Article
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol. 33; No. 2 | 2025

Study Effects of Erythromycin and the Role of Vitamin C on Enzymes Glutathione and Catalase in Male Albino Mice

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دراسة تأثير عقار الأرثرو مايسين ودور فيتامين سي على انزيمي الكلاتاثيون والكتاليز في ذكور الفئران البيضاء

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Accepted: 27/5/2025 **Published:** 30/6/2025

ABSTRACT

Background:
This study examined toxic effect of a member of the macrolide antibiotic family, erythromycin, on the levels of certain enzymes, namely glutathione (GSH) and catalase (CAT), and investigated the protective role of Vitamin C in mitigating its toxicity in male albino mice (*Mus musculus*).

Materials and Methods:
A total of 25 male albino mice were used in the experiment and divided into five groups. The first group served as the control. The second and third groups (treatment groups) were administered erythromycin at doses of 15 mg/kg and 30 mg/kg, respectively. The fourth and fifth groups (protective groups) were given Vitamin C at a dose of 100 mg/kg along with erythromycin. The experiment lasted for 14 days.

Results:
A significant decrease in GSH and CAT levels was observed in the treatment groups: (31.2 ± 0.3 µmol/L, 402.9 ± 15.08 U/L) for the second group and (32.1 ± 0.1 µmol/L, 374.9 ± 17.9 U/L) for the third group, which received erythromycin at doses of 15 mg/kg and 30 mg/kg, respectively, compared to the control group at a significance level of ($P \leq 0.05$). However, the fourth and fifth groups, which were administered vitamin C along with erythromycin, exhibited an increase in enzyme levels, with no significant differences compared to the control group at the same significance level.

Conclusion:
Erythromycin exerts a dose-dependent toxic effect on the levels of glutathione and catalase enzymes in the serum of albino mice. Additionally, vitamin C has a protective role in reducing the drug's toxicity when administered together.

Key words:
Erythromycin; Vitamin C; CAT; GSH; Liver enzymes.

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INTRODUCTION

When drugs are used in combination with certain vitamins in some therapeutic strategies, the purpose is either to enhance effectiveness or to reduce side effects. Erythromycin is one of the widely used drugs today, as it is used to treat infections such as middle ear infections, respiratory tract infections, and skin infections [1]. This drug is a broad-spectrum antibiotic that has been used for over six decades against various types of bacteria, such as staphylococci and Gram-positive bacteria [2]. The drug was first discovered in the 1960s, and due to its antimicrobial properties, it was used as an alternative to penicillin for treating infections. However, the emergence of side effects initially raised concerns about drug hypersensitivity. To overcome this issue, the chemical composition of the drug was modified to make it more suitable for human use [3]. In addition to structural modifications, therapeutic strategies were developed to eliminate or reduce the side effects associated with erythromycin use. One such approach includes taking dietary supplements alongside the treatment [4]. With the rise of antibiotic resistance and drug hypersensitivity issues, attention has shifted toward alternative medicine and the use of natural products as substitutes or complementary treatments to chemical compounds to minimize their side effects [5].

Among widely used dietary supplements, vitamin C is a well-known antioxidant that plays a crucial protective role against oxidative agents generated within the body due to biological activities, particularly peroxides and oxygen and nitrogen radicals [6]. The vitamin acts as a cofactor in neurotransmission, protects the skin from oxidation by preserving the melanin layer, and enhances iron absorption in the intestines. vitamin C is considered one of the most essential micronutrients due to its electron-donating property, which helps protect against free radicals. Additionally, it serves as a protective agent against drug-induced toxicity in the liver and plays a role in regulating gene expression and hormone synthesis [7].

Glutathione (GSH) is an enzyme synthesized in the liver and nerve cells, functioning as a potent antioxidant against free radicals (ROS) and (NOS). It is composed of three amino acids: glutamate, cysteine, and glycine. GSH plays a crucial role in maintaining the integrity of the cell membrane, red blood cells, and structural proteins within cells. Additionally, it is involved in gene regulation and protein expression [8]. Recently, GSH has been explored in combination with certain nanocompounds for targeting tumors in various types of cancer, such as breast cancer. This approach has shown effectiveness in inhibiting cancerous cells [9].

Catalase is an enzyme found in nearly all cells, known for its high efficiency in breaking down peroxides and preventing the harmful effects of their accumulation. It decomposes hydrogen peroxide (H2O2) into water and oxygen, thereby protecting cell membranes from oxidation and subsequent damage. Additionally, catalase helps reduce complications of diabetes by minimizing its impact on the kidneys and cardiovascular diseases, working in conjunction with peroxidase and glutathione enzymes [10].

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MATERIALS AND METHODS

- **Animals Used in the Experiment**

We used 25 male albino mice aged between 11 to 12 weeks and weighing between 218 to 268 grams. They were fed a diet consisting of various grains along with milk powder.

- **Experiment design**

The animals were randomly divided into five groups as follows:

First group (control group): Administered a saline solution at a concentration of 0.5 ml.

Second group (treatment group): Given erythromycin at a concentration of 15 mg/kg only.

Third group (treatment group): Given erythromycin at a concentration of 30 mg/kg.

Fourth group (protective group): Administered erythromycin at a concentration of 15 mg/kg along with vitamin C at 100 mg/kg.

Fifth group (protective group): Given erythromycin at a concentration of 30 mg/kg along with vitamin C at 100 mg/kg.

The drug dosage was determined based on reference [11], while the vitamin dosage was adopted from reference [12]. The experiment lasted for two weeks.

- **Blood drawing and animal sacrifice**

After anesthetizing the animals, they were sacrificed, and a 5 ml blood sample was collected via cardiac puncture. The blood was transferred into specialized tubes and centrifuged at a speed of 4000 rpm to separate the serum, which was then stored at a temperature of 4°C.

- **Determination of Catalase Level in Blood Serum**

The reagents were prepared in advance following the method described in reference [13]. The reaction involves the interaction between ammonium molybdate and hydrogen peroxide to produce a yellow color with absorbance at a wavelength of 374 nm. A correction factor is added to account for the interference from proteins in the serum. The reaction proceeds as shown in the following equation:

$$2H_2O_2 + \text{Catalase} \longrightarrow 2H_2O + O_2$$

- **Determination of Glutathione (GSH) Level in Blood Serum**

The method outlined in reference [14] was used to estimate the GSH concentration in the serum. The principle of the method relies on the reducing activity of the compound 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which reduces the sulfhydryl group in glutathione, resulting in the formation of a yellow compound. The intensity of the color is directly proportional to the amount of GSH in the serum, and it can be measured at a wavelength of 414 nm.

- **Statistical Analysis**

The program SPSS V. 25 was used to find the variance and LSD (Least Significant Difference) to determine the significant differences between the studied means [15].

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RESULTS AND DISCUSSION

The results of the study, shown in Table (1), indicated a significant decrease at the 5% probability level in the levels of the catalase (CAT) and glutathione (GSH) enzymes in the serum of the mice in the treatment groups that received the drug alone at concentrations of 15 mg/kg and 30 mg/kg, compared to the first control group. This finding is consistent with [16], which indicated a decrease in enzyme levels with oxidative stress, leading to changes in the activities of the catalase and glutathione enzymes in the liver. This can be explained by the oxidative stress caused by the drug, which leads to tissue damage in the heart with the accumulation of ROS, and thus increases toxicity within the tissues [17]. Furthermore, erythromycin is widely used as an antibiotic for various human diseases, such as middle ear infections and respiratory tract infections. There is increasing evidence pointing to the toxicity of macrolide derivatives on the liver and other vital tissues [18].

Several factors contribute to the exacerbation of liver disorders, including the excessive use of medications, which affects the liver's antioxidant enzymes, leading to a decrease in their levels. This, in turn, increases oxidative stress and the formation of free radicals, causing inflammation and tissue damage [19]. Antibiotics are often used in high quantities, and with the increased therapeutic resistance of Staphylococcus aureus, the dosage has to be raised with each administration, which increases the likelihood of side effects and drug hypersensitivity, particularly to macrolide compounds. Consequently, this affects the biochemical processes of antioxidant enzymes like peroxidase and catalase, which are a common link between chronic liver damage and liver fibrosis [20].

There are several defense mechanisms in the body to eliminate free radicals and harmful compounds, including non-enzymatic antioxidants such as vitamins C and E. Another type of antioxidants is enzymatic antioxidants like catalase, glutathione, peroxidase, and others, which help rid the body of harmful molecules and compounds, thereby maintaining natural balance and protecting against oxidative stress [21].

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Table (1) Toxicity of Erythromycin and the Protective Role of Vitamin C on Catalase (CAT) and Glutathione (GSH) Enzyme Levels in Male White Mice.

Groups	GSH /mg/mmol	CAT /mg/mmol
Control Group (G1)	3.9 22 ± 0.3 21	459.1 72 ± 14.2 21
Therapeutic Group (G2) - Erythromycin (15 mg/kg)	2.8 31 ± 0.3 21	402.9 32 ±15.08 1
Therapeutic Group (G3) - Erythromycin (30 mg/kg)	2.1 32 ± 0.1 21	374.9 02 ±17.9 21
Protective Group (G4)- Erythromycin (15 mg/kg) + Vitamin C (100 mg/kg)	3.532 ± 0.7 21	422.9 32 ±24.2 21
Protective Group (G5)- Erythromycin (30 mg/kg) + Vitamin C (100 mg/kg)	3.1 32 ± 0.3 21	408.88 2 ±33.8 21
P. value	0.0002	0.002
LSD	0.3746	33.9315

As for the fourth and fifth groups (protective groups), no significant differences were observed in the levels of glutathione and catalase, especially in the fourth group. The fifth group did not show any notable significant difference at the $P \leq 0.05$ level when compared to the first group (control), as can be seen in Table (1).

Modern medicine has taken a new approach in establishing therapeutic protocols based on combining chemical drugs and dietary supplements to eliminate associated side effects. Among these practices is the use of vitamin C with erythromycin in mice, as seen in study [22], which led to a reduction in the toxic effects of the drug on liver cells. Especially as ascorbic acid (Vitamin C) is a non-enzymatic antioxidant. The results of this study align with what has been found, as Vitamin C helps protect cell membranes and strengthens the immune system to combat inflammation, particularly in the mucous membranes of the respiratory system, reducing bacterial infections and communicable diseases.

Vitamin C works to prevent the depletion of the GSH enzyme in the liver by mediating the removal of harmful molecules produced from reactions within the body. This helps preserve and maintain the internal environment of cells and lipid membranes [23]. Vitamin C plays an important role in converting certain amino acids into neurotransmitters, as well as protecting cellular membranes from the harmful effects of high blood sugar levels. Additionally, it activates vitamin B and converts cholesterol in the liver into fatty acids [24]. Vitamin C also regulates genetic processes and is found in all body cells at higher concentrations than in plasma. This vitamin is relatively safe when taken in appropriate doses [25].

In a recent study [26] conducted outside the body on a red blood cell model to prevent the hemolysis of red blood cell membranes due to oxidative factors and to extend the storage period of blood from donors for transfusions, it was found that treatment with Vitamin C at a

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concentration of (100) micromoles, along with other compounds, prevents hemolysis and reduces the effects of hydrogen peroxide, thus increasing the storage period of the blood [26].

CONCLUSIONS

Erythromycin has a toxic effect, depending on the dose used, on the levels of glutathione and catalase enzymes in the blood serum, which leads to the appearance of side effects on the liver, blood vessels, and heart of white rats. Additionally, vitamin C plays a protective role in reducing the toxicity of the drug when used alone, as the vitamin works to reduce oxidative stress, thereby protecting both CAT and GSH from declining levels, considering them as enzymatic antioxidants.

Conflict of interests.

There are non-conflicts of interest.

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الخلاصة

المقدمة:

تطرقت الدراسة الى اختبار التاثير السمي لاحد افراد عائلة الادوية الميكروليدية (الارثرومايسين) على مستويات بعض الانزيمات الكلوتاثيون (GSH) والكاتليز (CAT) ودور فيتامين C كعامل وقائي للحد من التأثير السمي في ذكور الفئران البيضاء *Mus musculus*.

طرق العمل:

استخدمت في التجربة 25 ذكر من الفئران البيضاء و قسمت الى خمس مجاميع كالاتي المجموعة الاولى تمثل السيطرة اما المجموعتين الثانية الثالثة (العلاجية) جرعت الارثرومايسين بتركيز (15 mg/kg) و (30 mg/kg) على التوالي اما المجاميع الوقائية الرابعة و الخامسة فقد جرعت كلا المجموعتين فيتامين C بتركيز (100 mg/kg) بالاضافة للعقار. استمرت التجربة لفترة 14يوم.

النتائج:

سجلت مستويات الكلاتوثيونGSH والكتاليز CAT انخفاض معنوي بلغ (21 0.3 ± 2.8 31)، (1 15.08± 32 402.9) للمجموعة الثانية و(21 0.1 ± 2.1 32) ، (21 17.9± 02 374.9) للثالثة التي جرعت العقار فقط بتراكيز (15 mg/kg) و (/kg) 30 mg على التوالي مقارنة مع السيطرة عند مستوى احتمال ($P\leq0.05$) . اما المجاميع الوقائية الرابعة و الخامسة التي اعطيت الفيتامين بتركيز (100 mg/kg) بالاضافة للارثرومايسين فقد سجلت ارتفاع في مستويات الانزيمات و لم تسجل اي فروق معنوية فيها عند المقارنة مع المجموعة الاولى السيطرة عند نفس مستوى الاحتمال .

الاستنتاجات:

أن عقار الارثرومايسين له تأثير سمي حسب الجرعة المستخدمة على مستويات انزيمات الكلاتوثايون والكاتاليز في مصل الدم للفئران البيضاء، كما ان هناك دور وقائي لفيتامين C للتقليل من الاثر السمي للعقار عند استخدامهما معا.

الكلمات المفتاحية:

الارثرومايسين ;فيتامين C ; CAT ;GSH ;انزيمات الكبد