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HEMATOPOETIC STEM CELL RESPONSE TO STIMULATION OF EXTRACTS FROM ANIMAL MATERIALS

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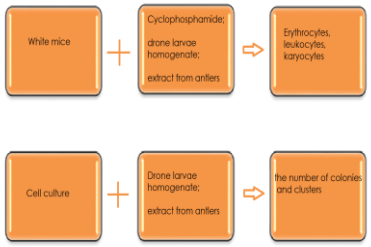
Graphical abstract



Abstract

The article presents the results of the research hemostimulating activity of aqueous extracts of antler young Siberean stag and drone larvae homogenate. These substrates were obtained from raw materials of animal origin. Altai Krai and Altai Republic are subjects of the Russian Federation which is the place of production of the raw material. Experiments were conducted in two stages. The first stage - in vitro, which included a research of experimental substrates on the culture of mouse marrow cells. During the experiments were obtained different results. We counted the number of colonies grown in cell culture for this. The second stage of experimenters - in vivo. It included an assessment of the myeloprotector on model of cytostatic myelosuppression of mice and analysis of bone marrow and peripheral blood.

Keywords: Hemostimulating activity, siberean stag, drone larvae homogenate

1.0 INTRODUCTION

Degenerative diseases (Alzheimer's disease, Parkinson's disease, hepatic cirrhosis) are a serious problem in modern society. Process degeneration dominates the process of recovery and regeneration in humans in health. This is due to the failure and incompetence of the deep mechanisms of regulation. Regeneration is a complex integrated process, which is implemented by a variety of intermediaries and regulatory mechanisms. Against the background of what has been said a certain interest antlers of Siberian stag. This is a prime example of the high activity of the regeneration phase of active growth antlers of Siberian stag increased by 2-3 cm every day. The activity due to the high rate of proliferation and differentiation of stem cells. This occurs when there is a sufficient amount of nutrients and regulators - hormones, growth factors.

Each year deer shed their antlers in winter, but in spring and summer, they grow again become greater than before. This genetic program activated every year, it has considerable resistance to various failures and breakdowns in the genetic apparatus. Since we do not have information about the transformation of antlers stem cells into cancer or a higher incidence of tumors compared to other mammals, although the rate of growth antlers of Siberian stag large. This is possible only if there is a strong regulatory system that is resistant to various types of genetic damage. Unfortunately, these regulatory mechanisms have not been investigated so far, despite the long history of the use of deer antlers. Not found active ingredients - growth and differentiation factors that are responsible for the growth of antlers of siberian stag. In this connection, the search for active substances previously unknown and are able to stimulate the activity of stem cells to promote their

release from the bone marrow and the entry to the affected organ or tissue is an urgent task for Regenerative Medicine. Antlers of Siberian stag are very interesting object of scientific research. They possess anti-inflammatory, antioxidant and hepatoprotective effects [6, 7]. The history of their use in countries such as China, Korea and Japan goes back several thousand years, but the study of antlers of Siberian stag are still relevant. They have a regenerative, immunomodulatory [6], hypotensive, adaptogenic effects; they are able to stimulate the proliferation of leukocytes and fibroblasts[7]. Aminoglycans selected from antlers can stimulate the regeneration of cartilage tissue [8]. Polypeptides have immunomodulatory capacity [6]. It shows that these peptides are capable of stimulating the proliferation of fibroblasts, epithelial progenitors, T - and B - lymphocytes, red blood cells, and stimulate phagocytic activity of lymphocytes. The Russian Federation is the world's largest supplier of products and maral breeding is well developed. Altai Krai and Altai Republic is one of the leading regions in terms of maral breeding, antlers of siberian stag for us is a renewable source of raw materials. Every year in the Altai Krai and Altai Republic produced 63 tons of antlers, of which only 5% is used for domestic needs, the rest is exported. The homogenate drone larvae is a promising product of beekeeping. It's obtained by grinding the larvae of drones. Thus composed drone larvae homogenate contain a wide range of nutrients: growth factors, hormones, vitamins, minerals, amino acids and phospholipids [9]. Many years of experience with the drone homogenate in China and the results of its impact leaves no doubt the feasibility and effectiveness of this product. Growth and differentiation factors contained in the drone homogenate mechanisms of action that have not been studied in detail so far can serve as a tool to explore the possibilities of stimulation of the regeneration of tissues, organs, and drug development corresponding destination. Virtually no studies on the activity of the homogenate drone larvae, despite the fact that it is a very promising product beekeeping comprising a wide range of biologically active molecules. Bee larvae in the development process undergoes a metamorphosis and is transformed into an adult bee. This process occurs due to processes of cell differentiation and transdifferentiation larvae, and they in turn are activated under the influence of various growth factors and hormones. The biologically active molecule responsible for these processes have not yet been found. In this regard, a study was conducted hemostimulating activity the homogenate drone-brood in vivo and in vitro to in order to assess the prospects for further work in this direction. It is known that the formation of new blood cells is possible thanks to the pool of poorly differentiated stem cells in the bone marrow. Stem cells being exposed to growth factors further differentiate into germ cells of hematopoiesis. Therefore, assessing the effect of extracts from antlers of Siberian stag and the homogenate drone-brood in vitro model can identify their effect directly on the stem cells, and in animal experiments to evaluate hemostimulating activity.

2.0 EXPERIMENTAL

For research use white mice weighing 22-25g. Animals were kept in vivarium under stationary conditions where they received a standard diet and water ad libitum. Myelosuppression caused by a single intraperitoneal administration of cyclophosphamide at a dose of 80 mg / kg in a volume of 0.2 ml. It was 6 experimental groups of animals. The mice of the first group were control group. The second group consisted of mice received a single injection of cyclophosphamide intraperitoneally. Mice of the third group were injected once cyclophosphamide and drone larvae homogenate 0.2 ml intragastrically daily for 5 days. The fourth group received homogenate drone larvae of the above-described scheme.In the fifth group modeled myelosuppression and intragastrically an extract from antlers of young Siberian stag 0.2 ml intragastrically for 5 days. Counts the total number of red blood cells, total number of white blood cells, total number of karyocytes. For in vitro experiments homogenate drone larvae and extract from antlers was filtered through a membrane filter. Further dilutions of the homogenate were prepared drone larvae and antler extract in a ratio of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048. To study the effect homogenate drone larvae and antler extract the formation of granulocytic colonies was prepared the nutrient medium of the following formulation: 79% RPMI-1640, 1% methylcellulose, 20% fetal calf serum, 280 mg / ml L-glutamine, 4 mM 2-mercaptoethanol; 50 mg / l gentamicin. The concentration of viable elements adjusted to 2×10(5) to 1 ml of substratum. Next a substratum gently aspirated and not adhered to the plastic during the incubation cells were washed twice. Concentration not adherent viable bone marrow nuclears adjusted to 2 × 105 to 1 ml of the composition: 89% RPMI-1640, 1% methylcellulose, 10% fetal calf serum, 280 mg / ml L-glutamine, 50 mg / l gentamicin 1 IU / ml recombinant erythropoietin. 0.2 ml of cell suspension were placed in 96-well plates. Each line correspond to his own breeding tablet. The first line corresponds to the number of pure homogenate, the second - 2-fold dilution, third – 4-fold etc. until 2048-fold dilution, respectively. Drone larvae homogenate added 5% of total wells. Similar actions were carried out with an extract from antlers. The control plate was planted without drone larvae homogenate and extract from antlers. This study granulocyte colony plates were cultured for 7 days in a CO2 incubator at 37. ° C, 5% CO2 and 100% humidity, and in the case of erythroid colonies plates incubated 3 days under the same conditions. After incubation the number of colonies and clusters were counted. Statistical processing was performed using the U-test Mann-Whitney. The level of statistical significance was considered significant if the probability of error does not exceed 0.05.

3.0 RESULTS AND DISCUSSION

The group of myelosuppression received cyclophosphamide was noted a reduction of the total number of white blood cells up to 23% and 15% respectively of the targets on 3 and 5 day, and then began to rise, and CPR for 10 hours reached 69% of baseline values (Figure 1). The introduction of homogenate on the background of myelosuppression on day 3 resulted in an even greater inhibition of leukocyte germ (to 18.5% of the control). Further, in the treatment of homogenate were recorded much more rapid in comparison with the group myelosuppression recovery total number of white blood cells, the total number of white blood cells on day 10 reached 121% of the control. Intake extract of antlers on the background of myelosuppression caused by 3 day almost complete lack of white blood cell (10% of control), then the rate began to rise, reaching a 10 day 172% relative to baseline values. When the antler extract administered to mice whom does not conducted myelosuppression at 3d day caused an increase in white blood cells, for 5-8 hours index was above 150% on day 10 and the total number of white blood cells reached 193% of control values.

A total number of karyocytes in the group with myelosuppression throughout the experiment was significantly lower than the control group (Figure 2). The group that received cyclophosphamide and homogenate the total number of karyocytes was higher than the corresponding values of the group with myelosuppression, but significantly lower than background values, there were observed on 8th days maximum the total number of karyocytes - 89% of the baseline value, then the figure dropped to 67%.

The group that received an extract antler with cyclophosphamide on 3d day showed a stronger bone marrow suppression, in comparison with a group of myelosuppression; on 5th day indicators were equal; on day 8 was observed jump the total number of karyocytes to 80% of control, and on day 10 - 30% reduction from the background values. In the group receiving only homogenate the total number of karyocytes on 8th day it was lower than the other groups at 10th day and reached their exceeded 70% of control values. The group receiving antler extract, marrow depression was observed, with a minimum value (30%) for 5 days, on 8th - 10th day indicator has reached 70% of control values. Graph in Figure 3 shows the granulocyte colony growth.

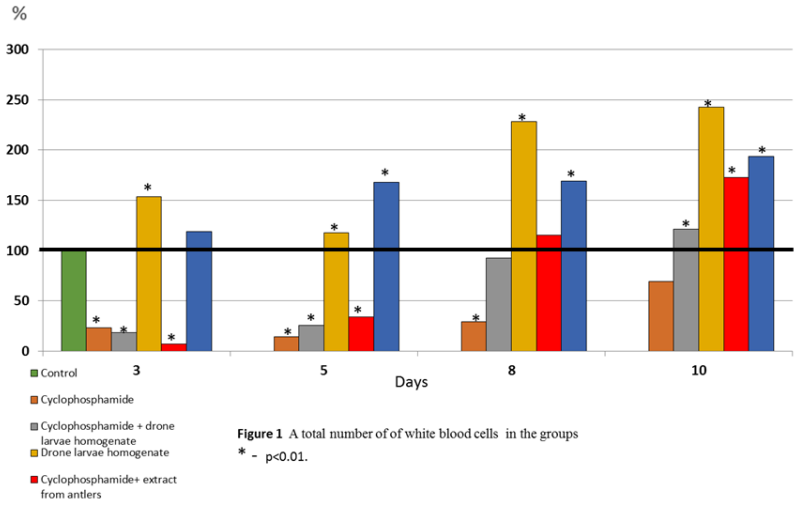


Figure 1 A total number of white blood cells in the group

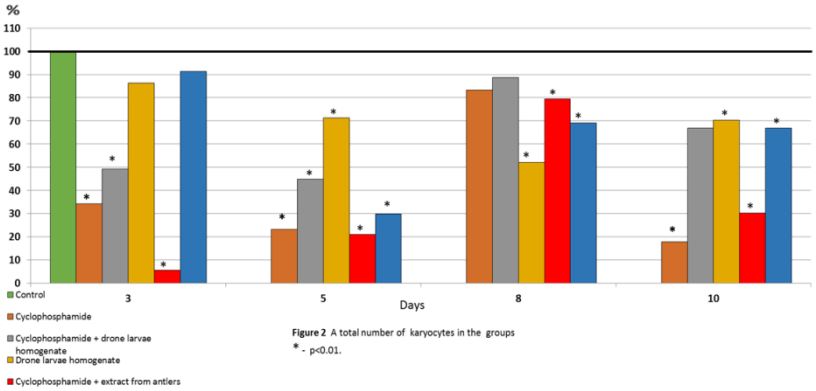


Figure 2 A total number of karyocyte in the group

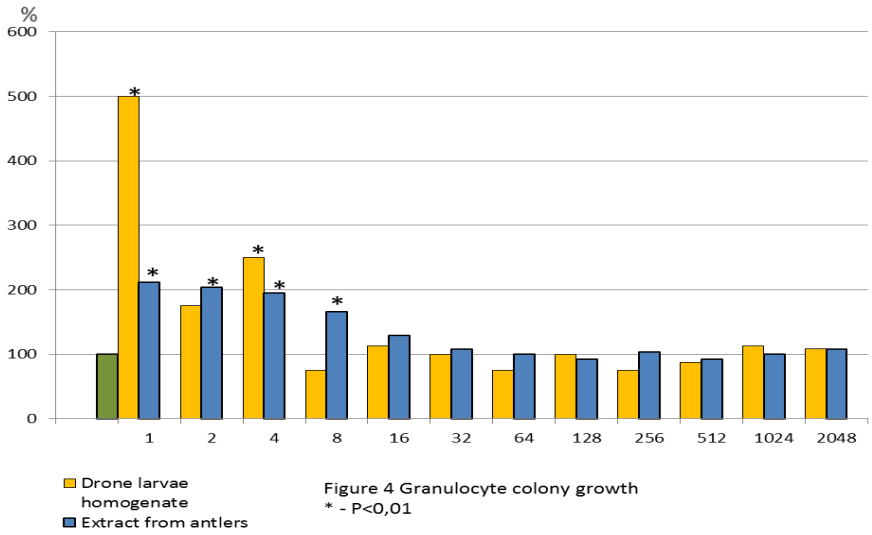


Figure 3 Granulocyte colony growth

The number of colonies at the initial extract homogenate drone-brood addition was 5 times the baseline values. Stimulates the activity continued until dilution 4 times, then the number of cell colonies was at the level of the background.

At the same time stimulating activity of the extract from antlers of Siberian stag were more resistant to dilution. This value is slightly decreased and persisted until the breeding 8 times, which was 170% from the background.

Source extract of antlers of Siberian stag showed less stimulation of colony formation than the the homogenate drone-brood - 2.8 times higher than the background values. However, the stimulating effect persists up to sixteen fold dilution, where the number of colonies was 1.5 times greater than baseline values.

4.0 CONCLUSION

In this study, PJ90 was isolated from forest soil in Suranaree University of Technology, Thailand. Based on morphological characteristics and 16S rRNA gene analysis, PJ90 might be classified as *Streptomyces triostinicus*. This strain has been shown to produce antimicrobial compounds which is mainly active against Gram-positive bacteria and yeasts. The study of this isolate could be further explored for the development of new antibiotic drugs to treat infectious diseases causing by pathogenic and drug-resistant strains.

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