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Research Paper

Morphological and functional state of immune organs in rats with experimental type 1 diabetes mellitus (DM-1)

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Abstract: *The work is devoted to the study of morphological and functional state of immune organs - thymus and spleen, in rats Wistar with experimentally induced type 1 diabetes mellitus (DM-1) and to examine the effectiveness of phytopreparation BNO 10.30 (Imupret). For the experiment were involved 20 rats, which were divided into 4 groups: group 1 - control group of healthy rats, 2 - control group that received "Imupret", 3 - rats with experimental DM-1, 4 - rats with experimental diabetes, treated with the drug "Imupret." Functional changes in the immune organs were evaluated by the results of morphometric analysis; morphological picture was evaluated by histological changes. As a result, in animals with DM-1 were found initial pathological changes of these organs: increased volume of white pulp in the spleen, thymus medulla increase, which indicates the initial inflammatory and degenerative processes. According to the results of the correlation analysis there was established a direct relationship between the presence of DM-1and the pathological changes of spleen and thymus (r = 0,529, p <0.05). In this way, the results demonstrate the importance of early diagnostics of immune organs in patients with type-1.*

Key words: *type 1 diabetes, spleen, thymus, hystological changes, morphometric analysis.*

I. INTRODUCTION

During the past 20 years the number of patients with type 1 diabetes mellitus (DM-1*)* in the world increased almost in 3 times ahead in terms of the prevalence of all non-communicable diseases. The questions of the pathogenesis and treatment of type-1diabetes are still valid. Current medical therapy of DM-1 is unable to prevent the progression of clinical symptoms and its complications, despite the achievements in medicine of recent years. One of the major problems of endocrinology and related medical specialties is the early detection of pathological changes of internal organs in patients with DM-1 [5, 3]. It is known that disturbances of metabolism and immunity in patients with DM-1are closely related [4, 6]. In this case, the synthesis of antibodies and phagocytic activity of macrophages and leukocytes decrease. In conditions of chronic hyperglycemia, in organs of the immune system, including the spleen and thymus, appear morphological changes characterized by the development of secondary immunodeficiency with weakening of cell-mediated and humoral immunity, cytokine imbalance, violation migratory activity of cells [1, 2]. It should be noted that the problem of morphological changes of immune organs has not found sufficient coverage in the literature and requires further study for more active development of preventive and therapeutic measures. Therefore, it was actually to study the phytopreparation BNO 10.30 (Imupret), Bionorica (Germany).

The aim of this study was to investigate morphological changes in the spleen and thymus by histological changes and morphometric analysis in rats with experimentally induced DM-1 and to examine the influence of phytopreparation BNO 10.30 (Imupret) on these immune organs.

II. MATERIALS AND METHODS

The study was conducted in intact Wistar male rats (only 20 animals), weighing 130-150 grams, with the experimental type-1 diabetes. Experimental diabetes type-1 in rats was induced by a single intraperitoneal streptozotocin administration (S0130, Sigma-Aldrich Co. LLC, USA) in a dose of 55.0 mg / kg, which was diluted in 0,1 M citrate buffer, pH 4.5. Rats of the control group were injected intraperitoneally 0.5 ml of 0.1 M citrate buffer, pH 4.5. After four weeks of diabetes, the rats per os administered drug "Imupret" of 0.05 ml / animal three times a day for 14 days. Functional changes in immune organs evaluated the results of morphometric analysis. There were qualitative histological changes (appearance of germinal centers in

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lymphoid tissue, changes in the density of lymphoid tissue). In our studies the quantitative parameters were: changes in the relative value of the white and red pulp of the spleen (%), changes in the relative ratio of cortex and medulla spleen (%). Photos were received on microscope Olympus BX 51.

Morphometric analysis performed by the software Carl Zeiss (AxioVision SE64 Rel.4.9.1), magnification × 400. All rats were divided into 4 groups: group 1 - control group of healthy rats, 2 - control group that received the drug "Imupret", 3 - rats with experimental DM-1, 4 - rats with experimental DM-1, which received drug "Imupret." Statistical analysis of the data was performed using the program Statistica 6.0. Results were compared using Student's t-test. Results are presented as the average value (M) and standard error (± m). The difference was considered statistically significant when P <0,05.

III. Results

In the thymus of the control group - 5 animals (25%), were clearly recorded cortical and medulla parts and capsule of lobes. Cortex is represented by numerous precursors of T-lymphocytes and cells at different stages of antigen-independent differentiation. Medulla contains differentiated T-cells and Hassall's corpuscles. Hassall's corpuscles are built with reticuloendothelial cells, which may contain derivatives of apoptotic cells and can be an indicator of immune and age-related changes (Fig 1.a, b).

The study shows, that spleen in control group has no histopathological changes. The capsule and trabeculae are intact; they have fibroblasts, hemocapillars and arterioles. Stroma of spleen is represented with reticuloendothelial cells, among which are clusters of lymphoid tissue (white pulp). Besides erythrocytes, there registered a significant number of leukocytes and macrophages. There were recorded only a few clusters of macrophages. White pulp is presented by lymphoid nodules, containing lymphocytes and macrophages. The processes of recycling of damaged red blood cells and other blood cells occur in the red pulp, in the white pulp appears the immune response to antigens. Under the conditions of immune response (bacterial and viral antigens, autoantigens) there was marked increased volume of white pulp in the parenchyma of the spleen. Simultaneously, it can be registered increased density of activated macrophages and products after lysis of erythrocytes - hemosiderin and others. Based on these changes, we argue about functional changes in the organ, especially about the inflammatory process (fig.1 c, d).

Fig. 1. The thymus of control group. 1a - cortical substance, moderate density lymphocytes isolated reticuloendothelial cells. 1b - medulla, stromal components, single Hassall's corpuscles. Note: Hassall's corpuscles; lymphocyte. Hematoxylin-eosin.

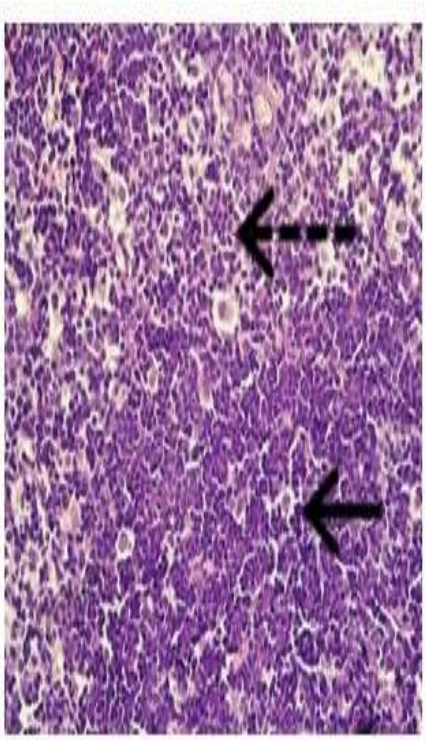


Fig.1 a

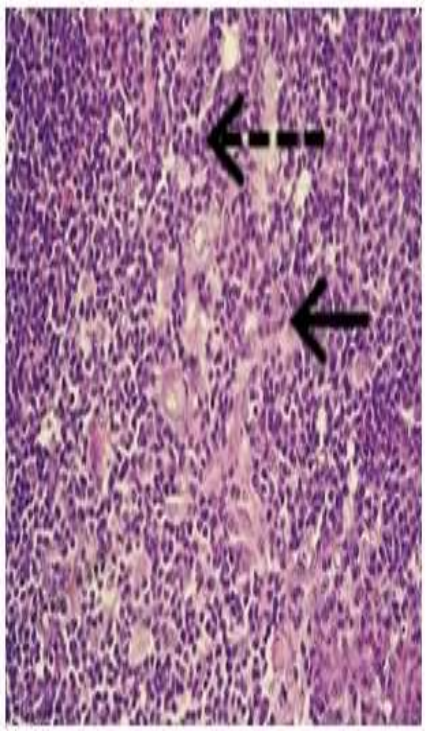
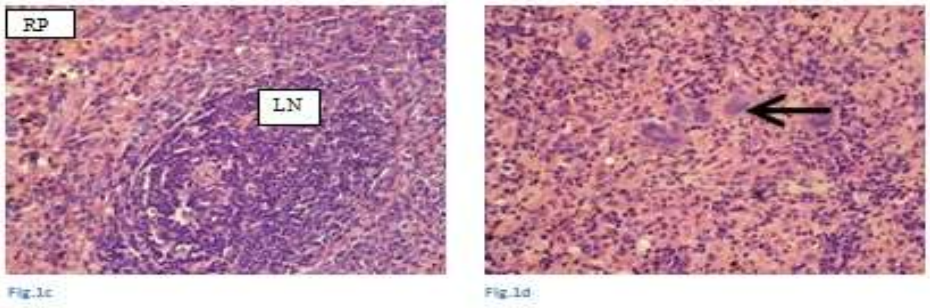


Fig.1b



The spleen of control group. 1 c - lymphoid nodule represented by accumulation of lymphocytes in the parenchyma of the spleen. 1d - red blood cells, white blood cells and macrophages in the red pulp. Note: LN - lymphatic nodule; RP - red pulp; macrophages. Hematoxylin-eosin.

It was noted an increased size (volume) of medulla in the thymus by 15% (Table 1) in the group of rats with experimental DM-1 - 5 animals (25%). We can say about absence of histological disorders of thymus in rats of this group and changes are recorded only in zones of antigen-independent differentiation of lymphocytes. Increased density of lymphocyte may indicate the formation of a class of immune cells. There were signs of immune activation in spleen. On the morphological level it had manifested in the following changes: an increased relative representation of white pulp in the parenchyma by 20% compared with the control (Table 2), increased density of white blood cells in the red pulp, lymphoid nodules were around the secondary nodules (activation of germinal centers). Secondary nodules concentric increased the size of primary nodules. The density of lymphocytes increased. Some nodules had apoptotic cells, which can be interpreted as a manifestation of damaged cells recycling. There were no activated macrophages, which indicate no bacterial immune response in the white pulp of the spleen. Some samples had hemosiderin deposits, resulting from increased utilization of red blood cells.

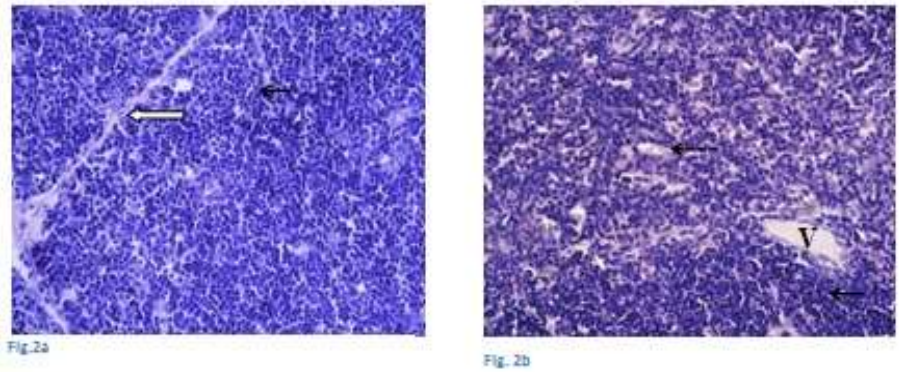


Figure 2 a, b. Thymus of rats with DM-1: **2a**- normal structure of cortical substance, there are isolated reticuloendotheliocytus and intact particles of septum. **2b** - medulla, increased density of lymphocytes, isolated Hassall's corpuscles. Note: Hassall's corpuscles; septum of particles; V - venules; lymphocyte. Hematoxylin-eosin.

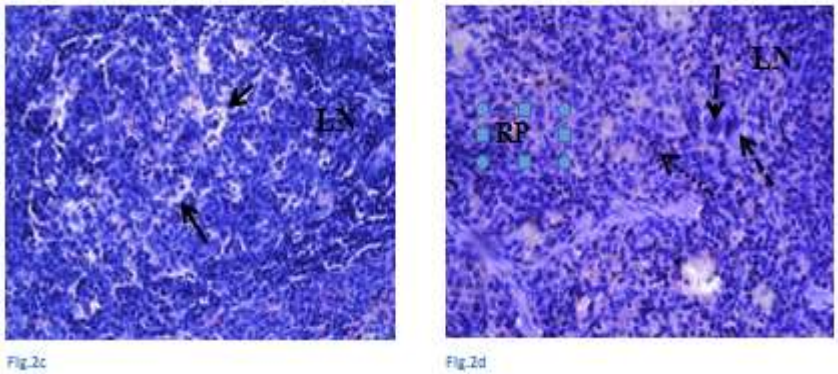


Figure 2 c, d. The spleen of rats with DM-1: 2c. - Pronounced activation of lymphoid nodules, increased density of lymphocytes and cluster of apoptotic lymphocytes. 2d. - secondary lymphoid nodules in the red pulp. Note: LN - lymphatic nodule; RP - red pulp; lymphocyte with signs of apoptosis; hemosiderin deposits; macrophage. Hematoxylin-eosin

In group with experimental DM-1- 5 rats (25%), which received "Imupret", there was recorded density of lymphocytes, particularly in the cortex of the thymus. The volume of cortex in thymus increased by 15% compared to the control. There were only isolated Hassall's corpuscles. There was no histological pathology. Density and volume of lymph nodes in spleen were slightly lower, than in diabetic rats without treatment.

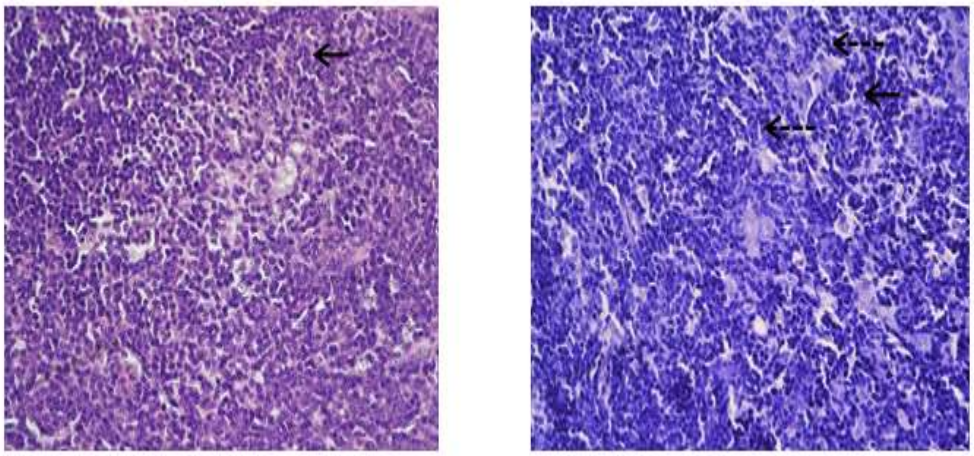


Figure 3 a, b. Thymus of rats with DM-1, which received "Imupret". Fig. 3a - density of lymphocytes in cortex, substance without structural defects. Fig. 3b - medulla, increased density of lymphocytes, Hassall's corpuscles. Note: Hassall's corpuscles; lymphocyte. Hematoxylin-eosin.

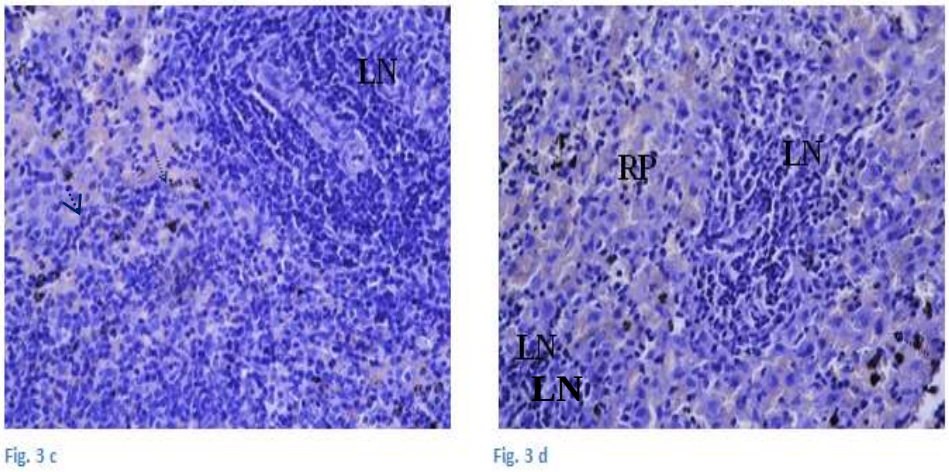


Figure 3 c, d. The spleen of diabetic rats, which received "Imupret. 3c - signs of activation of lymphoid nodules, moderately increased density of lymphocytes. 3d - germinal center of lymphoid cells in the red pulp. Note: LN - lymphatic nodule; RP - red pulp; hemosiderin deposits. Hematoxylin-eosin

In the control group, which received the "Imupret" - 5 animals (25%), histological structure of the thymus was the same as in control group without "Imupret". In the spleen, lymph nodes in the periphery noted signs of immune activation (reaction), reflected in a quantitative increase in the ratio of white to red pulp (% white pulp increased by 20%) - Table 2. We can assume that Imupret has immunostimulatory effects.

Table 1. Changes of medulla volume of the thymus

Group	Volume of medulla (%) in the thymic parenchyma
Intact rats	25,5±9,6
Intact rats + Imupret	26,5±7,7
Rats with experimental DM-1	29,1±8,1
Rats with experimental DM-1 + Imupret	21,6±7,2

Table 2. Changes in volume of white pulp in spleen

Group	The relative volume of white pulp (%) in the parenchyma of the spleen	p-value
Intact rats	28,8±11,5	-
Intact rats + Imupret	40,1±11,3	0,01
Rats with experimental DM-1	32,1±7,7	0,17
Rats with experimental DM-1 + Imupret	38,4±11,3	0,06

IV. CONCLUSIONS

It was shown the occurrence of lesions in the central (thymus) and in peripheral (spleen) immunogenesis organs, as the result of experimentally induced DM-1. In thymus and spleen of diabetic rats were recorded an increased population of leucocytes, lymphocytes, apoptotic cells and the relative volume of these organs became bigger, which indicates their antigenic stimulation in response to the disease. Subsequently, in the spleen and thymus of rats with DM-1 was observed the development of secondary immunodeficiency with the weakening of cell-mediated and humoral immunity. In the group of rats with DM-1, which received Imupret, it contributed to the restoration of cell structure of thymus and spleen due to immune-stimulating action. This demonstrates the ability of the Imupret to influence on key parameters of immunity, which is especially important for patients with DM-1.

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