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Effect of methotrexate and aspirin interaction and its relationship to oxidative stress in rats

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Abstract

This study aims to investigate the effect of aspirin in methotrexate toxicity (increase or decrease) relating to oxidative stress and histopathological changes of the liver and kidney in male rats. Twenty rats were divided into equal 4 groups, the first is considered control group, the second was treated with methotrexate in a dose of 10 mg/kg, the third was treated with methotrexate and aspirin in doses of 10 and 30 mg/kg respectively, the fourth was treated with aspirin alone with a dose of 30 mg/kg. All doses were given by daily oral dosage for 4 constitutive days. The result revealed a significant decrease in the concentration of both glutathione and albumin and a significant increase in the concentration of both malondialdehyde and ALT enzyme in the two groups treated with methotrexate alone or in combination with aspirin as compared to the control group. The histopathology revealed that the severity of lesions was in the group of methotrexate with aspirin, group of methotrexate only and a group of aspirin respectively, which are representing by coagulative necrosis and hypertrophy of hepatocytes in the liver while the lesions of kidney were atrophy of some glomeruli and renal cystic formation. The study concludes that aspirin increases the toxic effect of methotrexate at the level of oxidative stress concomitant with the occurrence of hepatic and renal toxicity.

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Introduction

Methotrexate is used in the treatment of cancer and psoriatic rheumatoid arthritis Methotrexate began and extended to be used for chemotherapy (1). It is efficient for the therapy of breast, lung and bladder cancer, as well as other blood cell cancer (2). Mechanism of action of Methotrexate is competitive inhibition of dihydrofolate reductase enzyme (DHFR) cause inhibiting of folic acid, some proteins and nucleic acid synthesis (3). Because of its ability to inhibit the binding of interleukin-1 beta to the receptors of cell surface and selective down-regulation of activation of B-cells and T-cell, therefore, it is used for treatment of rheumatoid arthritis (4). Methotrexate has a toxic effect on the liver, kidney and other organs. The most common adverse effects of methotrexate to the kidney because of its excretion and it metabolite in the liver leading to hepatotoxicity.

The inhibition of methotrexate to immunity appears to increase the infections because of low white blood cell count leading to acute pneumonitis and rarely pulmonary fibrosis (5). It has been reported the present of methotrexate crystals in the renal tubules especially when it is administrated at high doses cause severe renal toxicity and acute renal failure considering the most important side effect (6). Methotrexate increases the reactive oxygen species in renal tissues; therefor it has been reported to reduce the methionine synthesis and antioxidant enzymes such as catalase and glutathione peroxidase (7).

Aspirin metabolize by carboxylesterase to salicylic acid which has several pharmacological interactions with another drugs such as antacid, charcoal and methotrexate. Most of

these interactions occur because of ability of aspirin to displace drugs from albumin binding. It is metabolized in different pathways, therefore, it may effect on metabolizing of another drugs (8). Caution should be taken when high dose of aspirin given with another drug which leads to increase or decrease another drug concentration. That type of interaction can be expected if the pharmacokinetics and pharmacodynamics of drug are known. The most important interaction occurs when it is given with another cytotoxic drug that may end to death (9). The present study aims to investigate the effect of aspirin in methotrexate toxicity (increase or decrease) relating with oxidative stress and histopathological changes in liver and kidney in male rats.

Materials and methods

Wister male rats aged 1-2 months and weight between 150-200 gram raised in special cages inside the animal house of the College of Veterinary Medicine, University of Mosul providing water and food along the experiment time.

Drugs and chemicals

methotrexate tablet is from EBEWE pharma Ges Company, Austria. It was grounded then used in the form of powder. Aspirin powder is from sanofi-aventis Company, France. The doses were accounted according to the weight of the animal. The drugs were dissolved with distilled water. The volume of drug administration was 2 ml/kg body weight, using the gavage needle of the oral administration.

Experimental design

In this experiment, 20 male rats were randomly distributed to 4 groups. The first one was considered control group which was given only distilled water orally. The second group was given methotrexate orally at dose 10 mg/kg. The third was given methotrexate orally at 10 mg/kg and aspirin at dose 30 mg/kg and the fourth was given aspirin alone in a dose of 30 mg/kg orally. The doses were continued for four consecutive days. The doses and period conducted according to a pilot study.

Blood collection

The animals were anesthetized by ether and the blood was drawn by micropipette from the eye orbit, then placed in clean sterile plastic tubes and left until the blood clot for 35 minutes in a room temperature, then centrifugation at 3000 rpm for 15 minutes for serum isolation and then frozen in -20°C until biochemical measurements.

Histopathogical examination

The necropsy was performed in the end of the experiment to study the microscopic pathological changes of the liver and kidney. The organs were removed quickly and washed with tap water. They were then transferred to special containers to preserve the samples in 10% neutral buffered formalin solution for three days, then washed in tap water, later the specimen crossing in alcohol (70°, 80°, 90°, 96°, 100°), xylene, waxed in the paraffin form of patterns, slides by microtome to 5 microns with a thickness of 4-5 µm were cut from each block, and then stained with hematoxylin and eosin. Light microscope was used for investigation and effected areas were photographed (10).

Biochemical measurements

The level of glutathione was measured in the serum by the method of measurement (11) at a wavelength of 412 nm, whereas the malondialdehyde was measured by the method of (12) at a wavelength of 530 nm. ALT enzyme, Albumin and total protein were measured by using kits from Biolabo company - France.

Statical analysis

The data were analyzed by using SPSS program, one-way analysis of variance then applied Least significant difference (LCD) for all groups of animals.

Results

The level of glutathione showed significant decrease in the groups of methotrexate alone and in combination with aspirin, while malondialdehyde recorded significant increase in the same groups as compared with the control group (Table1).

The groups of methotrexate with and without aspirin showed significant increase in ALT enzyme as compared with control group while there were no significant changes in total protein while the albumin concentration appeared significant decrease in methotrexate with and without aspirin as compared to control group (Table 2).

Table 1: Concentration of glutathione and malondialdehyde in the serum of rat treated by methotrexate with and without aspirin

Treatment Mg/Kg	Glutathione Mmol/L	Malondialdehyde Mmol/L
Control	4.7±1	0.1±0.03
Methotrexate 10 mg/kg	1.7* ±0.06	1.8* ±0.07
Methotrexate 10 mg/kg + Aspirin 30 mg/kg	1.1* ±1.1	3.14* ±0.04
Aspirin 30 mg/kg	3.9±0.9	0.26±0.07

Each group consists of 5 rats. Data expressed as mean and stander error.
* means significant difference from control group ($p < 0.05$)

Table 2: concentration of ALT enzyme, total protein and albumin in the methotrexate with and without aspirin

Treatment	ALT u/l	Total protein g/l	Albumin g/l
Control	10±0.9	6.62±0.8	4.96±1
Methotrexate 10 mg/kg	24*±3	6.84±0.9	3.9* ±0.8
Methotrexate 10 mg/kg +Aspirin 30 mg/kg	26*± 2.2	6.82±1	2.62* ±1.2
Aspirin 30 mg/kg	9±1	6.48±2	5.07 ±2

Each group consists of 5 rats. Data expressed as Mean and stander error.
* means significant difference from control group ($p < 0.05$)

The results of histopathology revealed that the lesions were more severe in the group of (methotrexate and aspirin), the group of (methotrexate only) and the group of (aspirin only) respectively compared with the control group (Figure 1). The lesions of the group of (aspirin only) were cell swelling of hepatocytes and stenosis and un-arrangement of sinusoids in the liver (Figure 2), while the lesions of kidney were cell swelling of the epithelium lining renal tubules and stenosis of the lumen of renal tubules (Figure 3). The lesions of the group of (methotrexate only) were congestion of central vein, portal vein and sinusoid and un-arrangement of it. There is hypertrophy of many hepatocytes, cell swelling and necrosis of some hepatocytes in the liver (Figure 4). The lesions of kidney were hemorrhage, cell swelling and coagulative necrosis of epithelium lining renal tubules (Figure 5). The lesions of group of (methotrexate and aspirin) represented by coagulative necrosis of hepatocytes, hypertrophy of many hepatocytes, severe congestion of central vein and portal vein, hemorrhage and un-arrangement of sinusoids in the liver (Figure 6), while the lesions of kidney were interstitial nephritis, severe congestions, hemorrhage in the glomeruli, atrophy of some glomeruli and cystic formation, and acute cell swelling of epithelium lining renal tubules (Figure 7**).**

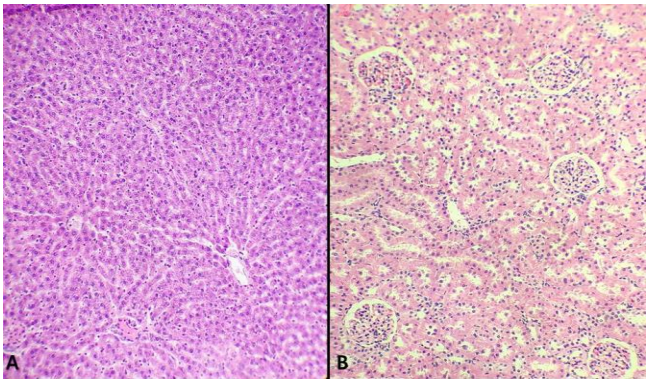

Figure 1: Photomicrograph of control group (A): liver section of rat, H&E 100x. (B): kidney section of rat, H&E, 100x.

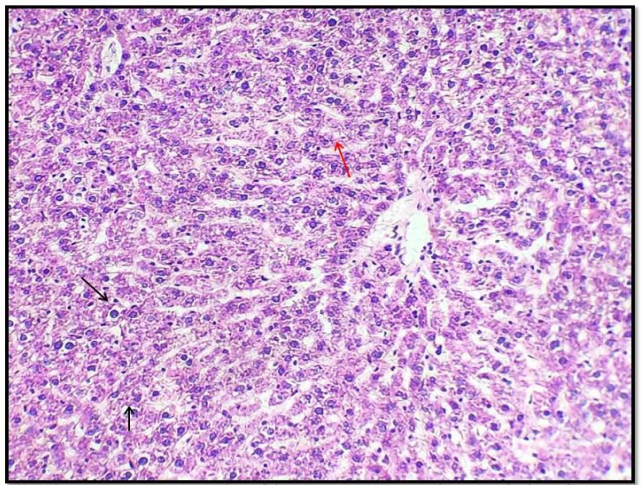

Figure 2: Photomicrograph of liver section of rat of the group of (aspirin only) shows cell swelling of hepatocytes (arrow) and stenosis and un-arrangement of sinusoids (red arrow). H&E, 200x.

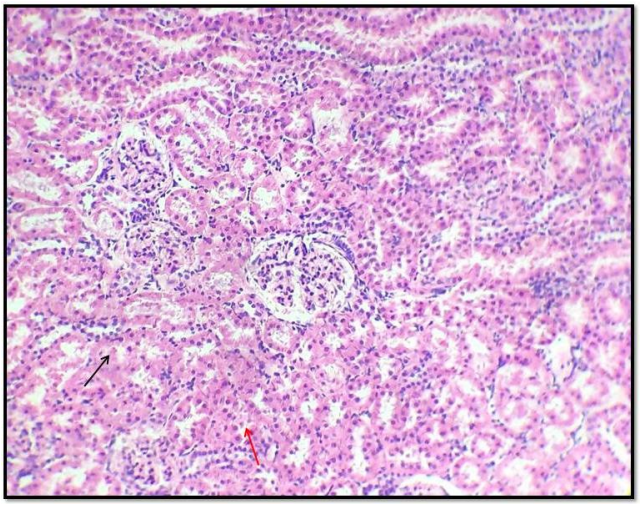

Figure 3: Photomicrograph of liver section of rat of the group of (aspirin only) show cell swelling of epithelium lining renal tubules (arrow), stenosis of the lumen of renal tubules (red arrow). H&E, 200x.

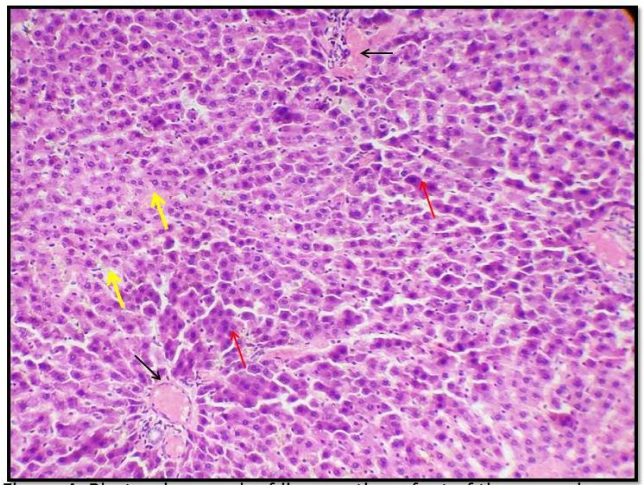

Figure 4: Photomicrograph of liver section of rat of the group of (methotrexate only) shows congestion of central vein, portal vein (arrow) and sinusoid and un-arrangement of it, hypertrophy of many hepatocytes (red arrow) and cell swelling and necrosis of some hepatocytes (yellow arrow). H&E, 200x.

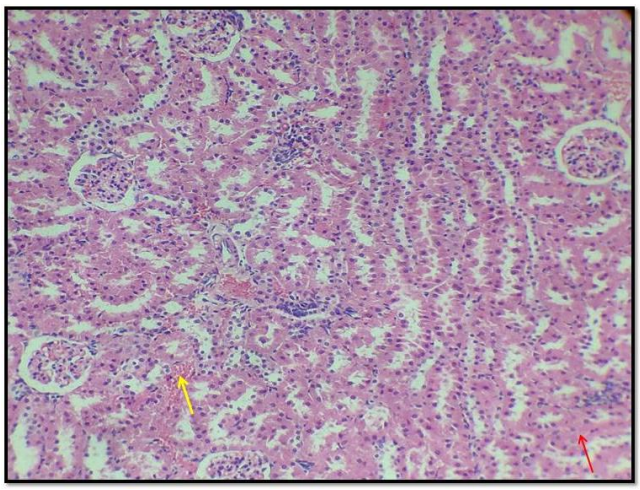

Figure 5: Photomicrograph of kidney section of rat of the group of (methotrexate only) shows cell swelling and coagulative necrosis of epithelium lining renal tubules (red arrow) and hemorrhage (yellow arrow). H&E, 100x.

Discussion

The kind of drugs interaction includes direct effect and influence on absorption, metabolism, excretion, protein binding, or interference at the cell level (13). One of the reasons for increasing the toxic effect of methotrexate when given with aspirin is that the interaction between the two drugs at the level of competition for protein binding, where methotrexate is associated with albumin by 35-50%, and when given at a high dose, especially with the aspirin that leads to competition between them, and removal of methotrexate from the albumin binding lead to increase concentration of free methotrexate in the plasma with a slow withdrawal rate of methotrexate from the tissues (14). Second reason for increasing toxicity of methotrexate due to aspirin reduces the excretion of unbounded methotrexate also aspirin reduces the excretion of unbounded methotrexate in 66% in urine from the distal tubules (15).

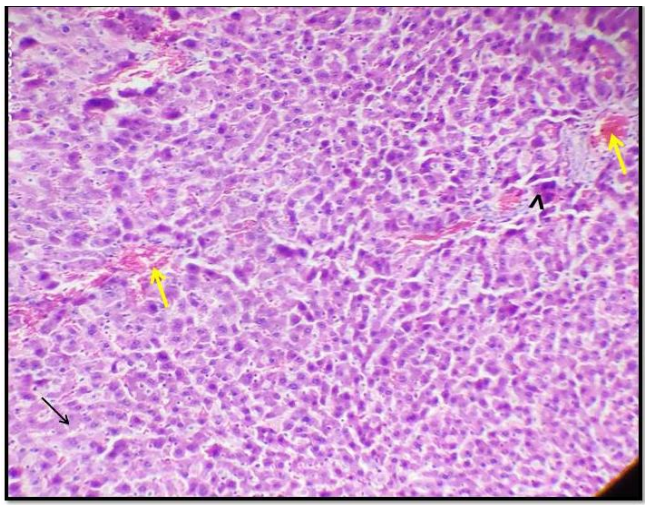

Figure 6: Photomicrograph of liver section of rat of group of (methotrexate with aspirin) shows coagulative necrosis of hepatocytes (arrow), hypertrophy of some hepatocytes (arrow head) and congestion of central vein and portal vein (yellow arrow). H&E, 200x.

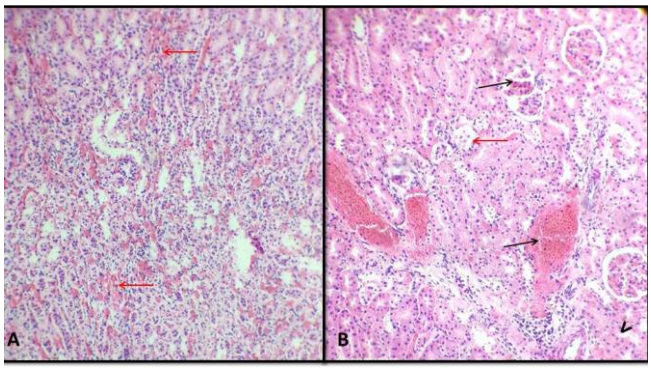

Figure 7: Photomicrograph of kidney section of group of (combination of methotrexate with aspirin) (A): interstitial nephritis, severe congestions and hemorrhage (red arrow), H&E. 90x. (B): severe congestions and hemorrhage in the glomeruli (arrow), atrophy of some glomeruli and cystic formation (red arrow) and acute cell swelling of epithelium lining renal tubules (arrow head). H&E, 100x.

Methotrexate inhibits the oxygenation of cancer cells by isolating the mitochondria from normal cells and leads to energy reduction by uncoupling oxidative phosphorylation with phosphates it means methotrexate has the same effect on healthy cells especially in high concentration (16). The reduction in NADPH and malic NADPH effects on the state of sustainability of the reduced form of the glutathione which is considered an important antioxidant for the cell and its decrease level in the cell leads to the case of oxidation of unsaturated fatty acids and this leads to the oxidative phosphorylation of fats and increase the MDA (17), that may be a reason for the decreased glutathione in the two groups of methotrexate alone and in combination with aspirin.

Methotrexate acts as inhibitor of dihydrofolate reductase affecting on the production of folic acid and works to prevent the manufacture of certain amino acids and protein synthesis (18), where it effects on the liver functions and leads to a decrease in the albumin production in addition to injury of glomeruli causing increase albumin filtration in urine represented by present of interstitial nephritis (19). One consequence of the albumin decline is present of a high free binding plasma drug which increases the methotrexate toxicity.

It is likely that the causes of hepatic injury in methotrexate aspirin interaction are the main metabolite of methotrexate which oxidizes within the hepatic cells and transformed into polyglutamate. Prolonged administration or high methotrexate concentration results in the accumulation of this compound (polyglutamate) in the hepatocytes combined by a decrease in folate levels (20). This high concentration of polyglutamate causes prolongation of the intracellular drug therefor, hypertrophy of the hepatic cells is seen as a compensatory mechanism for toxicity as well as it is considered as cellular adaptation (21). It also indirectly inhibits thymine and purine, which inhibits DNA synthesis in the cells. In addition, methotrexate cause disturbances in the resistance of the blood vessels of the pre-glomeruli, causing direct damage to the glomerulus and cause the obstruction of the tubules (22).

Main methotrexate metabolite is also deposited in the renal tubules leading to necrosis of it (23), as well as the role of mitochondria in the development of renal toxicity including electron transfer chain, cytochrome 450, NADPH, oxides, and zanethin dehydrogenase, in addition to the inhibitory role of methotrexate in the manufacture of nucleic acids, proteins, lipids, and other cellular structures, that elevation of malondialdehyde is one of its indicators (24).

Conclusion

This study concludes that given aspirin with methotrexate causes increase in toxicity of methotrexate on the level of oxidative stress accompanying with hepato-renal toxicity.

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Conflict of Interest

There is no any conflict of interest.

Reference

1. Van Lumig PP, Menting SP, Van Den Reek JM. An increased risk of non-melanoma skin cancer during TNF-inhibitor treatment in psoriasis patients compared to rheumatoid arthritis patients probably relates to disease-related factors. J Euro Acad DermatVener. 2015;29(4):752-60. https://doi.org/10.1111/jdv.12675
2. Cojoc M, Mäbert K, Muders MH. Role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. Sem Cancer Biol. 2015;1(31):16-27. https://doi.org/10.1016/j.semcancer.2014.06.004
3. Martinez-Outschoorn UE, Peiris-Pages M, Pestell RG. Cancer metabolism: A therapeutic perspective. Natu Rev Clin Onco 2017;14(1):11. https://doi.org/10.1038/nrclinonc.2016.60
4. Stapf M, Teichgraeber U, Hilger I. Methotrexate-coupled nanoparticles and magnetic nanochemothermia for the relapse-free treatment of T24 bladder tumors. Inter J Nanom. 2017;12:2793. https://doi.org/10.2147/ijn.s120969
5. Luo D, Carter KA, Miranda D. Chemophototherapy: an emerging treatment option for solid tumors. Advan Sci. 2017;1(1):4. https://doi.org/10.1002/advs.201600106
6. Li X, Abe E, Yamakawa Y. Effect of administration duration of low dose methotrexate on development of acute kidney injury in rats. J Kidney. 2017;2(130):2472-1220. https://doi.org/10.4172/2472-1220.1000130
7. Saka S and Aouacheri O. The investigation of the oxidative stress-related parameters in high doses methotrexate-induced albino Wistar rats. J Bioequiv. 2017;9:372-6. https://doi.org/10.4172/jbb.1000327
8. Rainsford KD. Pharmacokinetics and metabolism of the salicylates in aspirin and related D. CRC Press. 2016;19:148-222. https://doi.org/10.1201/9780203646960.ch4
9. Hennessy S, Leonard CE, Gagne JJ. Pharmacoepidemiologic methods for studying the health effects of drug-drug interactions. Clin Pharma Therap. 2016;99(1):92-10. https://doi.org/10.1002/cpt.277
10. Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. 3rd ed. New York: McGraw-Hill; 1986. https://doi.org/10.1016/s0031-3025(16)39410-7
11. Sedlak J and Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analy Bioch. 1968;1(25):192-205. https://doi.org/10.1016/0003-2697(68)90092-4
12. Buege JA, Aust SD. Microsomal lipid peroxidation in methods in enzymology. Acad Press. 1987;52:302-310. https://doi.org/10.1016/s0076-6879(78)52032-6
13. Burger DM, Brake LH, Aarnoutse RE. Mechanisms of drug interactions I: Absorption, metabolism, and excretion. Mechanisms Models Drug Inter. 2018:15-47. https://doi.org/10.1007/978-3-319-72422-5_2
14. Humana P, Cham Hall JJ, Bolina M. Interaction between low-dose methotrexate and nonsteroidal anti-inflammatory drugs, penicillins, and proton pump inhibitors: A narrative review of the literature. Anna Pharm. 2017;51(2):163-78. https://doi.org/10.1177/1060028016672035
15. Berthier J, Arnion H, Saint-Marcoux F. Multidrug resistance-associated protein 4 in pharmacology: Overview of its contribution to pharmacokinetics, pharm. Pharmaco. Life Sci. 2019;231:116540 https://doi.org/10.1016/j.lfs.2019.06.015

تأثير التداخل بين الميثوتريكسيت والأسبرين وعلاقته بالإجهاد التأكسدي في الجرذان

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الخلاصة

استهدفت هذه الدراسة التحري عن تأثير الأسبرين في سمية الميثوتريكسيت (زيادة او نقص) وعلاقته بمستوى الإجهاد التأكسدي والتغيرات المرضية في كبد وكلى الجرذان. قسمت عشرون جرذا إلى ٤ مجاميع متساوية واعتبرت المجموعة الأولى مجموعة سيطرة وعوملت المجموعة الثانية بالميثوتريكسات بجرعة ١٠ ملغم/كغم وعوملت المجموعة الثالثة بالميثوتريكسيت والأسبرين بجرعات ١٠ و ٣٠ ملغم/كغم على التوالي، اما المجموعة الرابعة فأعطيت الأسبرين وحده بجرعة ٣٠ ملغم/كغم. أعطيت جميع الجرع يوميا عن طريق الفم لمدة ٤ أيام متواصلة. أظهرت النتائج انخفاض معنوي بتركيز الكلوتاثايون والألبومين وارتفاع معنوي بتركيز المالونداليهايد وإنزيم ALT في كل من المجموعة المعاملة بالميثوتريكسيت وحده ومجموعة الميثوتريكسيت مع الأسبرين بالمقارنة مع مجموعة السيطرة. اظهرت نتائج التغيرات المرضية النسيجية أن الآفات كانت أكثر شدة في المجموعة المعاملة بالميثوتريكسيت مع الأسبرين والمجموعة المعاملة بالميثوتريكسيت لوحده ثم المجموعة المعاملة بالأسبرين لوحده على التوالي و تمثلت الآفات بالنخر التجلطي وضخامة الخلايا الكبدية في الكبد بينما كانت آفات الكلى هي ضمور بعض الكبيبات وتكون الاكياس الكلوية. تستنتج هذه الدراسة أن الأسبرين يزيد من التأثير السام للميثوتريكسيت من خلال زيادة الإجهاد التأكسدي المصاحب لحدوث التسمم الكبدي والكلوي.

16. Phull AR, Nasir B, Abdul Haq I. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Inter. 2018;1,281:121-36. https://doi.org/10.1016/j.cbi.2017.12.024
17. Qiu H and Schlegel V. Impact of nutrient overload on metabolic homeostasis. Nut Rev. 2018;20(9):693-707. https://doi.org/10.1093/nutrit/nuy023
18. Rushworth D, Mathews A, Alpert A. Dihydrofolate reductase and thymidylate synthase transgenes resistant to methotrexate interact to permit novel transgene regulation. J Bio Chem. 2015;18,290(38):22970-6. https://doi.org/10.1074/jbc.c115.671123
19. Ahmed MA. Tayawi HM. Ibrahim MK. Protective effect of Silymarin against kidney injury induced by carbon tetrachloride in male rats. Iraqi J Vet Sci. 2019;33(1):127-130. Doi: 10.33899/ijvs.2019.125529.1051
20. Abraham, A. Folate analogs Synthesis of folate and antifolate poly gamma glutamates by [(9-fluorenylmethoxy)oxy]carbonyl chemistry and biological evaluation of certain methotrexate polyglutamate polylysine conjugates as inhibitors of the growth of H35 hepatoma cells. J Med Chem. 1990;33(2):711-717. http://dx.doi.org/10.1021/jm00164a038.
21. Rashid, S. Comparative pharmacokinetic study of theaflavin in healthy and experimentally induced liver damage rabbits. Iraqi J Vet Sci. 2019;33(2):235-242. Doi: 10.33899/ijvs.2019.162962
22. Yang SL, Zhao FY, Song H. Methotrexate associated renal impairment is related to delayed elimination of high-dose methotrexate. Sci World J. 2015:1-8. https://doi.org/10.1155/2015/751703
23. Garcia H, Leblond V, Goldwasser F. Renal toxicity of high-dose methotrexate. Neph Therap. 2018;14:S103-13. https://doi.org/10.1016/j.nephro.2018.02.015
24. Al-abdaly YZ, Al-Kennany ER, Al-Hamdany EK. Concomitant occurrence of oxidative stress with sustanon in male rats. Basrah J Vet Res. 2018;17(3): 137-147. http://www.basjvet.com/wp-content/uploads/2019/04/136-147.pdf