Study the Effects of Methotrexate with and without Vitamin A on Some Biochemical and Histological Parameters in Male Rabbits

دراسة تأثيرات الميثوتركسيت مع وبدون فيتامين أ على بعض المعايير الكيموحيوية والنسجية في ذكور الارانب

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Abstract

The present study aims to evaluate the effects of methotrexate (MTX) with and without vitamin A (Vit. A) on some biochemical parameters and histological structure in male rabbits liver. Twenty male rabbits weighing 1250-1480 gm were divided into four equal number groups. The first group was given 2 ml distilled water as control group. The second group was given MTX (20 mg/kg), the third group was given Vit. A (5000 IU), while the fourth group was given MTX (20 mg/kg) +Vit. A (5000 IU) in alternative days. Following four weeks of treatment, lipid profile total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), [low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)]; in addition to thyroid hormones triiodothyronine (T_3) and thyroxin (T_4)] and liver enzymes [glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT)] were determined in the serum. Also, the histological examination of liver of all the experimental groups were carried out. The results were revealed that the treatment with MTX caused a significant $P \leq 0.05$ increases in TC, HDL, LDL, T_4, and GPT when compared with the control group. The treatment with Vit. A did not cause any significant $P \geq 0.05$ differences in all the studied parameters. The MTX+Vit. A treated group showed a significant $P \leq 0.05$ increases only in GPT compared with the control group; while a significant $P \leq 0.05$ decreases was found in TC, HDL, T_3, T_4, and GOT when compared with the MTX treated group. The histological examination of the liver sections showed that MTX administration caused major histological changes in comparison with the control such as inflammatory cell infiltrations, vascular congestion, sinusoidal dilatation and granular degeneration of hepatocytes. Treatment with Vit. A showed a typical structure in liver tissue. While in MTX+Vit. A group, the histological changes were less severe than those in the MTX treated group; these changes were granular degeneration of hepatocytes and sinusoidal dilatation at low levels. The overall results of this study confirmed that administration of Vit. A decreased the side effects of MTX; this protective effect of Vit. A may have clinical applications in chemotherapy.

Keywords: methorexate, vitamin A, lipid profile, thyroid hormones

الملخص

تهدف الدراسة الحالية الى تقييم تأثيرات الميثوتركسيت MTX مع وبدون فيتامين أ في بعض المعايير الكيموحيوية والتركيب النسجي للكبد في ذكور الارانب. استخدم عشرون ذكراً من الارانب تتراوح اوزانهم بين 1250-1480 غم وقسمت الى اربعة مجاميع متساوية. اعطيت المجموعة الاولى 2 مل من الماء المقطر كمجموعة سيطرة، اعطيت المجموعة الثانية MTX (20 ملغم/كغم)، اعطيت المجموعة الثالثة Vit.A (5000 وحدة دولية)، بينما اعطيت المجموعة الرابعة MTX + Vit. A بين يوم ويوم. بعد اربعة اسابيع من المعاملة، قيمت صورة الدهون [TC, TG, HDL, LDL, VLDL]؛ بالاضافة الى هرمونات الدرقية [T_3, T_4] وانزيمات الكبد [GPT, GOT]. فضلا عن اجراء الفحص النسجي للكبد للمجاميع التجريبية. اظهرت النتائج بان المعاملة مع MTX سبب زيادة معنوية في مستويات TC, HDL, LDL, T_4, GPT عند المقارنة مع مجموعة السيطرة. بينما المعاملة مع Vit. A لم تسبب اي فروقات معنوية في جميع المعايير المدروسة. اظهرت المجموعة المعاملة مع MTX+Vit. A زيادة معنوية فقط في مستوى GPT عند المقارنة مع مجموعة السيطرة؛ بينما وجد نقصان معنوي في مستويات TC, HDL,T_3, T_4,GOT عند المقارنة مع المجموعة المعاملة مع MTX. اظهر الفحص النسيجي لانسجة الكبد في المجموعة المعاملة مع MTX بان هناك تغيرات نسجية كبيرة بالمقارنة مع مجموعة السيطرة مثل ارتشاح الخلايا الالتهابية، احتقان دموي، توسع الجيبانيات بالاضافة للتنخر الحُبيبي للخلايا الكبدية. اظهرت الارانب المعاملة مع Vit. A فقط مظهر مثالي لنسيج الكبد، بينما في المجموعة المعاملة بـ MTX+Vit. A كانت التغيرات النسجية على مستوى اقل مقارنة مع المجموعة المعاملة مع MTX ؛ وتضمنت هذه التغيرات التنخر الحُبيبي للخلايا الكبدية وتوسع الجيبانيات بمستويات اقل. النتائج الاجمالية لهذه الدراسة اكدت أن تجريع Vit. A قلل من التأثيرات الجانبية لل MTX ؛ ان هذا التأثير الوقائي ل Vit. A ممكن تطبيقه في المعالجة الكيميائية.

الكلمات الدالة: الميثوتركسيت، فيتامين أ، صورة الدهون، هرمونات الدرقية

Introduction

Methotrexate (MTX), which is a folic acid antagonist, is used commonly 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 low doses [1].The clinical use of MTX is limited due to dose-dependent hepatotoxicity, strong evidences support a role for reactive oxygen species (ROS) in the pathogenesis of MTX damages [2]. Administration of MTX induces oxidative stress and significantly reduces antioxidant enzymes such as superoxide dismutase; however, it is known that cells are protected against oxidative stress by the action of certain enzymes, vitamins, and other substances, collectively known as antioxidants [3].

Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations without harmful side effect [4]. Vitamin A is a potent antioxidant and acts as a scavenger of free radicals either independently or as a part of large enzyme system [5].

The lipid profile in serum includes total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) [6]. Cholesterol is found as a chief constituent of several lipoproteins particles namely HDL and LDL [7]. The body gets triglycerides from external sources by high-fat food, and after absorbed through intestinal transmitted into the blood stream mediated chylomicrons, then transported to storage sites molecules as lipoproteins [8]. High-density lipoprotein is the smallest and most dense lipoproteins particle, diameter ranges between 7-12 nm [9], they move the cholesterol from tissues and blood to the liver to be destroyed and excreted in liquid gall bladder [10]. Low-density lipoprotein is made in the liver and in the epithelial cells of the gut. Function of LDL is transfer the cholesterol from the liver to the tissues [10]. Very low density lipoprotein is a lipoprotein with diameter 30-80 nm, synthesized mainly by the liver but may also be synthesized to a lesser degree by the intestine [9]. It is the major carries of endogenous TG and acts to transfer TG from the liver to the peripheral tissue [11].

The thyroid hormones, thyroxine (T_4) and triiodotyronine (T_3) are iodinated compounds, synthesized by the thyroid gland. Up to 80% of the T_4 is converted into T_3 by peripheral organs such as the liver, kidney and spleen [12]. Synthesis, mobilization, and degradation of lipids are controlled by thyroid hormones, because lipids are the major calirogenic molecules [13]. Moreover, thyroid hormones are important factors for the other physiological phenomena such as growth, puberty, and mental development [14].

The liver has a wide range of physiological functions, including detoxification of endo-and xenobiotic compounds, homeostatic regulation of the plasma concentration of a multitude of metabolites, and synthesis of many proteins that circulate in the blood [15]. Liver enzyme levels are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases, liver enzymes are released into the blood in increasing amount when the liver cell membrane is damage. Glutamic pyruvic transaminase (GPT), also known as alanine aminotransferase (ALT), is an enzyme found in the liver. The levels of this enzyme are accordingly more specific indicators of liver injury [16]. Glutamic oxaloacetic transaminase (GOT), also known as aspartate aminotransferase (AST), is an enzyme found in the mitochondrion and cytoplasm of all liver cells. The evaluation of GOT activity is a basic procedure for the diagnosis and the monitoring of hepatocellular disorders [17]. The most serious side effect of MTX therapy is hepatic toxicity, it has been reported that liver damage may occur as well in particular high doses or following chronic administration of MTX [18].

The present study aims to study the effect of MTX on some biochemical parameters (lipid profile, thyroid hormones, and liver enzymes) and the histological structure of the liver in male rabbits, and to investigate the protective effects of Vit. A on these parameters and reducing the toxic effect of MTX on the liver.

Materials and Methods

Animals and Experimental Design

Twenty local male rabbits (*Oxyctolagus ciniculus*) weighing1250-1480 gm were obtained from Biotechnology Research Center, Al-Nahrain University. They were housed in plastic cages in a room under standard environmental conditions (26 ± 2°C; 12/12 h light/dark cycle); feed and water were provided ad libitum. The animals were allowed to acclimatize for two weeks before beginning the experiment. Following acclimatization, the rabbits were divided into 4 groups, each group including 5 animals, as follows:-

Group 1: (Control): Rabbits were given 2 ml of distilled water intraperitoneally on alternative days for 4 weeks.

Group 2: (MTX): Rabbits were given MTX (20 mg/kg) intraperitoneally on alternative days for 4 weeks.

Group 3: (Vit. A): Rabbits were given Vit. A (5000 IU) orally on alternative days for 4 weeks.

Group 4: (MTX+Vit. A): Rabbits were given MTX (20 mg/kg) intrapertonieally + Vit. A (5000 IU) orally on alternative day for 4 weeks.

Blood Collection

At the end of the experimental period and after overnight food deprivation, blood samples were taken from the rabbits by heart puncture into plastic tubes. After centrifugation (3000 rpm, 10 minutes), sera were separated for biochemical assays.

Lipid Profile Estimation

Serum lipids concentrations were spectrophotometrically estimated using commercial kits (BIOLABO S.A., France). Both TC and TG were measured enzymatically [19], whereas HDL-C is estimated by precipitation technique [20]. According to Friedewald equation [21], VLDL-C and LDL-C were calculated as: VLDL-C = TG/5 and LDL-C = TC - (VLDL-C + HDL-C).

Determination of Thyroid Hormones

Using mini VIDAS, Biomerieux (France); Serum T_3 and T_4 concentrations were determined for the rabbits according to the manufacturer recommended procedure, by using Biomerieux T_3 and T_4 kit [22].

Estimation of Liver Enzymes Activity

Enzymatic colorimetric method was followed in estimation of GPT and GOT activity [23]. The enzyme activity was assayed with Randox kit.

Histological Examination

Histological examination was done by isolating the liver from the male rabbits directly after killing by heart puncture. The liver was placed in Petri dish containing physiological solution (normal saline) for removing blood and connective tissues which attack to it, and then it was kept in a container containing Bouin's solution for 24 hours; after that replaced with alcohol 70% and kept for preparing tissue slides. The preparation of histological sections depended on standard methods of [24].The sections were examined under light microscope, and then photographs are taken from the microscope immediately by digital camera under magnification 40x, 100x.

Statistical Analysis

The results were analyzed statistically by using the Statistical Analysis System (SAS, 2004) [25]. Data were reported as mean ± SE. Completely randomized design-CRD (ANOVA table) and Duncan multiple a ranges were used for comparative between means. The significant level was set as $P \leq 0.05$.

Results and Discussion

Serum Lipid Profile

Results of serum lipids profile of the four experimental groups were illustrated in Table (1). There was a significant $P \leq 0.05$ increases in serum TC level in the MTX treated group in comparison with the control group. This result was in agreement with [26] who found that the treatment of the mice with MTX caused a significant increase in TC. It has been stated that treatment with MTX caused increases in serum cholesterol, the change appeared to be an associated with reduction in systemic inflammation [27]. In other study, Abdul-Barry and Al-Naama (2009) [28] mentioned that significant increases in TC level might be attributed to the high exposure of free radicals which might be stimulated the rate limiting enzyme hydroxyl-methyl-glutaryl CoASH reductase which was responsible for liver cholesterol synthesis. As result MTX, which responsible for production of free radical, cause an elevation in cholesterol level by stimulate this enzyme. The results revealed no significant $P \geq 0.05$ differences between each of the Vit. A treated group and the MTX+Vit. Treated group when compared with the control group, while a significant $P \leq 0.05$ decrease was found in the MTX+Vit. A treated group in comparison with the MTX treated group. This may be attributed to the effect of Vit. A acts as scavenger of free radical which produces by MTX and this reduced the synthesis of cholesterol. In a previous study [29], it has been reported that supplementation with Vit. A reduced the level of serum cholesterol in hypertension patients.

No significant differences in TG level was found between the three groups (MTX treated group, Vit. A treated group and MTX+Vit. A treated group) when compared with the control group. Also, there was no significant difference in the MTX+Vit. A treated group in comparison with the MTX treated group. A similar result was also reported by [30] who found that the treatment with single dose of 10 mg/Kg MTX caused no significant differences in TG level. While [31] noted that the treatment of patients with active rheumatoid arthritis by MTX

caused a significant increase in TG level. In a previous study [32], it has been stated that the treatment with Vit. A caused a significant decrease in TG level when compared with the control. This refers to the ability of antioxidant Vitamin to reduced serum TG level. These differences in results may be due to the dose, duration which used in the experiments.

The results revealed that the treatment with MTX caused a significant P≤0.05 increases in serum HDL level when compared with the control group; while there were no significant P≥0.05 differences between each of the Vit.A treated group and the MTX+Vit. A treated group when compared with the control group. On the other hand, the treatment with MTX+Vit. A caused a significant P≤0.05 decrease in level of serum HDL-C in comparison with MTX treated group. A previous study [31] reported that the treatment of patients with active rheumatoid arithritis by MTX caused a significant increase in HDL level. The results showed that there were a significant P≤0.05 increase in serum LDL level in the MTX group when compared with the control group. This increment was supposed to be related to the oxidative stress and lipid peroxidation induced by MTX. The lipid peroxidation was reported to produce high level of oxidized LDL [32]. There were no significant P≥0.05 differences between each of the Vit. A treated group and the MTX+Vit. A treated group when compared with the control group. On the other hand, there was no significant P≥0.05 differences in the MTX+Vit. A treated group in comparison with the MTX treated group. The oxidative stress effect of MTX was counteracted by the antioxidant effect of Vit.A. However, the antioxidant activity of Vit.A was not reported to decrease the level of oxidized LDL [33]. This may explain why Vit. A administration did not significantly decreases LDL level. The results showed that there were no significant P≥0.05 differences in serum VLDL level between the three groups when compared with the control group. Also, there was no significant P≥0.05 differences in the MTX+Vit. A treated group in comparison with the MTX treated group.

The group treated with MTX showed a significant increase in LDL level, this increment was supposed to be related to the oxidative stress and lipid peroxidation induced by MTX. The lipid peroxidation was reported to produce high level of oxidized LDL [32].The oxidative stress effect of MTX was counteracted by the antioxidant effect of Vit. A. However, the antioxidant activity of Vit. A is not reported to decrease the level of oxidized LDL. [33].This may explain why Vit. A administration did not significantly decrease LDL level. No significant differences were found in VLDL level between the MTX treated group and control, and this agreement with changes in TG level because VLDL was the major carrier of endogenous TG and act to transfer TG from the liver to the peripheral tissue [11].

Table (1): Effect of MTX (20 mg/Kg), Vit.A (5000 IU) and MTX+Vit. A on serum lipid profile in male rabbits.

Groups	Serum Lipid Profile (mean±SE)				
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	62.03±1.68^{b}	115.00±10.44^{a}	28.93±2.49^{b}	9.94±0.86^{b}	23.00±2.09^{a}
MTX	109.35±22.98^{a}	115.75±18.42^{a}	37.75±7.28^{a}	48.43±20.11^{a}	23.17±3.69^{a}
Vit.A	46.93±3.52^{b}	96.53±14.16^{a}	20.65 ±0.83^{b}	7.02 ±0.83^{b}	19.31±2.83^{a}
MTX+Vit.A	72.65±11.38^{b}	103.28±5.79^{a}	27.12±3.31^{b}	27.03±10.05^{ab}	20.64±1.16^{a}

» Means carrying similar small letters indicate a non-significant difference P≥0.05

» Means carrying different small letters indicate a significant difference P≤0.05

Thyroid Hormones Levels

Thyroid hormones levels of the four experimental groups included in this study were indicated in Table (2). The results showed no significant P≥0.05 differences in serum T_3 level between the three groups (MTX treated group, Vit. A treated group and MTX+Vit.A treated group) when compared with the control group. A significant P≤0.05 decreases was found in the MTX+Vit.A treated group in comparison with the MTX treated group. The results showed that there was a significant P≤0.05 increases in serum T_4 level in the MTX treated group when compared with the control group; while there were no significant P≥0.05 differences between each of the Vit.A treated group and the MTX+Vit.A treated group when compared with the control group. A significant P<0.05 decreases was found in the MTX+Vit.A treated group in comparison with the MTX treated group. The liver injury induced by MTX was supposed to be the reason behind the significant increase of T_4 level. This probably reflect the reduction in the D1, D3 groups of deiodinase enzyme (which were found in the liver and kidney) that were responsible for the conversion of $T_4 \rightarrow T_3$ beside the reduction of hepatocellular thyroid-binding globulin as suggested by other studies [34].The significant changes in T_4 level in the different groups were not the case with the T_3 level, this can explained by the suppression of conversion of $T_3 \rightarrow T_2$ along the conversion of $T_4 \rightarrow T_3$ in the liver [35]. These

results were suggested that the oxidative stress and lipid peroxidation of MTX was the reason behind the altered T_4 level and this effect was suppressed by antioxidant effect of Vit. A.

Table (2): Effect of MTX (20 mg/Kg), Vit. A (5000 IU) and MTX+Vit. A on serum thyroid hormones levels in male rabbits.

Group	Thyroid Hormones Levels (mean± SE)	
	T_3 (nmol/L)	T_4 (nmol/L)
Control	2.43 ± 0.17^{ab}	23.25 ± 1.43^{b}
MTX	3.01 ± 0.47^{a}	44.24 ± 7.23^{a}
Vit.A	1.84 ± 0.23^{b}	22.24 ± 3.72^{b}
MTX+Vit.A	2.10 ± 0.24^{b}	25.35 ± 3.41^{b}

» Means carrying similar small letters indicate a non-significant difference P≥0.05
» Means carrying different small letters indicate a significant difference P≤0.05

Liver Enzymes Activity

Results of liver enzymes activity of the four experimental groups included in this study were shown in Table (3). The statistical analysis of the results revealed that the level of serum GPT was significantly P≤0.05 increased in the MTX treated group with 20 mg/kg and in the MTX+Vit.A treated group when compared with the control group. This result was in agreement with that of other studies which found that the treatment with MTX caused a significant elevation in GPT level in mice [26] and rats [36]. The GPT was a cytosolic enzyme of the hepatocyte and an increase in its level in serum reflects a leakage in plasma membrane permeability, which in turn, was associated with cell death, and considered being one of the indicators of liver necrosis [2]. Also, the elevation of enzyme level could be attributed to the damage structural integrity of the liver, possible by oxidative stress and lipid peroxidation; the lipid peroxidation causes disruption of the membrane bilayer and cell integrity and eventually necrosis that leads to leakage of this enzyme into the blood [37]. In agreement with a previous study [38], the present study showed that there was a non-significant P≥0.05 increased in serum GPT level in the Vit.A treated group when compared with the control group. Also, the results showed a non-significant P≥0.05 decreases in GPT level in MTX+Vit.A treated group in comparison with MTX treated group. This could be due to the dose of Vit.A which may be did not suitable for significant preventing hepatotoxic effect of MTX and the reduction in GPT level may belong to antioxidative properties of Vit.A which act to reduced lipid peroxidation in cell membrane of hepatocyte.

The results showed that there was a non-significant P≥0.05 increases in serum GOT level of the MTX treated group when compared with the control group. This was agreement with [39], while other study [37] revealed that the treatment with MTX caused a significant elevation in GOT level; these differences in results may be due to the dose and duration which used in the experiments. The level of serum GOT did not decreasing significantly P≥0.05 in the Vit. A treated group and in the MTX+Vit.A treated group when compared with control group. These agreement with [38], while it disagreement with [5]. These differences may be due to the high dose of Vit.A (15000 IU/Kg) which used in that study. A significant P≤ 0.05 decreases was found in serum GOT level in the MTX+Vit.A treated group when compared with MTX treated group. While in a previous study [40], no significant change was found. This difference in the results may be due to the low used doses of Vit.A which was not suitable to reduced MTX toxicity. It has been reported that Vit. A improvement the liver enzyme GOT; also, it contributes to membrane stabilization [41]. So the protective action of Vit. A related to cell membrane stabilizing effect that reduced the leakage of GOT enzyme to the blood stream.

Table (3): Effect of MTX (20 mg/Kg), Vit. A (5000 IU) and MTX+Vit. A on serum activity of GPT and GOT in male rabbits.

Group	Serum Enzymes Activity (means± SE)	
	GPT (U/L)	GOT (U/L)
Control	23.00 ± 3.70^{b}	15.34 2.27^{ab}
MTX	52.06 ± 6.82^{a}	19.28 ± 2.65^{a}
Vit.A	27.60 ± 3.19^{b}	11.90 ± 2.03^{b}
MTX+Vit.A	45.62 ± 4.36^{a}	13.00 ± 1.09^{b}

» Means carrying similar small letters indicate a non-significant difference P≥0.05
» Means carrying different small letters indicate a significant difference P≤0.05

Histological Study

The histological examination of the liver from the control group, Figure (1) showed normal shape of liver parenchyma tissue, which consist from the hepatic lobule with central vein and intact hepatocytes arranged as trabeculi or cords around central vein as well as having sinusoids on form cavities located between hepatocytes.

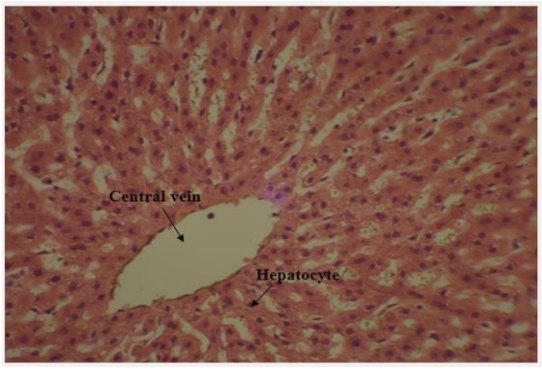

Fig. (1): Cross section in control liver of male rabbit showing normal structure appearance with presence of central vein and sheets of hepatocytes. (H & E, 40 X)

Histological evaluation of liver tissues from the administrated-MTX- rabbits revealed that there were major histological changes such as inflammatory cell infiltrations, vascular congestion and sinusoidal dilatation. In addition, necrosis of hepatocytes was evident which represented by cloudy swelling cells, Figures (2,3). The emergence of inflammatory cells in liver caused by damage of hepatocyte which may result from immunological reasons; a previous study [42] stated that the oxidative stress which results from accumulation of free radicals cause damage to hepatocyte and lipid peroxidation in cell membrane and that lead to immunological and inflammatory respond. The granular degeneration and vascular congestion which were observed in the liver tissue are agreement with [43] who noted that the treatment of rats with 20 mg/kg of MTX causes histological liver changes such as focal inflammatory cell, granular degeneration and vascular congestion. These changes may be due to the poor drainage bloody result by blockage of venous liver causing disruption of the flow of blood through hepatocyte. The widespread necros was which is noticed in the liver tissue is in agreement with [37] who found that the treatment of rabbits with 0.25 mg/kg/day of MTX in a period for 8 weeks caused necrosis in liver section, also a study by [18] referred to that the treatment of rats with MTX causes necrosis and that belongs to accumulation of polyglutamate forms of the drug in hepatocyte which decreases hepatocellular folic acid levels and leads to hepatocyte necrosis. Also, the lipid peroxidation which is caused by MTX treatment leads to disruption of the memberane bilayer and cell integrity and eventually hepatic necrosis [36].

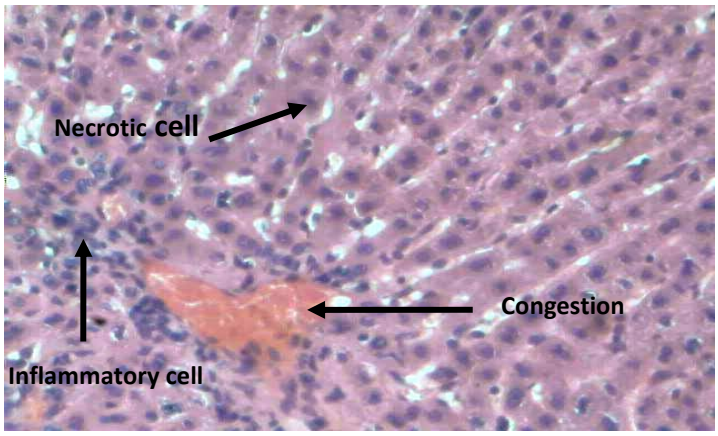

Fig. (2): Cross section in liver of male rabbit treated with MTX (20 mg/kg) showing degeneration and necrosis with heavy inflammatory cell infiltrate with congestion of blood vessel. (H & E, 40 X)

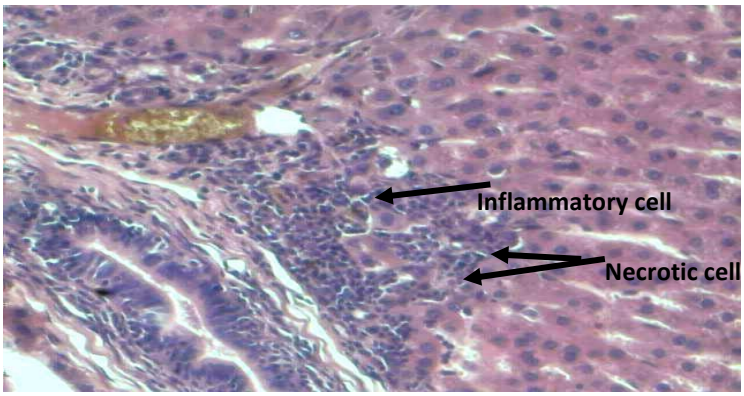

Fig. (3): Cross section in liver of male rabbit treated with MTX (20 mg/kg) showing widespread necrosis and degeneration with heavy inflammatory cell infiltrate mainly in portal area. (H & E, 100X)

Rabbits treated with Vit. A, Figure (4), showed a typical structure of liver tissue. The liver lobule was hexagonal in shape; at the center of the lobule, there was a central vein; the hepatocytes were organized into cords one cell thick, separated by hepatic sinusoids. These findings refer to the treatment with Vit. A don't cause damage to liver.

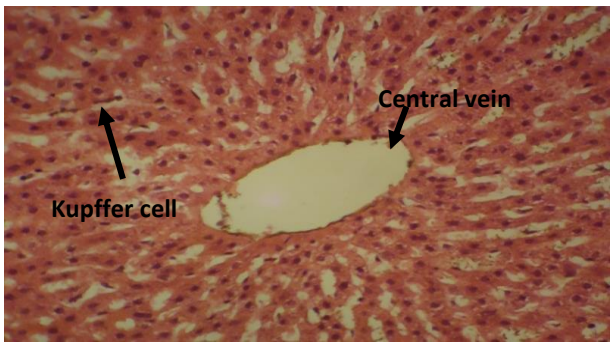

Fig. (4): Cross section in liver of male rabbit treated with Vit.A (5000 IU) showing normal appearance of hepatocyte with central vein. (H & E, 40X)

Regarding the MTX+Vit. A group, the histological changes were slight than those in the MTX treated group. In this group, granular degeneration of hepatocytes (swelling of the cells with accumulations of substances in the cytoplasm) and sinusoidal dilatation were observed at low degree in comparison with MTX treated group. Also, loss of architecture was found, Figures (5,6). These findings were similar to that reported by [44] who noted that the treatment of rats with 12 mg/kg of MTX in alternative days for one week caused histological change and the treatment of MTX+Vit.A caused reduction in tissue damage. Also, [40] noted that the co administration of Vit.A along with MTX reduce the histopathological effect of MTX. This appears wise to speculate that Vit. A posse's hepatoprotection against MTX- induced hepatotoxicity. Also, Vit. A contributes to membrane stabilization [41], so the protective action of Vit.A may also be related to this liver cell membrane-stabilizing. The antioxidant properties of Vit. A are probably the contributing factor for this hepatoprotection by reduced the oxidative stress caused by MTX and reduce tissue damage.

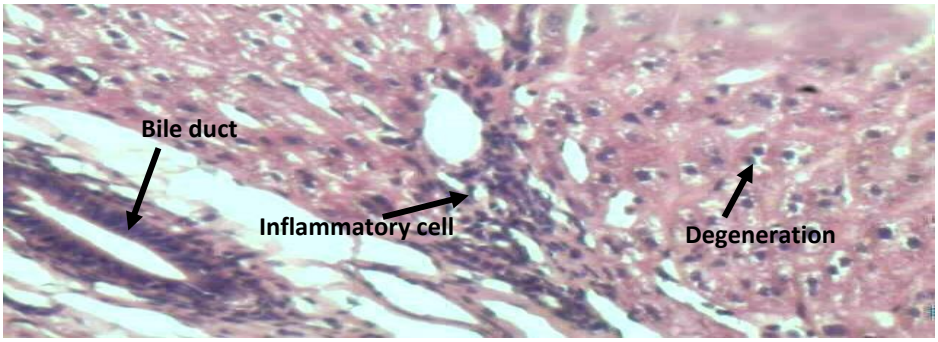

Fig. (5): Cross section in liver of male rabbit treated with MTX+Vit.A showing mild degeneration change, loss of architecture and mild inflammatory cell infiltrate near the portal area. (H & E, 100 X)

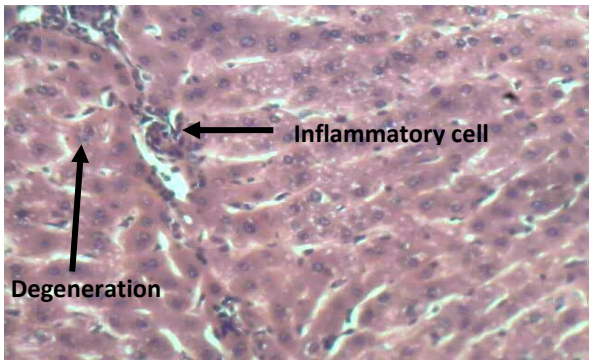

Fig. (6): Cross section in liver of male rabbit treated with MTX+Vit. A showing mild degeneration change of hepatocyte with still mild inflammatory cell infiltrate. (H & E, 100 X)

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