

Hystological Changes Of The Spleen In Experimental Rheumatoid Arthritis And Morphological Alteration After Pathogenic Treatment

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ABSTRACT

After the use of anti - inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil, regeneration processes were recorded in the structure structures of the White - breed rats, the mass of the spleen was on average-1205±7.12 mg in 18-month-old white-breed rats, and on average-1138±6.21 mg in 24-month-old white-breed rats. The diameter of the venous sinuses in the red pulp was 0.38±0.01 µm in 18-month-old rats and 0.25±0.05 µm in 24-month-old rats. The area of venous sinuses was reported to have decreased from an average of - 74,381±1.19 mkm² in rats at 18 months compared to rats in the rheumatoid arthritis-calling group, to an average of 69,251±0.99 mkm² in rats at 24 months. The total area of red pulp was 984-35.24±1.97 mkm² in 18-month rats, 875-61. 19±2.01 mkm² in 24-month rats.

Keywords: spleen, rheumatoid artgritis, complex Freund's adjuvant, autoimmune disease, meloxicam, pomegranate seed oil.

1. INTRODUCTION

Rheumatoid arthritis (RA), an autoimmune disease, significantly affects all body systems. This indicates a positive correlation between the presence of RA and several other diseases and disorders [6,8]. Therefore, with RA, the risk of cardiovascular issues changes. Over 10 years, the risk of developing cardiovascular pathology in RA patients is 1%, but chronic inflammation adversely affects the endothelium, leading to its dysfunction, which significantly increases the risk of cardiovascular disease. Additionally, RA contributes to cardiovascular complications due to changes in the local architecture of large vessels. Specifically, subclinical damage to the common carotid artery is observed with prolonged RA. Furthermore, patients with RA show increased brain hyperperfusion and a rise in arterial hypertension. The risk of atherosclerosis also increases [6,9].

Pomegranate extract has an anti-inflammatory effect in experimental arthritis. Pomegranate juice has anti-inflammatory and chondroprotective effects. The result of systematic reviews showed that studies of humans, animals and in vitro showed the positive effects of pomegranate on clinical signs, inflammatory and oxidative factors of rheumatoid arthritis. Pomegranate is able to overcome its complications by reducing inflammation and oxidative stress. Punicalagin, an active substance derived from pomegranate peels, in the mouse model of collagen-inducing arthritis, is an inhibitor of joint inflammation, damage to the tissue of the spine and destruction of systemic bones. Punicalagin may be one of the natural therapeutic compounds that can relieve the development of rheumatoid arthritis by modulating the NF - αB pathways by suppressing inflammation and migration of fibroblast-like synoviocytes. Punicalin has had beneficial effects on IL-1 β - and TNF - α-stimulated chondrocytes and lower metabolic disorders [1,14].

Studies have shown that pomegranate peel can be used as a preventive treatment for arthrosis. A herbal preparation consisting of Propolis extract, pomegranate peel, and aglianico grape extract (PPP) (4:1:1) has a therapeutic effect in the mouse model of collagen-triggering arthritis by lowering levels of cytokines IL-17, IL-1 β. The combination of collagen, nanohydroxyapatite and grape seed, pomegranate peel and plant extracts of Jabuticaba bark offers a promising strategy for developing new biomaterials to regenerate bone tissue. An experimental study found that a collection of dry powder of

pomegranate concentrate, Eucommiae Cortex and Achyranthis Radix 5:4:1 (g / g) had strong anti-osteoarthritis effects in a surgically induced rabbit osteoarthritis model [3,4,7].

The result of a large number of experiments on animals showed that pomegranate peels, enriched with bioactive compounds such as phenols and flavonoids, have a strong antioxidant effect and have a therapeutic effect in rheumatoid arthritis. The anti-arthritis potential of the butanol fraction of *Punica granatum* extract may be due to the presence of active phytochemicals such as flavonoids, irioid glycosides, and phenolic compounds. A systematic review showed reliable evidence of the efficacy of pomegranate in osteoarthritis [2,7].

Experimental studies have found chondroprotective effects of oral consumption of pomegranate fruit extract in the post-traumatic osteoarthritis model. The results of a randomized placebo-controlled study showed that pomegranate extract relieved disease activity and improved some biomarkers of inflammation and oxidative stress in the blood in patients with rheumatoid arthritis. The results of clinical studies have shown that pomegranate juice consumption can improve physical function and stiffness, reduce the activity of enzymes that break down mountain tissue, and increase antioxidant status in patients with osteoarthritis of the knee [6,15].

According to a literature analysis, the scarcity of information about the morphological characteristics of the spleen in patients with rheumatoid arthritis and the lack of study of spleen morphometrics in patients with this disease, as well as data on the effectiveness of the use of pomegranate danagi oil in the correction of rheumatoid arthritis, justify the relevance of this study.

The purpose of the study

In experimental rheumatoid arthritis and the study of morphological, morphometric and immunogystochemical indicators of the spleen of non-white rats under the influence of an anti-inflammatory nonsteroidal agent and pomegranate seed oil.

2. MATERIAL AND METHODS

Morphological studies of this experimental rheumatoid arthritis related to spleen tissue were carried out in the research laboratory of immunohistochemistry and pathogistology located in the vivarium and simulation center of the Bukhara State Medical Institute.

In order to carry out experimental studies, 100 mixed-sex, 18-24-month-old white rats weighing 350-500 g were selected. All laboratory animals were obtained from the same vivarium and were performed on non-white rats aged 18-24 months. These adult (18-24 months old) white outbred rats were kept under standard vivarium conditions with relative humidity (50-60%), temperature (19-22°C) and light regime (12 h dark and 12 h light).

N.A. Nuraliev in the formulation of a standard vivarium food ration for laboratory animals. and all. (2016) based on the recommendations in the methodological manual.

Animals in the experiment were divided into 3 groups:

first group control group-laboratory animals (n=20) fed with standard vivarium ration, healthy rats;

the second group - laboratory animals (n=40) were fed with a standard vivarium diet, in which rheumatoid arthritis with a blanket Freund was called;

the third group - laboratory animals (n=40) were fed standard vivarium ration, infected rats were treated with NAID for 2 weeks. (meloxicam 1 mg / kg once a day for 14 days).

Our first group was a control group, 20 white non-breeding bats selected to compare results with the rest of the groups and housed in separate cells.

In the remaining 80 rats, rheumatoid arthritis was called by the method of the adjuvant Freund in laboratory conditions.

Required materials:

Complex Freund's Adjuvant (CFA): an oily mixture containing mycobacteria (*Mycobacterium tuberculosis*). It was administered in special doses.

Animals: mostly 18-24 months old, healthy white broodless rats.

Injection equipment: fine needles of 27-30 sizes.

Injection site: usually injected into the muscles of the hind limbs of the animal (intra-muscular) or under the tail.

We treated rats with rheumatoid arthritis, taken as Group 3 in our experiment, by giving NAID for 2 weeks. To do this, we gave rats meloxicam 1 mg / kg once a day for 14 days.

All experimental white-breed rats were decapitated on an empty stomach in the morning at the end of the experiment, and the organs were measured.

3. RESULTS

The analysis of the macroscopic, microscopic, organometric and histomorphometric indicators of the spleen, isolated from white non-breeding rats called experimental rheumatoid arthritis, was studied in the experiment. Macroscopically, when the spleen organ was seen the glare of the capsule covering the top of the organ was reduced, the spleen color was found to be darker red than the control group. The Shape of the spleen is usually found to be slightly rounded at the edges in a conical shape with a rim.

Macroscopically, it can be seen that the spleen sizes have become relatively large, no other manifested changes have been detected. When the resulting micropreparations are analyzed, it is determined that the capsule surrounding the spleen tissue from the outside has become unevenly thickened, these changes were observed in almost all micropreparations of the spleen. Specific changes in the white pulp of the spleen, such as a decrease in the amount of lymphocytes, an increase in the amount of blood in the blood vessels of the spleen, that is, signs of dimming, were observed. Inside the vessels, signs of adhesion of small blood-shaped elements with each other and to the vascular wall were identified. Red pulp, plasma components that passed from the vascular interval to the tissue in the stroma were identified, and the accumulation of many eroded blood-shaped elements in the red pulp was found especially in the accumulation of yellow tongues formed from the accumulation of erythrocytes, that is, the accumulation of hemosiderin pigments. It was also found that in the red pulp, lymphocytes and macrophages accumulate scattered and concentrated. When white pulp is seen, Primary and secondary lymphatic nodes with developed areas are visible. In the Perarterial lymph mucosa, lymphocytes are visible, developing in the marginal and mantle areas, with slightly increased lymphocyte content and surface area. The area of reproduction is seen to develop lymphocytes.

In the 18-month-old white non-breeding rats in the experimental group, it weighed $1,257 \pm 8.15$ mg and in the 24-month-old rats $1,203.79 \pm 5.63$ mg. In 18-month-old rats, the high severity of the spleen means the activity of their immune system and the development of spleen tissue. In rats at 24 months of age, however, a decrease in the severity of the spleen indicates a decrease in immunity and changes in the structure of the spleen as age progresses. The morphology of the red pulp varies from its area in 18-month-old white zotless rats to its area of 115398.23 ± 2.36 mkm² and in 24-month-old rats to 995237.31 ± 2.45 mkm², spleen bands and venous sinus area have been observed to expand, the diameter of venous sinuses is 0.48 ± 0.01 in 18-month-old rats and 0.31 ± 0.03 Mkm² in 24-month-old rats it has been found to be $81,747 \pm 1.21$ Mkm² in bats and $73,118 \pm 1.11$ mkm² in 24-month-old bats.

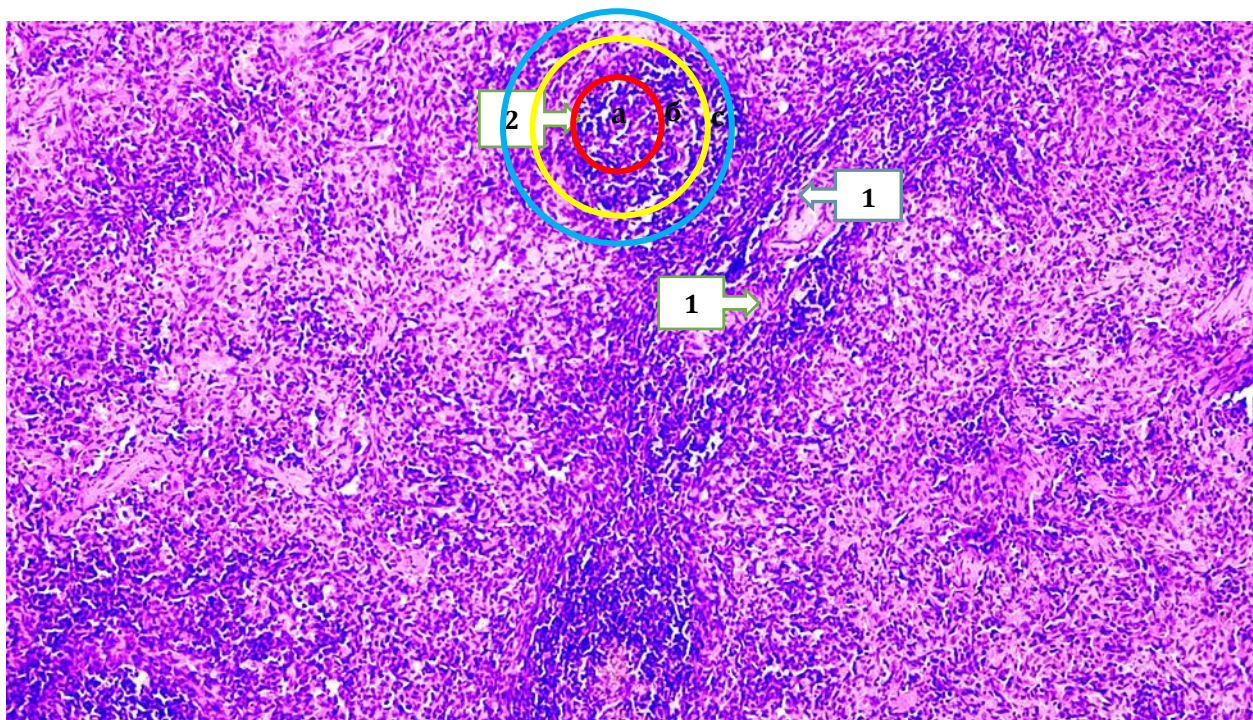


Figure 1. Microscopic view of the 18-month-old white broodless rat spleen in rheumatoid arthritis in an experiment. It is stained with hematoxylin-eosin. 4x20 enlarged. 1-we see an increase in the yield of the vascular walls located in the female pulp, and its surroundings are surrounded by foci rich in leukocytes; 2 - white pulp; s-marginal Zone; B - increased mantle zone; a - decrease in the volume of the reproduction Center.

This is not due to age-related involution processes, but rather a change resulting from pathology. Reticular fibers and cells

have increased significantly, and their macrophages have been found to be involved in active phagocytosis processes. In the experimental group, there was an increase in active motile cells in the spaces between blood cells, erythrocytes, granulocytes and circular mononuclears.

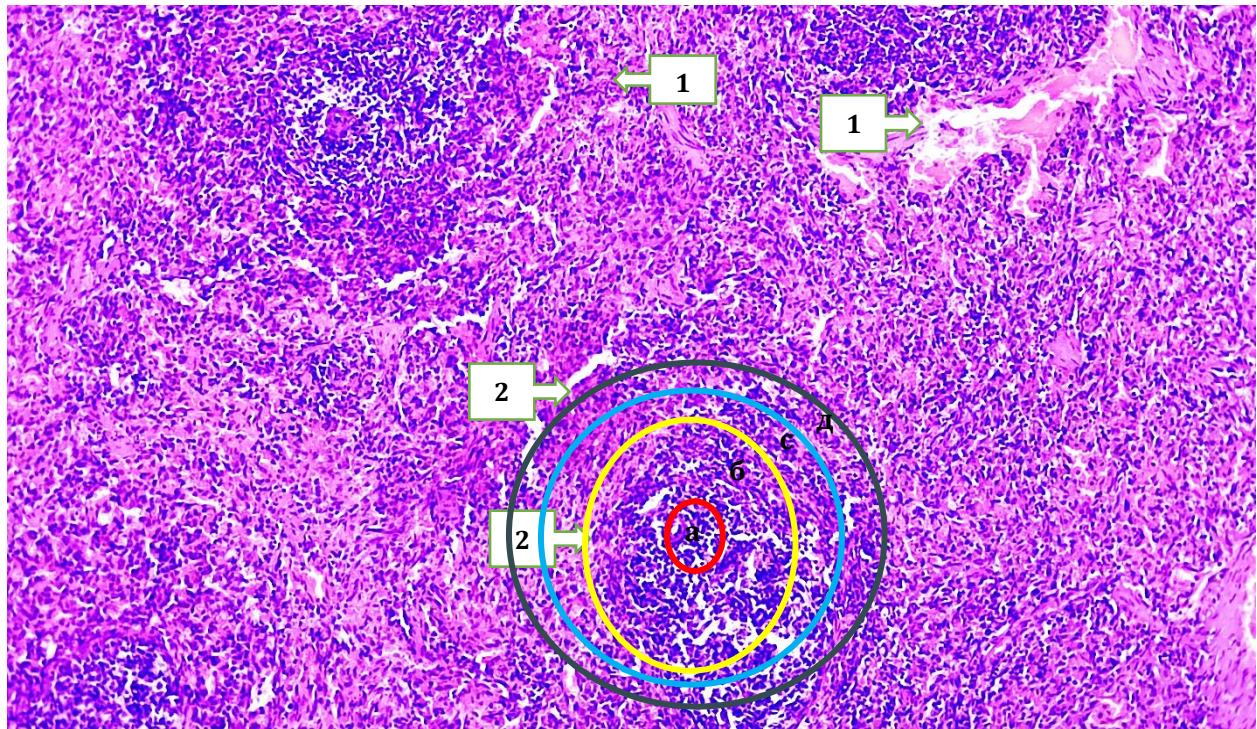


Figure 2. Under experimental conditions, rheumatoid arthritis is called, a microscopic view of the 24-month-old white non-breeding rat spleen of Group 2. It is stained with hematoxylin-eosin. 4x20 enlarged. 1-we see an increase in the volume of the vascular walls located in the female pulp and its surroundings are surrounded by leukocytes; 2 - the formation of cracks in the red pulp; 3 - white pulp; a - a decrease in the volume of the reproduction Center(in terms of the cell number); B - an increase in the mantle zone; s-marginal Zone; d-periarterial lymphatic

High levels of proliferation of lymphoid nodules and T-zone T-lymphocytes were also observed, along with migration of plasma cells from the outside with antigen-specific differentiation. There was also an increased phagocytic activity of old and damaged erythrocytes in red pulp macrophages and an increase in hemosiderin deposits. The area of the white pulp of the experimental group was also observed to be enlarged at age two in non-white rats, and these indicators are: 18-month-old rats at $81673.12 \pm 3,11 \text{ mkm}^2$, and 24-month-old rats at $67249.54 \pm 2, 17 \text{ mkm}^2$.

The process of recovery in the spleen of white-breed rats after treatment with NAID was recorded in all zones, the indicators approached normal, but did not fully recover. When macroscopically seen, almost no macroscopic changes are detected when compared to the control group. Microscopically, it was found that the spleen capsule was thickened every time in the various micropreparations from which it was obtained. An increase in the relative amount of lymphocytes in the reproductive tract from lymphatic nodes has been found. Symptoms of venous fullness in the vessels are not detected, it was mainly found that there is an increased arterial blood flow. A decrease in tumors in the stroma was found in the red pulp an increase in the amount of lymphocytes and macrophages was found.

General condition of the spleen:

The Shape of the spleen has been restored, shrinking in size. The weight was $1205 \pm 7.12 \text{ mg}$ in 18-month-old rats, and esamg in 24-month-old rats. These rates are significantly reduced compared to the case of rheumatoid arthritis ($1257 \pm 8.15 \text{ mg}$ vs. $1203.79 \pm 5.63 \text{ mg}$), but slightly higher than normal rates (conditionally $1197 \pm 7.13 \text{ mg}$ vs. $1119.67 \pm 2.33 \text{ mg}$).

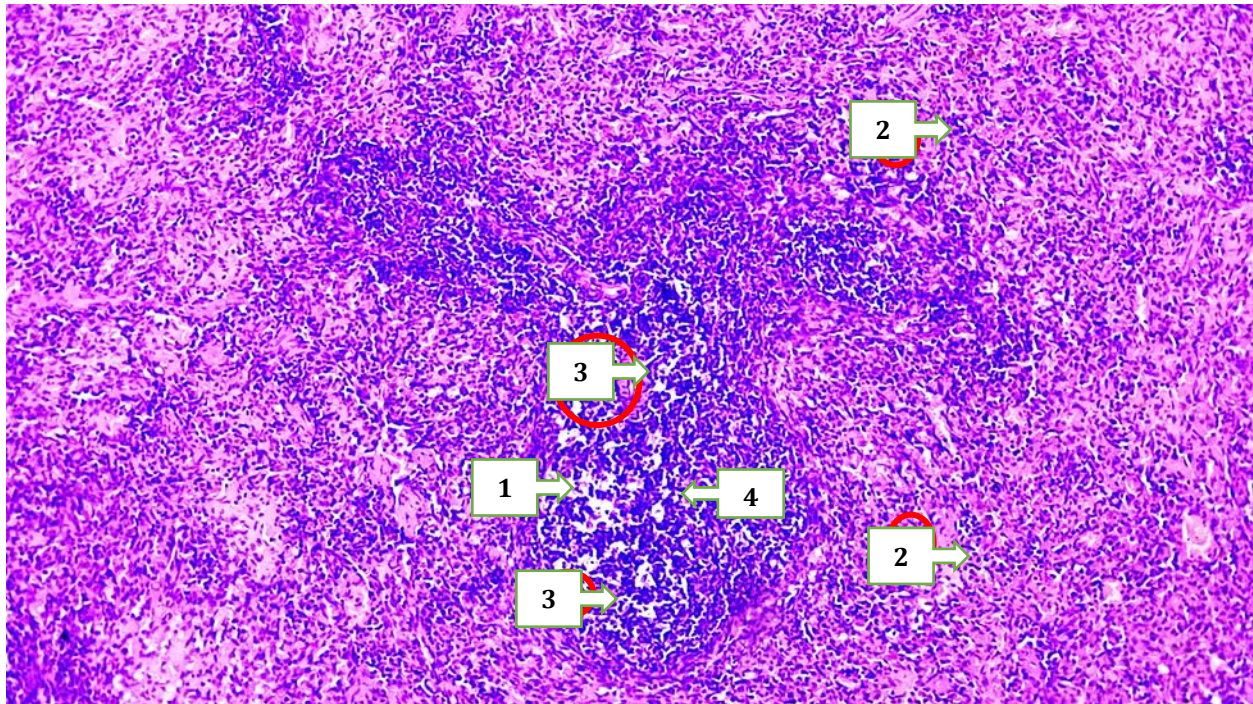


Figure 3. Microscopic appearance of the white non-breeding rat spleen of Group 3 at 18 months under the influence of NAID (anti-inflammatory nosteroid agent) in rheumatoid arthritis. It is stained with hematoxylin-eosin. 4x20 enlarged. 1-reduced diameter of the white pulp 2 - decreased volume of venous sinuses located in the red pulp ; 3 - blood vessels located in the white pulp; B-reduced lymphocytes around the germinative Center.

Changes in red pulp:

Spleen bands and venous sinuses decreased in size, venous sinuses decreased in area and diameter (3 figure):

Venous sinuses diameter: $0.38 \pm 0.01 \mu\text{m}$ in 18-month-old rats; $0.25 \pm 0.05 \mu\text{m}$ in 24-month-old rats, and their area is $74,381 \pm 1.19 \mu\text{m}$ in 18-month-old white non-breeding rats, and $69,251 \pm 0.99$ in 24-month-old rats

it was found to have decreased to mkm^2 .

The total area of red pulp is also reduced:

$98435.24 \pm 1.97 \text{ mkm}^2$ in 18-month rats; $87561.19 \pm 2.01 \text{ mkm}^2$ in 24-month rats.

Also, in macrophages, the activity of the phagocytosis process is impaired, the number of hemosiderin deposits is reduced. Changes in white pulp:

The area and diameter of the white pulp showed signs of recovery:

Area: $79563.18 \pm 1.32 \text{ mkm}^2$ in 18-month rats; $63215.27 \pm 1.25 \text{ mkm}^2$ in 24-month rats.

Diameter: $1256.32 \pm 5.47 \mu\text{m}$ in 18-month-old rats; MGM in 24-month-old rats. In lymphoid nodules and T-zones, T-lymphocyte proliferation and plasma cell antigen-specific differentiation processes are somewhat reduced but maintained to the point where they can provide an immune response.

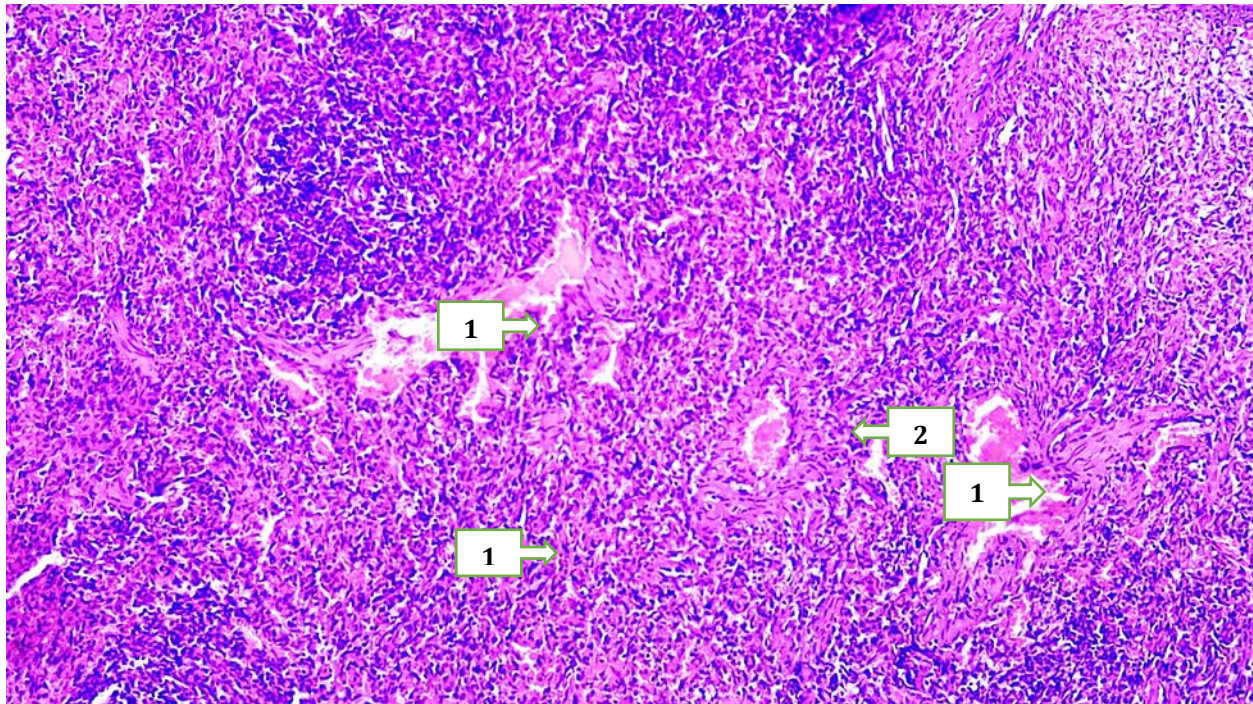


Figure 4: microscopic view of the 24-month-old white non-breeding rat spleen of Group 3 under the influence of NAID (anti-inflammatory nonsteroidal agent) in rheumatoid arthritis. It is stained with hematoxylin-eosin. 4x20 enlarged. 1 - decrease in the volume of sinusoids located in the red pulp; 2-decrease in the volume of the vessel wall in the red pulp.

After the use of anti - inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil, the diameter of the germative centers of the white pulp of the spleen of white non - brood rats decreased when compared with those of rats called rheumatoid arthritis, and in 18-month-old white non-brood rats, the size ranged from 136 μm to 154 μm , on average-145.24 \pm 1.12 μm , and in 24-month-the mold was found to average-139.78 \pm 1.01 μm .

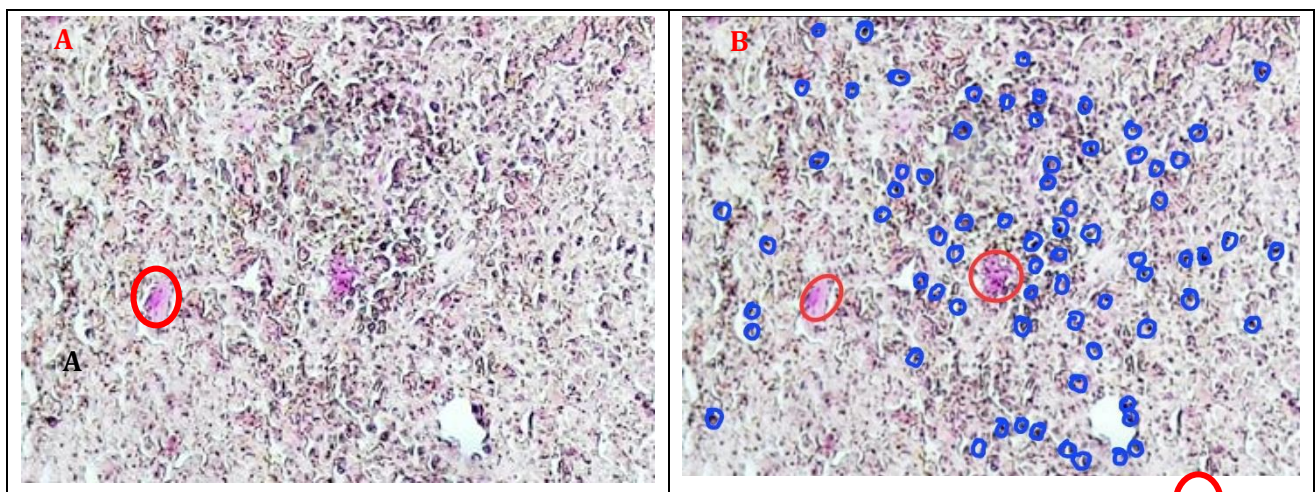


Figure 5. A) microscopic view of the spleen of an 18-month-old white broodless rat affected by an anti-inflammatory nosteroid agent (meloxicam) and pomegranate seed oil in rheumatoid arthritis. Painted in Van-Gison. Image enlarged 200 times. B) 1-marked with Red-collagenization of sinusoids; macrophages in Blue. Area-1036250 px²

When the number of macrophages in the lymphatic follicular marginal area was compared to that of arthritis-called rats, it was observed that 18-month-old white zotless rats decreased from 123.4 \pm 1.3 to 95.2 \pm 1.07 respectively, and 24-month-old white zotless rats decreased from 98.4 \pm 1.08 to 57.3 \pm 1.1 respectively. According to the analysis of the results of the study, rheumatoid arthritis is called decrease in the number of macrophages in the marginal area of the white pulp of the spleen of white non-bred rats, which indicates a significant degree of destructive effect of rheumatoid arthritis on the structure

structures of the spleen. The use of an anti-inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil has led to a revival of morphological indicators of the spleen of white-breed rats.

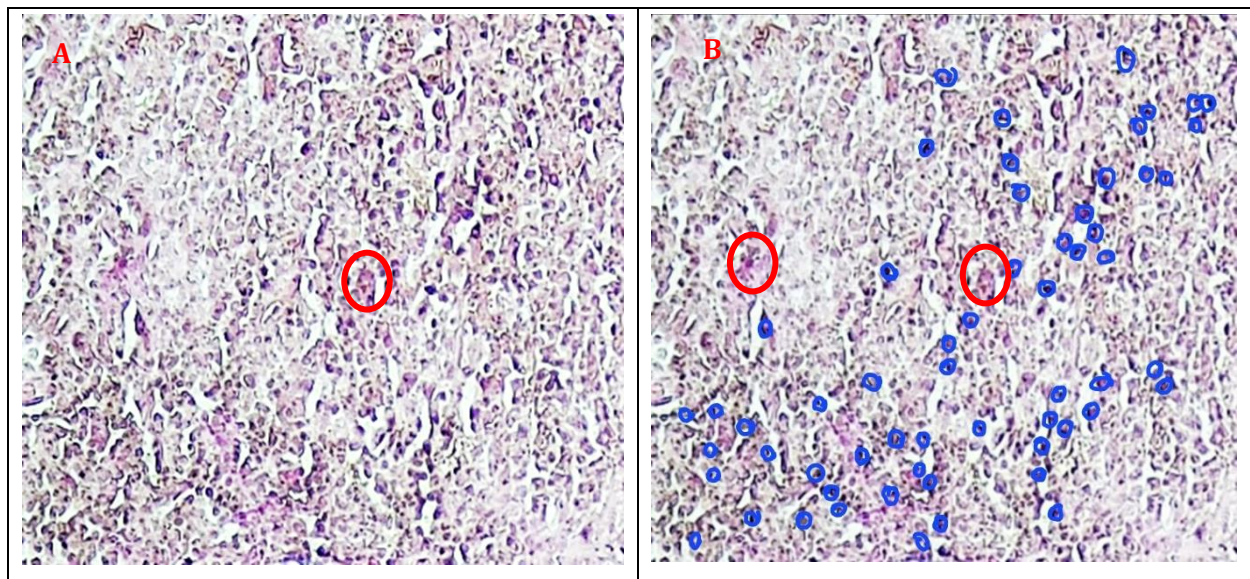


Figure 6. A) microscopic appearance of a 24-month-old white purebred rat spleen affected by an anti-inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil. Painted in Van-Gison. Image enlarged 200 times. B) 1-collagenization of sinusoid atrophy (marked in red) in red pulp.; Macrophages in blue. Area-1036250 px²

After rheumatoid arthritis was called and an anti-inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil were applied, signs associated with regeneration were also noted in the germinative centers of the lymphatic follicles of the white pulp of the White zotless rat spleen.

4. CONCLUTIONS

After the use of anti - inflammatory nonsteroidal agent (meloxicam) and pomegranate seed oil, regeneration processes were recorded in the structure structures of the White - breed bats, the mass of the spleen was on average-1205±7.12 mg in 18-month-old white-breed rats, and on average-1138±6.21 mg in 24-month-old white-breed rats. The diameter of the venous sinuses in the red pulp was 0.38±0.01 µm in 18-month-old rats and 0.25±0.05 µm in 24-month-old rats. The area of venous sinuses was reported to have decreased from an average of - 74,381±1.19 mkm² in rats at 18 months compared to rats in the rheumatoid arthritis-calling group, to an average of 69,251±0.99 mkm² in rats at 24 months. The total area of red pulp was 984-35.24±1.97 mkm² in 18-month rats, 875-61. 19±2.01 mkm² in 24-month rats. These positive changes in the structural structures and functional state of the spleen of white-breed rats indicate the effectiveness of anti-inflammatory nonsteroid agent (meloxicam) and pomegranate seed oil in improving the state of the immune system in rheumatoid arthritis.

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