discussion. Monitoring of spatial learning process of two animal groups in the elevated maze showed that animals of group I (control group of 3-month-old mice) when placed in the maze for the first time, needed the help of the experimenter only in two trials of the first testing day. Later they independently opened up the new environment and demonstrated study activity. On the 5th-day, mice of the I control group completely opened up the maze, made no significant errors, and the passage time significantly decreased. On the 6th and 7th days, all mice of this group identified the shortest way to the target and spent an average of 0.20±0,04 minute. At the end of the experiment, the majority of the animals could pass the maze in 5-6 sec.

Animals of group II (experimental group of 3-month-old mice) compared to the control group showed restriction of movement. On the first day of the experiment mice of this experimental group were not able to learn the way leading to the nest, even from the last platform. On the 2d, 3-4th days mean the number of errors decreased and mice reached the target-nest less than in 1 minutes. Mice of experimental group increased the rate of path recognition, mostly, from the middle part of the maze. Though, despite the visible improvement of the spatial learning process, on the 5-6th days mean the number of errors gradually increased and on the 7th day almost approached the error number in the 1st day. Moreover, the meantime of crossing the maze increased to 2,50±0.51 minute on the 7th day of the experiment. Despite the visible improvement in the spatial learning process on 3-4th days results obtained from the II experimental group of animals differed significantly from the control group's results in both studied parameters (number of errors and time needed for crossing the maze) (Fig. 1).

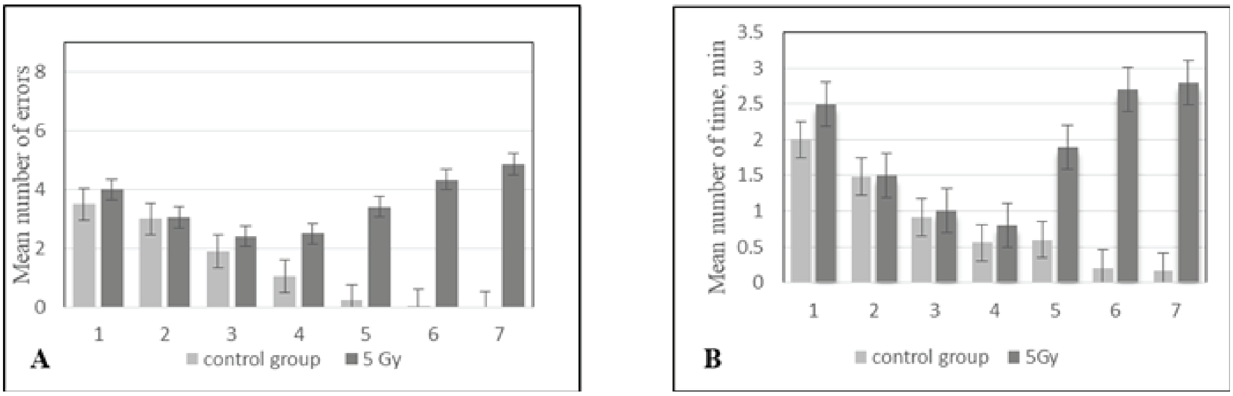

Fig. 1. Effect of gamma-radiation on the cognitive parameters of 3-month-old white mice after 48 hours of irradiation (during the 1-week period)
A - Average number of errors in control and experimental mice (groups I, II);
B- Average time to cross the maze (min) in control and experimental mice (groups I, II)

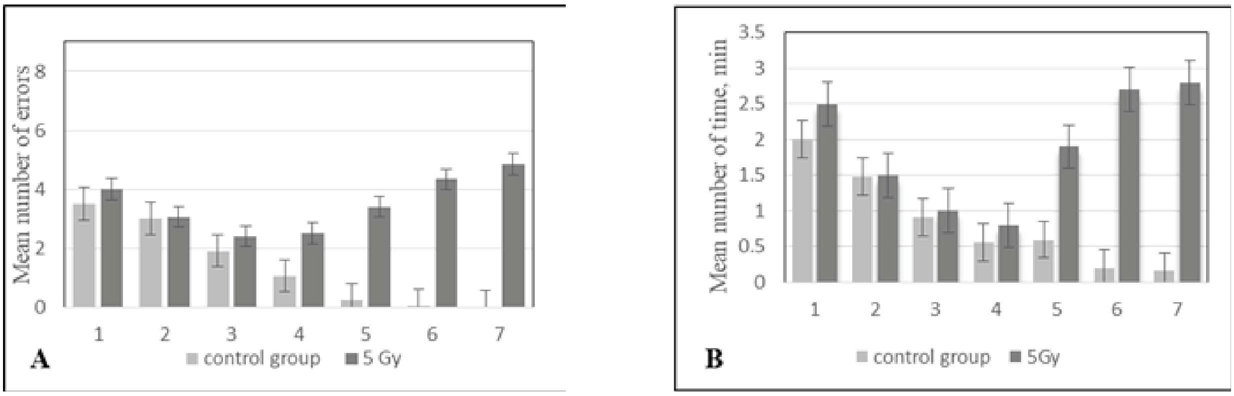

Fig. 2. Effect of gamma-radiation on the cognitive parameters of 3-month-old white mice after one month of irradiation (during the 1-week period)
A - Average number of errors in control and experimental mice (groups I, II);
B- Average time to cross the maze (min) in control and experimental mice (groups I, II)

The same test was carried out in the experimental groups after one month of irradiation. During 1-4 days of the experiment there was no significant difference between the control and experimental group in the average number of attempts needed for crossing the maze and number of errors, but from the 5th-day number of errors and average time increased (Fig. 2).

The same test was carried out in 1-year old mice. In animals of control group III on the first day of the observation number of mean errors and meantime for crossing the maze, accordingly, were equal to 3.85±0.06 and 2.04 minutes. Later they independently opened up the new environment and the number of errors decreased and on the 6th and 7th days, mice found the shortest way leading to target and spent an average of 0.19 sec.

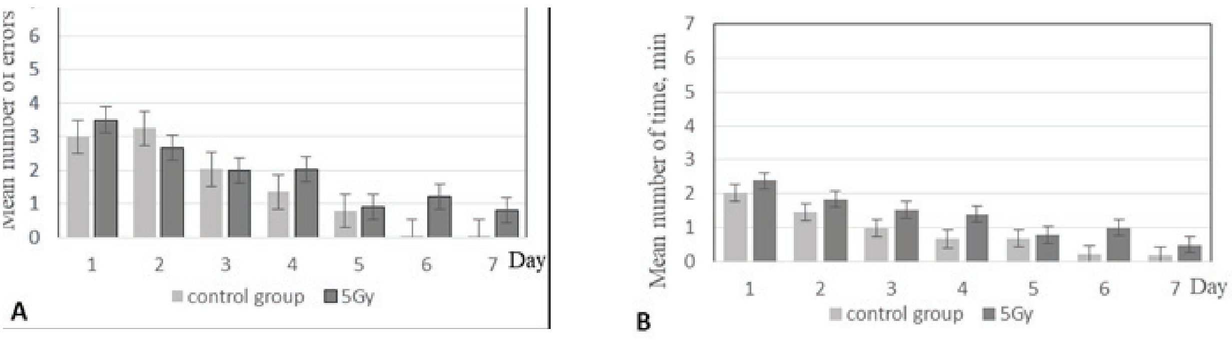

Fig. 3. Effect of gamma-radiation on the cognitive parameters of 1-year old white mice after 48 hours of irradiation (during the 1-week period)
A - Average number of errors in control and experimental mice (groups I, II);
B- Average time to cross the maze (min) in control and experimental mice (groups I, II)

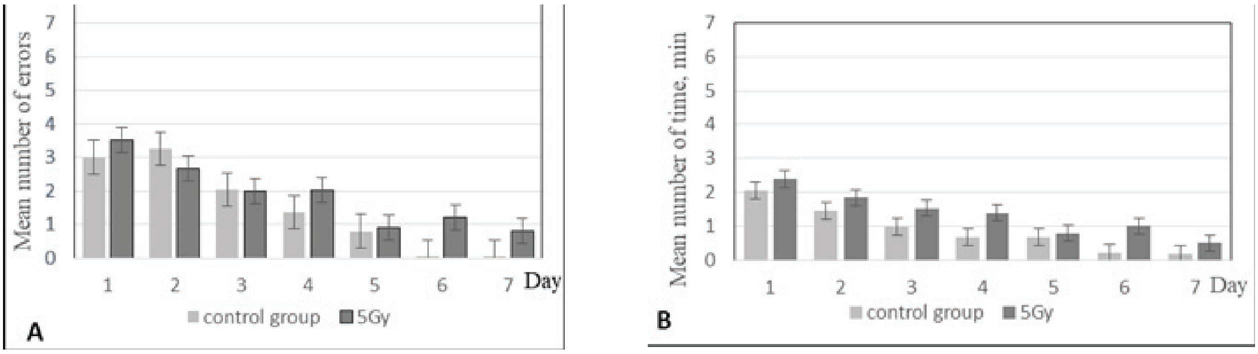

Fig. 4. Effect of gamma-radiation on the cognitive parameters of 1-year old white mice after one month of irradiation (during the 1-week period)
A - Average number of errors in control and experimental mice (groups I, II);
B- Average time to cross the maze (min) in control and experimental mice (groups I, II)

Animals of the experimental IV group showed decreased number of errors and on the 5th day of the experiment improvement of the learning process and the total average time: mice reached the target-nest in 0.79±0.3 minute and the number of errors was the same as in the control group. On 6-7th days improvement of the learning process and of the total average time needed for crossing the maze continued to decrease (Fig. 3). The same results were obtained after one month of irradiation (Fig. 4).

The results of the study indicate that ionizing irradiation with total dose 5 Gy results in a delayed spatial learning process. In different age groups of mice, the effect of radiation on the delay of the spatial learning process is different - it is especially pronounced in young (3-month-old) mice. In the old (1-year-old) mice delaying effect of the radiation on the cognitive functions was insignificant. As seems from the results of our experiment, aged animals turned out to be more radioresistant. These data match the literature data on the age dependence of radiation-induced cognitive dysfunction; epidemiological studies revealed that the risk for cognitive dysfunctions is higher during prenatal and childhood irradiation [2,12].

Conclusion. Using a laboratory white mouse model, we showed that ionizing radiation exposure causes spatial memory and behavior changes in different age groups of animals – aged mice turned out to be more radio-resistant. A study of cognitive parameters revealed that gamma irradiation can be considered as a factor inducing radiation aging, which confirms the modern view that radiation accelerates aging and mortality.

Age-related radioresistance plays an especially major role in the early stage of post-radiation recovery. Though, the late radiation aging effect formation is especially pronounced in young animals.

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SUMMARY

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The aim of the study was to establish the dependence of memory processes and learning ability in gamma-irradiated white mice on the age and period after irradiation.

The 3-month and 1-year-old male mice (Mus musculus) were used in the study. Mice whole-body irradiation was performed at a dose of 5 Gy with 137Cs by using a "Gamma-capsule-2". Spatial learning and formation of memory were estimated in the elevated-type multi-way maze and elevated plus-maze. Experiments were carried out 48 hours and 30 days after irradiation for seven days (five trials each day). The number of errors (deviations from optimal trajectory) and total time for crossing the maze were calculated.

The results of the study indicate that ionizing irradiation with a total dose of 5 Gy results in a delayed spatial learning process, causes spatial memory and behavior changes in different age groups of animals – aged mice turned out to be more radio-resistant. Age-related radio-resistance plays an especially major role in the early stage of post-radiation recovery. Though, the late radiation aging effect is especially pronounced in young animals.

Keywords: ionizing radiation, cognitive parameters, spatial learning, elevated plus maze.

РЕЗЮМЕ

ВЛИЯНИЕ ИОНИЗИРУЮЩЕГО ИЗЛУЧЕНИЯ НА КОГНИТИВНЫЕ ПАРАМЕТРЫ У БЕЛЫХ МЫШЕЙ

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Целью исследования явилось установление зависимости когнитивных параметров гамма-облученных белых мышей от возраста и периода после облучения.

В исследовании использовались трехмесячные и годовалые самцы мышей (Mus musculus). Облучение всего тела мышей суммарной дозой 5 Гр проводили с помощью ^{137}Cs в «Гамма-капсуле-2». Способность к пространственному обучению и формированию памяти оценивались в крестообразном лабиринте приподнятого типа. Исследования проводили спустя 48 часов и 30 дней после облучения в течение семи дней (пять тестов каждый день). Рассчитывали количество ошибок (отклонения от оптимальной траектории) и общее время прохождения лабиринта.

Результаты исследования показали, что ионизирующее облучение общей дозой 5 Гр приводит к замедлению процесса пространственного обучения, вызывает изменения пространственной памяти и поведения у разных возрастных групп животных - старые мыши оказались более радиорезистентными. Возрастная радиорезистентность играет особенно значимую роль на ранней стадии пост-радиационного восстановления. Однако формирование эффекта позднего радиационного старения, особенно ярко выражено у молодых животных.

რეზიუმე

მაიონიზებელი გამოსხივების გავლენა კოგნიტურ პარამეტრებზე თეთრ თაგვებში

ს. კალმახელიძე, დ. მუსერიძე, ხ. ლომაური, მ. გოგებაშვილი, თ. გაბუნია, თ. სანიკიძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; ი. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, საქართველო

კვლევის მიზანს წარმოადგენდა გამა-დასხივებული თეთრი თაგვების მეხსიერებისა და სწავლის უნარის დამოკიდებულების დადგენა ასაკზე და დასხივების შემდგომი პერიოდის ხანგრძლივობაზე.

კვლევაში გამოყენებული იყო სამი თვის და ერთი წლის მამალი თაგვები (Mus musculus). თაგვების მთელი სხეულის დასხივება საერთო დოზით 5 Gy ხორციელდებოდა ^{137}Cs–ით, „გამა კაფსულა-2"-ის გამოყენებით. სივრცითი სწავლისა და მეხსიერების ფორმირების უნარი შეფასდა ამაღლებულ ჯვარედინ ლაბირინთში. ექსპერიმენტები ჩატარდა დასხივების 48 საათის 30 დღის შემდეგ, შვიდი დღის განმავლობაში (ხუთი ტესტი ყოველდღე). გამოთვლილი იყო შეცდომების რაოდენობა (გადახრები ოპტიმალური ტრაექტორიიდან) და ლაბირინთის დასრულების საერთო დრო.

კვლევის შედეგებმა აჩვენა, რომ მაიონიზებელი გამოსხივება საერთო დოზით 5 Gy აფერხებს სივრცითი სწავლების პროცესს, ცხოველ-

თა სხვადასხვა ასაკობრივ ჯგუფებში - ასაკოვანი თაგვები უფრო რადიორეზისტენტული აღმოჩნდა. ასაკთან დაკავშირებული რადიორეზისტენტობა განსაკუთრებულ როლს ასრულებს პოსტრადიაციული აღდგენის ადრეულ სტადიაზე. თუმცა, გვიანი რადიაციული დაზერების ეფექტის ფორმირება განსაკუთრებით გამოხატულია ახალგაზრდა ცხოველებში.

DEVELOPMENT OF FORMULATION AND TECHNOLOGY OF FOAMING AGENT FROM MASTIC (PISTACIA LENTISCUS L.) GUM

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Helicobacter pylori (H. pylori) and its eradication problem is the subject of intensive research. Since its discovery (1982), this gastropathogen has been considered a serious public health problem due to its association with dyspepsia, gastritis, gastric and duodenal ulcers, and gastric carcinoma. Helicobacter pylori is detected in 50-80% of Asia's population, 70-90 percent of Africa, 30% of USA, 70% of Eastern Europe, 30-50 percent of Western Europe, and 20% of Australia's population. H. pylori infection is influenced by age, ethnicity, gender, region, and socioeconomic level [1,5,10].

Numerous investigations on the efficient eradication of H. pylori have been conducted in vitro and in vivo trials since its discovery. Proton pump inhibitors, several antibiotics, bismuth salts, and other substances were investigated. However, research show that these medication regimens are ineffective. The increase of H. Pylori antibiotic resistance is the most significant issue impeding successful therapy. Among the difficulties are the following:

- side effects of proton pump inhibitors and antibacterial drugs - cytotoxicity to the intestinal flora and general toxicity to the body [3,4,10];
- negative attitude towards taking tablets and capsules by patients and the prescription's vulnerability [2].

As a consequence, it is critical to develop local antibacterial, targeted delivery formulations for H. pylori eradication that offer extended action and are characterized by high bioavailability up to H. pylori's localization in the stomach mucosa based on the biologically active ingredient [6].

The evergreen plant Mastic gum (Pistacia lentiscus L., family Anacardiacae, genus Pistacis L.) was discovered to exhibit bactericidal effect against 11 strains of Helicobacter pylori. The European Medicines Agency (EMA) certified Pistacia lentiscus L. gum as a herbal medicine in 2015 for two therapeutic indications: moderate dyspepsia and skin inflammation/minor wound healing. Poor solubility and biological permeability, on the other hand, greatly decrease its healing capability [7].

As a result, the present challenge is to create a mechanism that allows for enhanced medication penetration across the epithelial barrier in the stomach. In this aspect, foams are very interesting.Foams are light systems, unlike solid medicinal forms, they do not swell, on the contrary, they grow in volume, completely covering the mucous membrane. Foams are considered as an alternative to solid and liquid therapeutic forms, they do not require taste correction, are designed for delivery a healing substance through the skin and mucous membranes, and for effective treatment [2,8,9].

The aim of the research was to determine the formulation of the innovative medicinal form - foam system from Mastic gum and to develop the technology.

- To achieve is the goal we have to solve the following tasks:
- Determining the formulation of the foaming powder composition containing Mastic gum based on biopharmaceutical studies;
- Development of foaming powder technology containing Mastic gum;
- Study of physico-chemical and technological characteristics of foaming powder;

Material and methods. Mastic gum, foaming agents, foam stabilizing agents, foaming structure - polyol group substances.

Biopharmaceutical, physico-chemical and technological methods of analysis were used in the research process.

Results and discussion. In the first stage, the foaming substances and their optimal concentration were determined. Surfactants were used for this purpose: (surfactants) sodium lauryl sulfate (SLS), lecithin, egg white protein, sodium dodecyl sulfate (SDS). During the research, the ability of the above substances (foaming agents) to foam, both individually and in combinations, was studied. The results are shown in Figs 1 and 2.