The effect of vitamine A and glutamine on methotrexate induced hepatotoxicity in rats

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تأثير فيتامين A والكلوتامين على سمية الكبد المحدثة بالميثوتركسيت في الجرذان تأثير

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Abstract

Methotrexate is one of the most effective and commonly used medicines to treat various forms of arthritis and other rheumatic conditions. It is known as a disease-modifying antirheumatic drug (DMARD) because it not only decreases the pain and swelling of arthritis but also can reduce damage to joints and the risks of long-term disability.

Method: Twenty eight male rats, were taken from the research center in the College of Medicine at the University of Kufa. These animals were equally divided into four groups, Each of 7 rats, the first group received distilled water added equivalent fluid (corn oil , NaOH and citric acid) and served as control. The second group received methotrexate at dose of 2.5 mg/BW orally twice daily by gavage, the third group received methotrexate and vitamin A at doses of 2.5 mg /BW + 50000 IU respectively orally twice daily by gavage and the forth group received methotrexate and glutamine at dose 2.5 mg/BW + 500 mg /BW twice daily respectively. All groups dosing for period 30 days.

At the end of the experiment, all rats were anesthetized using ether and drawed blood 4ml from heart using a 23-gauge needle syringe then killed and take piece of liver and placed in formalin 10 % for histopath examination. The blood placed in centrifuge 3000/minute for 10 minutes to obtain serum for analyzing liver function test (alkaline phosphatase, aspartate amino transferase (AST) and alanine amino transferas (ALT) measurements and bilirubin)

Result: In animal treatment with methotrexate only and those treated with MTX and vit A and glutamine there was no significant difference in serum ALT, serum AST and Bilirubin when compared with control group but ALP elevated in MTX with vit A treated rats and no significant difference in MTX and MTX+glutamine treated groups but showed amyloid changes liver and congestion in methotrexate treatment rat compared with control and no damage in liver of MTX with vit A and glutamine treated groups

Conclusion: liver enzymes could be normal despite histopathic change in methotrexate treated animals.

الخلاصة

ميثوتركسيت من الأدويةِ الأكثر فاعليةِ والمستعملةِ عموماً لمُعَالَجَة الأشكالِ المُخْتَلِفةِ مِنْ إلتهابِ المفاصل والحالات الروماتزميةِ الأخرى، المعروف بدواء معالج مرضِ روماتزميِ (DMARD) لأنه لَيسَ فقط يقلل الألمَ وورمَ إلتهابِ المفاصل لكن أيضاً يُمْكِنُ أَنْ يُحوّلَ ضرر إلى المفاصلِ وأخطارِ العجزِ الطويل المدى.

الطريقة: استعملت ثمانية وعشرون جرذ من الذكور، أُخِذَ مِنْ مركزِ البحوث في كليَّةِ الطبِّ في جامعةِ الكوفة. هذه الحيواناتِ قُسّمتْ إلى أربع مجموعاتِ،عدد كُلّ مجموعة 7 جرذانِ، إستلمتْ المجموعةَ الأولى (السيطرة) ماءاً مُقَطَّراً مضافَ اليه سائلاً مكافئاً (زيت ذُرة، NaOH وحامض الستريك). إستلمتْ المجموعةُ الثانيةُ الميثوتركسيت بجرعةِ مِنْ 2,5 ملغم/وزن الجسم عن طريق الفم مرَّتين في اليوم باستعمال قسطرة، إعطيت المجموعةَ الثالثةَ الميثوتركسيت وفيتامين A بجرعة 2,5 ملغم/وزن الجسم + 50000 وحدة دولية على التوالي، والمجموعة الرابعة جرعت بالميثوتكسيت والكلوتامين بجرعةِ 2,5 ملغم /وزن الجسم + 500 ملغم/وزن الجسم. وللفترةِ 30 يوم.

في نهايةِ التجربةِ، كُلّ الجرذان كَانت تخدرَ بإستعمال المخدر الايثر ويسحب منها دمّ 4 مل مِنْ القلبِ باستعمال سرنج مقياسِ 23 ويوضعَ الدمّ في جهاز الطرد المركزي 3000 / دقيقة لفترة10 دقائقِ للحُصُول على المصلِ لتَحليل إختباراتِ انزيمات وظائف الكبد ثمّ تقتل الحيوانات ويأخذ قطعة من الكبدِ وَتضع في الفورمالينِ 10 % لفحصِ النسيجي.

النتائج: وجد ليس هناك تغيير معنوي بين الانزيمات الوظيفية في مصل AST وBil و ALT في الكبد لكل المجاميع المعالجة بالميثوتركسيت فقط والعلاج بالميثوتركسيت مع الفيتامين A والكلوتامين بينما اظهر ارتفاع في مستوى ALP في مجموعة المعالجة بالميثوتركسيت مع فيتامين A فقط ولا يوجد تغيير في مستواه في المجاميع الاخرى ولكن في الفحص النسيجي لاحظ هناك تغير في نسيج الكبد وظهور الاحتقان وارتشاح البروتينات بين خلايا الكبد في الجرذان المعالجة بالميثوتركسيت فقط بينما في المجاميع التي عولجت بالميثوتركسيت مع فيتامين A والكلوتامين لم يظهر اي تغير في نسيج الكبد.

الاستنتاجِ: ان انزيمات الكبد ممكن تبقى طبيعية بالرغم من التغيرات النسيجية في الحيوانات المعالجة بالميثوتركسيت.

Indroduction

Methotrexate is one of the most effective and commonly used medicines to treat various forms of arthritis and other rheumatic conditions. It is known as a disease-modifying antirheumatic drug (DMARD) because it not only decreases the pain and swelling of arthritis but also can reduce damage to joints and the risks of long-term disability. (1)

Methotrexate is a drug that inhibits the vitamin folic acid. It is used in low doses for patients with rheumatoid arthritis or psoriasis and in high doses as chemotherapy for cancer patients (2). Surprisingly, started early in the course of the disease, methotrexate is nearly as effective as the biologic agents recently introduced for the treatment of rheumatoid arthritis and is commonly administered in combination with either biological agents or other small molecule antirheumatic drugs. (3) As currently used, As currently used, low-dose methotrexate is safe and Well tolerated. Because of its efficacy and safety, low- dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis not responsive to nonsteroidal anti-inflammatory drugs alone (4).

These mechanisms of action and the role of methotrexate in the suppression of rheumatoid arthritis are reviewed. At the doses commonly used for the treatment of rheumatoid arthritis, the bioavailability of Oral methotrexate varies considerably between individuals, but in general is in the range of 70% ,and food does not significantly affect uptake of the drug. There is some evidence that at higher doses oral bioavailability declines, phenomenon on most likely due to the fact that uptake of methotrexate from the gastrointestinal tract is mediated by a saturable transporter, reduced folate carrier 1 (RFC1).(5)

Not surprisingly, common side effects of MTX treatment are myelosuppression and gastrointestinal toxicity. Renal and liver damage may occur as well, in particular after high dose and/or chronic administration of MTX. Combination of MTX with several other drugs has proven to increase the therapeutic efficiency and/or the toxicity in vitro and in vivo. The clinical significance of many of these interactions remains to be determined (6). Due to its potentially toxic effect on the liver, additional hepatotoxic medicinal products should not be taken during treatment with methotrexate unless clearly necessary and the consumption of alcohol should be avoided or greatly reduced. One has to take into consideration, however, that coadministration of NSAIDs and methotrexate may involve an increased risk of toxicity. The steroid dose can be reduced gradually in patients who exhibit therapeutic response to methotrexate therapy. (7) . In adults with severe, acute rheumatoid arthritis who are unresponsive or intolerant to conventional therapy, 7.5mg orally once weekly or divided oral doses of 2.5 mg at 12 hour intervals for 3 doses (7.5mg) as a course once weekly. The dose may be increased to 20 to 25 mg a week over time if needed (8.)

Material and method:

Animals:

The animals which were used in this study were Wistar albino male rats weighing (250 – 300) g their age between 4-5 months. The rats were maintained in cages in the animal care facility, subjected to alternate 12-hour periods of dark and light. Food and water were supplied ad libitum during all experiments.

Twenty eight male rats were equally divided into four groups, Each of 7 rats, the first group received distilled water added equivalent fluid (corn oil , NaOH and citric acid) and served as control. The second group received methotrexate at dose of 2.5 mg/BW orally twice daily by gavage, the third group received methotrexate and vitamin A at doses of 2.5 mg /BW + 50000 IU respectively orally by gavage and the forth group received methotrexate and glutamine at dose 2.5 mg/BW + 500 mg /BW respectively. All groups dosing for period 30 days.

At the end of the experiment, all rats were anesthetized using ether and drawed blood 4ml from heart using a 23-gauge needle syringe then killed and take piece of liver and placed in formalin 10 % for histopath examination. The blood placed in centrifuge 3000/minute for 10 minutes to obtain serum for analyzing liver function test (alkaline phosphatase, aspartate amino transferase (AST) and alanine amino transferas (ALT) measurements and bilirubin)

Chemicals and drugs

Methotrexate 2.5 mg/BW tablet the manufacturer by (Ebewe; Austrialia), Vitamine A 50000 IU capsule manufacture by (Alesgandderia; Egypt) and glutamine 500 mg tablet manufacture by (UT; Holland) , were purchased from a local pharmacies. Corn oil , NaOH and citric acid . kits of enzyme

preparation of drug doses:

Methotrexate:

The doses that used for methotrexate group were prepared by dissolving 2.5 mg in 0.5 ml of NaOH and added 71 ml distilled water that given daily orally at dose level 0.1 ml/ 100g body weight

Vitamine A:

The doses that used for vitamine A group were prepared by dissolving 50000 IU in 70 ml of corn oil that given daily orally at dose level 0.1 ml/ 100g body weight .

Glutamine:

The doses that used for glutamin group were prepared by dissolving 500 mg in some drops (0.5 ml) of citric acid and added 5 ml distilled water that given daily orally at dose level 0.1 ml/ 100g body weight.

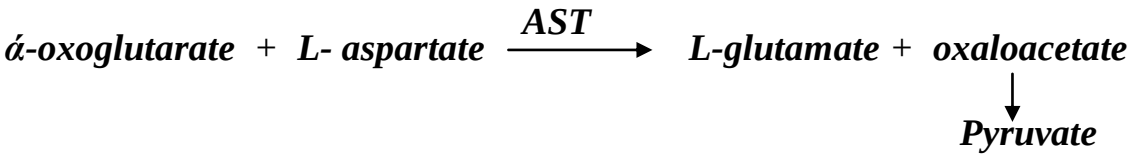
Biochemical test:

AST and ALT and AL Determination of the plasma concentrations of the liver enzymes ALT , AST and AL were measured in serum samples obtained from all groups of rats. Activities were expressed as IU L–1. The measurements were done in accordance with the methods of the diagnostic kits (Biolabo Reagents, Maizy, France).

Determination of aspartate and alanine transaminase activities:

Principle

Aspartate transaminase (AST) or glutamate oxaloacetate transaminase (GOT) catalyzed the irreversible transfer of an amino group from aspartate to ά-oxoglutarate forming glutamate and oxaloacetate .



Aspartate transaminase is measured by monitoring the concentration of decarboxylated pyruvate that react with 2-4 DNPH (2,4-dinitrophenyl-hydrazine)forming the colored hydrazone.

Alanine transaminase (ALT) or glutamate pyruvate transaminase (GPT) catalyzed the reversible transfere of an amino group from alanine to ά-oxoglutarate forming pyruvate and glutamate.

Alanine transaminase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine .

ALT and AST activities were measured in serum and supernatant according to the method of Reitman and Frankel, 1957. The absorbance was read against blank at 540 nm by using visible spectrophotometer.

$$\text{Calculation (IU/L)} = \frac{\text{Test} - \text{Control}}{\text{Stander} - \text{Blank}}$$

Determination of Alkaline phosphatase activity:

Principle

Colorimetric determination Alkaline phosphatase activity was achieved according to the following reaction :

ALP

Phenyl phosphate ⟶ phenol + phosphate

The phenol librated is measured in the presence of 4-amino-antipyrine and potassium ferricyanide. ALP activity was measured in serum and supernatant according to the method of Belfield and Goldberg, 1971 in which absorbance of colored mixture was measured spectrophotometrically at 510 nm according the equation:

$$\text{Calculation (IU/L)} = \frac{\text{Test} - \text{Control}}{\text{Standard} - \text{Blank}} \times \text{n}$$

n = 142 IU / L

Histopathological preparation

Section from different groups were prepared by routine techniques . the liver were fixed in neutral buffered formalin (10%). 5-6µm sections were stained by H&E (hemotoxylin and eosin stains), change in liver tissue were diagnosed through examination of section by light microscope.

Statistical Analysis

A computer program (SPSS 13.0) was used for statistical analysis. Values of $p < .05$. All results were expressed as means + standard error (SEM).

Results

In methotrexate only group 2.5 mg/BW there is no significant difference between all parameters GPT, GOT and ALP when compared with control group $p>0.05$ and Comparing the control group with groups treatment with methotrexate 2.5mg/BW + glutamate 500mg /BW and with vitamine A 50000 IU showed no significant difference $p>0.05$ (table -1).

Table -1-effect methotrexate , MTX+vit A and MTX +glutamine on serum AST,ALT, ALP and Bil level IU/L of different experimental groups

group n=7	GPT	GOT	ALP	Bil
Control	100.50 ± 1.50	224.0 ± 17.00	783.5 ± 16.5	0.6 ± 0.0
Methotrexate	116.3 ± 14.3	208.4 ± 4.4	783.0 ± 43.6	0.8 ± 0.12
Methotrexate+ vit A	135 ± 15.00	208.5 ± 8.5	1230.0± 20.00*****	0.7 ± 0.1
Methotrexate+ glutamine	140 ± 5.7	230.0 ± 15.0	748.3 ± 30.3	0.6 ± 0.1

n = number of animal
* significant P-value ≤ 0.05
mean ±SE

Light Microscopic Evaluations in the livers of rats from methotrexate treated showed congestion and amyloid(Figure 2) compared with control group (figure-1), while MTX-treated rats with glutamine and vitmine A hepatocytes showed a normal histological appearance (Figure 3), but congestion was observed in glutamine treated group.

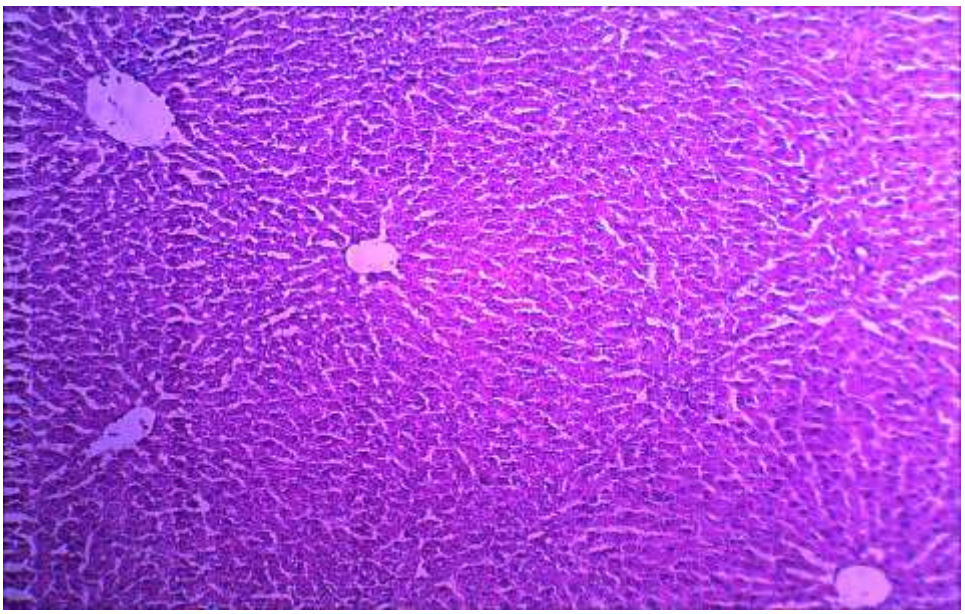


Figure -1 **Normal histological appearance of liver in control (H&E stain, 40x)**

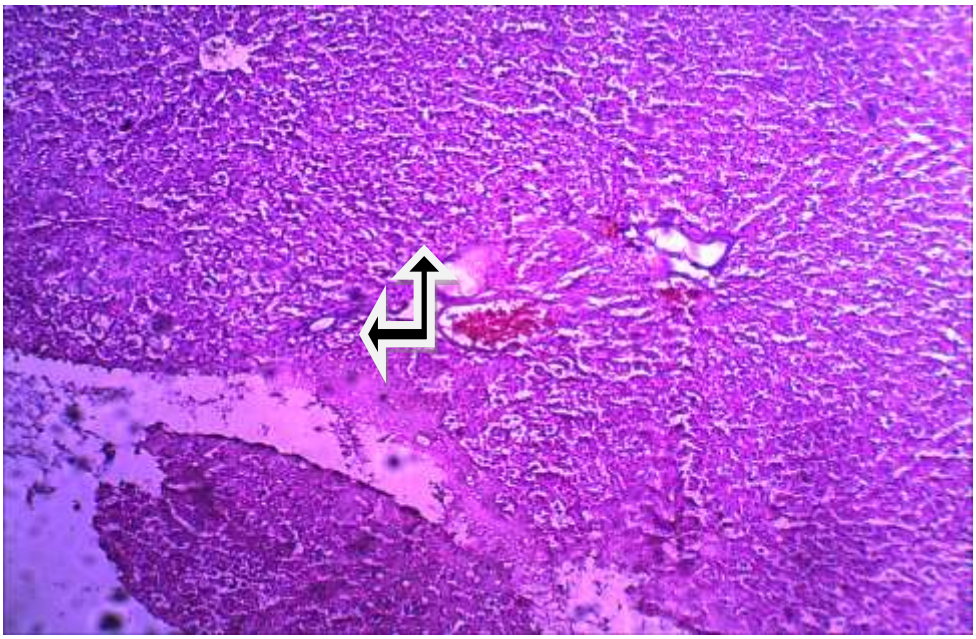


Figure 2- congestion and amyloidal appearance in liver in methotrexate treated group(H&E stain, 40x)

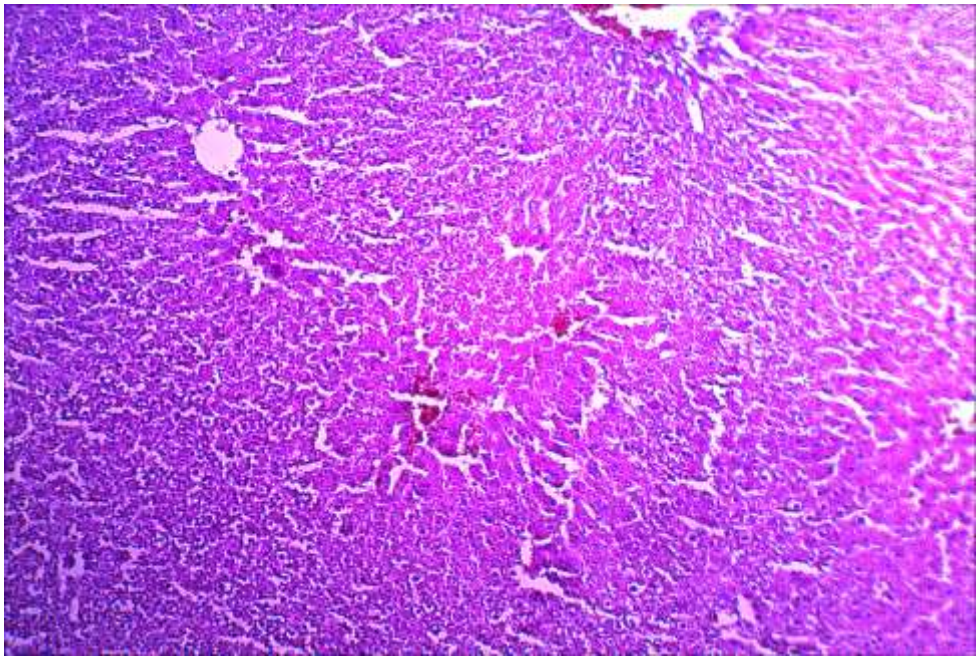


Figure- 3.—Normal histological appearance of liver in MTX+ glutamine and MTX+ vit A group (H&E stain, 40x)

Discussion

In this study no significant changes in liver enzyme namely AST, ALT and Bilirubin between different groups was noted, probably because of the low dose used of methotrexate (2.5 mg/BW) for 30 days treatment period these finding were similar to that reported by Roenigk HH Jr, et all. 1988(9) who observed serum aminotransferase concentrations are not elevated in association with the hepatotoxicity ascribed to long-term treatment with methotrexate for psoriasis.
while the ALP level was no significantly changed in MTX and MTX + glutamine groups but was elevated in MTX + vit A group this result is similar to that reported by Eldon A and Shaffer, 2009. (10) who observed that Alkaline phosphatase levels increase up to 3 times normal in many liver disorders and infiltrative disorders like amyloidosis.

In this study, the level of aminotransfere was not changed significantly, yet, there was a significant histopathological change in methotrexate treated group. Therefore, liver function tests are not reliable predictors of histological changes in treatment groups . THE same WA re also ported by R. F. Willkens, et all 1990, (11) he found that histological abnormalities were not predicted by liver function test changes.

Regarding histopatholgical changes significant changes in the form of congestion with amyliod changes were observed in methotrexate only treated group this result was similar to the study by Hall, et all .1991 (12) who observed histopathological damage in the liver with lower doses of methotrexate for longer durations . While in animal treated with MTX and vit A and glutamine no significant change were observed, these finding are similar to that reported by Yeshwanth R, et all, 2010 (13) and the study of the effect of glutamine on methotrexate toxicity by Rubio, Isabel T. MD et all. 1998(14) ,the hepatoprotective potential of vit A and glutamine could be attributed to their antioxidant effects protecting the liver from oxidation damage of methotrexate.

Conclusion

liver enzymes could be normal despite histopathic change in methotrexate treated animals.

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