

Effect of Methotrexate on the Liver Enzymes and Lipid Profile in Adult Female Albino Mice

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Abstract:

Methotrexate (MTX) was used for treatment of malignancies and now is widely used in treatment of rheumatoid arthritis. In this research the evaluation of the effects of MTX on some liver enzymes and lipid profile was studied. Twenty four adult female mice divided into three groups (8 mice each). The first two groups were treated with MTX while the third group was used as a control. MTX was intraperitoneally given at 50 µg/ml and 75 µg/ml to the first and second groups respectively for 35 days ,whereas the control group was intraperitoneally injected with normal saline. The results showed a significant ($p<0.05$) increase in serum levels of glutamic oxaloacetate transaminase (GOT) , glutamic pyruvic transaminase (GPT), Alkaline phosphatase (ALP) , total cholesterol and triglyceride (TG), however high density lipoprotein (HDL) showed a significant ($p<0.05$) decrease in mice treated by MTX when compared with control group .

Key words : (MTX) Methotrexate, (GOT) glutamic oxaloacetate Transaminase, (GPT)Glutamic Pyruvic Transaminase, (ALP) alkaline phosphatase, (TG) triglyceride, (HDL) high density lipoprotein, (LDL-c) low density lipoprotein-cholesterol .

Introduction:

Methotrexate (MTX) is commonly used as a cytotoxic agent in the treatment of leukemia and other malignancies as well as in the inflammation diseases such as psoriasis and rheumatoid arthritis in lower doses [1]. With the widespread use of MTX, although hepatotoxicity is the most important potential major side effect [2]. Methotrexate used for cancer chemotherapy are well known to produce a cute toxic side effects in multiple organ systems. The most common target organs are bone marrow, gastrointestinal tract, mucosal membrane, and hair follicles [3]. It has been reported that liver damage may occur as well in particular high doses or following chronic administration of MTX [4]. However,long-term

administration of MTX is associated with an increase risk of liver damage. The risk for development of liver disease is not often predicted with the use of standard liver enzymes and thus, patients receiving MTX must be monitored routinely by examination of liver biopsies [5].

The mechanisms of action of MTX are complex, and proposed to inhibits purine and pyrimidine synthesis, which accounts for its efficacy in the therapy of cancer as well as for some of its toxicities. Certain aspects of MTX toxicities are also attributed to adenosine release [6]. As MTX promotes adenosine release acting at its receptor and mediates the immunologic and anti-inflammatory effects of MTX in the treatment of

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rheumatoid arthritis [7]. One long-term adverse effect of MTX is hepatotoxicity [8]. In rheumatoid arthritis patients receiving doses of MTX, a biopsy should be performed if a patient develops persistent abnormalities on liver blood tests. The suggestion is that biopsies be performed after every 1 to 2 g of cumulative MTX therapy [9]; [10], because liver function tests were not an effective form of monitoring for methotrexate hepatotoxicity [11]. The aim of this research is to study the effects of chemotherapy drug (methotrexate) on liver enzymes and lipid profile levels in mice.

Materials and Methods:

Twenty four adult female albino mice with 8-10 weeks of age, and 25-30 grams of weight were used in this study. All mice were provided with food and water *ad libitum*. The mice were divided into three groups, (each composed of eight mice). The first and second groups received intraperitoneal injections of MTX at 50 µg/ml and 75 µg/ml respectively, whereas control group was given normal saline for 35 days. The room temperature was maintained at (24±2) °C, and the animals were exposed to 14 hours light 10 hours darkness program.

Methotrexate was obtained from (Hexal company) at concentration of (50mg /2ml), the stock solution of MTX was (25mg/ml), and from it the concentration 50µg/ml, 75µg/ml were prepared to be used in this study.

Biochemical examination

1- Lipid profile

Determination of total cholesterol, triglyceride and HDL in the serum were measured by enzymatic kit method produced by biomerux (France) according to [12].

Determination of liver enzymes

Glutamic Oxaloacetate Transaminase (GOT) & Glutamic Pyruvic Transaminase (GPT)

The GOT and GPT enzymes activity were evaluated in mice serum by using enzymatic colorimetric kit method (Randox company) and according to [13]. The absorbency was measured at 546 nm by using spectrophotometer.

ALP (alkaline phosphates)

To estimate the activity of the ALP enzymes, procedure of [14] was used.

Statistical Evaluation

Data were analyzed by SPSS version 10 with analysis of variance with ANOVA- test. Data are presented as means ± SD and the level of significance was equal to less than 0.05 [15].

Results and Discussion:

In the present study the result showed a significant ($p < 0.05$) increase in serum levels of liver enzymes (GOT, GPT, ALP), in mice treated by MTX comparing with control group (Table 1). This finding suggests that MTX can inflame the liver and causing abnormal serum levels of liver enzymes.

Table (1): Serum levels of liver enzymes in mice treated with MTX and control

Groups	GOT IU/ml (mean±SD)	GPT IU/ml (mean±SD)	ALP IU/ml (mean±SD)
Control	A 214.63±16.19	A 62.07±9.41	A 74.22±11.83
MTX (50µg/Kg)	B 287.05±23.37	B 88.05±14.47	B 134.27±21.07
MTX (75µg/Kg)	B 291.05±32.92	B 92.91±13.06	B 141.84±34.20

Differences A,B are significant (P<0.05) in compared rows

It was reported that the increase of GOT ,GPT serum levels may be due to the cytotoxic effect of MTX on liver cells. This may lead to increase the permeability of liver cell membrane , causing the movement of high quantity of these enzyme to blood serum .This explains the increases of the enzyme level in blood serum and decreases in liver after using these toxic agents [16] . Kremer *et al.*[10] demonstrated that hepatotoxic effects are associated with long-term use and high doses of MTX and are common in patients taking a daily dose.However in patients with rheumatoid arthritis, the treatment with MTX; lead to persistent elevation in liver enzymes. Methotrexate is also known to be widely used as a therapeutic agent in different diseases, and connected with various side effects, including liver toxicity. The mouse model used in this study clearly demonstrate the toxic effects of this drug as there is an increase in the death rate, as well as the significant increase of GOT and GPT levels. It was reported that MTX administered in conjugation with either nicotinamide or methionine, the rise in the death rate and in GOT and GPT levels is markedly reduced [17]. Griffith *et al.* [18] reported that MTX is effective in rheumatoid arthritis but it can induce abnormalities in liver function test. The study is also showed that folate supplementation can reduce the incidence of elevated liver enzymes

during MTX treatment. Hepatic damage is also reported to be in relation to MTX use and combined with the elevation of liver enzymes,GOT and GPT released into the blood [19] . Moreover Robert *et al.* [20] found that ALP and transaminase were significantly increased in patients at the time of starting (or restarting) MTX therapy. The elevation of ALP level is correlated with the severity of the toxic effect of MTX. Palazzi *et al.* [21] found that the administration of MTX at 10 mg caused an increase in transaminase. Also the administration of folinic acid with 7.5mg of MTX was reported to increase transaminase enzyme. However the reduction in MTX dose with folate administration were unable to normalize serum transaminase. While the use of ursodeoxycholic acid progressively reduced liver enzymes to normal levels. Belinsky *et al.* [22] using mice, reported that long-term administration of MTX for management of chronic inflammatory disease is associated with risk of liver damage, MTX-induced liver inflammation may be mediated by elevation of complement pathway gene expression . Complement is produced by hepatocytes secreted in high amounts into the blood. Activation of the complement pathway results in a cascade of liver enzymatic events producing amplification of response. The results of the present study are also showed a significant (p<0.05)

increase in serum levels of total cholesterol and triglyceride, while high density lipoprotein (HDL) level was significantly ($p < 0.05$) decreased in mice treated with MTX in comparison to control group (Table 2). Our data indicate that MTX is associated with increase levels of total cholesterol, TG and decrease level of HDL, all

of these parameters are important aspects and cause increasing the development of atherosclerosis and subsequently clinically overt cardiovascular disease.

Table (2): Levels of lipid profile in mice treated with MTX and control

Groups	T.Chol. mg/dl (mean±SD)	T.G mg/dl (mean±SD)	HDL mg/dl (mean±SD)
Control	A 122.30±21.07	A 97.04±18.63	A 29.50±4.28
MTX (50µg/Kg)	B 243.85±26.92	B 189.55±21.92	B 16.22±4.01
MTX (75µg/Kg)	C 266.71±37.82	C 204.62±41.04	B 15.29±5.03

Differences A,B,C are significant ($P < 0.05$) in compared rows

Similarly Georgiadis *et al.* [23] demonstrated that post MTX treatment the levels of cholesterol, triglyceride and low density lipoprotein-cholesterol (LDL-c) were significantly elevated when compared to the control values, the inflammation reported after the treatments in all of these experiments is responsible for the elevation of these lipid profile parameters. Belinsky *et al.* [22] reported that 35 genes involved in fatty acid synthesis were upregulated in the MTX-exposed samples and this was in consistent with frequent steatosis associated with MTX exposure. Fatty metamorphosis is a hallmark of MTX-induced hepatotoxicity. Also van Ede *et al.* [24] found that the use of MTX can cause a folic acid deficiency with subsequently higher homocysteine levels and, thereby, increasing the risk of cardiovascular disease by increasing levels of total cholesterol, LDL-c, TG and reduction in serum HDL-c level

which is in consistence to the finding reported in the present study.

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تأثير عقار الميثوتركسيت على انزيمات الكبد وصورة الدهون في اناث الفئران البيض البالغة

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الخلاصة:

استخدم عقار الميثوتركسيت لعلاج الامراض السرطانية ويستخدم الان بشكل واسع في علاج التهاب المفاصل . تم في هذا البحث تقييم تأثير الميثوتركسيت على بعض انزيمات الكبد وصورة الدهون في الدم . استخدمت في البحث اربعة وعشرون أنثى من الفئران البيض البالغة. قسمت الحيوانات الى ثلاث مجموعات (8 فئران لكل مجموعة) . عوملت اول مجموعتين بالميثوتركسيت بينما المجموعة الثالثة استخدمت كسيطرة . تم إعطاء الميثوتركسيت داخل الخلب البريتوني وبجرع مقدارها 50 و 75 مايكروغرام / مل الى المجموعة الاولى والثانية على التوالي لمدة 35 يوم، بينما حققت مجموعة السيطرة بالمحلول الملحي الفسيولوجي داخل الخلب البريتوني. أظهرت النتائج ارتفاعا معنويا في مستوى كل من انزيمات الكبد GOT و GPT و ALP بالاضافة الى الكوليسترول والكليسيريدات الثلاثية ، في حين اظهر البروتين الدهني عالي الكثافة انخفاضا معنويا في الفئران المعاملة بالميثوتركسيت مقارنة مع مجموعة السيطرة .