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Study of the hemostimulating activity of «Apicaine-R»

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Abstract

Introduction. Currently, the problem of treatment of oncological diseases is becoming relevant in medicine and pharmacy. According to the World Health Organization, the number of cancer patients is increasing every year by 10 million people. In turn, oncological diseases account for about 15 % of cases of mortality of patients from their total number, and 40 % of patients die in the first year after the diagnosis. Recovery cases from oncopathology is about 26 %. Socioeconomic relevance in solving the optimal use of limited resources of funding sources makes it necessary to conduct comprehensive medical and pharmaceutical research, including analysis of the laws of epidemiology MN.

Materials and Methods. Pharmacological, logical, mathematical-statistical, system-analytical, retrospective and comparative methods of analysis were used while conducting of researches. The study of the specific hemostimulating activity of the medicine "Apicaine-R" on the basis of bee venom was conducted on a model of myelosuppression caused by cyclophosphan.

Results and Discussion. It has been established that during 1991-2016, the incidence rate of the population in MN increased from 303.7 per 100 thousand to 315.4 per 100 thousand, or 3.4 per cent (Fig. 1). At the same time, the highest levels of SAR incidence were registered in 2010-2013 (341.2-360.3 cases per 100 thousand). It should be noted that in 2015-2016 there was a tendency for a slight increase in the incidence of cancer (by 0.57%).

In the general structure of the mortality rate of the population of Ukraine and the primary disability of the adult population, MN occupy the second place. Thus, in Ukraine, 87.468 people died in 2012, 80,526 in 2013, 67,189 in 2014, and 66,017 deaths in 2015, 78,700 in 2016. It should be noted that over One third of the deceased from MN was the population of the working age.

By studying the specific hemostimulating activity of the “Apicaine-R” on the basis of bee venom, it was found that the medicine has a stimulating effect on bone marrow bloodstream processes in mice while simulating a model of myelosuppression induced by cyclophosphan.

Conclusions. Taking into account the above, organizations providing affordable and effective medical and pharmaceutical assistance to patients with MN in the next few years will be relevant and should be considered at different levels of management of the domestic health care system.

The hemostimulating activity of the “Apicaine-R” on the basis of bee venom on the model of myelosuppression induced by cyclophosphan was studied firstly. The medicine “Apicaine-R” in doses of 0.5 mg/kg and 2.5 mg/kg effectively affected the processes of bone marrow hematopoiesis, which indicates the effective protection of the hematopoiesis system from the damaging effects of cyclophosphan. A further study of the “Apicaine-R” medicine for possible using in clinical practice is an actual.

Key words: indicators of cancer incidence, treatment of malignant neoplasms, medicine "Apicaine-R" on the basis of bee venom, hemostimulating activity.

INTRODUCTION

Currently, the problem of treatment of oncological diseases is becoming relevant in medicine and pharmacy. According to the World Health Organization, the number of cancer patients is increasing every year by 10 million people [1]. In turn, oncological diseases account for about 15 % of cases of patient’s death from their total number. Moreover, 40 % of patients die in the first year after diagnosis, and convalescence from cancer pathologies is about 26 % [2].

The "Declaration of the Rights of Cancer Patients" adopted at the European Conference "Support for Cancer Patients" (1991) and the Paris Charter for Combating Cancer (2000) defines the right of every oncologic patient to lead a full-fledged life in conditions that provide them dignity, special treatment, medical, psychological, social and labor rehabilitation [1, 2]. Over recent years, malignant neoplasms (MN) have been one of the leading places in the overall structure of morbidity and mortality of the population, both in Ukraine and worldwide. According to the literature data, the pharmaceutical provision of this group of patients requires the involvement of enormous financial resources [3, 4]. Socioeconomic relevance in solving the optimal use of limited resources of funding sources makes it necessary to conduct comprehensive medical and pharmaceutical research, including analysis of the laws of epidemiology MN. The study was conducted on the basis of officially published data on the morbidity and mortality of patients with MN for 1991-2016 according to the National Cancer Registry of Ukraine bulletin [5]. In the course of research, logical, mathematical-statistical, system-analytical, retrospective and comparative analysis methods were used.

It has been established that during 1991-2016, the incidence rate of the population in MN increased from 303.7 per 100 thousand to 315.4 per 100 thousand, or 3.4 per cent (Fig. 1).

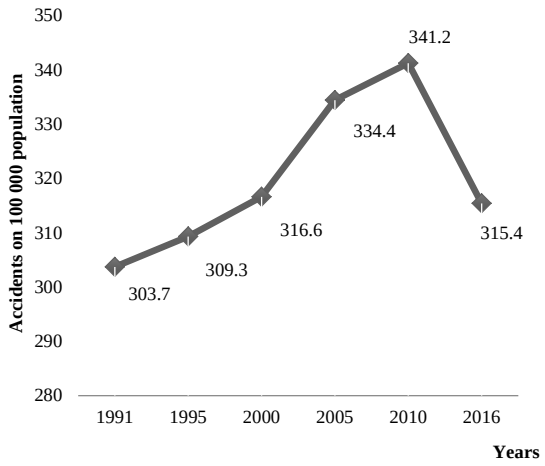

Fig. 1. Dynamics of indicators of morbidity of the population of Ukraine in MN during 1991-2016 (per 100 thousand population)

At the same time, the highest levels of SAR incidence were registered in 2010-2013 (341.2-360.3 cases per 100 thousand). It should be noted that in 2015-2016 there was a tendency for a slight increase in the incidence of cancer (by 0.57%).

According to the European database "Health for All", the prevalence of MN in Ukraine is 2.4% of the population, in the countries of the European Region of the WHO – 2.3%, in the EU countries - 2.7%. In 2016, the contingent of patients with MN in Ukraine was 985.4 thousand people. Compared to 2015, it has increased by 23.6 thousand.

In the general structure of the mortality rate of the population of Ukraine and the primary disability of the adult population, MN occupy the second place. Thus, in Ukraine, 87.468 people died in 2012, 80,526 in 2013, 67,189 in 2014, and 66,017 deaths in 2015, 78,700 in 2016. It should be noted that over One third of the deceased from MN was the population of the working age.

The standardized mortality rates of the population from the ZN in Ukraine during the period of 1991-2016 tended to decrease (-11.7 %), which coincides with the European tendency (-12.1%) and the tendency in EU countries (-14.2 %). At the same time, standardized indicators of mortality rates of the population of working age population in Ukraine are 35.6% higher than in the EU countries and 27.3% are the average indicators in the WHO European Region as a whole. Ukraine occupies the sixth place in terms of the standardized indicator of mortality of the working-age population from oncological pathology.

Taking into account the above, organizations providing affordable and effective medical and pharmaceutical assistance to patients with MN in the next few years will be relevant and should be considered at different levels of management of the domestic health care system.

The first results of study of the influence of bee venom on tumor cells were published in 1950 by Havas. After 30 years, other groups of scientists began to conduct research on the cytotoxicity of bee venom on various lines of tumor cells.

The effectiveness of bee venom is due to the properties of the active substances in its composition, in particular, melittin - a cell membrane modifier responsible for stimulating activity of adrenal hormones, phospholipase A2 and hyaluronidase, apamin - a natural neuromodifier, as well as peptide, etc. (histamine, catecholamine and polyamines).

Recently, the number of scientific publications has significantly increased with a description of the perspective effects of bee venom on various tumor cell lines, showing not only the results of bee venom action, but also the characteristics of signaling pathways through which the poison inhibits cell proliferation [8-12].

Malignant blood diseases (hemoblastosis, leukemia) affect people of all ages, including the elderly and newborns, are equally often seen in men and women.

Leukemia is characterized by the uncontrolled growth of blood cells in the bone marrow. It is an extensive group of diseases, different in their etiology. In leukemia, a malignant clone can come from both immature hematopoietic cells of the bone marrow, and from developing and normal blood cells.

The International Agency for Research on Cancer (IARC) provided data showing that about 12.7 million people were sick with cancer and about 7.6 million died from cancer. Malignant blood diseases such as leukemias, Hodgkin's lymphomas, non- Hodgkin's lymphomas and myelomas account for about 9 % of all cancers in the economically developed countries of the world.

The molecular mechanisms of the antileukemic activity of differential polyphenols of bee honey on various cell lines (HL-60, 562, U937, NALM-6, YCUB) were studied *in vitro* experiments.

There are no data about effect of bee venom on leukemia *in vitro* and *in vivo* experiments in the available literature [13-17].

Analyzing the foregoing, a new anti-anxiety medicine "Apicaine-R" was developed (Patent for invention No. 111273 UA "Lyophilized medicine for injection") on the basis of bee venom substance at the National University of Pharmacy (Kharkiv, Ukraine) under the direction of Academician of the Ukrainian Academy of Sciences, Doctor of Pharmacy, Professor A.I. Tikhonov.

The purpose of our work is to study the specific hemostimulating activity of the medicine "Apicaine-R" on the basis of bee venom using a model of myelosuppression caused by cyclophosphan.

MATERIALS AND METHODS.

The experiments were conducted on 100 nonlinear adult mice of males weighing 20-25 g.

Animals were obtained from the nursery of laboratory animals of PE "Dali-2001" (Kiev). During the experiment, animals were kept in a vivarium at the temperature of 18-20 °C, humidity of 50 – 60 %, natural "day-night" light mode, in plastic boxes, on a standard diet.

Researches were approved by the State Committee on Bioethics of the State Enterprise "State Scientific Center of Drugs and Medical Products". All studies were conducted in compliance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" [18].

Table 1. The effect of the medicine on the total number of megakaryocytes

Control intact	Control pathology	Dose, 0.5 mg/kg	Dose, 2.5 mg/kg	Dose, 5.0 mg/kg
2 days				
119.0 ± 5.88	53.30 ± 5.68*	52.50 ± 12.59*	107.3 ± 14.48	155.0 ± 36.36**
7 days				
139.8 ± 11.12	63.80 ± 5.36*	74.50 ± 6.77*	74.30 ± 6.75*	37.50 ± 6.85*,**
14 days				
145.0 ± 8.71	96.00 ± 5.25*	83.75 ± 10.19*	97.50 ± 12.44*	93.75 ± 43.75

Note: * - $p \leq 0.05$ comparative to intact control.
**- $p \leq 0,05$ comparative to positive control.

The medicine “Apicaine-R” on the basis of bee venom in the form of injection is used in researches. It is developed in the laboratory of "Parenteral and oral liquid dosage forms", National University of Pharmacy.

Animals were divided into 5 groups: the 1st group is a control, the 2nd is an untreated control, the 3rd, 4th, and 5th are experimental groups. In each group there were 15 mice.

The cyclophosphan was chosen to simulate a bone marrow suppression. It is an alkylating and antimetabolic cytostatic which is compromise process of DNA and RNA synthesis, the vital activity and mitosis of blood cells, especially in the lymphoid tissue and bone marrow [19].

Cyclophosphan (Endoxan®, ser. 41029 C, shelf life until 09. 2017 u /, produced by Baxter Oncology, Germany) was introduced to mice at a dose 200 mg/kg intraperitoneally in a volume of 0.2 ml. The indicated dose and the scheme of administration of cyclophosphan correspond to the literature data on its using as an inhibitor of hemopoiesis in experiments on rats [20].

The medicine was introduced to mice of the experimental groups during of 14 days daily, once a day, the animals received 0.2 ml of subcutaneous injections of the medicine “Apicaine-P” in doses of 0.5, 2.5 and 5 mg/kg (according to the active substance).

Selected doses of the medicine are in the range of their effective doses in various experiments on animals [20-23].

As a control served: a group of untreated rats, which were administered cyclophosphan according to the indicated scheme without treatment, as well as a group of intact rats, whose indicators were used as a physiological norm. Animals of both control groups were injected 0.2 ml of physiological solution of sodium chloride during 14 days subcutaneously.

The effectiveness of the medicine was evaluated by the criteria of morphological indicators of the bone marrow: 2, 7 and 14 days (5 mice from each group).

A momentary decapitation was used for animals removing from the experiment. Bone marrow was aspirated from the femur of mice and count the total number of megakaryocytes and myelogram counts. Megakaryocytes were counted in the Fuchs-Rosenthal count chamber. Bone marrow smears for counting of myelogram were fixed in a May-Grunwald fixative and repainted by Romanowsky stain [24]. For the calculation, 500 nucleated cells were differentiated and the absolute values obtained were expressed as a percentage of the total number of cells [25].

Microscopic researches were carried out using a microscope BIMAM R-12.

Statistical processing of the results of the bone marrow researches was performed using the nonparametric method of Mann-Whitney; the other results were calculated according to the methods of the variance analysis generally accepted in pharmacology. The probability of the results obtained was estimated at a significance level of at least 95 % ($p \leq 0.05$) [26, 27].

RESULTS AND DISCUSSION.

The effect of the medicine on the number of megakaryocytes

Introduction of cyclophosphan to mice caused a statistically significant decrease in the total number of megakaryocytes on the 2nd and 7th day. On the 14th day there is a tendency to increase the total number of megakaryocytes (Table 1, Fig. 2).

The introduction of the medicine to animals in dose of 0.5 mg/kg caused a similar change during the observation period. A dose of 2.5 mg/kg caused a decrease in the total number of megakaryocytes on the 7th day, with a further increase on the 14th day, compared with the intact control. In the group receiving the drug at a dose of 5.0 mg / kg, the total number of megakaryocytes increased in three times as compared with the group treated with cyclophosphan, with a further sharp decrease in megakaryocytes compared with the intact control and positive control. On the 14th day there is a tendency to an increase in the total number of megakaryocytes in all experimental groups as compared with the control group (Table 1, Fig. 2).

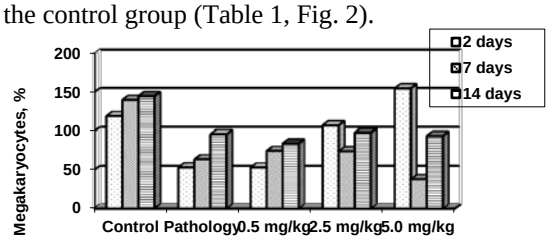

Fig. 2. The effect of the medicine on the level of megakaryocytes in the blood of mice exposed to cyclophosphan.

The effect of the medicine on the myelogram of mice

A single injection of cyclophosphan in a dose of 200 mg/kg caused to the suppression of bone-marrow hemopoiesis. There was a decreasing in lymphocytic, erythrocyte and monocytic pool. On the 2nd day there is a sharp decrease in promyelocytes, myelocytes and metamyelocytes, the number of which reaches a normal value on the 7th day. Banded neutrophils are decreased by 2 times on the 2nd day, and on the 7th and 14th day their number was increased by 1.5 times. The decrease in cells of the

erythroid series is observed on the 2nd and 7th day by an average of 2-5 times, with further normalization on the 14th day. The number of monocytes and lymphocytes is varied within the physiological norm (Fig. 3-17).

The introduction of the medicine during 14 days at a dose of 0.5 mg/kg against the background of myelosuppression caused by cyclophosphan is presented in Figures 3-17. On the 2nd day there is a decrease in the banded neutrophils with their further increase by 2 times on the 7th and 14th day, compared with the control group. There is a statistically significant increase in the number of lymphocytes (by 5 times), with further normalization till the end of experiment.

At the introduction of the medicine to mice at a dose of 2.5 mg/kg, a suppression of the lymphoid lineage and increasing in the erythroid lineage was observed compared with the control group and untreated control group. On the 7th day there is a recovery of all lineages. An exception is the increase in the number of banded neutrophils compared with the control group.

The introduction of the medicine to mice at a dose of 5.0 mg/kg caused a similar pattern of changes in the number of banded neutrophils. The number of erythroid row in this group also varied compared with the control group. There was an increase in lymphocytes on the 2nd day, compared with the control group and further nominalization to the control values on the 7th and 14th day (Fig. 3-17).

Thus, the introduction of the medicine “Apicaine-R” provided a stimulating effect on the processes of bone marrow hematopoiesis under the conditions of myelosuppression, caused by cyclophosphan, more pronounced with the introduction in doses of 0.5 and 2.5 mg/kg.

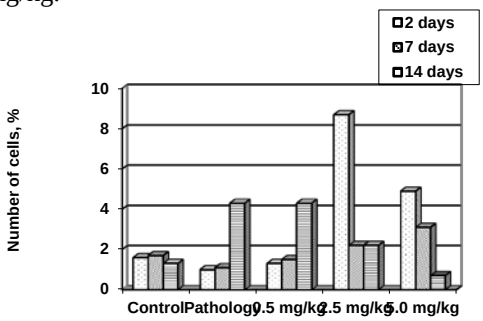

Fig. 3. The effect of the medicine on the number of myeloblasts in the bone marrow of mice exposed to cyclophosphan.

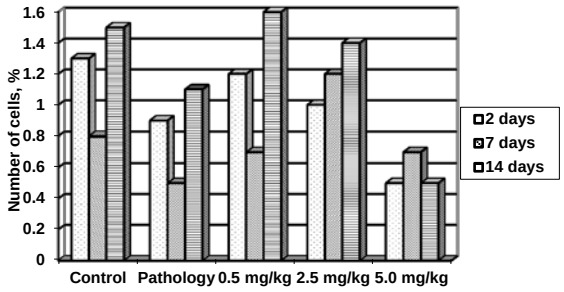

Fig. 4. The effect of the medicine on the number of promyelocytes in the bone marrow of mice exposed to cyclophosphan.

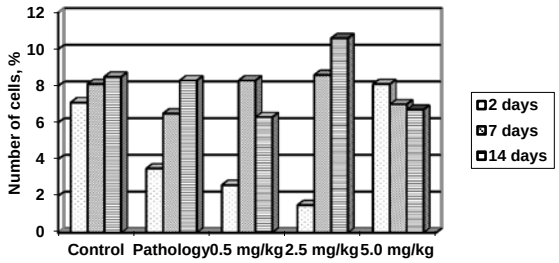

Fig. 5. The effect of the medicine on the number of myelocytes in the bone marrow of mice exposed to cyclophosphan.

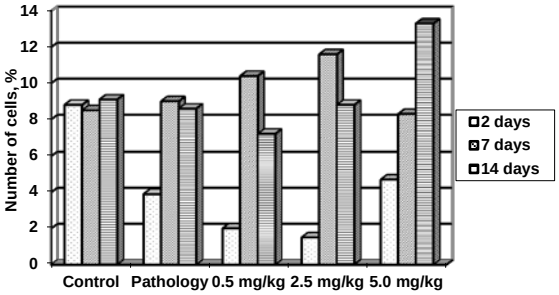

Fig. 6. The effect of the medicine on the number of metamyelocytes in the bone marrow of mice exposed to cyclophosphan.

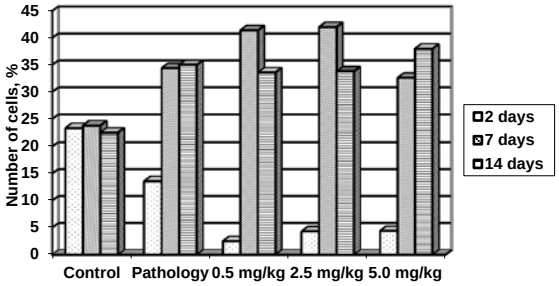

Fig. 7. The effect of the medicine on the number of banded neutrophiles in the bone marrow of mice exposed to cyclophosphan.

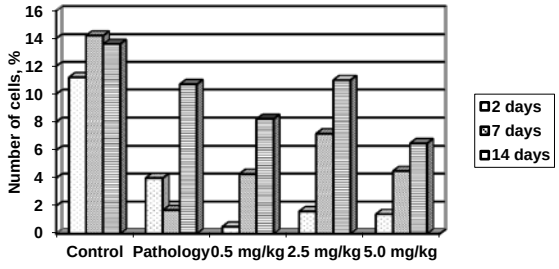

Fig. 8. The effect of the medicine on the number of segmentonuclear neutrophils in the bone marrow of mice exposed to cyclophosphan.

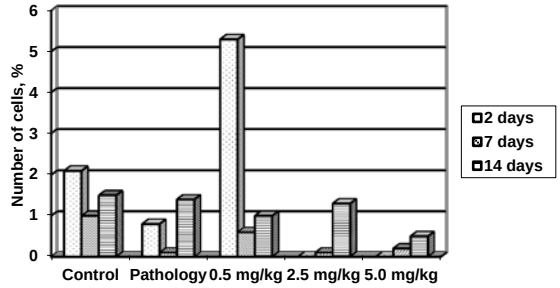

Fig. 9. The effect of the medicine on the number of eosinophils in the bone marrow of mice exposed to cyclophosphan.

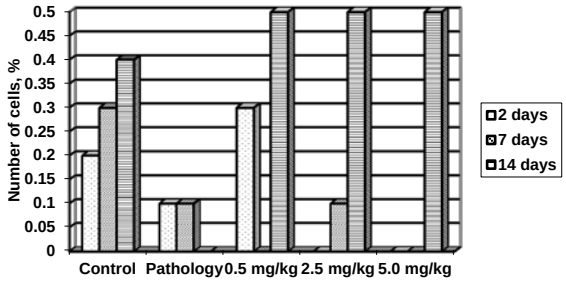

Fig. 10. The effect of the medicine on the number of banded basophils in the bone marrow of mice exposed to cyclophosphan.

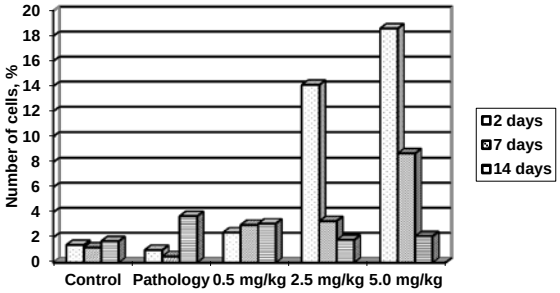

Fig. 11. The effect of the medicine on the number of erythrocytoblasts in the bone marrow of mice exposed to cyclophosphan.

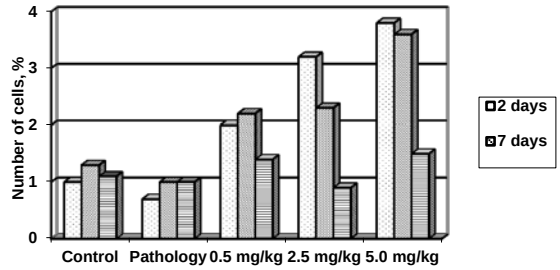

Fig. 12. The effect of the medicine on the number of pronormoblasts in the bone marrow of mice exposed to cyclophosphan.

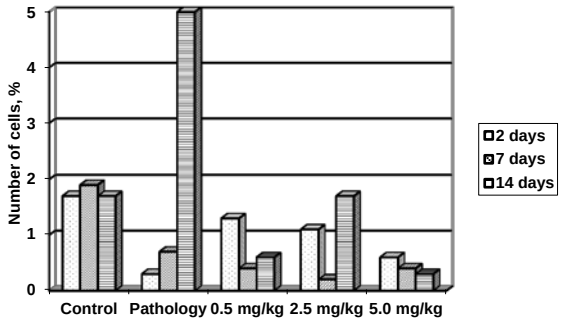

Fig. 13. The effect of the medicine on the number of normoblastic basophils in the bone marrow of mice exposed to cyclophosphan.

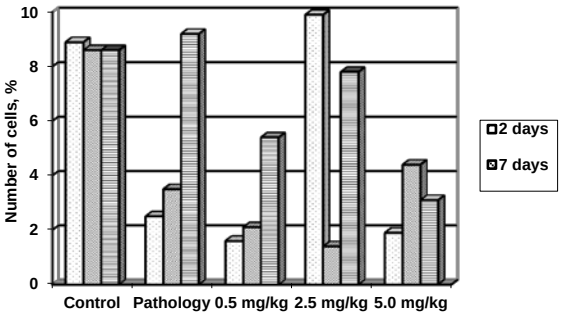

Fig. 14. The effect of the medicine on the number of polychromatophilic normoblasts in the bone marrow of mice exposed to cyclophosphan.

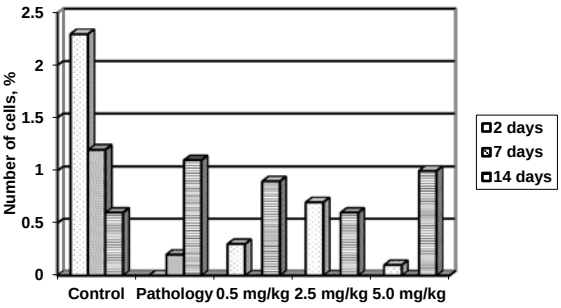

Fig. 15. The effect of the medicine on the number of oxyphilic normoblasts in the bone marrow of mice exposed to cyclophosphan.

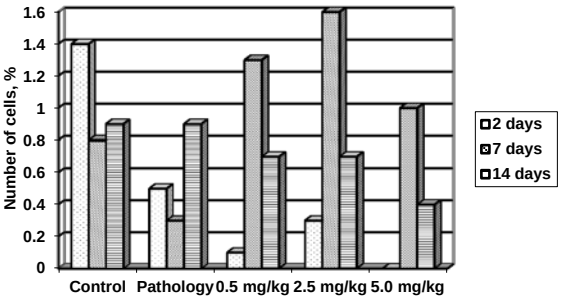

Fig. 16. The effect of the medicine on the number of monocytes in the bone marrow of mice exposed to cyclophosphan.

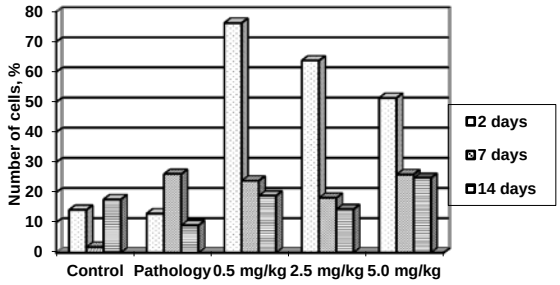

Fig. 17. The effect of the medicine on the number of lymphocytes in the bone marrow of mice exposed to cyclophosphan.

DISCUSSION

As a result of the conducted researches, it follows that the medicine "Apicaine-R" has a stimulating effect on the bone marrow hematopoiesis in mice when simulating their model of myelosuppression caused by cyclophosphan.

CONCLUSIONS

For the first time, the hemostimulating activity of the medicine "Apicaine-R" on the basis of bee venom was studied on a model of myelosuppression caused by cyclophosphan.

The medicine "Apicaine-R" in doses of 0.5 mg/kg and 2.5 mg/kg effectively affected the processes of bone marrow hematopoiesis, which indicates the effective protection of the hematopoietic system from the damaging effects of cyclophosphan.

The further investigating of the medicine "Apicaine-R" for possible use in clinical practice is actual.

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