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Original paper

# Morphological parameters of spleen and thymus of the male rats on the basis of the hirudological influence of *Hirudo verbana*

**Ruslan AMINOV[1], Alina AMINOVA[1], Lyudmyla MAKYEYEVA[2]**

[1]Cellular and Organism Biotechnology Laboratory, Faculty of Biology, Zaporizhzhia National University, Zhukovsky Street 66, 69600 Ukraine
[2]Department of Histology, Cytology and Embryology, Zaporizhzhia State Medical University, Zaporizhzhia, Maiakovskyi Avenue 26, 69035 Ukraine

Corresponding Author: Ruslan Aminov; e-mail: 91_amin_91@ukr.net

**ABSTRACT.** The aim of this work is to study the morphological parameters of the spleen and thymus of rats along with hirudological influence from *Hirudo verbana*. The work was carried out on 40 male outbred laboratory rats weighing 280–300 grams, age 6.5–7 months. Animals that had passed the quarantine regime and had no external manifestations of the disease were used for the study. Manipulations with animals were performed in compliance with regulated norms and rules for the treatment of laboratory animals. For the study, two groups of animals were formed – control and experimental. After fixation of rats, the medical leech *Hirudo verbana* with an average weight of 0.8 ± 0.1 g was put on the animals of experimental group. The place of setting is the coccyges zone. Four times with an interval of one time per week. Then the animals were decapitated and the spleen and thymus were removed for histological examination. The white pulp of the spleen was analyzed – the area and diameter of the lymphoid follicles and central arteries, the number of lymphocytes per unit area (400 μm$^2$) in the lymphoid follicles, in the thymus – the number of cells per unit area, both in the cortex and in the medulla. Under the influence of biologically active substances of the medicinal leech in the white pulp of the spleen, the size of the lymphoid follicles and central arteries increases, the number of lymphocytes per unit area (400 μm$^2$) of the lymphoid follicle increases, and in the thymus, both in the cortex and in the medulla, the number of cells increases per unit area (400 μm$^2$), the predominance of the cortical over the medullar ones. As a result of the obtained experimental study, we revealed the immune stimulating effect of *Hirudo verbana* on the organs of the immune system of laboratory rats: thymus and spleen.

**Keywords:** medicinal leech, hirudological influence, morphogenesis, spleen, thymus

## Introduction

As a result of environmental degradation, the use of xenobiotics, the increase of number of allergies, viral and bacterial diseases, immunodeficiencies of various etiologies, has led to heightened interest of biologists and immune morphologists to find different methods of influencing the immune system [1–3]. Basically, how they affect the structure of the thymus as an key central organ and the spleen as an important peripheral organ of the immune system seem to be worth of consideration [4, 5]. As long as under the influence of various adverse factors primarily disrupts the structure of lymphoid organs of the immune system, changes the relationship of immune competent cells, the content of these biologically active substances [6,7]. Also, immune deficiency is manifested by dysfunction of cellular and humoral parts of the immune system [8]. Researchers have found that removal of the spleen leads to the development of accidental involution of the thymus and suppression of immunity [9]. The complex anatomical structure of the structural components of the white pulp of the spleen, cortical and medullar substance of the thymus, which have both T. and B. zones, creates favorable conditions for cell cooperation in the immune response [10]. Researchers proved that the spleen and thymus of rats have a similar histological structure to that of humans. The spleen is an organ of hematopoiesis

and immune protection. In rodents, it is a universal organ of hematopoiesis, where erythrocytes and granulocytes are produced, as well as an important organ of lymphocyte formation and immune response. The spleen supports cellular and humoral immune responses, innate and acquired immunity, quantitative and qualitative composition immune cells of blood, lymph and other lymphoid organs [11]. Therefore, the study of the morphological structure of the spleen and thymus is an urgent task in testing various methods of influencing the immune system [12]. One of such methods of influencing the immune system is hirudological influence. It is widely used in veterinary medicine and agriculture. In veterinary medicine it is used in the treatment of mastitis and increasing reproductive capacity in cows [13–17], in the treatment of vascular diseases of horses, endometriosis, intoxication, hematomas in cats and dogs [18–21] and other domestic animals [22]. The researchers registered a positive effect of hirudological influence on goat breeding: increased appetite, improved fur condition, disappearance of cracks on the horns and hooves, body weight, milk yield and the number and body weight of their offspring. In goats there was a migratory redistribution of blood lymphocytes with their temporary deposition in places of attachment of leeches, increase in phagocytotic activity of blood neutrophils in animals [23]. In our previous studies, we showed a positive hirudological effect on myeloid and lymphoid blood cells of laboratory rats [24–26]. It was important to study the lymphoid organs of laboratory animals after hirudological exposure due to such a wide effect of hirudological influence on the organism and immunity of animals.

## Materials and Methods

*Animals and housing conditions*

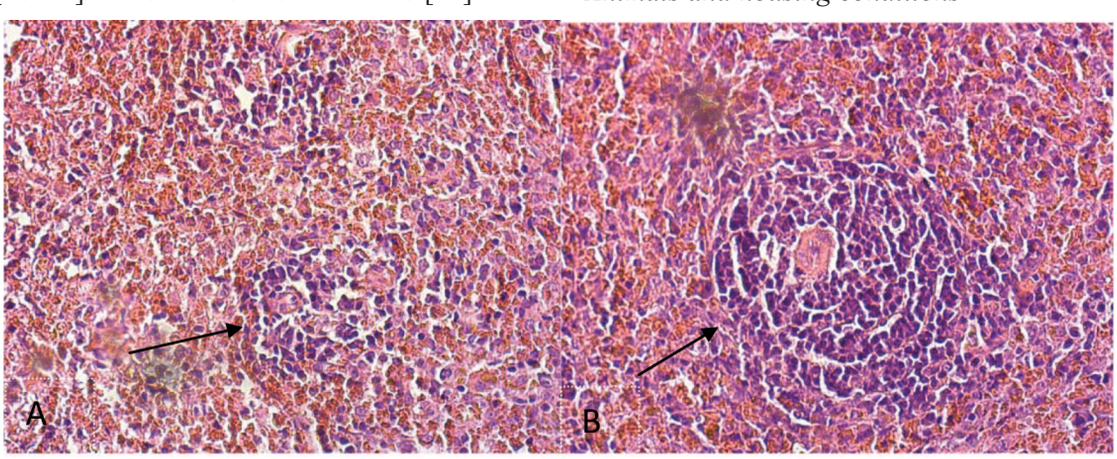


Figure 1. Lymphoid follicle of the white pulp of the spleen of male laboratory rats of control (A) and experimental (B) groups. Staining: hematoxylin and eosin

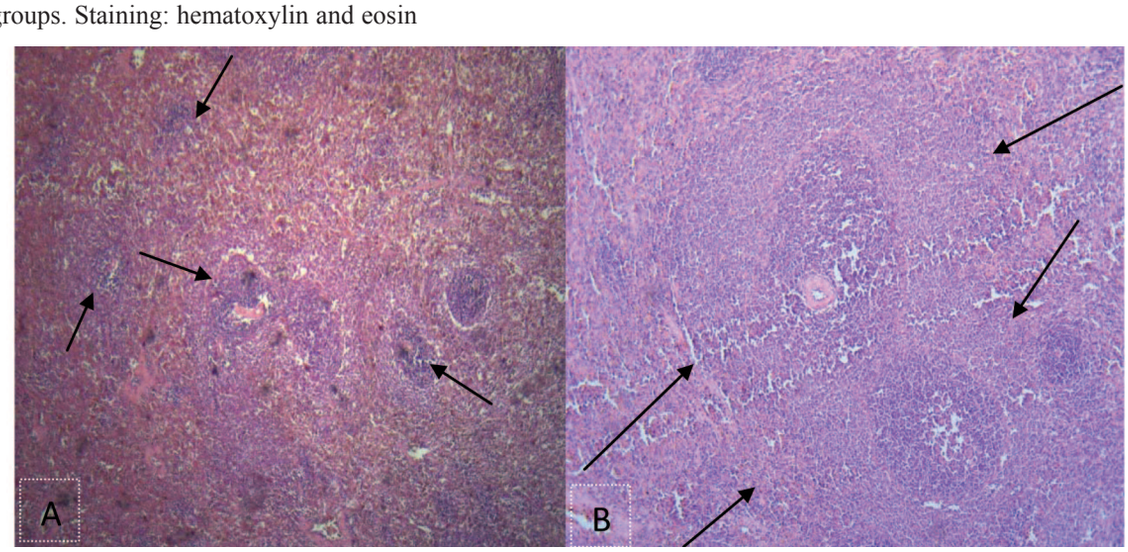


Figure 2. White and red pulp of the spleen of male laboratory rats of control (A) and experimental (B) groups. Staining: hematoxylin and eosin

Table 1. Changes in lymphoid follicles of the spleen of male rats under the influence of *Hirudo verbena*

| Groups of animals | Area lymphoid follicle, μm$^2$ | Diameter lymphoid follicle, μm | The number of lymphocytes in the lymphoid follicle per 400 μm$^2$ | Area central artery lymphoid follicle in μm$^2$ |
|---|---|---|---|---|
| Males control | 14507.1±1324.2 | 137.8±8.3 | 15.08±0.52 | 831.5±61.2 |
| Males experiment | 19931±1455.2* | 157.2±10.1* | 19.83±0.63* | 994.7±66.3* |

* – $P < 0.05$ in comparison with the control group

The work was performed on 40 white nonlinear male laboratory rats weighing 280–300 g, aged 6.5–7 months. Animals were kept in vivarium on a standard diet in individual cages. The study was conducted in accordance with ethical standards and recommendations for the humanization of work with laboratory animals, which are reflected in the ”European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes”, the Law of Ukraine ”On the Protection of Animals from Animals Handling”.

*Experimental procedures*

Animals were divided into control and experimental groups. An attachment of one hungry leech *Hirudo verbana* to experimental male rats (Carena, 1820) weighing 0.8 ± 0.1 grams was performed four times, once a week for each rat. Each time, a new hungry leech was taken for an attachment. The attachments were carried out on the coccyges zone. Then the rats were taken out of the experiment by decapitation, abiding by ”Methodological recommendations for the withdrawal of laboratory animals from the experiment”.

*Histological examination*

The spleen and thymus were removed and fixed in a 10% solution of neutral formalin, pieces of organ were embedded in paraffin blocks. Serial sections of the entire organ of the spleen and thymus were made using a microtome Thermo Scientific HM 325 with a thickness of 4–6 μm. To study the structural components of the spleen and thymus, histological sections were stained with hematoxylin and eosin. Details of the histological structure of the organs were studied using a cytomorphological complex based on an Axiocam digital camera and a Carl Zeiss Primo Star microscope, where digital photographs of micropreparations were taken. In which, the area and diameter of lymphoid follicles in the white pulp of the spleen, the area, diameter and thickness of central arteries and the number of lymphocytes in lymphoid follicles per unit area (400 μm$^2$), the number of cells per unit area in the cortical and medullar zones were determined. Computer program analysis of histological specimens was performed using the Zeiss ZEN 2011 program.

*Statistical analysis*

Descriptive data was expressed as mean and standard deviation. Values were calculated using the computer program SPSS v.21.0. (IBM SPSS Statistics., USA) software. Normality assumptions were checked using Shapiro-Wilk test. To compare the mean values with, the parametric Student’s t-test was used. Differences were considered to be statistically significant at $P$ values of less than 0.05 ($P < 0.05$).

## Results and Discussion

In the analysis of the lymphoid follicle of the spleen in the experimental group of animals, the white pulp predominated in comparison with the control group of animals (Fig. 1 and 2).

As a result of the study of the number of lymphocytes per unit area (400 μm$^2$) in the white pulp of the spleen registered a significant increase in lymphocytes compared with the control group of animals ($P < 0.05$; Tab. 1).

Also, increase of the other indicators of the lymphoid follicle in the experimental group compared with the control, namely its area and diameter, was registered ($P < 0.05$; Tab. 1). In the study of central arteries in lymphoid follicles in the experimental group we noticed the increase of their parameters: diameter and thickness, as well as the increase of the area of the periarterial zone due to possible enhancement of lymphoid infiltration, indicating activation of differentiation of T-cell pool

Table 2. Changes in the number of lymphocytes in the cortical and brain substances of the thymus of male rats under the influence of *Hirudo verbena*

| Groups of animals | The number of lymphocytes in the cortical substance of the thymus per 400 μm$^2$ | The number of lymphocytes in the brain substance of the thymus per 400 μm$^2$ |
|---|---|---|
| Males control | 15.36±0.22 | 8.07±0.62 |
| Males experiment | 18.25±0.27* | 13.11±0.44 |

* – see: table 1

of lymphocytes (Tab. 1). Our results of white pulp of the spleen after immunomodulator is confirmed by other researchers. For example, the effects of imunophan and gamavit were described, which led to an increase in the relative area of white pulp [7]. It was experimentally proven that in the offspring of female laboratory rats, which were injected with humid compounds, there is activation of hematopoiesis and increased mitotic activity in the spleen, increased number of vascular components [7].

When analyzing the morphology of the structure of the central lymphoid organ – the thymus of male rats, we registered mostly physiological involution of it in the control group of animals compared with the experimental, in which the histological structure of the organ was completely restored (Fig. 3).

It looked like a fully functional organ of the immune system, which may indicate an immunomodulatory property of hirudological effects. Which is able due to the influence on the morphogenetic function (control and regulation of histogenesis) of the immune system to start the functional recovery of the lymphoid organ. This assumption is also confirmed by analysis of matters of thymus (Tab. 1).

There is the increase in cortical substance over the medullar one. Also in the cortical and medullar zones of the experimental group compared with the control the number of lymphoid cells per unit area increased ($P < 0.05$) (Tab. 2), which indicates an immune stimulating hirudological effect on the development of lymphoid organs of the immune system. Our results are consistent with other researchers who, when using imunophan in laboratory rats, observed a predominance of cortical substance due to a decrease in medullar matter and an increase in the total number of lymphocytes in the thymus [27].

In conclusion, white pulp is predominant in the spleen of the experimental group, an increased number of lymphocytes per unit area (400 μm$^2$) of lymphoid follicle; area and diameter of the lymphoid follicle; area, diameter and thickness of the central arterioles in the white pulp of the spleen compared with the control group of animals $P < 0.05$. The analysis of the thymus in the experimental group of animals revealed the predominance of cortical substance over the medullar compared with the control group. In males of the control group, the thymus in volutes, is replaced by adipose tissue, the

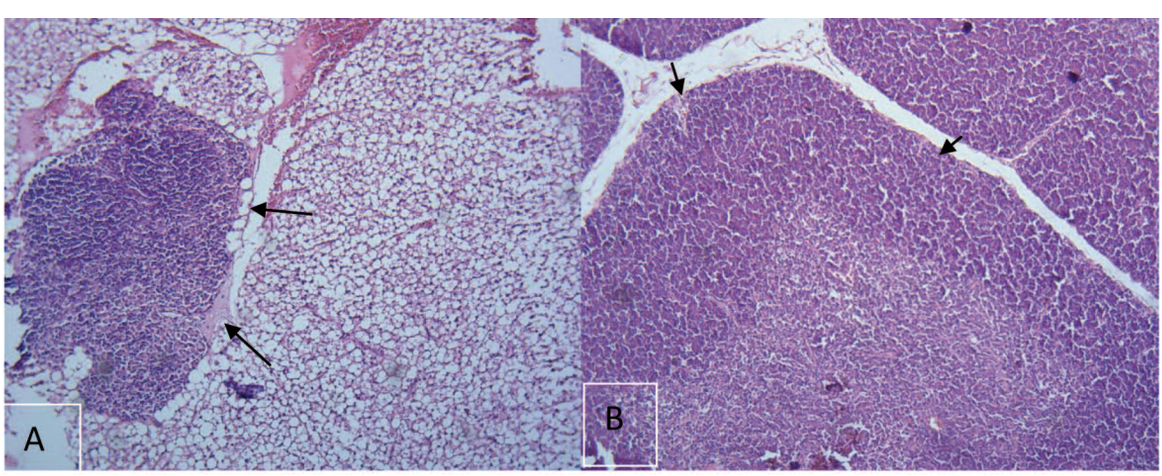


Figure 3. Thymus of male laboratory rats of control (A) and experimental (B) groups. Staining: hematoxylin and eosin

structure is lubricated, there are no epithelial cords. Compared with the experimental group in which the body is fully functioning according to histological parameters.

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