Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index *eISSN: 3107-0426*

Original article

Ameliorative Effects of Pistacia Atlantica on Methotrexate Induced Haematological Toxicity in Albino Rats. Part 1: Model Establishment

Anwar Abdalmula[1]*, Amaal Alhameedi[2], Ayiman Alamari[3], Fahima Alnagar[4]

[1]*Department of Physiology, Biochemistry and Nutrition, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya*
[2]*National Authority for DNA Research and Analysis, Tripoli, Libya*
[3]*Department of Pharmaceutical Technology, College of Medical Sciences and Technologies, Tripoli, Libya*
[4]*Department of Biochemistry, Faculty of Medicine, University of Tripoli, Tripoli, Libya*
Email. *a.alessawi@uot.edu.ly*

Keywords.
Methotrexate, Pistacia Atlantica, Haematological Changes, Albino Rats

Abstract

Methotrexate (MTX) is an antifolate drug widely used in cancer chemotherapy and treatment of rheumatoid arthritis and other inflammatory diseases. The haematological toxicity of MTX is one of its major side effects. The present study is the first of a 2-part series investigating the ameliorative effects of *Pistacia Atlantica* on MTX-induced haematological toxicity in albino rats. This first part establishes the MTX-induced haematological toxicity model in albino rats in terms of MTX dosage and rats' gender preference that is more susceptible to induction, while the second part evaluates the ameliorative effects of *P. Atlantica* on this model. A total of 30 rats were divided into 6 groups with 5 rats/group. Fifteen females and 15 males were each allocated to 3 groups: G1, G2, G3. The G1, G2 and G3 groups of both genders were injected intraperitoneally once with 40 mg/kg, 20 mg/kg and 10 mg/kg MTX, respectively. Blood samples were collected from all rats before MTX induction (baseline or day 0) and 7 days post-MTX induction (euthanasia day or day7) to determine haematological indices. Both rat genders that received low (G3) and medium (G2) MTX doses showed a reduction in RBC counts, Hb concentration and HCT values and an elevation of MCH, MCHC, MPV and PLCR values. Only the female group that received low MTX doses showed elevation in MCV, RDW-CV, RDW-SD, PLT counts and PCT values. The low dose of MTX also showed high PDW and P-LCC in both genders. The haematological changes observed in this study suggest that the female gender of rats and the low dose of MTX are the best variables to be used in the next trial in part 2 of this series.

Introduction

Methotrexate (MTX) is a structural analogue of folic acid act as a competitive inhibitor of the dihydrofolate reductase enzyme that catalyses the reduction of dihydrofolate to tetrahydrofolate, the active form of folic acid required in dividing cells to generate the DNA monomer thymidine monophosphate and is essential for cell replication and synthesis of DNA and RNA [1]. This anti-proliferative property made MTX at high doses a valuable chemotherapy agent against a variety of malignant disorders [2]. In addition to the anti-cancer effect, MTX at low doses has anti-inflammatory and immunosuppressive actions and is widely used as a first-line treatment in autoimmune [3] and inflammatory diseases such as rheumatoid arthritis [4], psoriasis [5] and Crohn's disease [6]. These MTX actions contribute to a variety of mechanisms, such as promotion of adenosine release that has potent inhibitory effects on nearly all inflammatory cell types [7], nitric oxide synthase uncoupling that increases the sensitivity of T cells to apoptosis in response to methotrexate stimulation [8], expression of certain long non-coding RNAs that induce apoptosis [9] as well as inhibition of specific pathways such as Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway, which stimulates production of a variety of inflammatory signals [10, 11].

Although the use of MTX has therapeutic benefits in many diseases, its use has adverse effects such as haematological toxicity [12], hepatic toxicity [13], renal toxicity [14], pulmonary toxicity [15, 16], neurotoxicity [17], increased infection risk [18-20], dermatological toxicity [21] and gastrointestinal side effects [22]. The haematological toxicity of methotrexate includes myelosuppression [23], leukopenia [24], thrombocytopenia [25], neutropenia [26], pancytopenia [24] and megaloblastic anaemia [12, 27]. The present study is the first of a 2-part series investigating the ameliorative effects of *P. Atlantica* on methotrexate induced haematological toxicity in albino rats. This first part of these manuscripts establishes the haematological toxicity model in terms of selecting the methotrexate dosage and gender of rats, while the second part evaluates the ameliorative effects of *P. Atlantica* on this model.

Methods

Ethical approval

All experimental protocols were approved by the Bioethics Committee at the Biotechnology Research Centre (BEC-TR) under reference number (001.A.26.41).

Received: *08-07-2026 -* ***Accepted:*** *06-09-2026 -* ***Published:*** *14-09-2026*

Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index *eISSN: 3107-0426*

Animals and Study Design

The study was conducted for seven days at the small animal house of the Faculty of Medicine, University of Tripoli, Tripoli, Libya. Thirty healthy adult albino rats of both sexes were used for the study and maintained under standard laboratory conditions and fed *ad libitum* standard commercial rat feed and clean drinking water. The experiment was designed by injecting three different doses of methotrexate (50 mg/2 ml injectable solution, Hikma Specialised Pharmaceuticals, Cairo, Egypt) into 30 albino rats (15 females and 15 males) allocated randomly into 6 groups with 5 rats in each group. Both female and male rat groups were allocated into 3 groups; group 1 (G1), group 2 (G2) and group 3 (G3) of both genders received single intraperitoneal injections of 40 mg/kg, 20 mg/kg and 10 mg/kg methotrexate, respectively.

Blood samples were collected from the retro-orbital plexus at two time points: before methotrexate injection (day 0 time point) and after 7 days of injection (euthanasia day or day 7 time point). The ethylene diamine tetra acetic acid (EDTA) - anti coagulated blood was used to determine the hematological parameters of total white blood cells count (WBC, $x10^3/mm^3$), red blood cell count (RBC, $x10^6/mm^3$), haemoglobin concentration (Hb, g/dl), haematocrit (HCT, %), Mean Corpuscular Volume (MCV, fL), Mean Corpuscular Haemoglobin (MCH, pg), Mean Corpuscular Haemoglobin concentration (MCHC, g/dl), Red Cell Distribution Width- Coefficient of Variation (RDW-CV, %), Red Cell Distribution Width-Standard Deviation (RDW-SD, fL), Platelet count (PLT, $x10^3/mm^3$), Mean Platelet Volume (MPV, fL), Platelet Distribution Width (PDW, fL), Plateletcrit (PCT, %), Platelet Large Cell Count (P-LCC, $x10^3/mm^3$), and Platelet Large Cell Ratio (PLCR, %).

Statistical Methods

Results are expressed as mean ± SEM. Data were analysed using GraphPad Prism statistical software (version 6.07; GraphPad Software Inc., La Jolla, CA, USA). Statistical analysis of data between groups was performed using Wilcoxon matched-pairs signed-rank tests, and statistical significance was accepted at $p < 0.05$.

Results

The mean ± SEM values of the total WBC counts are shown in Figure 1. Most of the rat groups showed a lower total WBC count after 7 days of methotrexate injection than on day 0. However, this drop was not statistically significant except in the male rat group, which received the medium dose of methotrexate.

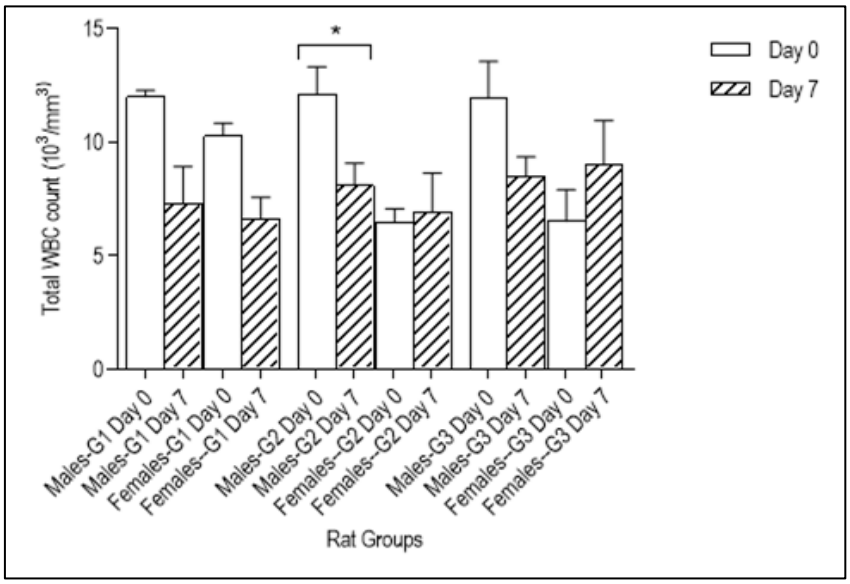

Figure 1. The total WBC count in the blood of MTX-treated rats.

*Blood samples were collected at 2 time points (0 and 7 days), and the total WBC counts were determined. Except for the G2 male rat group, which received 20mg/kg methotrexate, no significant difference in the total leukocyte numbers was detected between days 0 and 7 of the tested rat groups. Blood values were analysed by paired Wilcoxon tests, and each column represents the mean ± SE of 5 rats, with * representing $P < 0.05$.*

The mean ± SEM values of the erythrocyte indices are shown in Figure 2. There was a reduction in RBC counts, Hb concentration and HCT values on day 7 compared to day 0 in the G2 and G3 groups of both genders. On the other hand, there was an elevation in MCH and MCHC values in the groups of G2 and G3 of both genders and an elevation in MCV, RDW-CV and RDW-SD values in the groups of G3 of the female groups. The groups of G1 in both genders did not show significant differences in the tested erythrocyte parameters.

Received: *08-07-2026 -* ***Accepted:*** *06-09-2026 -* ***Published:*** *14-09-2026*

Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index eISSN: 3107-0426

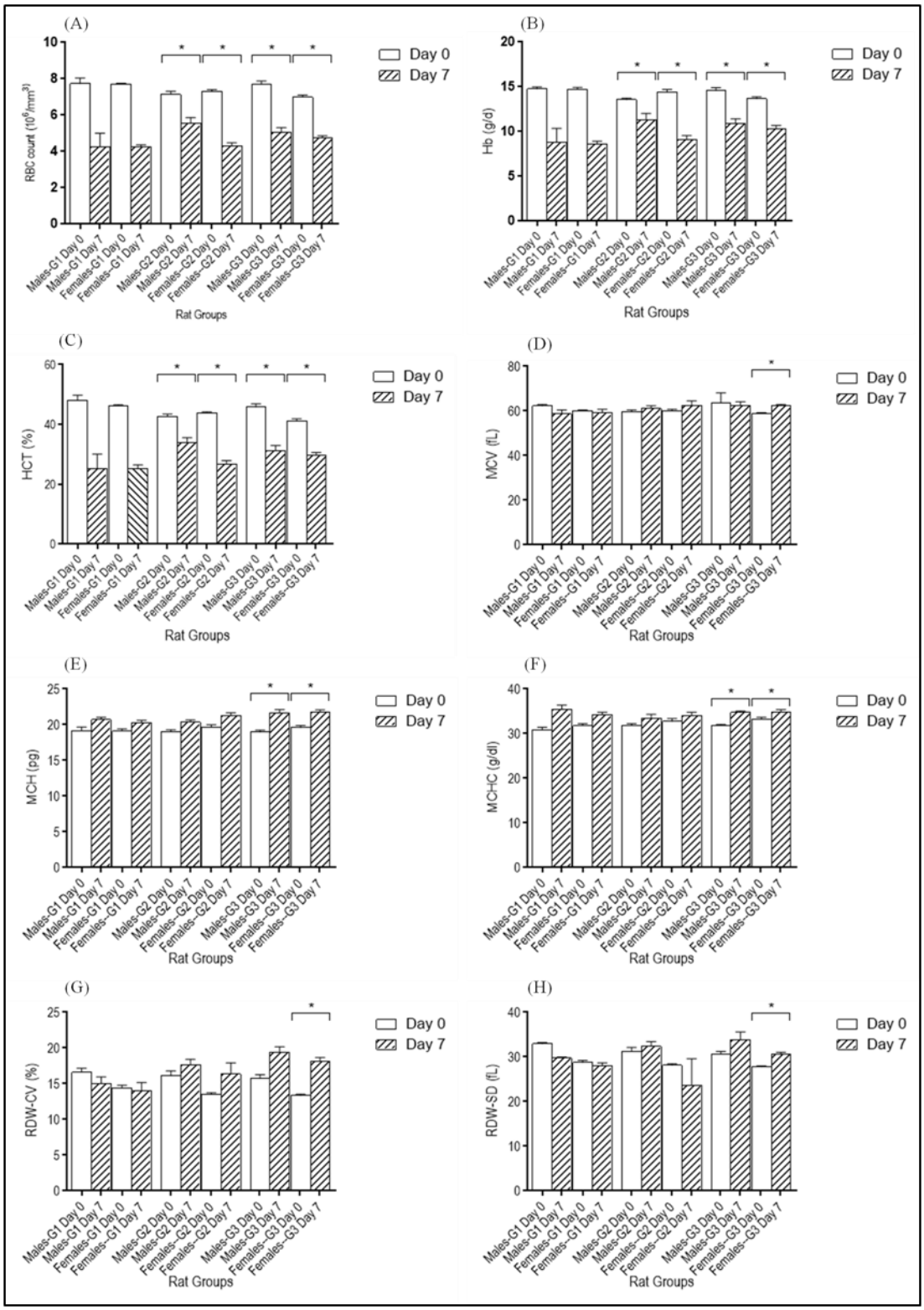

Figure 2. The values of the erythrocyte indices in the blood of MTX-treated rats.

*Blood samples were collected at 2 time points (0 and 7 days), and the RBC counts (A), Hb concentration (B), HCT (C), MCV (D), MCH (E), MCHC (F), RDW-CV (G) and RDW-SD (H) values were determined. There was a reduction in G2 and G3 regarding RBC counts, Hb concentration and HCT; but an elevation in the MCH and MCHC values of both genders, and an elevation in the female group of G3 regarding the MCV, RDW-CV and RDW-SD values. The G1 group in both genders did not show significant differences in the tested erythrocyte parameters. Blood values were analysed by paired Wilcoxon tests, and each column represents the mean ± SE of 5 rats, with * representing $P < 0.05$.*

Received: 08-07-2026 - **Accepted:** 06-09-2026 - **Published:** 14-09-2026

Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index eISSN: 3107-0426

The mean ± SEM values of the platelet indices are shown in Figure 3. There was an elevation in the platelet indices on day 7 compared to day 0 in the females of group G3 regarding PLT counts and PCT values; and in both genders of the same group regarding PDW and P-LCC. The elevation of MPV and PLCR values was determined in both genders of groups G2 and G3. The groups of G1 in both genders did not show significant differences in the tested platelet parameters.

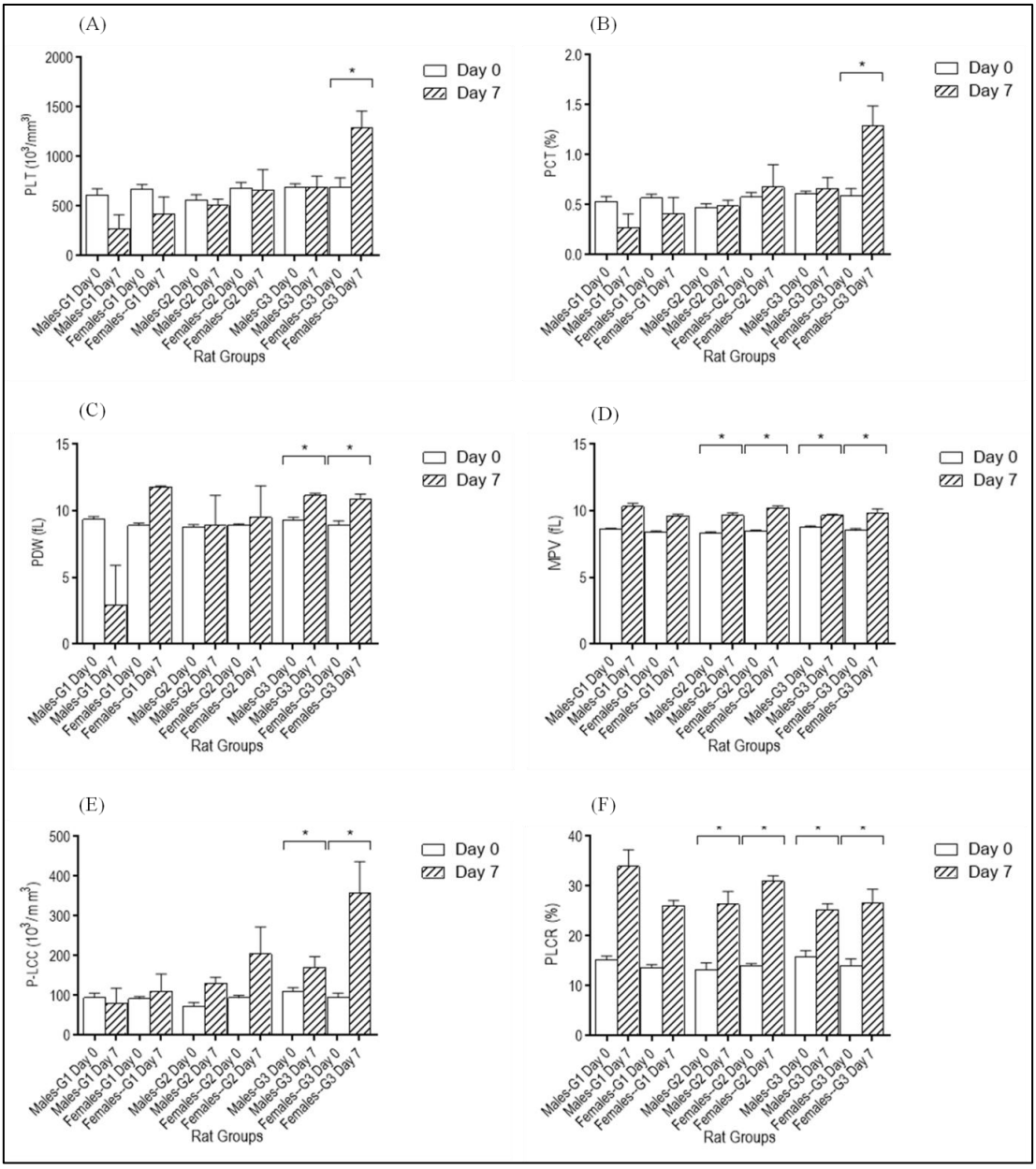

Figure 3. The values of the platelet indices in the blood of MTX-treated rats.

*Blood samples were collected at 2 time points (0 and 7 days), and the PLT counts (A), PCT (B), PDW (C), MPV (D), P-LCC (E) and PLCR (F) values were determined. There was an elevation in the female group G3 regarding PLT counts and PCT, and in both genders of the same group regarding PDW and P-LCC. The elevation of MPV and PLCR values was determined in both genders of groups G2 and G3. The groups of G1 in both genders did not show significant differences in the tested platelet parameters. Blood values were analysed by paired Wilcoxon tests, and each column represents the mean ± SE of 5 rats, with * representing P < 0.05.*

Discussion

In human medicine, gender affects the pathophysiology and response to therapy in many diseases [28-30]. In order to establish an animal model to study the effect of a therapeutic agent, the gender of the animal used and the dosage of the inducer are among the important variables to be considered in model design. This study aimed to determine which animal gender is most susceptible to different doses of MTX for establishing a haematological toxicity model and subsequently to evaluate the ameliorative effects of *P. Atlantica* on this model. After 7 days of MTX injection with different doses into both rat genders, the animals in this study showed the expected haematological responses to MTX induction. Changes in

Received: *08-07-2026 -* ***Accepted:*** *06-09-2026 -* ***Published:*** *14-09-2026*

Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index eISSN: 3107-0426

erythrocyte indices were observed consistent with the expected drop in folic acid levels known to induce megaloblastic anaemia. These changes include reduction in the RBC counts, Hb concentration and HCT values; and abnormally large size of RBC reflected by elevation of MCV, with high MCH, MCHC, RDW-CV and RDW-SD values. These changes were attributed by some authors to the ineffective erythropoiesis observed in megaloblastic anaemia [31, 32]. Thrombocytopenia was also observed in this study.

It was manifested through the high elevation of MPV, which reflects large platelet sizes due to megakaryocytic activity of bone marrow [33], high PDW, which suggests a wide range of platelet sizes due to abnormal platelet production and high P-LCR, which is directly related to PDW and MPV and inversely related to platelet count [33]. Thrombocytopenia is observed in megaloblastic anaemia as a consequence of hypoproduction or ineffective thrombocytosis [34, 35]. However, the platelet counts and PCT values did not significantly differ between groups except in one female group exposed to the low dose of methotrexate, which showed a higher platelet count. This might be attributed to reactive thrombosis to anaemia, as seen with iron deficiency anaemia or haemolytic anaemia [36]. In regards to the leukopenia observed as MTX side effects [24],the total WBC counts in this study dropped in all groups after 7 days of MTX injection; however, this drop was not statistically significant except in one group. Although the rats that received the large dose of MTX showed similar trends of haematological changes observed in rats that received the low and medium doses, these changes were not statistically significant. This might be attributed to the unfortunate death of one rat in the male group, which reduced the sample size and compromised the statistical power of the findings for that cohort.

Conclusion

In conclusion, both rat genders that received low and medium MTX doses showed a reduction in RBC counts, Hb concentration and HCT values, and an elevation in MCH, MCHC, MPV and PLCR values. However, only the female group that received low MTX doses showed an elevation in MCV, RDW-CV, RDW-SD values, PLT counts and PCT. The low dose of MTX also showed high PDW and P-LCC in both genders. Having said that, this study showed that the female rat group that received the low dose of MTX was the most severely affected group, which suggests that the female gender and the low dose of MTX are the suitable variables to be used for the upcoming trial in the second part of this series.

Conflict of interest. Nil

References

1. Rodwell VW. Metabolism of purine & pyrimidine nucleotides. In: Harper's Illustrated Biochemistry. New York: McGraw Hill; 2023.
2. Mantadakis E, Cole PD, Kamen BA. High-dose methotrexate in acute lymphoblastic leukemia: where is the evidence for its continued use? Pharmacotherapy. 2005;25(5):748-55.
3. Cipriani P, et al. Methotrexate: an old new drug in autoimmune disease. Expert Rev Clin Immunol. 2014;10(11):1519-30.
4. Œwierkot J, Szechiński J. Methotrexate in rheumatoid arthritis. Pharmacol Rep. 2006;58(10086):473-92.
5. Czarnecka-Operacz M, Sadowska-Przytocka A. The possibilities and principles of methotrexate treatment of psoriasis – the updated knowledge. Adv Dermatol Allergol. 2014;31(6):392-400.
6. Lémann M, et al. Methotrexate in Crohn's disease: long-term efficacy and toxicity. Am J Gastroenterol. 2000;95(7):1730-4.
7. Cronstein BN, Sitkovsky M. Adenosine and adenosine receptors in the pathogenesis and treatment of rheumatic diseases. Nat Rev Rheumatol. 2017;13(1):41-51.
8. Spurlock CF 3rd, et al. Increased sensitivity to apoptosis induced by methotrexate is mediated by JNK. Arthritis Rheum. 2011;63(9):2606-16.
9. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012;81:145-66.
10. Malemud CJ. The role of the JAK/STAT signal pathway in rheumatoid arthritis. Ther Adv Musculoskelet Dis. 2018;10(5-6):117-27.
11. Thomas S, et al. Methotrexate is a JAK/STAT pathway inhibitor. PLoS One. 2015;10(7):e0130078.
12. Singh YP, et al. Low-dose methotrexate-induced pancytopenia. Clin Rheumatol. 2007;26:84-7.
13. Curtis JR, et al. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. Ann Rheum Dis. 2010;69(1):43-7.
14. Grönroos M, et al. Methotrexate induces cell swelling and necrosis in renal tubular cells. Pediatr Blood Cancer. 2006;46(5):624-9.
15. Kim YJ, Song M, Ryu JC. Inflammation in methotrexate-induced pulmonary toxicity occurs via the p38 MAPK pathway. Toxicology. 2009;256(3):183-90.
16. Ameen M, et al. Pneumonitis complicating methotrexate therapy for pustular psoriasis. J Eur Acad Dermatol Venereol. 2001;15(3):247-9.
17. Brugnoletti F, et al. Recurrent intrathecal methotrexate induced neurotoxicity in an adolescent with acute lymphoblastic leukemia: serial clinical and radiologic findings. Pediatr Blood Cancer. 2009;52(2):293-5.
18. Altz-Smith M, Kendall LG Jr, Stamm AM. Cryptococcosis associated with low-dose methotrexate for arthritis. Am J Med. 1987;83(1):179-81.
19. Aglas F, et al. Interstitial pneumonia due to cytomegalovirus following low-dose methotrexate treatment for rheumatoid arthritis. Arthritis Rheum. 1995;38(2):291-2.

Received: *08-07-2026 -* ***Accepted:*** *06-09-2026 -* ***Published:*** *14-09-2026*

Libyan Med J. 2026;18(9):606-611
https://doi.org/10.69667/lmj.26910
Libyan Medical Journal
https://lmj.ly/index.php/ojs/index eISSN: 3107-0426

20. McLean-Tooke A, et al. Methotrexate, rheumatoid arthritis and infection risk – what is the evidence? Rheumatology. 2009;48(8):867-71.
21. Truchuelo T, et al. Focal skin toxicity related to methotrexate sparing psoriatic plaques. Dermatol Online J. 2010;16(6):16.
22. Ben-Lulu S, et al. Dietary transforming growth factor-beta 2 (TGF-β2) supplementation reduces methotrexate-induced intestinal mucosal injury in a rat. PLoS One. 2012;7(...).
23. Mori S, et al. Factors associated with myelosuppression related to low-dose methotrexate therapy for inflammatory rheumatic diseases. PLoS One. 2016;11(4):e0154744.
24. Gutierrez-Ureña S, et al. Pancytopenia secondary to methotrexate therapy in rheumatoid arthritis. Arthritis Rheum. 1996;39(2):272-6.
25. Paul M, et al. Methotrexate promotes platelet apoptosis via JNK-mediated mitochondrial damage: alleviation by N-acetylcysteine and N-acetylcysteine amide. PLoS One. 2015;10(6):e0127558.
26. Pamuk ON, et al. Do impaired memory, cognitive dysfunction and distress play a role in methotrexate-related neutropenia in rheumatoid arthritis patients? A comparative study. Rheumatol Int. 2013;33:2631-5.
27. Griffiths C, et al. A systematic review of treatments for severe psoriasis. Database Abstr Rev Eff (DARE). 2000.
28. Cassese M, Zuber V. Clinical trials and gender medicine. Ann Ist Super Sanita. 2011;47:100-3.
29. Leitner MK, Kautzky-Willer A. Gender-specific differences in age-associated endocrinology. Z Gerontol Geriatr. 2013;46:505-10.
30. Regitz-Zagrosek V, Seeland U. Sex and gender differences in clinical medicine. In: Regitz-Zagrosek V, editor. Sex and gender differences in pharmacology. Berlin: Springer; 2012. p. 3-22.
31. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clin Med Res. 2006;4(3):236-41.
32. Srinivasan A. Failure of erythrocyte production. In: Kamat DM, Frei-Jones M, editors. Benign hematologic disorders in children: a clinical guide. Cham: Springer; 2021. p. 369-83.
33. Bashir AB, et al. Role of platelet indices in patients with dengue infection in Red Sea State, Sudan. Int J Sci Res. 2015;4(1):1573-6.
34. Nishikawa M. Thrombocytopenia due to deficient platelet production. Nihon Rinsho. 2003;61(4):575-80.
35. Baig MA. Platelet indices – evaluation of their diagnostic role in pediatric thrombocytopenias. Int J Res Med Sci. 2015;3(9):2284-9.
36. Keung YK, Owen J. Iron deficiency and thrombosis: literature review. Clin Appl Thromb Hemost. 2004;10(4):387-91.

Received: *08-07-2026 -* ***Accepted:*** *06-09-2026 -* ***Published:*** *14-09-2026*