ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Effect of Folic Acid on Blood Picture in Female Rabbits Treated Experimentally with Methotrexate

Afaf Farooq Mawlood[1], Fidan Fikrat Ahmed[2], Ayoub Ali Hussein AL-Bayti[3*], Kasim Sakran Abass[4]

General Directorate for the education of Diyala, Iraq[1]
Medical Laboratory Techniques, Kirkuk Health and Medical Technical College[2,3]
Northern Technical University, Iraq. Department of Anatomy, 4Veterinary Medicine College, University of Kirkuk, Iraq[4]

Corresponding Author: 3*

ABSTRACT— Methotrexate (MX) is a drug with multiple physiological effects on the body. The objective of this search is to evaluate the enhancing action of folic acid on the blood profile of MX-treated rabbits. 30 female rabbits were divided into 3 groups and each group consisted of 10 animals. CON group, which included healthy rabbits, did not receive any treatment, while the MX group included animals which were injected intraperitoneally with methotrexate once a week. Finally, FO-MX rabbits received a combined dose of oral folic acid with methotrexate. The duration of the experiment lasted 4 weeks and after completion the animals were slaughtered and the marrow was collected. The results demonstrated that MX caused megaloblastic anemia which was characterized by erythroid hyperplasia, megaloblastoid polychromatophilic rubricytes, macromyelocytes and meta-myelocytes. While the FO-MX group showed a good prognosis and improved health. We concluded that co-administration of FO with MX ameliorated the adverse changes of MX, so we recommend that further studies be conducted to evaluate and understand the mechanism of effects of these drugs against the toxicity of the MX.

KEYWORDS: megaloblastic anemia, methotrexate, folic acid.

1. INTRODUCTION

Methotrexate (MX), a folic acid oppositionist, is utilized as an illness modifying anti-rheumatic drug concomitantly with NSAIDs at low doses [1], [2]. As well as participating in the treatment of several cancers [3]. Together with the extended usage of MX, a clear-defined toxicity features evidenced [4]. Experimentally, poisonous side effects of MX have been shown in many animals such as rats, rabbits, and even dogs [5]. Patients taking MX may have to discontinue treatment due to adverse effects, which in low doses are usually mild, self-limiting, or preventable, but may be more severe in some patients [6], [7]. Folate supplementation has been shown to be effective in preventing MX toxicity resulting from folic acid antagonism [8]. Folic acid is recognized as a water-soluble B vitamin and has been considered as member of B complex [9]. It is naturally present in a balanced diet and its intake is very necessary for humans health because it prohibits megaloblastic anemia, neural tube birth defects, heart diseases, changes in cognitive functions, osteoporosis and many cancers [10], [11]. Therefore, we conducted this research to know and evaluate the effect of co-administration of folic acid with methotrexate on female rabbits.

2. Materials and Methods

2.1 Chemicals

Methotrexate injection, USP, 25 mg/ml, pet pharmacy was used. Whilst dietary folate supplements tablets

(1333 mcg DFE) obtained from Spring Valley Company (USA).

2.2 Animals and experiment design

Thirty female rabbits were used in this experimental study. Their ages ranged between 10-11 months and weighed 1000-1200 g. They were kept in suitable environmental conditions in terms of temperature, exposure to light and were housed in plastic cages, with easy access to food and water. Rabbits were conditioned for one week before starting the experiment. They were divided into 3 groups with 10 rabbits in each, as shown in table 1.

Table 1: Experimental groups and dosages.

Groups	Dosages
CON	Included rabbits without any treatments as control.
MX	Rabbits were given an intraperitoneal injection of methotrexate at a dose of 20 mg/kg once a week lasts 4 weeks [12].
FO-MX	Rabbits were co-treated with oral folic acid at a dose of 5 mg/kg [**13**] and methotrexate at 20 mg/kg was administered intraperitoneally for 4 weeks.

2.3 Bone Marrow Examination

After sacrificing the animals, the femur was broken after cleaning the surrounding tissues, and a special needle was inserted into it to prepare bone marrow smears with the two slides technique as mentioned previously by Thrall [14]. The syringe was attached and negative pressure was applied. Once the marrow was collected, the marrow drop was allowed to spread smoothly on a glass slide. The spreader slide was then placed directly on top of the droplet with little pressure allowing the drop to spread smoothly before the two glass slides were withdrawn. After air drying, slides were stained with Giemsa stain and examined under an oil immersion lens.

2.4 Blood smear examination

A blood film was made on a slider and left to air dry, then smeared with Leishman's stain and examined by oil immersion as previously described [15]. The prepared blood smear was then poured over a sufficient amount of Leishman's spot and allowed to act for 2 minutes. The stain on the slide was then diluted with Leishman's buffer for at least 8 minutes, washed in distilled water, and dried in air. It was examined under oil emission to explain the differential shape and size of RBCs.

3. Results and discussion

In the results of the control rabbits (figure 1), normal bone marrow tissue with moderate hematopoietic cellular elements was seen. Meanwhile, there were no lymphocytes, plasma cells, or their precursors. Whereas for the MX rabbits section, megaloblastic adiposity was seen, as in figure 2, as well as a clear erythroid hyperplasia as in figure 3. In the rabbits of the same MX group a large number of vacuolated hyper-segmented neutrophils were observed (figure 4). In addition to vacuolated granulocyte precursor cells (figure 5). As for rabbits that were dosed with folic acid in the FO-MX group, a slight decrease in myeloid cells and neutrophils and a decrease in bands as well as mature neutrophils were seen, with a mild myeloid hyperplasia (figure 6). The cytological findings of MX group may be consistent with megaloblastic anemia.

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

This mostly results from inhibition of DNA synthesis in RBCs [16]. Most of the reasons for this inhibition are folate deficiency caused by the antagonist folic acid MX, an immunosuppressive drug. The granulocytic neutrophils in this group show large amounts of the highly detailed nutrients. This is thought to be due to the maturation of the nucleus of giant myeloid cells leading to the development of a large number of hyper-fractionated neutrophils [17], [18]. Several previous studies confirmed that hematological toxicity is one of the serious complications commonly observed with high doses of MX. Among these adverse complications is thrombocytopenia followed by rapidly progressive leukopenia, as well as megaloblasts [19], [20]. This damage may increase with the combined use of other drugs, and folic acid deficiency, when feeding rabbits exposed to MX with folic acid has given very positive results. In figure 7, the peripheral blood film of the control group showed red blood cells of normal size, shape, and count. As for the rabbits that were dosed with methotrexate, the peripheral blood film showed megaloblastic changes characterized by oval, macrocytic red cells, polychromatophilic, marked anisocytosis and poikilocytosis, and hyper-segmented neutrophiles (figure 8). All these changes in characteristics are consistent with other studies in mice indicating a case of megaloblastic anemia in this group [21]. When the animals were supplemented with folate, the blood film became closer to the control group (figure 9). This indicated that providing additional folic acid to those with a deficiency in the body corrected the deficiency. Untreated folate deficiency can lead to megaloblastic anemia and pancytopenia [22], [23].

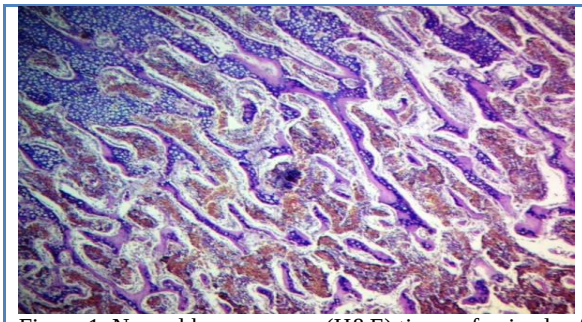

Figure 1: Normal bone marrow (H&E) tissue of animals of the CON group reveals cellular hematopoietic elements and bone trabeculae , scale bar(SB) = 100 µm.

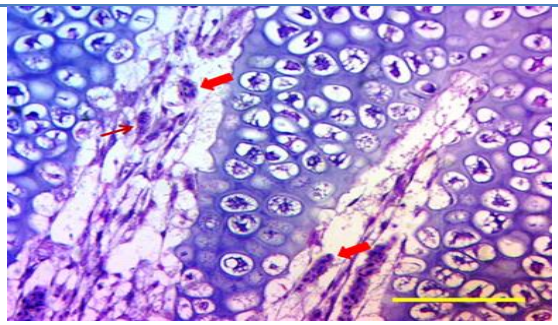

Figure 2: Bone marrow tissue (Gimsa) of MX rabbits, manifests megaloblastic adiposity (red arrows). Where there is a metarubricytic macrocytic (arrow) with a peanut -shaped nucleus at top left, SB = 50µm.

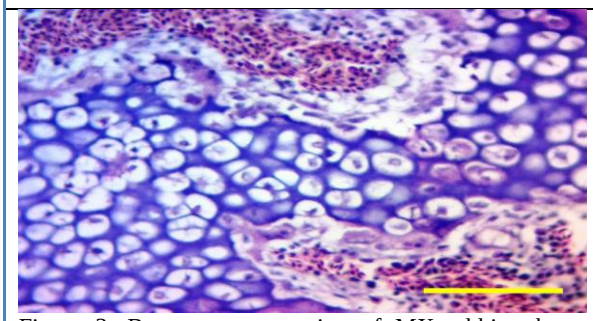

Figure 3: Bone marrow section of MX rabbits shows erythroid hyperplasia (prorubricytes and basophilic rubricytes with dark blue color cells), SB = 50 µm.

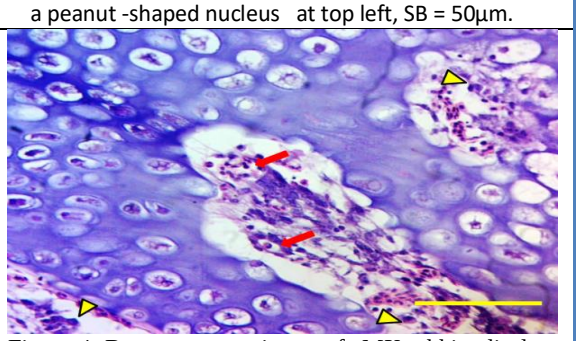

Figure 4: Bone marrow tissue of MX rabbits displays a large vaculated hypersegmented neutrophils (red arrows), with megaloblastoid polychromatophilic rubricytes (yellow head arrows) , SB = 50µm.).

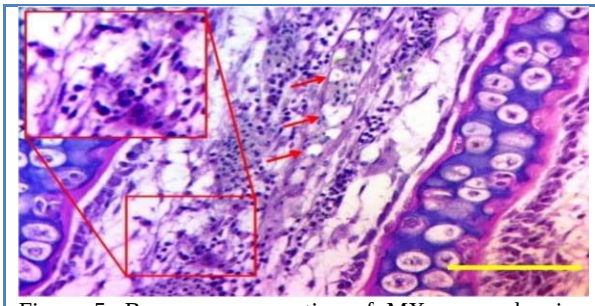

Figure 5: Bone marrow section of MX group showing vacuolated granulocytic precursor cells (red arrows). The red square box indicates that macromyelocytes and metamyelocytes presented very clearly after treatment SB = 50µm.

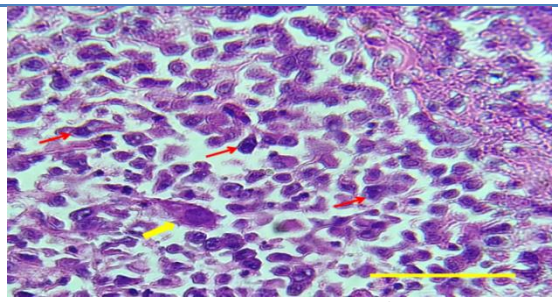

Figure 6: Bone marrow section of group FO-MX showing increase in the neutrophils metamyelocyte (red arrows) and there is a decrease in bands as well as mature neutrophils. There is a mild myeloid hyperplasia (yellow arrows) indicting for formation new cells and decrease in the number of neutrophils ,SB = 75µm).

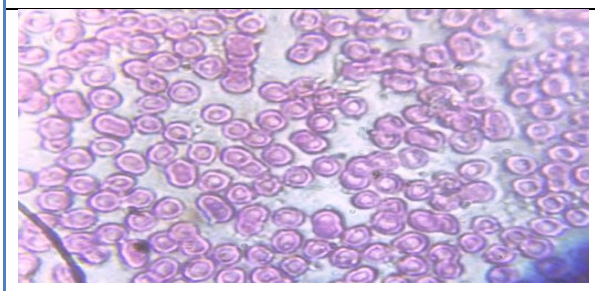

Figure 7: Peripheral blood film of CON group stained by Leishman's stain showed the normal size, shape, and number of red blood cells.

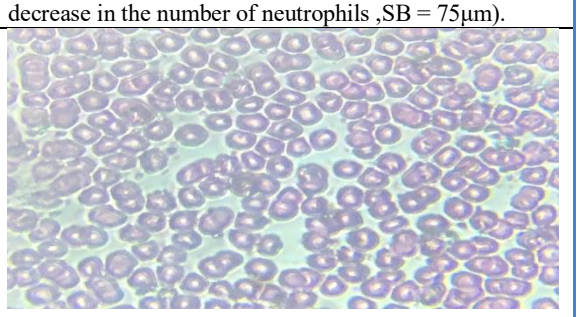

Figure 8: Peripheral blood film of MX group (megaloblastic anemia) stained by Leishman's stain.

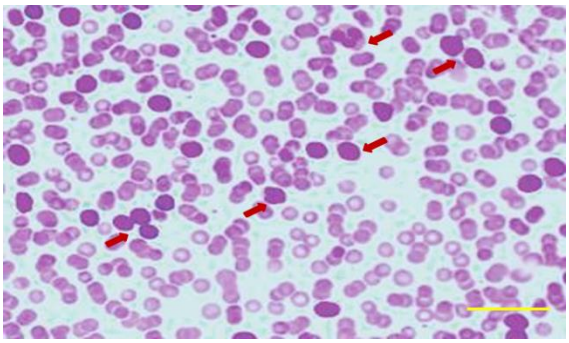

Figure 9: Peripheral blood film of FO-MX group with normal morphology of erythrocytes. However there is still decrease in the number of the erythrocyte, the section shows numerous early myeloid precursors and normoblasts (yellow arrows), SB = 50µm.

4. Conclusions

We concluded that an intraperitoneal injection of methotrexate once a week for one month has toxic effects on the blood, but when combined treatment with folic acid, these damages are noteworthy reduced.

5. References

[1] Cantini F, Niccoli L, Capone A, Petrone L, Goletti D. Risk of tuberculosis reactivation associated with traditional disease modifying anti-rheumatic drugs and non-anti-tumor necrosis factor biologics in patients with rheumatic disorders and suggestion for clinical practice. Expert opinion on drug safety. 2019 May 4;18(5):415-25.

[2] Sharma UR, Rathnakaran AN, Raj BP, Padinjakkara G, Das A, Vada S, Mudagal MP. The positive

effect of pregnancy in rheumatoid arthritis and the use of medications for the management of rheumatoid arthritis during pregnancy. Inflammopharmacology. 2021 Apr 12:1-4.

[3] Kaur L, Sohal HS, Kaur M, Malhi DS, Garg S. A mini-review on nano technology in the tumour targeting strategies: drug delivery to cancer cells. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2020 Nov 1;20(17):2012-24.

[4] Young EP, Cheng WS, Bernhardt MB, Wang LL, Rainusso N, Foster JH. Risk factors associated with delayed methotrexate clearance and increased toxicity in pediatric patients with osteosarcoma. Pediatric blood & cancer. 2020 Apr;67(4):e28123.

[5] Patel NN, Ghodasara DJ, Pandey S, Ghodasara PD, Khorajiya JH, Joshi BP, Dave CJ. Subacute toxicopathological studies of methotrexate in Wistar rats. Veterinary World. 2014 Jul 1;7(7).

[6] Gisondi P, Altomare G, Ayala F, Bardazzi F, Bianchi L, Chiricozzi A, Costanzo A, Conti A, Dapavo P, De Simone C, Foti C. Italian guidelines on the systemic treatments of moderate-to-severe plaque psoriasis. Journal of the European Academy of Dermatology and Venereology. 2017 May;31(5):774-90.

[7] Friedman B, Cronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. Joint Bone Spine. 2019 May 1;86(3):301-7.

[8] Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nature reviews Rheumatology. 2020 Mar;16(3):145-54.

[9] Borah PK, Sarkar A, Duary RK. Water-soluble vitamins for controlling starch digestion: Conformational scrambling and inhibition mechanism of human pancreatic α-amylase by ascorbic acid and folic acid. Food chemistry. 2019 Aug 1;288:395-404.

[10] Cieślik E, Cieślik I. Occurrence and significance of folic acid. Pteridines. 2018 Dec 31;29(1):187-95.

[11] Acosta-Elias J, Espinosa-Tanguma R. The folate concentration and/or folic acid metabolites in plasma as factor for COVID-19 infection. Frontiers in pharmacology. 2020 Jul 16;11: 1062.

[12] Soliman ME. Evaluation of the possible protective role of folic acid on the liver toxicity ınduced experimentally by methotrexate in adult male albino rats. Egypt J Histol. 2009;32(1):118-28.

[13] Ghadhban RF, Alwan NA. The Role of Folic Acid on Some Physiological Parameters and Efficiency of Sperm in Male Rabbits. Systematic Reviews in Pharmacy. 2020;11(9):1003-7.

[14] Thrall, M. A. Veterinary Hematology and Clinical Chemistry. Lippencott Williams and Wilkins com. Philadelphia. (2004).

[15] Nivedhita G, Brundha MP. Analysis of Papanicolaou stain on peripheral smear compared to Leishman's stain: A prospective study. International Journal of Clinicopathological Correlation. 2020 Jul 1;4(2):40.

[16] Powers JM. Nutritional anemias. InLanzkowsky's Manual of Pediatric Hematology and Oncology 2022 Jan 1 (pp. 61-80). Academic Press.

[17] AKDAĞ İ, Ersoy A. Development of pancytopenia after single low-dose methotrexate therapy in patients with chronic kidney disease: a review of the literature. Turkish Journal of Internal Medicine. 2020;2(3):83-90.

[18] Khan KM, Jialal I. Folic Acid Deficiency. In: Stat Pearls. Stat Pearls Publishing, Treasure Island (FL); 2021. PMID: 30570998.

[19] Gaies E, Jebabli N, Trabelsi S, Salouage I, Charfi R, Lakhal M, Klouz A. Methotrexate side effects: review article. J Drug Metab Toxicol. 2012;3(4):1-5.

[20] Ahmadzadeh A, Zamani N, Hassanian-Moghaddam H, Hadeiy SK, Parhizgar P. Acute versus chronic methotrexate poisoning; a cross-sectional study. BMC Pharmacology and Toxicology. 2019 Dec;20(1):1-7.

[21] Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. Blood reviews. 2014 Mar 1;28(2):49-66.

[22] Attia AAA, Amer MAEM, Hassan M, Din SFG. Low serum folic acid can be a potential independent risk factor for erectile dysfunction: a prospective case-control study. Int Urol Nephrol. 2019 Feb;51(2):223-229.

[23] Torrez M, Chabot-Richards D, Babu D, Lockhart E, Foucar K. How I investigate acquired megaloblastic anemia. International Journal of Laboratory Hematology. 2022 Jan 3.