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Research Article

POSSIBLE CARDIAC ADVERSE EFFECTS OF THERAPEUTIC DOSES OF MACROLIDE ANTIBIOTICS (AZITHROMYCIN AND CLARITHROMYCIN) IN HEALTHY JUVENILE RATS: BIOCHEMICAL ASSESSMENT

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ABSTRACT

The macrolides antibiotics inhibit bacterial protein synthesis by an effect on translocation. They include erythromycin, azithromycin, clarithromycin, and roxithromycin . Their antimicrobial spectrum is varied. The drugs are associated with QT interval prolongation and cardiac dysrhythmias. This study was designed to determine whether or not a therapeutic oral dose of either azithromycin or clarithromycin administered for 5 or 10 days, respectively have cardiac adverse effects in healthy juvenile rats by assessing serum enzymes (CK-MB, LDH, AST and ALT), as markers of cardiac function. Twenty-eight healthy juvenile rats of both sexes weighing approximately 30gm were utilized and were randomly subdivided into 4 groups, control group orally-administered distilled water (DW) every 12hrs for 5 days via gavage tube, azithromycin suspension 12 mg/ kg every 12 hrs for 5 days via gavage tube, control group orally-administered DW every 12 hrs for 10 days via gavage tube and clarithromycin suspension 7.5 mg per kg for every 12 hrs for10 days via gavage tube. After scarification of animals by cervical dislocation, blood samples were taken by intra-cardiac puncture and utilized immediately to get serum in order to assess enzymes activities {heart creatin kinase isoform (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT)}.The results of the present study demonstrated that were significant increase in serum activities of both CK-MB and LDH in group of animals treated with therapeutic oral dose of (12mg/kg) azithromycin for 5 days compared to the corresponding serum enzyme activities of control animals. While, there were no significant increase in serum activities of both AST and ALT in group of animals treated with therapeutic oral dose of (12mg/kg) azithromycin for 5 days compared to the corresponding serum enzyme activities of control group. Moreover, in groups of animals treated with therapeutic oral dose of (7.5mg/kg) clarithromycin for 10 days concerning the effect of the intended drug on serum activities of both CK-MB and LDH, there was significant increase in serum activities of both CK-MB and LDH in clarithromycin-treated rats compared to the corresponding serum enzyme activities of control animals. But, there were no significant increase in serum activities of both AST and ALT in rats treated with therapeutic oral dose of (7.5mg/kg) clarithromycin for 10 days compared to the corresponding serum enzyme activities of control animals. The results of this study provide an evidence, for the first time, to our knowledge on the effect of treatment of healthy juvenile rats with either azithromycin or clarithromycin , where both drugs have adverse effects on cardiac tissue manifested by increase in the levels of cardiac markers, (serum enzyme activities) especially CK-MB and LDH.; while, there were no change in serum activities of both AST and ALT compared to control rats. Thus, further studies are needed to confirm such finding.

Key words: macrolides, cardiac adverse effects, biochemical markers, juvenile rats.

INTRODUCTION:

Macrolide antibiotics including erythromycin, azithromycin, clarithromycin, and roxithromycin; have been widely used for the treatment of infections caused by Gram +ve micro-organism by inhibiting bacterial protein synthesis through an effect on translocation[1]. Furthermore, they are generally well tolerated when used for the treatment of acute infections[2].

Their antimicrobial spectrum is varied; where, erythromycin is effective against Gram-positive bacteria and spirochaetes but not against most Gram-negative organisms, exceptions being N. gonorrhoeae and, to a lesser extent, H. influenzae. Mycoplasma pneumoniae, Legionella sp. and some chlamydial organisms are also susceptible[3].

Azithromycin is less active against Gram-positive bacteria than erythromycin but is considerably more effective against H. influenzae and may be more active against Legionella. It has excellent action against Toxoplasma gondii 4. Clarithromycin is two times active than erythromycin against H. influenza. It is also effective against Mycobacterium avium-intercellulare and it may also be useful in leprosy and against Helicobacter pylori[3, 4]

It had been demonstrated that azithromycin, clarithromycin, and erythromycin are associated with QT interval prolongation and cardiac dysrhythmias; where, these effects occur primarily in patients with either underlying cardiac disease or when they were administered with inhibitors of cytochrome P450 3A4 enzyme system in the liver and intestine, as co-administration of macrolides antibiotics with arrhythmogenic agents, such as terfenadine or astemizole, resulting in fatal ventricular arrhythmias, due to an increase in the concentrations of the unchanged drugs in plasma[5, 6]. Additionally, some macrolide antibiotics like erythromycin and clarithromycin possess arrhythmogenic activities and provoke Q-T interval prolongation with the resultant vatntricular arrhythmia[7-9]. In isolated heart preparations from guinea pigs and dogs, erythromycin was also shown to prolong the Q-T interval and action potential duration[10, 11]. To confirm a cardiac adverse effect of macrolide antibiotics, biochemical assessment of cardiac markers utilizing serum enzyme activities are needed.

OBJECTIVE:

This study was designed to determine whether or not a therapeutic oral dose of either azithromycin or clarithromycin administered for 5 or 10 days, respectively induced cardiac adverse effects in healthy juvenile rats by assessing serum activities of enzymes (CK-MB, LDH, AST and ALT), as markers of cardiac function.

MATERIALS AND METHODS:

Twenty-eight (4-week) healthy albino rats of both sexes weighing approximately 30 g were utilized in the study. They were obtained from the Animal Laboratory House of the College of Pharmacy, University of Baghdad. The animals were fed standard diet ad libitum and were free access to tap water. They were divided into four groups as follows: group I- Six juvenile rats were received orally-administered distilled water every 12hrs by gavage tube for 5 days. This group served as control group for rats received azithromycin; Group II- Eight juvenile rats were received azithromycin suspension 12 mg/ kg every 12 hrs for 5 days via gavage tube; Group III- Six juvenile rats were received orally-administered distilled water every 12hrs by gavage tube for 10 days. This group served as control group for rats received clarithromycin;

Group IV- Eight juvenile rats were received clarithromycin suspension 7.5 mg / kg for every 12 hrs for10 days via gavage tube.

One day after the end of each treatment period, the animals were sacrificed by cervical dislocation and blood samples were withdrawn by intra-cardiac puncture and utilized immediately to get serum after centrifugation for 10 minutes at 4,000 rpm which was utilized for the assessment of cardiac enzymes activities, {(heart creatin kinase isoform (CK-MB)[12]. lactate dehydrogenase (LDH)[13], aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[14]}.

Analysis of data was performed using Microsoft Excel version 2007 two - ways to analyze student t-test. The data were expressed as mean ± standard error of the mean (SEM). P value of less than 0.05 was considered significant.

RESULTS

Table 1 and figures 1 and 2 showed that there were significant increase ($P<0.05$) in serum activities of both CK-MB and LDH in group of animals treated with therapeutic oral dose of (12mg/kg) azithromycin for 5 days compared to the corresponding serum enzyme activities of control animals.

There were no significant increase ($P>0.05$) in serum activities of both AST and ALT in group of animals treated with therapeutic oral dose of (12mg/kg) azithromycin for 5 days compared to the corresponding serum enzyme activities of control group. (Tables 2 and figures 3 and 4).

Similar data were obtained in groups of animals treated with therapeutic oral dose of (7.5mg/kg) clarithromycin for 10 days concerning the effect of the intended drug on serum activities of both CK-MB and LDH, there were significant increase ($P<0.05$) in serum activities of both CK-MB and LDH enzymes in clarithromycin-treated rats compared to the corresponding serum enzyme activities of control animals. (Table 3 and figures 5and 6).

Moreover, there were no significant increase ($P>0.05$) in serum activities of both AST and ALT in rats treated with therapeutic oral dose of (7.5mg/kg) clarithromycin for 10 days compared to the corresponding serum enzyme activities of control animals. (Tables 2 and figures 7 and 8).

DISCUSSION:

Many reports had demonstrated that the cardiac adverse effects of macrolides antibiotics like azithromycin and clarithromycin is observed by means of ECG, where prolongation in the duration of action potential of the cardiac muscle was observed[15,16] which may occur because of potassium-channel suppression properties of these drugs, where, clarithromycin, roxithromycin and erythromycin are believed to have the greatest ability to reduce repolarization through potassium channel 1.

Moreover, by utilizing animal model, The possible cardiac adverse effect of macrolide group of antibiotic drugs like azithromycin and clarithromycin could be assessed by measuring cardiac markers (serum cardiac enzymes) such as CK-MB, LDH, AST and ALT.

The results of the present study showed that there were significant increase in the serum activities of CK-MB and LDH after treatment with either azithromycin or clarithromycin. (Tables 1,3 and figures 1, 2, 5 and 6), demonstrated myocardiocyte tissue damage due to either drug used compared to control juvenile rats; while, neither serum AST nor ALT activities were changed after treatment with each macrolide drug utilized in this study compared to control groups. Tables 2, 4 and figures 3, 4, 7 and 8.

Different myocardial markers were utilized to determine cardiac function; first, CK-MB resides in the cytosol and facilitates high energy phosphates into and out of mitochondria. It is distributed in a number of tissues including heart and skeletal muscle. In animal experiments, it was demonstrated that an increased in CK-MB activity in blood has been associated with evidence of irreversible cardiac injury (cell disruption)[17].

Second, lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to lactate. LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found predominately in blood serum. A high LDH-1 level to LDH-2 suggests MI[18].

Third, aspartate aminotransferase (AST), an enzyme was the first used. It is not specific for heart damage and it is also one of the liver function tests[18].

In conclusion, the results of this study provide an evidence, for the first time, to our knowledge on the effect of treatment of healthy juvenile rats with either azithromycin or clarithromycin on cardiac markers, where both drugs have adverse effects on cardiac tissue manifested by increase in serum enzyme activities especially CK-MB and LDH. Thus, further studies are needed to confirm such finding.

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Table 1: The effect of treatment with azithromycin on serum activities of CK-MB and LDH compared to control animals

Group	CK-MB (IU/L)	LDH (IU/L)
Control N= 6	115±7.13	461.50±19.24
Azithromycin N= 8	221.96±9.23*	629.88+11.75*

- Values are expressed as mean ± SEM.
- *: P<0.05, significant difference compared to control.
- N: number of animals.

Table 2: The effect of treatment with azithromycin on serum activities of AST and ALT compared to control animals

Group	AST (IU/L)	ALT (IU/L)
Control N= 6	33.1±3.2	23.9±3.5
Azithromycin N= 8	34±2.7 (NS)	27.9±2.1(NS)

- Values are expressed as mean ± SEM.
- NS: Non-significant difference with respect to control
- N: number of animals.

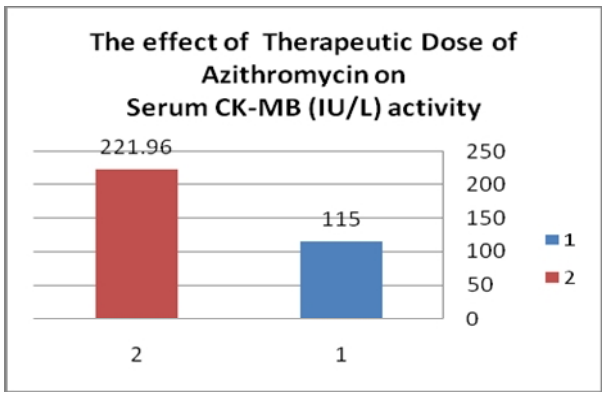

Figure 1: Histogram represents the effect of therapeutic dose of azithromycin 12mg/kg for 5 days (No. 2, red column) on serum CK-MB activity compared to control animals (No. 1, blue column).

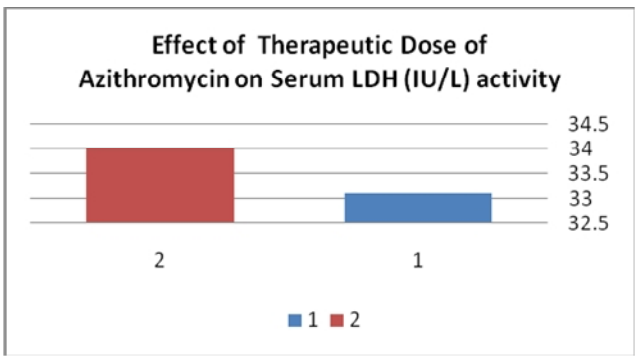

Figure 2: Histogram represents the effect of therapeutic dose of azithromycin 12mg/kg for 5 days (No. 2, red column) on serum LDH activity compared to control animals (No. 1, blue column).

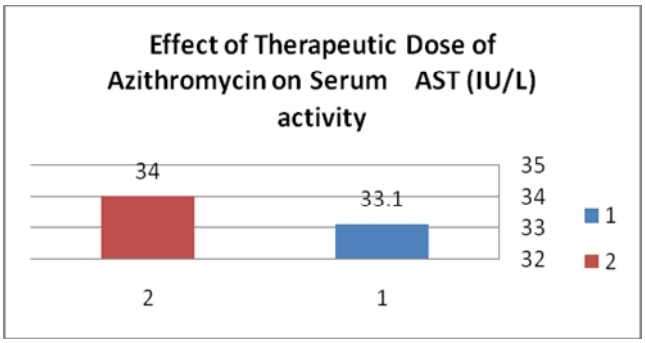

Figure 3: Histogram represents the effect of therapeutic dose of azithromycin 12mg/kg for 5 days (No. 2, red column) on serum AST activity compared to control animals (No. 1, blue column).

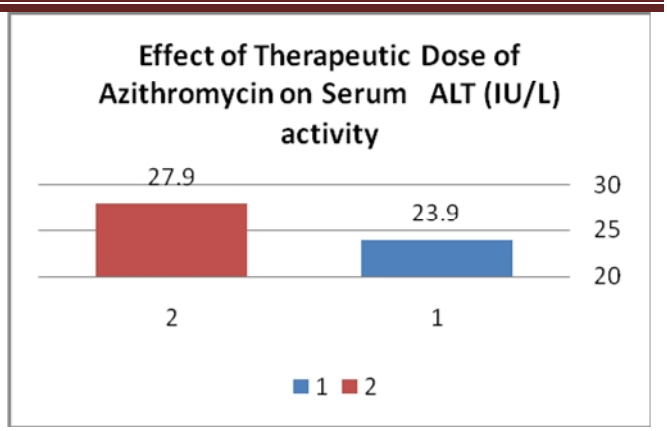

Figure 4: Histogram represents the effect of therapeutic dose of azithromycin 12mg/kg for 5 days (No. 2, red column) on serum ALT activity compared to control animals (No. 1, blue column).

Table 3: The effect of treatment with clarithromycin on serum activities of CK-MB and LDH compared to control animals

Group	CK-MB (IU/L)	LDH (IU/L)
Control N=6	120±6.4	440.7±9.8
Clarithromycin N=8	173.2±6.6*	678.3±11.23*

- Values are expressed as mean ± SEM.
- *: P<0.05, significant difference compared to control.
- N: number of animals.

Table 4: The effect of treatment with clarithromycin on serum activities of AST and ALT compared to control animals

Group	AST (IU/L)	ALT (IU/L)
Control	27.8±2.9	26.2±2.7
Clarithromycin	29.4±3.2 (NS)	26.3±2.5 (NS)

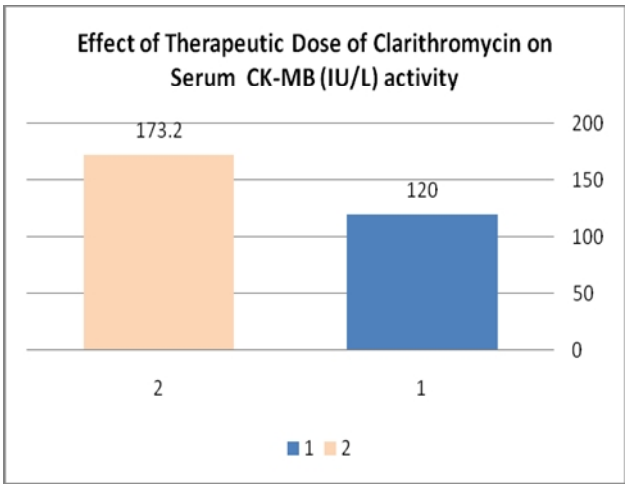

Figure 5: Histogram represents the effect of therapeutic dose of clarithromycin 7.5/kg for 5 days (No. 2, bright orange column) on serum CK-MB activity compared to control animals (No. 1, blue column).

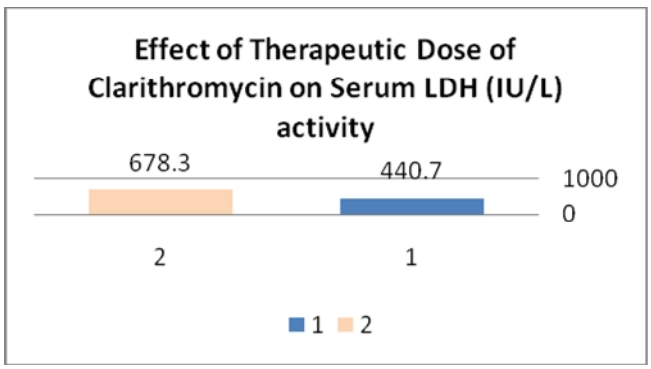

Figure 6: Histogram represents the effect of therapeutic dose of clarithromycin 7.5/kg for 5 days (No. 2, bright orange column) on serum LDH activity compared to control animals (No. 1, blue column).

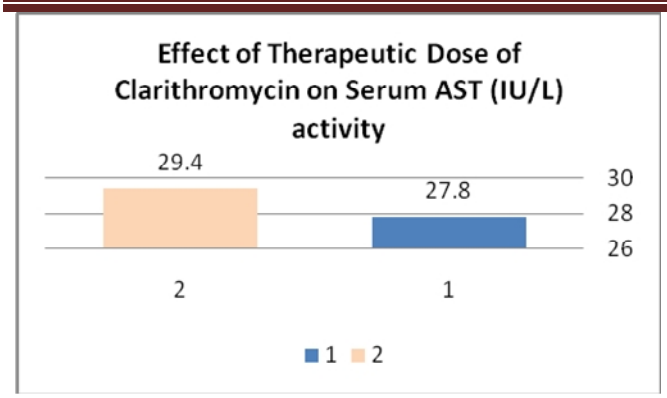

Figure 7: Histogram represents the effect of therapeutic dose of clarithromycin 7.5/kg for 5 days (No. 2, bright orange column) on serum AST activity compared to control animals (No. 1, blue column).

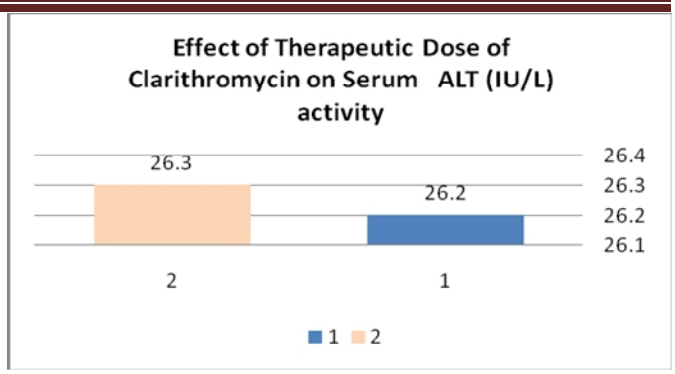

Figure 8: Histogram represents the effect of therapeutic dose of clarithromycin 7.5/kg for 5 days (No. 2, bright orange column) on serum ALT activity compared to control animals (No. 1, blue column).

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