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HISTOPATHOLOGICAL AND BIOCHEMICAL STUDIES OF METHOTREXATE HEPATOTOXICITY ON ALBINO RATS

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ABSTRACT

Methotrexate (MTX), the antiproliferative, anti-inflammatory, and immunosuppressive drug is one of the most effective drugs used for the treatment of a large number of solid tumors, hematologic malignancies, and autoimmune disorders. However, its significant hepatotoxicity limits its applicability, so this study was suggested to investigate the side effects of a high dose of MTX on the liver in experimental rats. Ten rats were divided randomly into two groups, including the control group and MTX-injected group. MTX group received a single dose of 40 mg/kg MTX intraperitoneally to induce liver injury. Physiological saline was injected into the control rats in the same manner. The period of the experiment was 14 days. At the end of the experiment, the rats were sacrificed. Sera and liver specimens were then collected for the evaluation of hepatic function by measurement of aspartate transaminase (AST) and alanine transaminase (ALT) serum levels and histological examination of liver tissues. The results showed that MTX administration induced a highly significant increase in serum AST and ALT levels. Additionally, the histopathological examination of livers indicated the presence of clear vacuoles in the hepatocytes, hydropic degeneration, and multi-focal necrosis. Additionally, there was mononuclear cell infiltration and Kupffer cellular hyperplasia. Congestion, desquamation of lining endothelial cells in some blood vessels, and haemorrhages were also detected. Therefore, we concluded that administration of high doses of MTX induced severe hepatotoxicity in experimental rats manifested by a significant increase of liver enzymes in serum and severe alteration in the liver histological structure.

Keywords: Methotrexate; hepatotoxicity; histopathological examination; hepatic enzymes.

INTRODUCTION

In the discipline of oncology, chemotherapy refers to medications used to treat cancer. Unfortunately, chemotherapy has several side effects, such as nausea and vomiting, alopecia or hair loss, and fatigue. Low leukocytic count and susceptibility to infections, low platelet count and bleeding problems, and low erythrocytic count and anemia can occur during chemotherapy. It can also cause mucositis, loss of appetite, pregnancy and fertility problems, bowel problems, and mental health problems (Janelsins *et al.*, 2011).

MTX, 2,4- diamino- N10-methyl ropylglutamic acid is a folic acid analog. In this drug, the NH2 and CH3 groups are

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linked to the C4 carbon and N10 hydrogen (Rahman and Chhabra, 1988). It is one of the most effective drugs used to treat a large number of solid tumours, hematologic malignancies, and autoimmune disorders (Purcell and Ettinger, 2003). Also, breast cancer, acute lymphocytic leukemia (ALL), osteogenic sarcoma, choriocarcinoma, lung cancer, and bladder carcinoma are well treated by MTX. In addition, MTX can be used for the treatment of primary CNS lymphoma and chronic myeloid leukemia (Grim *et al.*, 2003). It has been widely used for the treatment of psoriasis, rheumatoid arthritis, acute lymphoblastic leukemia, ectopic pregnancy, Crohn's disease, and ulcerative colitis (Herfarth, 2016).

MTX is considered an antiproliferative, anti-inflammatory, and immunosuppressive drug (Iwase *et al.*, 2015). It can act by competitive inhibition of dihydrofolate reductase. This enzyme is involved in the formation of tetrahydrofolate (Pountos and Giannoudis, 2017). The intracellular stores of tetrahydrofolate, which have a pivotal role in purine nucleotides and thymidylate synthesis, which are crucial for cell division and DNA synthesis are depleted as a result of this inhibition. Purine nucleotide and thymidylate synthesis inhibition during the S-phase of the cell cycle, ultimately prevents DNA synthesis, repair, and cellular replication (Wiczer *et al.*, 2016). Unfortunately, the significant hepatotoxicity of MTX limits its applicability (Duman *et al.*, 2013). Although MTX hepatotoxicity is still unclear, there have been a lot of trials to explain the drug's pathophysiological mechanisms to induce its hepatotoxicity. However, increased generation of reactive oxygen species (ROS), exhaustion of endogenous antioxidants, and augmented lipid peroxidation seem to play a crucial role in MTX hepatotoxicity (Mahmoud *et al.*, 2017). Consequently, this study was carried out to explore MTX hepatotoxicity in high doses and the possible mechanisms underlying its side effects on experimental rat models. Estimation of serum levels of AST and ALT and the histopathological examination of the liver was involved in this study.

MATERIALS AND METHODS

Animals:

Ten male rats weighing approximately 200-250g were bought from VACSERA, Helwan, Egypt. They were kept in cages under the standard room temperature and normal light/dark cycle in the laboratory of the Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University, Egypt. In addition, they had ad libitum access to commercial pellets and fresh drinking tap water during the period of the experiment.

Chemicals and kits:

Methotrexate was obtained as 50 mg/2ml injection vials from Mylan, Paris. ALT and AST kits were obtained from SPINREACT, Egypt.

Experimental design:

After the two weeks of accommodation, the animals have been separated into two groups, five rats in each cage. The control group in which the animals received I.P. normal saline single dose after seven days of the beginning of the study. The second group was the MTX group where the animals were injected with 40 mg/kg BW as a single dose of MTX intraperitoneally after seven days following the beginning of the study (Letertre *et al., 2020*). The duration of the whole experiment was two weeks. Ethically, all experimental protocols applied on the animals were approved by Assiut University

Sample Collection and Preparation:

All animals were sacrificed under the effect of anesthesia by chloroform inhalation after the experiment had been accomplished. After the animals had been completely anesthetized, blood samples were collected for serum preparation from the heart of each rat by using a 3 ml disposable syringe. Then, the collected blood was put in sterilized plain tubes and left for clotting in a slope

position at room temperature and centrifuged at 4,000 rpm for 15 minutes. After that, sera were aspirated by micropipette, distributed into Eppendorf tubes, and kept frozen at -20 °C till the time of analysis. Additionally, liver specimens were isolated and washed with normal saline. After washing, they were cut into pieces, placed in 10% phosphate-buffered formalin for fixation, and then underwent processing for histopathological examination.

Liver function tests:
The Central Lab. of Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University was the place where the liver function was estimated. Serum levels of AST and ALT were measured by using of 6705 UV |Vis Spectrophotometer (Murray and Kaplan, 1984).

Histopathological examination:
Tissue specimens were processed routinely, sectioned at 4µm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination by light microscopy (Olympus, CX,31; Tokyo Japan) and photographed using a digital camera (Toupview, LCMos10000KPA, China) (Bancroft and Stevens, 1997). The method of Kose *et al.* was used for the histopathological semiquantitative scoring of liver damage in this study with some modifications to evaluate the extent of damage. Microscopic damage was identified as absent (0), slight (1), moderate (2), and severe (3), for each finding (Kose *et al.*, 2012).

Statistical analysis:
Student's t-test was used in the statistical analysis, which was carried out by using the Prism program, version 5.01. The level of significance was set at $P<0.05$ and the data were presented as mean ± S.E.

RESULTS

Liver function tests (AST and ALT levels:
AST levels showed a significant increase in the MTX group 103.0 ± 1.58 when compared to the control one 85.40 ± 0.51. In addition, there was a significant increase in MTX-treated rats 67.32 ± 4.35 in comparison with control rats 34.88 ± 2.85 in ALT levels. AST and ALT levels are demonstrated in Figure 1.

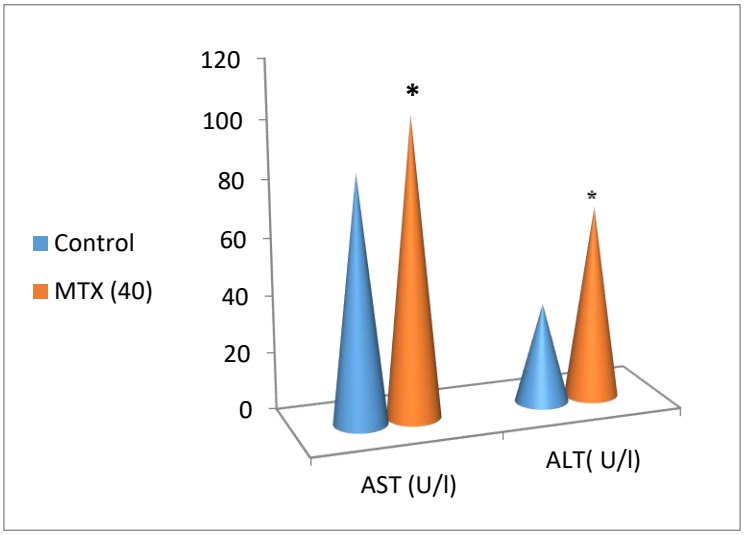

Figure 1: effect of MTX on liver function in the experimental groups. (*) means there was a significant difference from the control group at $P< 0.05$. Data were expressed as the mean ± S.E.

Histopathological findings:

The liver sections of control rats showed central veins surrounded by radiant polyhedral hepatocytes. The cells have prominent nucleoli inside a rounded nucleus which is surrounded by the cytoplasm. Hepatic sinusoids separate the cells from each other (figure 2A). On the other hand, the liver of the MTX-treated group revealed that I.P. injection of a single dose of 40 mg/kg MTX induced several pathological changes. These changes were categorized as necrobiotic changes (1.5 ± 0.2), cellular reactions (2.7 ± 0.2), and vascular changes (1.97 ± 0.5).

The necrobiotic changes (1.5 ± 0.2) exhibited in this group as the presence of clear fat vacuoles inside the hepatocytes of four rats. These vacuoles pushed the nucleus in some cases toward the periphery of the cell forming what is called the signet ring appearance (2B). Additionally, hydropic degeneration was observed in the hepatocytes of four animals with different degrees of severity. The severity of hydropic degeneration ranged from the presence of granulated cytoplasm in the hepatocytes to an empty cell with a nucleus only (figures 2C & D). As well as multi-focal necrosis was seen in three rats.

Concerning the cellular reaction (2.7 ± 0.2), there was mononuclear cell infiltration either periportal or perivascular, but mainly periportal in five rats of this group (figures 3 A, B & C). Kupffer cellular hyperplasia was also observed in two rats in this group (figure 3D).

The vascular changes (1.97 ± 0.5) include congestion of the blood vessels and hepatic sinusoids in the whole rats resulting in their dilatation and engorgement with blood (figures 4A, B & D). Desquamation of lining endothelial cells in some blood vessels was seen in four rats (figure 4 C). Also, haemorrhages were detected in three rats (figure 4 D).

Scoring of the histopathological findings of livers in the experimental groups was found in Figure 5.

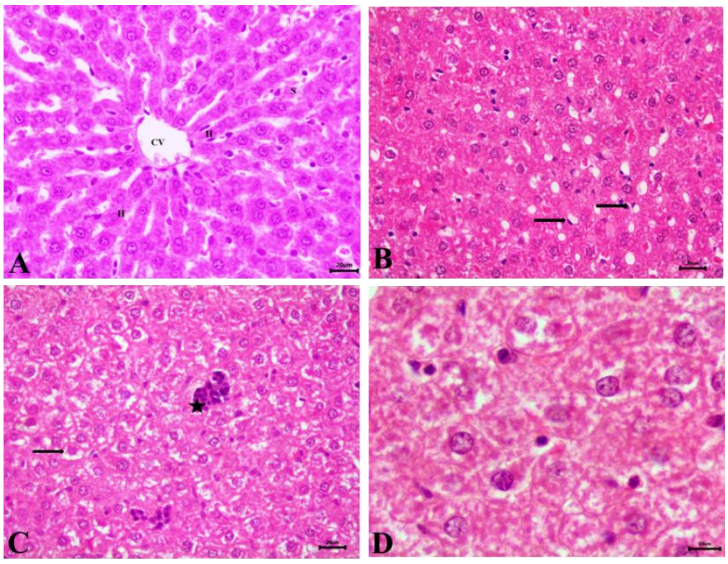

Figure 2: (A): liver from control group showing central vein (CV), hepatocytes (H), and hepatic sinusoids (S) (bar=20). (B): liver from MTX (40 mg/kg) administrated group showing presence of clear vacuoles inside the hepatocytes (arrows) (bar=20). (C): liver from MTX (40 mg/kg) administrated group showing severe hydropic degeneration in the hepatocytes (arrow) and kupffer cellular hyperplasia (star) (bar =20). (D): liver from MTX (40 mg/kg) administrated group showing severe hydropic degeneration with granulated cytoplasm (bar =10).

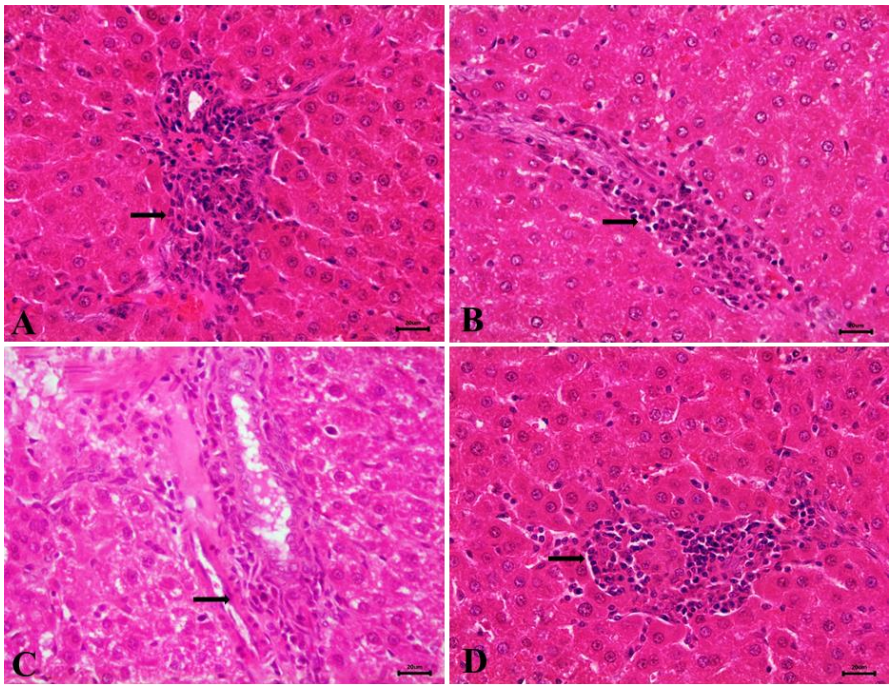

Figure 3: (A, B & C): livers from MTX (40 mg/kg) administrated group showing periportal mononuclear cell infiltration (arrow). (D): liver from MTX (40 mg/kg) administrated group showing kupffer cellular hyperplasia (bar=20).

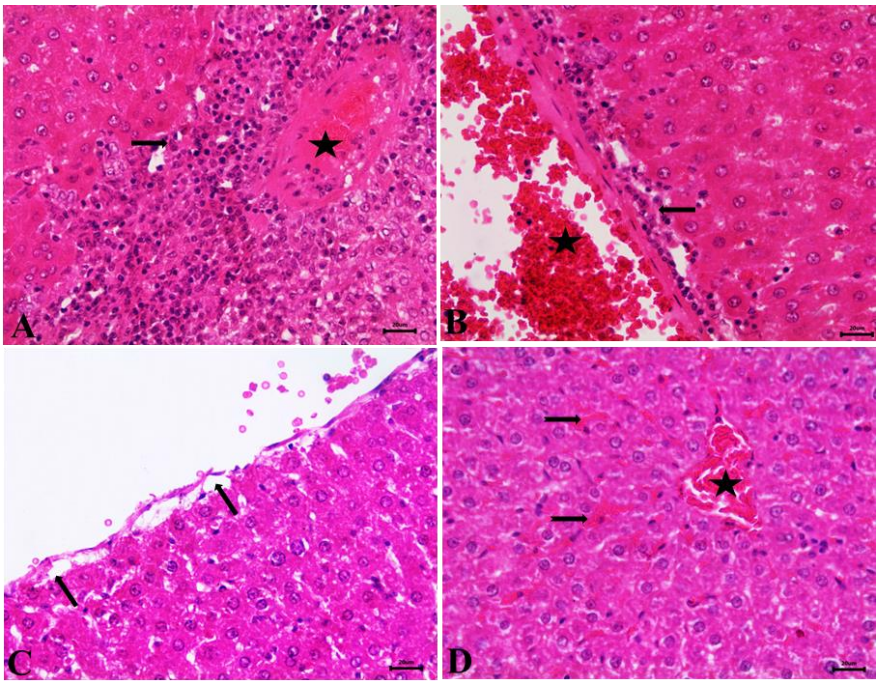

Figure 4: (A): liver from MTX (40 mg/kg) administrated group showing severe periportal mononuclear cell infiltration (arrow) and congestion (star) (B): liver from MTX (40 mg/kg) administrated group showing severe congestion of the blood vessel (star) and perivascular mononuclear cell infiltration (arrow). (C) liver from MTX (40 mg/kg) administrated group showing separation and desquamation of the endothelial cells of blood vessel wall (arrows). (D): liver from MTX (40 mg/kg) administrated group showing haemorrhage (star) and sinusoidal congestion (notched arrows) (bar=20).

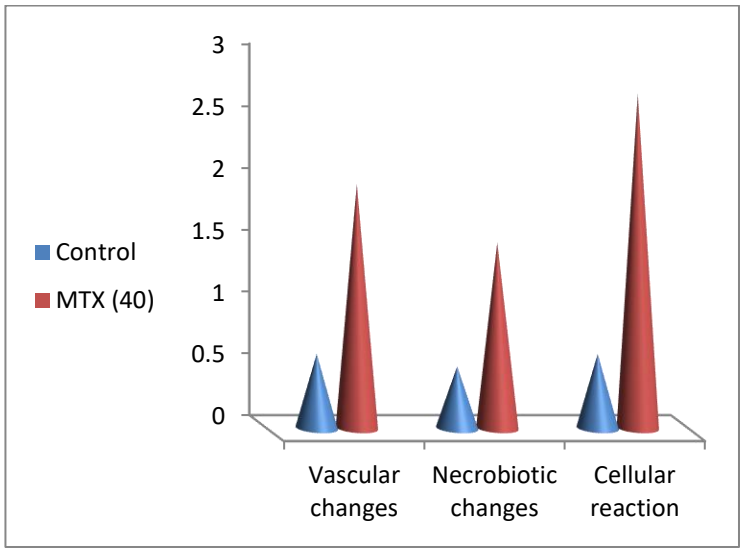

Figure 5: scoring of the histopathological findings of livers in the experimental groups. The level of significance was set at P<0.05. Data were expressed as the mean ± S.E.

DISCUSSION

MTX, the anti-neoplastic and immunosuppressive agent is used for the treatment of various cancers and chronic inflammatory diseases such as multiple sclerosis, sarcoidosis, psoriasis, rheumatoid arthritis, and Crohn's disease. Some systemic autoimmune diseases can be also treated with MTX (Pınar *et al.*, 2018). It acts by inhibiting dihydrofolate reductase, the essential enzyme in purines and pyrimidine synthesis (Al Maruf *et al.*, 2018). However, it induces several side effects on several tissues and organs. Hepatotoxicity is known to be one of the first side effects of MTX while the exact mechanism underlying this impact is still not fully understood (Karabulut *et al.*, 2020). Therefore, this experiment was suggested to quantify the hepatotoxicity of MTX via estimation of liver enzymes (AST and ALT) in addition to histopathological examination of liver tissue.

In the recent study, intraperitoneal injection of MTX induced serious liver damage demonstrated by a rise in AST and ALT levels that was extremely significant in the MTX group compared to the control. These enzymes are cytosolic in the hepatocytes and are believed to be the best indicators of liver necrosis. This is because the elevation in such enzymes' serum values reflects cell membrane leakage, which is associated with hepatocellular death (Hafez *et al.*, 2015). Several reports stated that MTX could cause cellular damage via its binding with dihydrofolic reductase leading to the prevention of folic acid to be converted to its active form, folinic acid. This inhibits nucleic acids and protein synthesis which in turn results in damage of organelles and cell membranes of hepatocytes allowing leakage of liver enzymes (Rizk *et al.*, 2018). Another hypothesis indicated that the production of ROS and the subsequent tissue destruction is thought to be one of the mechanisms of MTX drawbacks (Dhanesha *et al.*, 2015). Oxygen radicals and hydrogen peroxides can cause cell damage and release of liver enzymes from the hepatocytes into serum by binding to cellular macromolecules, particularly membrane lipids (Tousson *et al.*, 2014). In this context, it was mentioned that the increase in liver necrosis is characterized by an increase in enzymes leakage into the blood flow (Wambi *et al.*, 2008).

In addition, the marked MTX hepatic injury could be confirmed by its effects on liver histology. It induced several histopathological changes, summarized as hydropic degeneration and fatty degeneration, and multifocal necrosis. As well as mononuclear cell infiltration was also observed in the rats of this group. In

addition, there were congestion, desquamation of the endothelial cells of blood vessels' walls, and haemorrhages. In previous studies, there was dilation of the hepatic sinusoids, cellular infiltration, necrosis of hepatocytes, and congestion (Pinar *et al.,* 2018; Rizk *et al.,* 2018). Similar results were also reported by (Cure *et al.,* 2015).

There have been a lot of studies that interpret MTX-induced hepatotoxicity. One of these studies stated that most of the forms of MTX-induced tissue damage were attributed to the drug's promotion of free radical production (Khafaga and El-Sayed, 2018). Another study reported that due to the liver's role in the metabolism of toxins and drugs, it is one of the organs that are most adversely affected by any drug (Khadhim and Khudhair, 2018). MTX is oxidized by a soluble enzymatic system in the liver, where it is converted to its main extracellular metabolite, 7-hydoxymethotrexate. Another mechanism for MTX hepatotoxicity is that MTX is stored in a polyglutamated form inside cells (Tunalı-Akbay *et al.,* 2010). Accumulation of MTX polyglutamates due to long-term drug administration causes an increased intracellular content of the drug, a decrease in folate levels, and subsequent tissue damage (Prey and Paul, 2009).

CONCLUSION

We concluded that intraperitoneal injection of high doses of MTX induced severe hepatotoxicity in the experimental animals. This was clearly manifested by the presence of a significant increase of AST and ALT levels in serum and severe alterations in the liver histological structures.

Conflict of interest

The authors confirm that they do not have any conflicting interests.

Funding sources

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Ethical approval:

The authors declare that this study was approved by The Ethical Committee of The Faculty of Veterinary Medicine, Assiut University, Assit, Egypt, according to The OIE standards for the use of animals in research under the No. 06/2023/0076.

REFERENCES

Al Maruf, A.; O'brien, P.J.; Naserzadeh, P.; Fathian, R.; Salimi, A. and Pourahmad, J. (2018): Methotrexate induced mitochondrial injury and cytochrome c release in rat liver hepatocytes. *Drug and chemical toxicology,* 41**,** 51-61.

Bancroft, J. and Stevens, A. (1997): Theories and Practice of Histological Technique, 4th. *Chuchill Living Stone, Livingstone Edinburgh, London, New York.*

Cure, E.; Kirbas, A.; Tumkaya, L.; Cure, M.C.; Kalkan, Y.; Yilmaz, A. and Yuce, S. (2015): Protective effect of infliximab on methotrexate-induced liver injury in rats: Unexpected drug interaction. *Journal of cancer research and therapeutics,* 11**,** 164-169.

Dhanesha, M.; Singh, K.; Bhori, M. and Marar, T. (2015): Impact of antioxidant supplementation on toxicity of methotrexate: an in vitro study on erythrocytes using vitamin E. *Asian J Pharm Clin Res,* 8**,** 339-343.

Duman, D.G.; Kumral, Z.N.Ö.; Ercan, F.; Deniz, M.; Can, G. and Yeğen, B.Ç. (2013): Saccharomyces boulardii ameliorates clarithromycin-and methotrexate-induced intestinal and hepatic injury in rats. *British Journal of Nutrition,* 110**,** 493-499.

Grim, J.; Chládek, J. and Martínková, J. (2003): Pharmacokinetics and pharmacodynamics of methotrexate in non-neoplastic diseases. *Clinical pharmacokinetics,* 42**,** 139-151.

Hafez, H.M.; Ibrahim, M.A.; Ibrahim, S.A.; Amin, E.F.: Goma, W. and Abdelrahman, A.M. (2015): Potential

protective effect of etanercept and aminoguanidine in methotrexate-induced hepatotoxicity and nephrotoxicity in rats. *European journal of pharmacology*, 768**,** 1-12.

Herfarth, H.H. (2016): Methotrexate for inflammatory bowel diseases-new developments. *Digestive Diseases*, 34**,** 140-146.

Iwase, A.; Nakamura, T.; Nakahara, T.; Goto, M. and Kikkawa, F. (2015): Anti-Müllerian hormone and assessment of ovarian reserve after ovarian toxic treatment: a systematic narrative review. *Reproductive Sciences*, 22**,** 519-526.

Janelsins, M.C.; Kohli, S.; Mohile, S.G.; Usuki, K.; Ahles, T.A. and Morrow, G.R. (2011): An update on cancer-and chemotherapy-related cognitive dysfunction: current status. Seminars in oncology, Elsevier, 431-438.

Karabulut, D.; Ozturk, E.; Kuloglu, N.; Akin, A.T.; Kaymak, E. and Yakan, B. (2020): Effects of vitamin B12 on methotrexate hepatotoxicity: evaluation of receptor-interacting protein (RIP) kinase. *Naunyn-Schmiedeberg's archives of pharmacology*, 393**,** 2473-2480.

Khadhim, H.A. and Khudhair, A. (2018): The ameliorative effect of morin against methotrexate–Induced hepatotoxicity and some physiological and biochemical in male rats. *Plant Arch*, 18**,** 1503-11.

Khafaga, A.F. and El-Sayed, Y.S. (2018): Spirulina ameliorates methotrexate hepatotoxicity via antioxidant, immune stimulation, and proinflammatory cytokines and apoptotic proteins modulation. *Life sciences*, 196**,** 9-17.

Kose, E.; Sapmaz, H.I., Sarihan, E.; Vardi, N.; Turkoz, Y. and Ekinci, N. (2012): Beneficial effects of montelukast against methotrexate-induced liver toxicity: a biochemical and histological study. *The scientific world journal*, 2012.

Letertre, M.P.; Munjoma, N.; Wolfer, K.; Pechlivanis, A.; Mcdonald, J.A.; Hardwick, R.N.; Cherrington, N.J.; Coen, M.; Nicholson, J.K. and Hoyles, L. (2020): A two-way interaction between methotrexate and the gut microbiota of male sprague–dawley rats. *Journal of proteome research*, 19**,** 3326-3339.

Mahmoud, A.M.; Hussein, O.E.; Hozayen, W.G. and Abd El-Twab, S.M. (2017): Methotrexate hepatotoxicity is associated with oxidative stress, and down-regulation of PPARγ and Nrf2: Protective effect of 18β-Glycyrrhetinic acid. *Chemico-biological interactions*, 270**,** 59-72.

Murray, R. and Kaplan, A. (1984): Alanine aminotransferase. *Clinical Chemistry. Theory, analysis and correlation. Kaplan LA, Pesce AJ (Eds), CV Mosby St Louis*, 1090.

Pınar, N.; Kaplan, M.; Özgur, T. and Özcan, O. (2018): Ameliorating effects of tempol on methotrexate-induced liver injury in rats. *Biomedicine & Pharmacotherapy*, 102**,** 758-764.

Pountos, I. and Giannoudis, P.V. (2017): Effect of methotrexate on bone and wound healing. *Expert opinion on drug safety*, 16**,** 535-545.

Prey, S. and Paul, C. (2009): Effect of folic or folinic acid supplementation on methotrexate-associated safety and efficacy in inflammatory disease: a systematic review. *British Journal of Dermatology*, 160**,** 622-628.

Purcell, W.T. and Ettinger, D.S. (2003): Novel antifolate drugs. *Current oncology reports*, 5, 114-125.

Rahman, L.K. and Chhabra, S.R. (1988): The chemistry of methotrexate and its analogues. *Medicinal research reviews*, 8**,** 95-155.

Rizk, F.H.; Saadany, A.A.E.; Dawood, L.; Elkaliny, H.H.; Sarhan, N.I.; Badawi, R. and Abd-Elsalam, S. (2018): Metformin ameliorated methotrexate-induced hepatorenal toxicity in rats in addition to its antitumor activity: two

birds with one stone. *Journal of Inflammation Research*, 421-429.

Tousson, E.; Zaki, Z.T.; Abu-Shaeir, W.A. and Hassan, H. (2014): Methotrexate-induced hepatic and renal toxicity: role of L-carnitine in treatment. *Biomed Biotechnol*, 2, 85-92.

Tunalı-Akbay, T.; Sehirli, O.; Ercan, F. and Sener, G. (2010): Resveratrol protects against methotrexate-induced hepatic injury in rats. *Journal of Pharmacy & Pharmaceutical Sciences*, 13, 303-310.

Wambi, C.; Sanzari, J.; Wan, X.S.; Nuth, M.; Davis, J.; Ko, Y.-H.; Sayers, C.M.; Baran, M.; Ware, J.H. and Kennedy, A.R. (2008): Dietary antioxidants protect hematopoietic cells and improve animal survival after total-body irradiation. *Radiation research*, 169, 384-396.

Wiczer, T.; Dotson, E.; Tuten, A.; Phillips, G. and Maddocks, K. (2016): Evaluation of incidence and risk factors for high-dose methotrexate-induced nephrotoxicity. *Journal of oncology pharmacy practice*, 22, 430-436.

دراسات هستوباثولوجية وبيوكيميائية عن السمية الكبدية للميزوتريكسات على الجرذان البيضاء

ولاء حسن كامل ، مروه فاروق علي , صلاح محمد حسن عفيفي

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الميثوتريكسات (MTX)، وهو دواء مضاد للتكاثر، ومضاد للالتهابات، ومثبط للمناعة هو أحد الأدوية الأكثر فاعلية المستخدمة في علاج عدد كبير من الأورام الصلبة، والأورام الدموية الخبيثة، واضطرابات المناعة الذاتية. ومع ذلك، فإن سميته الكبدية الكبيرة تحد من قابليته للاستخدام. ولهذا تم اقتراح هذه الدراسة لفحص الآثار الجانبية للجرعة العالية من الميثوتريكسات على الكبد في فئران التجارب. تم تقسيم عشرة فئران بشكل عشوائي إلى مجموعتين، بما في ذلك المجموعة الضابطة ومجموعة تم حقنها بالميزوتريكسات. تلقت مجموعة الميثوتريكسات جرعة واحدة 40 مجم / كجم من الميثوتريكسات داخل الصفاق لاحداث الإصابة بالكبد. وتم حقن المحلول الملحي الفسيولوجي في الفئران الضابطة بنفس الطريقة. كانت فترة التجربة 14 يومًا. وفي نهاية التجربة، تم التضحية بالجرذان وجمع عينات من المصل وأنسجة الكبد لتقييم وظائف الكبد عن طريق قياس مستويات الأسبارتات ترانس أمينار (AST) والألانين ترانس أمينار (ALT) والفحص النسيجي لأنسجة الكبد. أظهرت النتائج أن حقن الميثوتريكسات أدى إلى زيادة ملحوظة للغاية في مستويات AST و ALT في الدم. بالإضافة إلى ذلك أظهر الفحص النسيجي المرضي للكبد وجود فجوات دهنية واضحة في خلايا الكبد، وتنكس مائي، ونخر متعدد البؤر. أيضا ، كان هناك انتشار للخلايا وحيدة النواة وتضخم خلايا كوبفر. كما تم اكتشاف احتقان و تقشر الخلايا البطانية في بعض الأوعية الدموية ونزيف. لذلك ، خلصنا إلى أن إعطاء جرعات عالية من MTX يسبب سمية كبدية شديدة في فئران التجارب تتجلى في زيادة معنوية في إنزيمات الكبد في المصل وتغير شديد في التركيب النسيجي للكبد.