

HEPATOTOXIC IMPACT OF ERYTHROMYCIN SUCCINATE AFTER ORALLY REPEATED EXPOSURE IN MALE ALBINO SWISS MICE (*Mus musculus*)

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ABSTRACT

The present study was aimed to determine the effects of erythromycin on the liver of albino Swiss mice. The study sample included 12 male mice divided into 3 groups. Mice of groups 1 and 2 were orally administrated with a daily dose 50 and 100 mg/kg bw erythromycin, respectively, while group 3 served as control and administrated with 0.1 ml distilled water. The treatment continued for 14 days . The results indicated a significant increase ($p<0.05$) in AST, ALT, and ALP activities of group treated with 100 mg/kg of erythromycin, compared with group treated with erythromycin 50 mg/kg . The study also showed several histological alterations in the liver tissues of animals treated with 50 mg/kg of the drug, including necrosis, hydropic degeneration, central vein congestion, and blood edema. Treatment with 100 mg/kg caused cell infiltration, cell vacuolation, as well as loss of radial arrangement of hepatocytes forming the liver tissue. It can be concluded that sub acute exposure to erythromycin exerts damaging effects on liver cells of treated mice.

Key words: liver, enzymes, degeneration, congestion, pyknosis

التميمي وظاهر

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التأثير السمي للارثرومايسين على الكبد بعد التعرض المتكرر فمويًا في الفئران البيض السويسرية (*Mus musculus*)

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المستخلص

هدفت الدراسة الى تحديد تأثير عقار الارثرومايسين Erythromycin في كبد الفئران البيض السويسرية. شملت عينة الدراسة 12 من الذكور جرى تقسيمها الى ثلاث مجاميع . جرعت المجموعة الاولى والثانية من الحيوانات فمويًا بجرعة (100,50) ملغم/كغم على التوالي مرة واحدة في اليوم. أما المجموعة الثالثة فقد جرعت 0.1 مل من الماء المقطر بوصفها مجموعة سيطرة. استمرت عملية التجريب 14 يوم متتالي . تم دراسة بعض المعايير الكيموحيوية، إذ تضمنت الدراسة قياس نشاط انزيمات AST و ALT و ALP لتحديد وظيفة الكبد. أشارت النتائج الى حصول ارتفاع معنوي ($P < 0.05$) في تركيز الانزيمات في المجاميع المعاملة بجرعة 50 ملغم/كغم بعد مدة التجريب، وقد سجل الجرعة 100 ملغم /كغم ارتفاعا معنويا عاليا في نشاط الانزيمات ايضا، مقارنة مع تركيز الانزيمات في مجموعة السيطرة. كما شملت الدراسة تشخيص التغيرات في نسجية المعدة بعد اجراء التقطيع النسجي، ومقارنة النتائج مع مجموعة حيوانات السيطرة. أظهرت النتائج حصول عدة تغيرات نسجية في كبد حيوانات المعاملة شملت تنخر وتنكس مائي، وحدوث احتقان في الوريد المركزي وظهور حالة الوذمة الدموية بعد المعاملة بالعقار جرعة 50 ملغم/كغم. أما المجموعة الثانية وبعد معاملتها بجرعة 100 ملغم /كغم منها، فقد اظهرت النتائج حدوث الارتشاح الخلوي، تفجى الخلايا، فضلا عن أنعدام الترتيب الشعاعي للخلايا الكبدية المكونه للنسيج من، مقارنة مع مجموعة السيطرة. يمكن الاستنتاج أن للارثرومايسين تأثيرا سلبيا في كبد الفئران المجرعة به.

الكلمات المفتاحية: الكبد، انزيمات، تنكس، احتقان، تغلظ النوى

INTRODUCTION

Erythromycin is an antibiotic that belongs to the macrolide group, with an inhibitory effect on bacterial RNA-dependent protein synthesis through binding to the ribosomal large subunit (14). Erythromycin is the most widely used antibiotic, exceeding even penicillin, against Gram negative and positive bacteria. Experiments on mice showed that erythromycin is a macrolide had activities against a wide spectrum of microbes and bacteria, despite that the active concentration which is necessary to inhibit or kill these microorganisms is unknown. The drug also has a suppressive effect against the transition of streptococcal and staphylococcal bacteria (3). Erythromycin was described to have a prolonged duration of high levels in the targeted tissues as compared to other drugs, leading to considering it as one of the most effective *in vivo* drugs against sensitive microbes. Therefore, erythromycin is one of the most commonly used medicines for the treatment of many diseases (10). Erythromycin has several side effects, especially to the stomach and the intestine, as exerted by vomiting and abdominal pain. The drug acts on the inhibition of bacterial growth through interference with bacterial protein synthesis. It is used to treat tonsillitis, pneumonia, bronchitis, gonorrhoea, pelvic inflammatory disease and urinary tract infection. Erythromycin causes severe damages to the stomach, as indicated by the occurrence of symptoms such as diarrhea (11). As well as, this medicine is secreted in mother's milk and easily passes through the placenta, while it is also found in low concentrations in the plasma of the fetus (15). This study was designed to detect the effects of erythromycin on a number of physiological parameters, which included the concentrations of the liver enzymes AST, ALT, and ALP, as well as the histological alterations in the liver of albino Swiss mice. The aim of this study is to demonstrate the toxic effect of erythromycin in the liver of male albino mice.

MATERIALS AND METHODS

Experimental design

Twelve male albino Swiss mice (*Mus musculus*) were used in the present study with an age range of 8-10 weeks and weight range

of 25-35 gm. The animals were obtained from the animal house of the Biotechnology Center, Al-Nahrain University. Erythromycin purchased from the State Company for Drugs Industry and Medical Appliances, Samarra (S.D.I Iraq) in tablets of 500 mg/kg concentration. Each mice in group 1 and 2 administered orally by gastric gavage 0.1 ml/log of body weight of the erythromycin solution which contained 0.5 mg and 1 mg representing the doses 50 and 100 mg/kg BW respectively for 14 days, while the third group considered as control one and dosed 0.1 ml/log BW distilled water.

Blood samples and analysis of enzymes

At the end of treatment, the blood was collected through cardiac puncture, poured into Eppendorf tubes, and centrifuged for 15 minutes. Serum was collected in tubes and stored until time of measuring the activity of AST, ALT, and ALP enzymes by the colorimetric method and using the special kit for this test (17).

Tissue preparation

The animals were dissected and the liver was carefully excised and washed by physiological saline and preserved in 10% formalin, the samples were dehydrated using serial dilutions of ethanol. The samples were then cleared with xylene and embedded in paraffin wax. Sections were mounted on glass slides, stained with Haematoxylin-Eosin, and examined and photographed using a microscope provided with a camera (8).

Statistical analysis

Statistical analysis of the data was performed by using ANOVA we applied factorial within completely randomized design with thirty replications. Least significant differences (LSD) were used to compare between means at 0.05 level.

RESULTS AND DISCUSSION

The current study demonstrated the extent of the effects of treatment with erythromycin on serum activity of AST, ALT, and ALP enzymes in the treated mice showed significant increases ($p < 0.05$) in the activities of these enzymes in the sera of mice groups treated for 14 days with 50 mg/kg and with 100 mg/kg, in comparison with those activities in the control group (Table 1). These results can be attributed to the effects of the drug on

liver function, as indicated by previously reported results (16) which showed that administration of 60 mg/kg erythromycin for 10 days and for 40 days increased the levels of AST and ALT, indicating the severe toxicity of this drug to the liver. Measurement of the levels of these enzymes is considered as an indication of the severity of the damage to the liver, as evidenced, for example, by the presence of higher levels of the AST cytoplasmic isoenzyme than those of the mitochondrial isoenzyme. Levels of this enzyme are increased during acute liver diseases (12) and during severe damages in the cells of the kidneys, pancreas, and red blood cells, while levels of ALT and AST increase in association with liver necrosis (9).

Table 1. Effects of erythromycin on the activities of AST, ALT, and ALP in the sera of albino Swiss mice (mean \pm standard error).

Group	mean \pm standard error		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	173.66	60.73 \pm	52.80 \pm 1.53
D.W	\pm 2.50	1.30	c
G1	202.15 \pm	77.34 \pm	66.34 \pm 1.10
50mg/kgBW erythromycin	3.06	2.13	b
G2	215.30 \pm	84.44 \pm	77.40 \pm 1.76
100mg/kgBW erythromycin	6.45c	2.55	a
LSD	3.841	4.919	4.961

Means with different letters within the same column are significantly different at $p < 0.05$.

Histopathological section of (Figure 1) which showed abnormal detect histological examination of the liver tissue of mice treated with 50 mg/kg erythromycin demonstrated several damaging impacts on the hepatocytes, such as degeneration and necrosis (Figure 2) as compared to the control group (Figure 1). These results are in agreement with previous report (20) mild necrosis in the hepatocytes of mice liver, which is due to toxic effects of the drug on these cells. Such a toxic effect is possibly due to the ability of erythromycin to bind to the sulfhydryl (SH) group in the proteins of the cell membrane and the other cellular proteins, leading to increased membrane permeability and inhibition of the ATPase-dependent transport. The impacts of

the drug might also be attributed to its ability to form oxygen free radicals that cause damages to lipids, proteins, and nuclear acids and, eventually, cell necrosis (18). The liver tissue sections of mice treated with 50 mg/kg also showed the occurrence of central vein congestion (Figure 3). The same treatment also caused vacuolation and swelling of hepatocytes as well as hemorrhage and blood congestion (Figure 4). The reason behind the appearance of vacuoles within the hepatocytes is the presence of hydropic degeneration, since toxins are known to cause inhibition of glycolysis and decreased levels of some essential substances (4,2). Toxins also cause inhibition of oxidative phosphorylation, leading to decreased ATP production and failure in the function of the sodium ion pump, resulting in flow of sodium and water into the cells and the extrusion of potassium from the cells. Such events lead to the swelling of the mitochondria and the endoplasmic reticulum, followed by isolation of ribosomes and the appearance of the cytoplasm filled with large and irregularly-shaped vacuoles (19). The occurrence of histopathological alterations in the liver tissue was more evident after treatment with 100 mg/kg erythromycin. Tissue sections of the liver showed other histopathological changes such as infiltration of inflammatory cells as a result of dilation and rupture of blood sinusoids in certain areas of the liver tissue, leading to unclear appearance of the sinusoids (Figure 5). Lymphocytes infiltration is caused by the acute inflammation resulting from the treatment with the drug (6,13). This effect occurs through the increased blood vessel permeability upon contraction of their endothelial cells in response to certain chemicals such as histamin or the loss of some desmosomes connecting these cells (1). Such events allow the blood cells to pass out of the vessel, especially neutrophils and monocytes that phagocytose the debris of the necrotic cells (7). In addition, (Figure 6) clarifies the vacuolation of hepatocytes along with increased necrosis and dilation of sinusoids. It was also possible to observe the change in the radial arrangement of the hepatocytes forming the liver tissue (Figure 7). The results of the present study agreed with those of a previous

study (5) that showed swelling of hepatocytes which has a harmful effect on the size of the blood sinusoids and causes loss of the radial arrangement of the liver cells. These effects are due to the ability of erythromycin to stimulate the synthesis of certain structural proteins. It was also possible to observe the precipitation of lipid droplets in the cytoplasm of the hepatocytes and the occurrence of fatty degeneration (Figure 8), which is caused by the clear degenerative and necrotic damages arising from the toxic effects of erythromycin on liver tissue. The study concluded that erythromycin has toxic effects on mice liver.

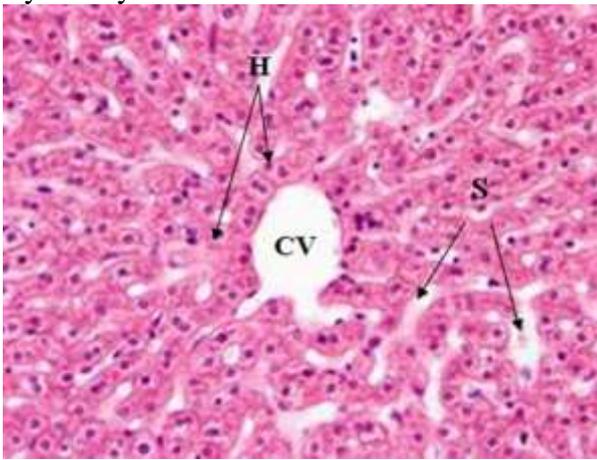


Figure 1. Section of mouse liver in the control group showing: Central vein (CV), Sinusoid (S), Hepatocytes (H) . 400X (H&E stain).

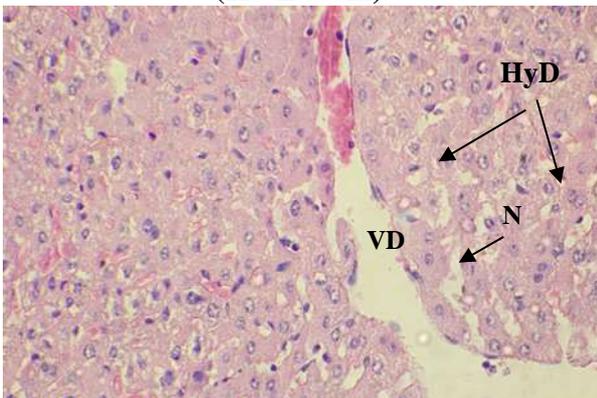


Figure 2. Section of liver tissue for Erythromycin -treated animals at a concentration of 50 mg / kg showing: Necrosis in hepatic cell (N), vasodilation (VD), Hydropic degeneration (HyD). 400X (H&E stain).

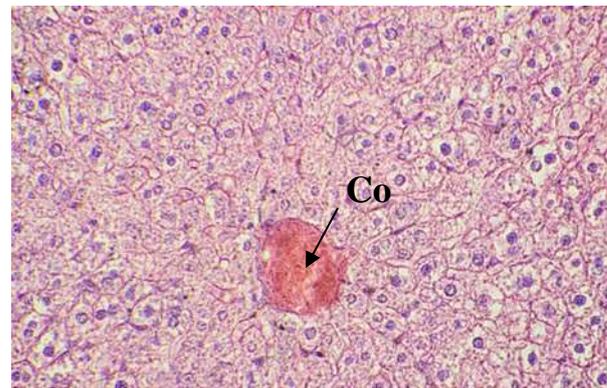


Figure 3 . Section of liver tissue for Erythromycin -treated animals at a concentration of 50 mg / kg showing : Congestion (Co) . 400X (H&E stain).

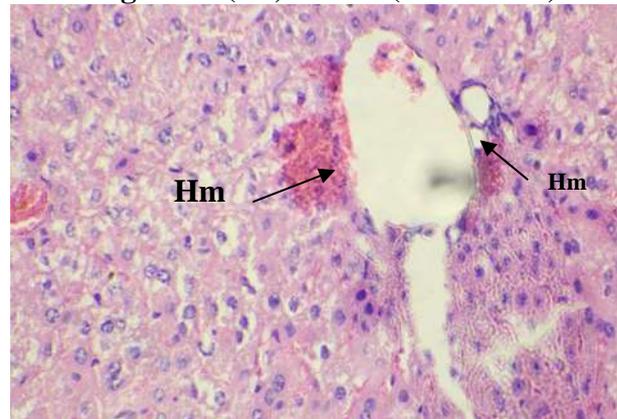


Figure 4. Section of liver tissue for Erythromycin -treated animals at a concentration of 50 mg / kg showing: Hemorrhagic (Hm). 400X (H&E stain).

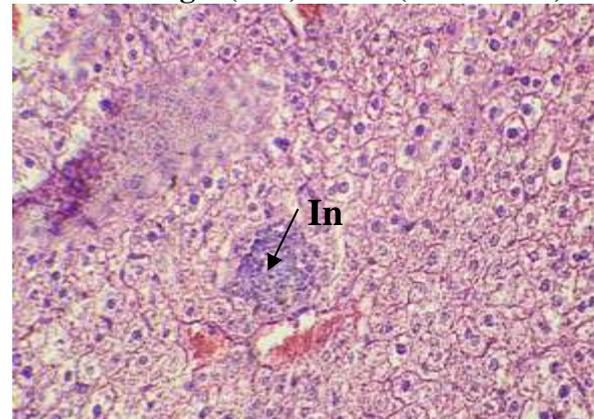


Figure 5. Section of liver tissue for Erythromycin -treated animals at a concentration of 1.0 mg / kg showing : Infiltration of lymphocytes (In) .400X (H&E stain).

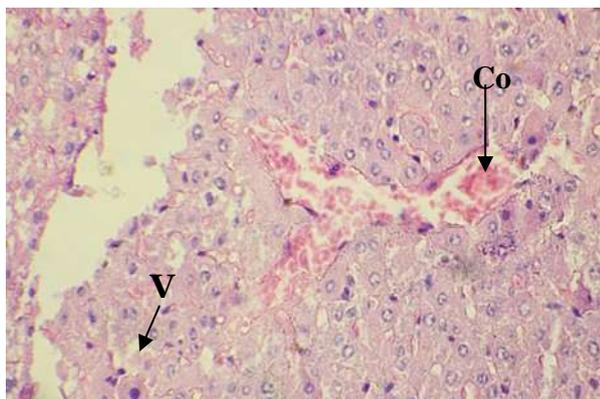


Figure 6. Section of liver tissue for Erythromycin -treated animals at a concentration of 1.0 mg / kg showing: Vacuolation (V), Congestion (Co). 400X(H&E stain).

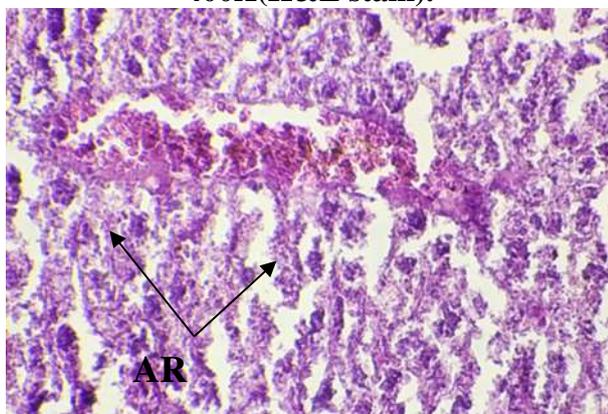


Figure 7. Section of liver tissue for Erythromycin -treated animals at a concentration of 1.0 mg / kg showing : Absent of Radial arrangement (AR). 400X(H&E stain).

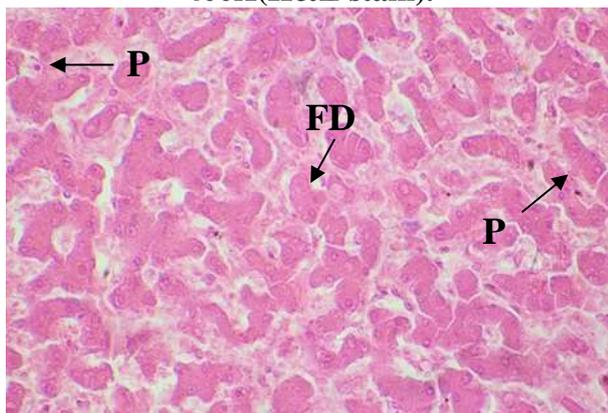


Figure 8. Section of liver tissue for Erythromycin -treated animals at a concentration of 1.0 mg / kg showing :Fatty Degeneration(FD), Pyknosis (P). 400X (H&E stain).

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