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Hematological and Histopathological Changes in the Spleen of Male Albino Rats (*Rattus norvegicus*) Treated with Meloxicam

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ABSTRACT

Meloxicam is a non-steroidal anti-inflammatory drug, which works by lowering the levels of hormones that cause inflammation and pain in the body. However, its effect on human organs is yet to be investigated. Therefore, this study was aimed at examining the hematological and histopathological impacts of meloxicam drug on the spleen of male albino rats (*Rattus norvegicus*). Male albino rats, weighing 180-200 g and aged 6-7 weeks, were divided into two groups of ten rats each; experimental and control. The control group was administered with a sterile normal saline solution, while the experimental group received a meloxicam solution, both for seven weeks. At the end of the experiment, the animals were sacrificed and the tissues were subjected to hematological and histological investigations. Some hematological parameters were determined. The results revealed histological changes in the spleen tissues of meloxicam-treated experimental rats, including the emergence of cellular degeneration and necrosis in both the white and red pulp. The presence of hemosiderin in the red pulp and cellular degeneration in the marginal zone as well as hyperplasia in the germinal center in the white pulp were also observed. Experimental animals administered with meloxicam revealed no significant changes in the number of red blood cells or packed cell volume in the hematological parameters. However, the platelets, white blood cells, and hemoglobin were significantly higher in the experimental group. The findings of this study revealed that meloxicam induces histological changes in the spleen architecture, as well as aberrant abnormalities in the various hematological parameters examined.

***Keywords*:** Hematological, Histopathological, Meloxicam, Rat, Spleen.

Introduction

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that is used to relieve pain in joint illnesses, cancer, rheumatoid arthritis, and Alzheimer's disease.[1-3] Prostaglandins are normally produced by the cyclooxygenase (COX)-1enzyme, which is present in various human tissues and they play an important role in maintaining the function of the organs in the body, blood hemostasis, and intestine integrity.[2,4] In a sick state, the COX-2 enzyme, which is responsible for prostaglandin synthesis, is expressed in the lining of blood vessels, and kidneys (PE2).[4,5] The therapeutic effect of most non-selective NSAIDs is achieved by reducing the level of prostaglandin synthesis via suppression of COX enzymes. There are two isoforms of the COX enzymes that have been discovered; COX-1 and COX-2.[1,6] When non-selective NSAIDs are used, both COX-1 and COX-2 are inhibited; however, COX-2 inhibition is more essential than COX-1 inhibition since COX-2 is generated in pathological conditions.[2] Therefore, it has become vital to utilize a medicine that inhibits COX-2 without affecting COX-1 levels. Meloxicam is used for this purpose because it is considered a selective inhibitor drug that inhibits COX-2 alone.[7] Meloxicam is a non-steroidal anti-inflammatory medicine that works by specifically inhibiting the COX-2 enzyme at low doses. This results in a reduction in prostaglandin production, which can cause fever, inflammation, and pain. Meloxicam in high doses may inhibit COX-1 causing a reduction in the level of prostaglandin production which has a great prophylactic and physiological importance.[8,9] Another study found that COX-2 is widely distributed in the vessels and architecture of the kidney, where it plays an important role in maintaining physiological balance, and that COX-2 inhibitors impair the structure and function of the kidney. It is, therefore, critical to comprehend the impact of selective COX-2 inhibitors, which are classified as a non-steroidal anti-inflammatory agent, on all vital organs, including the kidney.[10] However, using these types of drugs as NSAIDs is associated with a lot of risks to the liver, kidneys, and alteration of hematology.[7] When characterizing changes inside the lymphatic organs, such as the spleen, they must be broken down into portions and evaluated separately from one another using descriptive terms rather than interpretive phrases. The red pulp, white pulp, and per arteriolar lymphoid sheath are all parts of the spleen (PALS, T-cell area).[11,12]

When exposed to an immune influencing factor such as a medicine or microbial agent, lymph follicles and the marginal zone must have assessed changes in each section independently, including PALS size and density, cellular alterations with germinal center development, and decreased or enlarged lymphoid follicles.[13] The most reliable indicators in the measurement of toxicity are the development of the germinal center and follicle cellularity measurement and most of the time, it is difficult to identify some slight changes in the red pulp.[14] Studies are scarce about the effect of meloxicam drug on the histology of the spleen. Therefore, this study was conducted to identify the hematological indicators and histological spleen changes induced by the meloxicam drug.

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Materials and Methods

Experimental animal grouping and treatment

Ethical clearance for this study was obtained from the University of Baghdad, College of Science, with the Ethical Permission Number CSEC/1020/0032. A total of 20 matured albino male rats of 6-7 weeks old, weighing 180-200 g were used for conducting the research. The animals were divided into two groups of ten rats each, after one week of acclimatization to the new environment. Group 1 rats were administered with 1 mL/kg/bodyweight of sterile normal saline (0.9 % NaCl) using a 0.6 mm diameter catheter, straight into the stomach once a day for seven weeks.[15] On the other hand, Group 2 rats were administered orally, a dosage of 0.4 mg/kg BW of meloxicam solution (therapeutic dose) by a 0.6 mm diameter catheter directly into the stomach once daily for seven weeks. The medication solution was prepared by dissolving 0.4 mg of meloxicam powder (equivalent to a human therapeutic dose of 15 mg/kg) in 1 mL of distilled water as described by Carpenter.[16]

Histological preparation

At the end of the dosage period, the experimental animals (control and treated groups) were sacrificed under diethyl ether anesthesia. The spleen was removed; tissues were prepared and stained with hematoxylin and eosin.[17]

Hematological investigation

Hematological parameters such as hemoglobin (Hb), platelets count, packed cell volume (PCV), white blood cell (WBC) count, and red blood cell (RBC) count were determined using an automated cell counter micros ABX (Roche Diagnostic System, Montpellier, France).[18]

Statistical analysis

Hematological parameters collected were analyzed statistically using a t-test. The results were presented at a probability level less than 0.05 ($p \leq 0.05$) when comparing the two study groups.[19]

Results and Discussion

Histopathological analysis

Meloxicam; a selective COX-2 inhibitor was administered to the experimental male albino rats in the present study to explore its influence on the spleen architecture, as well as its effects on several blood parameters while utilizing the therapeutic dose. The results (Figure 1) obtained indicated that there were no histopathological alterations in the spleen slices of the control group when examined under a light microscope or conditions that express the appearance of the lesion. On the contrary, histological sections from the Group 2 rats revealed histological changes and areas of lesions, including the appearance of numerous macrophages and apoptotic cells in different areas in the red pulp as shown in Figure 2. Also, a marginal zone with necrotic cells and cell degeneration in the white pulp was observed. These findings revealed a high incidence of hemosiderin in red pulp degeneration cells and necrotic cells in the marginal zone (Figure 3). The most striking histopathological abnormalities in the treated group were germinal center hyperplasia and white pulp that was fused (Figure 4).

These observations are consistent with previous study.[20] Under the impact of meloxicam, there was a reduction in prostaglandin levels, resulting in widespread tissue necrosis and apoptosis. The meloxicam mechanism is exerted by the toxic effect of drug metabolites with increasing vasoconstriction causing blood vessel rupture. Because COX-2 suppression induced hemorrhage in the spleen's red pulp, the prostaglandin vasodilator was reduced.[21,22] According to some studies, bleeding is triggered by the renin-angiotensinogen system.[23] The spleen is also considered an important organ in evaluating the lesion related to treatment because it participates in the drainage of numerous substances, including drugs, and because of its relatively large size compared to other lymphoid organs. T and B cells in the spleen are similar to the xenobiotic detrimental effects or metabolism, causing apoptosis and necrosis. The establishment of the germinal center in the spleen is similarly affected, either increasing or decreasing depending on a variety of factors.[24] Due to an increase in the immunological response to antigen, the formation of secondary follicles in a white pulp with an increase in B-lymphocytes area and germinal center become more prominent. Therefore, the prominent rise that occurs in the white pulp area is sometimes indistinguishable from neoplasia.[25]

Hematological analysis

The values of RBCs and PCV were found to be non-significantly different, however, platelets and total WBCs were considerably higher ($p<0.05$) when compared to the control group, as was Hb content (Table 1). This study found evidence of meloxicam-stimulated hematological diseases, which is consistent with earlier research that has found meloxicam to be effective in triggering blood disorders.[26,27] NSAIDs create biochemical interference by inhibiting cyclooxygenase, which inhibits prostaglandin synthesis. This is the basis of the therapeutic effect but also has negative implications.[28] Because there were no significant differences in the % PCV or RBC count when animals in the control and experimental groups were compared. This could be attributed solely to the COX-2-selective meloxicam medication efficacy.[29] Hemostasis is dependent on the presence of two dynamic systems: leucocytes and hematopoiesis, for an organism's ability to react promptly to chemical poisoning.

However, increased levels of Hb, platelets, and WBCs were observed in the experimental animals treated with meloxicam, which is consistent with the findings of other studies that looked at the effects of various pharmaceuticals on animals that had been treated with them.[30,31] In the current study, the increase that appeared in the Hb value may be due to the increase in bone marrow activity under the influence of meloxicam. In addition, an increase in Hb may be associated with an increase in hemoglobin biosynthesis due to the presence of a high concentration of two substances (glycine and succinic). On the other hand, the higher platelet count in this study could be related to the increased activity of platelet-forming megakaryocytes that comes with greater bone marrow activity. The number of WBCs in the experimental male rats increased significantly in this study, which is consistent with the earlier research.[32] When an infection starts, the number of white blood cells increases.[11]

NSAID medication has been shown to improve immunological function in rats in some previous studies.[33] However, other research on steroid drugs has clarified their important role in treating a variety of pathological disorders, such as anti-inflammatory and immune-modulatory properties.[34] Steroids, such as glucocorticoids, have been utilized in immunological diseases due to their powerful immunosuppressive and anti-inflammatory effects, as well as Prednisone, which changes plasma cell development and hence the body's defensive mechanism against influencing factors.[35]

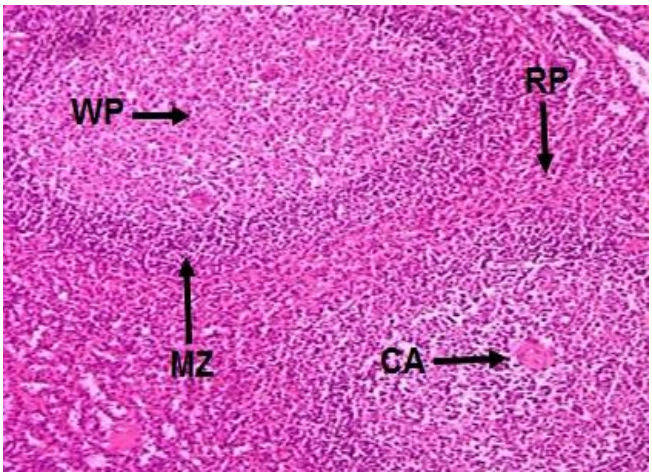

Figure 1: A cross section of spleen tissues in a male albino rat showing normal architecture. The section includes **WP:** White pulp; **MZ:** Marginal zone; **RP:** Red pulp, and **CA**: Central artery (H&E 40X).

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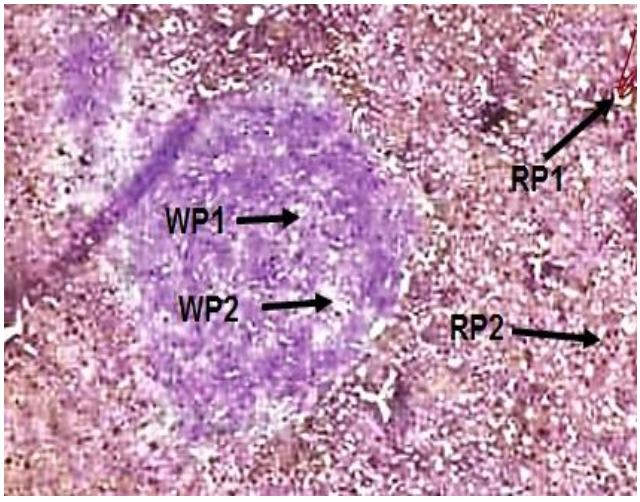

Figure 2: A cross section of spleen tissues of a male albino rat treated with meloxicam (0.4mg/kg BW). **RP1:** Apoptotic cells in red pulp; **PR2:** Massive macrophages in red pulp; **WP1:** Massive necrotic cells in white pulp; **WP2:** Degeneration cells in white pulp (H&E 40X).

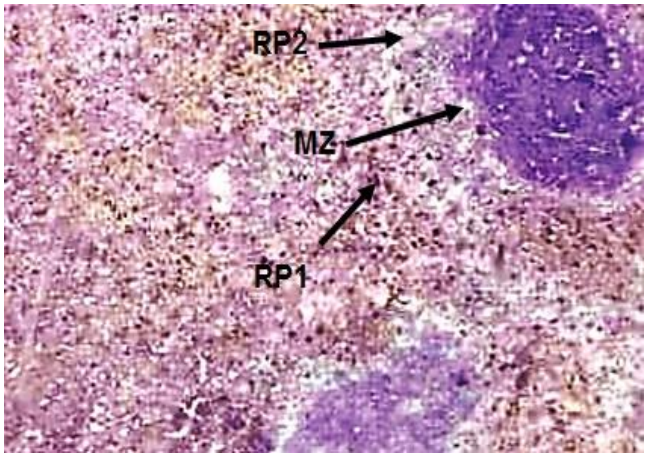

Figure 3: A cross section of spleen tissues of a male albino rat treated with meloxicam (0.4mg/kg BW). **RP1:** Hemosiderin prevalence in red pulp; **RP2:** Degeneration of cells in red pulp; **MZ:** Necrotic cells in marginal zone (H&E40X).

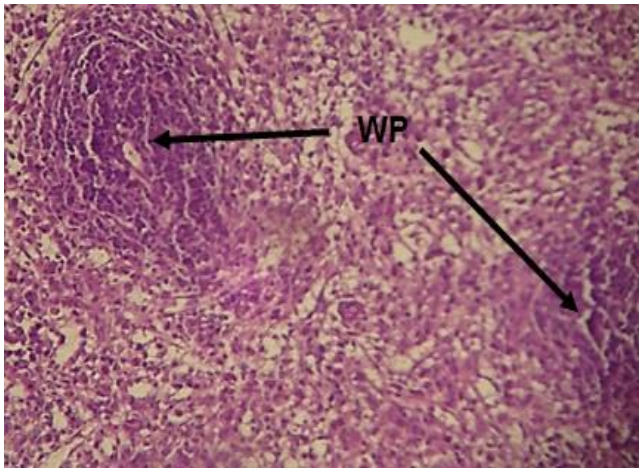

Figure 4: A cross section of spleen tissues of a male rat treated with meloxicam (0.4mg/kg BW. **WP:** Hyperplasia of germinal center with fused white pulp (H&E40X).

Table 1: Effects of Meloxicam solution (0.4 mg/kg BW) on the hematological parameters of albino male rats

Groups	RBCs ($\times10^{12}$/L)	WBCs ($\times10^{9}$/L)	PCV (%)	Hb (g/L)	Platelets ($\times10^{9}$/L)
Control	8.2±0.6	8.7±0.3*	46.2±1.4	12.3±0.2*	246.8±50*
Treated	8.8±0.3	15±1.3	49±1.6	17±0.5	645±170

Significant increase compared with the control group (*p<0.05)

Conclusion

The most important finding of this study is that meloxicam has the same harmful properties as commercial non-steroidal drugs, as it caused different damage in spleen tissues and abnormal hematological parameters in experimental male rats at therapeutic dose levels. As a result, meloxicam needs to be investigated further to determine its toxic efficacy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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