

## Evaluation of the Role of Vit C on Methotrexate Induced liver and Renal Toxicity

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### ABSTRACT

**Background :** Methotrexate (MTX) is one of the most effective drugs in cancer chemotherapy, as well as in the treatment of non-oncologic conditions such rheumatoid arthritis and psoriasis. It is one of the folic acid antagonists. However, The efficacy of MTX is often limited by it's severe side effects on the renal and liver tissue which may limit its use.

**Aim of Study:** The purpose of this study was to examine the histological alterations in the rat liver and renal structure after treatment with (MTX), as well as the potential protective impact of vitamin C. **METHODS:** An experimental study of eighteen (18) male albino rats which were randomly distributed into three groups. Each group consists of six animals. Group I was the control group. In Group II rats received a daily IM injection of MTX (5mg/kg b.w.) for seven days. Group III: For seven days, the animals were given MTX at the same doses, periods and modes of administration as before with concomitant vitamin C in a dose of (100 mg/day) was given. The mice were euthanized after blood samples were taken from the retroorbital venous plexus for biochemical tests of liver enzymes and renal function. Light microscopic examinations were performed on liver and kidney specimens.

**Results:** The current study found that (MTX) therapy caused significant damage to the rat liver and kidney, as seen by elevated liver enzyme levels and altered renal function tests. The central and portal veins were severely dilated and congested in sections of the liver, with patches of fatty degeneration readily visible. The kidney segment revealed glomerular atrophy, tubular dilatation, and degeneration. Rats given vitamin C concomitant with MTX, on the other hand, showed minor histological alterations.

**Conclusion:** Vitamin C appeared to have some protection against MTX poisoning by reducing earlier degenerative alterations.

**Keywords:** methotrexate, liver, renal , rat, vit C.

### تقييم دور فيتامين سي من تأثير سُمِّيَة عقار الميثوتريكسيت على الكبد والكلية

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### الخلاصة

**الخلفية :** الميثوتريكسيت هو أحد أكثر الادوية المستخدمة لعلاج السرطان ولعلاج انواع مختلفة من الامراض غير السرطانية كالتهاب المفاصل وداء الصدفية. وهو يعمل كمضاد للفولك اسيد مما يجعل استخدام الميثوتريكسيت محدودا بسبب تأثيراته السلبية على نسيج الكبد والكلية .

**الهدف من هذه الدراسة التجريبية :** هو لمعرفة الفوائد الوقائية لفيتامين سي من التأثيرات السمية للميثوتريكسيت على انسجه الكبد والكلية

**طريقة التجربة :** تم تقسيم ١٨ من جرذان الذكور من نوع albino الى ثلاث مجاميع سته لكل مجموعة.(المجموعة الاولى اعتبرت كمجموعة سيطرة .المجموعة الثانية اعطيت الميثوتريكسيت تحت الجلد وبجرعة 5 ملغ /كغم من وزن الجسم. لمدة سبعة ايام .المجموعة الثالثة اعطيت نفس الجرعة من الميثوتريكسيت لمدة سبعة ايام ولكن مع اضافته فيتامين سي بجرعه ١٠٠ ملغم يوميا

ثم قتلت كل الجرذان بعد السبعة ايام واخذت عينات من الدم من خلف محجر العين لغرض فحص وظائف الكبد والكلية ثم استأصلت الكلية والكبد واخذت عينات نسيجية منها وتم تحضيرها للفحص بالمجهر الضوئي. **النتائج:** في الجرذان المعالجة بالميثوتركسيت فقط شوهد تلف في انسجه الكبد والكلية مع زياده في انزيمات الكبد وخلل في وظائف الكلية. وظهر توسع واضح في اورده الكبد مع ترسب الشحوم في خلايا الكبد. اما في الكلية فلوحظ وجود ضمور في الحويصلات الكلوية وتوسع وتلف في انابيب النسيج الكلوي. بينما الجرذان التي اعطيت جرعه الفيتامين سي متزامنه مع العلاج بالميثوتركسيت لوحظ تغيرات طفيفه على انسجه الكبد والكلية.

**الاستنتاج:** فيتامين سي له القدره على وقايه انسجه الكبد والكلية من التأثير السمي للميثوتركسيت.

**الكلمات المفتاحية:** الميثوتركسيت ، الكبد، الكلية ، الجرذان ،فيتامين سي.

## INTRODUCTION

**M**ethotrexate (MTX) is known by its triple action, immunosuppressant, anti-inflammatory and folic acid antagonist, it interrupts cell cycle by blocking a number of folic acid cycle enzymes, prevents DNA synthesis, DNA repair, and cell division, acting as a "antiproliferative" agent<sup>1,2,3</sup>. One of the most effective cancer chemotherapy medications, it is used to treat acute lymphoblastic leukemia, osteosarcoma, breast cancer, head and neck malignancies, as well as non-oncologic conditions like rheumatoid arthritis and psoriasis<sup>1-6</sup>. However, severe side effects and toxic sequel, such as intestinal injury, central nervous system toxicity, and bone marrow toxicity, limit MTX's efficacy<sup>7,8</sup>. Because the kidneys are responsible for excreting more than 90% of MTX, nephrotoxicity is one of the major reasons for limiting its use. It has been observed that excessive dosages of it can cause acute renal failure<sup>9</sup>.

Several hypothesis have been put forward regarding the mechanism of MTX adverse effects. Oxidative liver damage was reported by some investigators as it leads directly to promotes lipid peroxidation by reducing antioxidant enzymatic defense capacity<sup>10</sup>, indirectly by inhibiting DNA synthesis in the liver and causing cellular arrest, which is linked to increased hepatocellular apoptosis<sup>10,11</sup>.

Extending the clinical usefulness of methotrexate is of great interest by combining it with other drugs to lessen its nephrotoxicity and hepatotoxicity<sup>9,12-14</sup>.

Vitamin C is an antioxidant that protects the body from the harmful effects of medicines. In various experimental studies, this vitamin has been found to be helpful in lowering oxidative renal and hepatic damage<sup>15-19</sup>. Antioxidant supplementation was found to result in higher survival duration, enhanced tumor responses, or both, as well as less toxicities than controls in many studies<sup>13-20</sup>.

As a result, the goal of this study is to see if Methotrexate-induced liver and kidney damage is morphologically protected by antioxidant effect of vitamin C.

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## MATERIALS AND METHODS

### Chemical Substances

Among the chemical compounds utilized in the experiment, Methotrexate Ebewe (50mg in 5ml, EBEWE pharma) available in the form of solution ready for injection, each 1ml of it contains 10 mg methotrexate. Vitamin C was provided by ALshahba LABS. ALEPPO -SYRIA. Tablets are available for purchase. dissolved daily in purified water and administered orally of 100mg/kg/day.

### Animals

Albino rats of Wistar strain, weighing between 150 – 210 g were used. Rats were individually weighed at the start of the experiment and housed in wire cages at room temperature. All of the experiments followed national criteria for the use and care of experimental animals.

### Design of an experiment

Eighteen (18) male albino rats were randomly assigned to three groups of six animals. Before the seven-day experiment began, each participant received four days of unrestricted access to food and water.

Group I (Control group) Once a day, the animals were given an equivalent dose of saline I.M.

The animals in Group II were given MXT alone as an i.m. injection once a day at a dose of 5 mg/kg.

Group III The identical dose, duration, and mode of administration of MTX were given to the animals at the same time. Vitamin C was also administered concomitantly at a dose of 100 mg/kg.

The MTX and vitamin C concentrations utilized in our investigation were based on the results of earlier research<sup>17,18</sup>.

At the end of experiment, the blood samples were collected ten hours after the last meal from retroorbital venous plexus for biochemical measurements, then an overdose of anaesthesia was used to sacrifice the animals. Histopathological examinations were performed on their kidneys and livers. Comparisons were made between the vitamin C group, the MTX-treated group, and the control group.

**Biochemical Analysis**

Samples of venous blood were drawn into tubes without any anticoagulant. Serum was centrifuged after clotting and maintained at -80 °C until analysis.

Blood samples used for measurements of :

- 1- Liver function tests: the level of aspartate transaminase (AST) and alanine transaminase (ALT) in the serum <sup>21</sup>.
- 2- renal function test: Creatinine and urea levels . All assay done by cobase c 111 analyzer

**Histopathological Processing**

All of the rats' kidneys and livers were fixed in a 10% formaldehyde solution, dried in graded alcohol, and embedded in paraffin. For light microscopy studies, 5 m thick The counter-stained sections were obtained, mounted on glass slides, and used hematoxylin and eosin <sup>22</sup>. A histopathologist coded the slides and analyzed them. The degree of bleeding, vacuolar degeneration, congestion, and necrosis in each section was assessed. The histopathologic score analysis was carried out in accordance with accepted practices, and the evaluation was provided as the average of the individual score grades for each of the following qualities. from 0 (no findings), 1 (mild), 2 (moderate), and 3 (severe) .

For kidney sections, degeneration in renal tubules, glomerular atrophy, cystic dilatation and tubular necrosis .

For liver sections, Hepatocytes with radial disruption around the central vein, sinusoidal dilatation, and nuclear alterations. <sup>23,24</sup>

**Statistical Analysis**

The mean and standard deviation (SD) of six observations were used to calculate all results.

**RESULTS**

**Biochemical Results**

Table (1) show s differences in the mean values of AST, ALT ,blood urea and serum Creatinine in group II and III when compared with the control group (Group I).

Group II & III revealed marked toxicity which showed a considerable increase in the mean values of AST, ALT,

blood urea, and serum creatinine when compared to the control group.

Table (1): Statistical analysis of liver and renal function tests for different groups of animals.

parameters	group		
	I	II	III
Urea(mmol/l)	6.8±0.1	10.1±0.21	8.2±0.12
Creatinine(mmol/l)	105.5±0.19	127.5±0.14	116.1±0.21
AST(unit/ml)	15±2.35	200± 1.45	137.1±0.15
ALT(unit/ml)	18.9 ± 2.58	84.6 ± 0.66	31.7±0.23

All data represented as mean values±SD (n=6/group ).

**Light Microscopic Evaluations**

Hepatocytes in the livers of control rats had a normal histological appearance, radially distributed in anatomizing cords that seemed to emanate from the central veins. Hepatocytes had polygonal shapes, rounded vesicular nuclei, and acidophilic cytoplasm in the center. Binucleate cells were found in some of the cells. Blood sinusoids, which were bordered by flat endothelial cells, separated the cords of liver cells (Figure 1a). In the MTX-treated (group II), sections of livers revealed variable structural changes of hepatic tissue. The main observations were marked dilatation and congestion of the central veins and blood sinusoids with moderate The radial pattern around the major vein has been disrupted (Fig 2a). Apoptotic cell foci were found inside the lobule on several slides. Some hepatocytes with vacuolated cytoplasm and pyknotic or karyolytic nuclei could be clearly seen. Also fatty changes with vacular degeneration can be observed (Fig 3a). Administration of vit C with MTX treatment exhibited marked reduction in the hepatic lesions. There is mild dilatation of central vein with minimal disruption of hepatocyte cord also cellular infiltration around the portal tract was present (Fig. 4a, -table 2).

Table (2):Comparison of mean values of the pathological lesions of rat's Livers in the 3 different groups.

LESIONS	GROUPS		
	CONTR OL	MTX	MTX+VIT C
Disruption of hepatocyte arrangement	0.0±0.00	1.9±0.22	1.1±0.12
Sinusoidal dilatation	0.0±0.00	2.5±0.20	2±0.00
Nuclear changes in hepatocyte	0.0±0.00	2±0.00	1±0.00

All data represented as mean values±SD (n=6/group)

Moreover, our study revealed that MTX is considered as nephrotoxic to renal system in rat due to histopathological alterations in Renal tissue. The transformations were dramatic. The tubular epithelial cells in the MTX group swelled significantly, with moderate to severe degeneration in renal tubules and desquamated cells within the tubule lumen in the corticomedullary junction (Fig. 2bA), also there is mild-moderate glomerular atrophy with sever tubular necrosis and cystic dilatation (fig 2b ).When compared to the third group (vit C and MTX group), where the changes were minor (Fig. 3b), or the control group, these changes were much more pronounced in the second group (MTX group) (table 3).

Table (3):Comparison of mean values of the pathological lesions of rat's kidneys in the 3 different groups.

Lesion	groups		
	Control	MTX treated	MTX+ VIT C
Degeneration in renal tubules	0.0±0.00	3.2±0.13	2.1±0.12
Tubular necrosis	0.0±0.00	2.9±0.00	1.2±0.00
Glomerular Atrophy	0.00±0.000	1.5±0.20	0.5±0.13
Dilatation-of tubules and Congestion	0.0±0.000	3.1±0.233	1.5±0.12

All data represented as mean values±SD (n=6/group)

## DISCUSSION

The drug methotrexate (MTX) is used to treat a number of immunological disorders and cancers. It is frequently used to treat psoriasis and rheumatoid arthritis. however, it includes a wide range of negative effects. Because it accumulates in the liver and is discharged in the kidneys, it can be hepatotoxic and nephrotoxic if used without supervision. Clinicians routinely use the medicine and want to lessen its adverse effects, particularly the hepatotoxic and nephrotoxic effects (18).

The effects of vitamin C (as an antioxidant) on MTX-induced liver and kidney damage in rats were studied in this work. We observed that rats given MTX + vitamin C had lower mean levels of ALT, AST, blood urea, and serum creatinine than rats given only MTX. Various investigations have shown that vitamin C protects against hepatic and renal damage (19,20).

Methotrexate was found to be nephrotoxic to the renal system in rats, as evidenced by the histopathological alterations in the glomeruli and tubular epithelium. The second group (MTX group) had more frequent and severe tubular necrosis of the kidney than the third group ( MTX and Vit C group) or the control group. Another researcher found that large doses of methotrexate can cause nephrotoxicity (21). Methotrexate has been shown to have a virulent effect on renal tubular cells, either through destruction of the tubular epithelium directly or through methotrexate precipitating in these tubules.(22).

In this investigation, glomerular atrophy and cystic dilatation of renal convoluted tubules with bleeding and congestion were found in the MTX group, although the severity was modest in the third group (MTX and vit C). This could be explained by the fact that as MTX levels rise in the body, it causes severe renal toxicity, culminating in the buildup of MTX crystals in the nephrones, which causes renal tubule dilation (23).

Administration of an antioxidant as adjuvant therapy may be promising in reducing the renal side effects of methotrexate (24) because the mechanism of MTX nephrotoxicity is thought to be triggered by the MTX-induced free radicals causing oxidative stress on kidney tissues (25).

Moreover, in the current study, MTX treated rats showed variable degrees of degenerative changes in the structure of hepatic tissue. This might explain the elevated levels of AST and ALT enzymes in the serum due to their leakage from injured liver cells. Some authors were in agreement with our findings (26). Degenerative findings were manifested by dilatation and congestion of the central and portal veins as well as congested sinusoids. The loss of fluid from the circulation and the engorgement of the arteries with RBCs might lead to congestion, whereas increased prostaglandin production that causes smooth muscle relaxation and subsequent vasodilation could also lead to dilatation ( 27).

Fatty deterioration and inflammatory cellular infiltration were seen in hepatocytes, particularly in the vicinity of the portal tracts. Some researchers identified the same observation .

stating that inflammation is a microcirculation reaction characterized by the movement of fluids and leukocytes into extravascular tissue from the circulation (28-30). Additionally, the buildup of fat in hepatocytes may be related to reduced protein synthesis brought on by rER destruction, which would then prevent the production of lipoproteins, which is involved in the transport of hepatic triglycerides to extra hepatic tissue and, as a result, fat accumulation in the cytoplasm (29). Previous experimental evidence has been proved



that vit C significantly decreased the levels of free radicals in the liver of mice treated with nickel (31). In the current research, concomitant administration of vit C with MTX was accompanied by marked protection of the liver and kidney from degenerative changes.

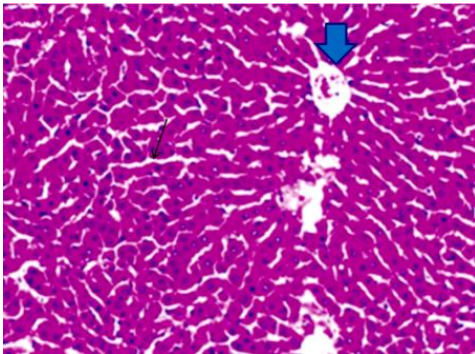


Fig 1a: liver histology in control rats Hepatocyte cords radiating from the center vein (thick arrow) and the blood sinusoids (thin arrow). (H&EX100).

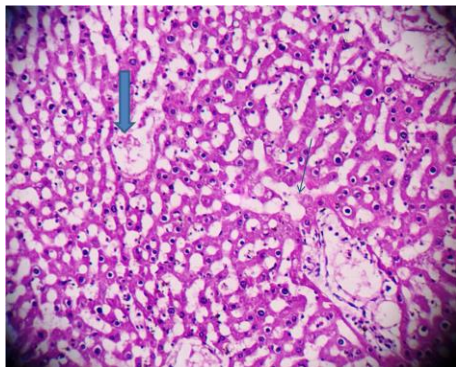


Fig 2a: Histopathological section of liver treated with MTX showing marked congestion of central vein (thick arrow) and sinusoid dilatation (thin arrow) with disturbed hepatocyte plate. (H&EX100).

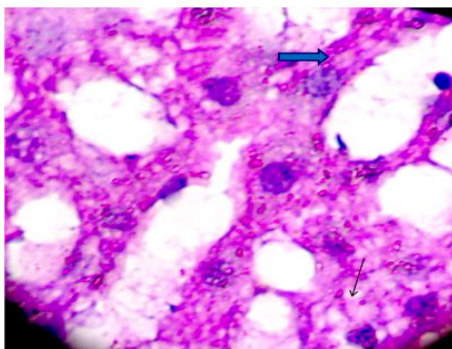


Fig3a: Section of liver treated with MXT showing vacuolar degeneration of hepatocytes (thick arrow) with mild fatty infiltration (thin arrow) (H&EX400)

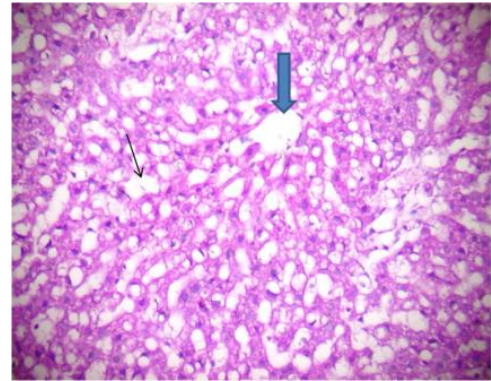


Fig4a: MTX and vit c treated groups (group III) showing mild dilatation of central vein (thick arrow) and sinusoids (thin arrow) (H&EX100).

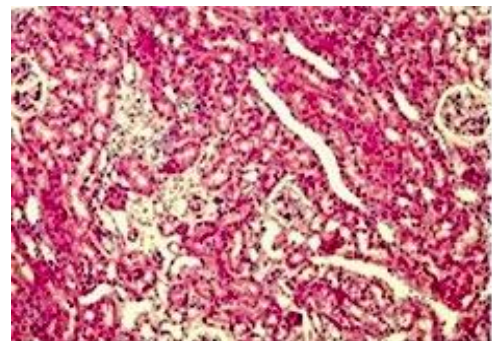
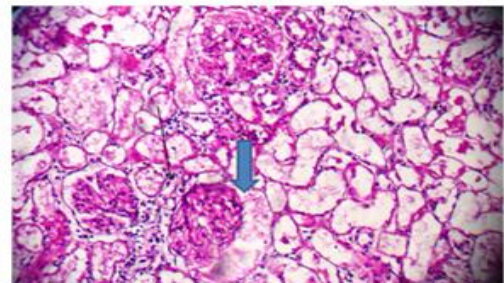
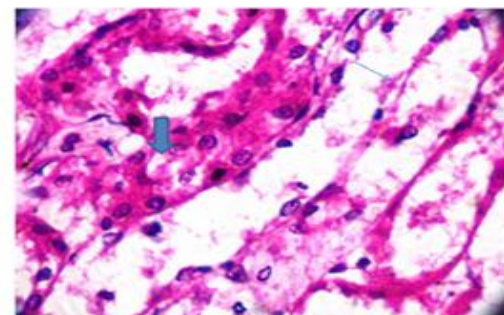


Fig 1b: Control kidney rat showing normal glomerulus and tubules. (H&E X100)



A



B

Fig2b: Sections of kidney treated with MTX (A) showing mild glomerular atrophy (thick arrow) with inflammatory cells infiltration (thin arrow) (H&E X100). (B) Tubular degeneration (thick arrow) and necrosis (thin arrow) with dilatation (H&E X400).

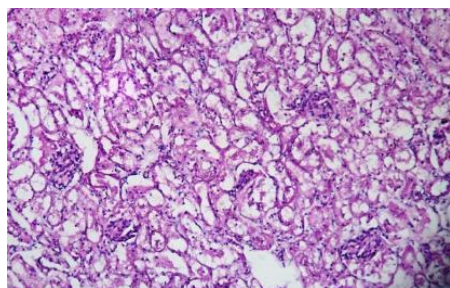


Fig 3b: MXT and Vit C groups (group III) showing mild degenerative and dilatation of glomeruli and renal tubules (H&EX100).

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