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Physiological effect of thiamin on cardiovascular system in adult rate treated with erythromycin

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Abstract

Background: Erythromycin is a common solution of macrolide antibiotics which has cardiovascular side effects, such as oxidative stress and myocardial damage. Thiamin (vitamin B1), which is an important coenzyme in energy metabolism, has anti-inflammatory and antioxidant properties. This research paper set out to examine the protective action of thiamin on cardiovascular toxicity induced by erythromycin in adult albino rats.

Methods: Four groups (n= 10 each) of male Swiss albino rats were assigned to control (normal saline), thiamin (400 µg/kg), erythromycin (400 mg/kg), and thiamin + erythromycin (2.4 µg/kg + 400 mg/kg) groups. Treatments were administered in two months orally. The level of malondialdehyde (MDA), glutathione (GSH), creatine kinase (CK), aspartate aminotransferase (AST), lipid profile, C-reactive protein (CRP) and interleukin-6 (IL-6 were measured by biochemical assay. The myocardium and aorta were examined histopathologically by use of the hematoxylin and eosin stains.

Results: MDA, CK, AST, CRP, IL-6, and induced dyslipidemia, and GSH was reduced ($p<0.001$ vs. control) on erythromycin treatment. The biochemical parameters were kept close to normal levels by thiamin supplementation and the changes caused by erythromycin were greatly decreased. It was found that histological sections of erythromycin-treated rats contained coagulative necrosis, fatty infiltration, inflammation and hemorrhage, and that thiamin co-treatment reinstated myocardial architecture with minor pathology.

Conclusion: The study indicates that supplementation of Thiamin has a protective effect on cardiovascular toxicity caused with erythromycin and improves antioxidant defenses, biochemical markers, and histological integrity. These results point to the use of thiamin as a possible complementary treatment to minimize cardiac adverse event of prolonged erythromycin consumption.

Keywords: Thiamin; erythromycin; cardiovascular toxicity; oxidative stress; histopathology

Introduction

Oxidative stress, inflammation, and metabolic dysregulation are major contributors to the development of cardiac dysfunction as cardiovascular diseases are identified as one of the major patterns of morbidity and mortality on the global arena (World Health Organization, 2023; Benjamin *et al.*, 2019) [21, 9]. The list of pharmacological agents that affect cardiovascular physiology directly or indirectly is very broad, encompassing the antibiotics that are frequently prescribed (Roden, 2004) [6]. Macrolide based antibiotics like erythromycin are also popular among them owing to their broad spectrum activity in bacterial action but there has been a concern about their possible negative effects on cardiac performance especially in susceptible individuals (Owens & Nolin, 2006) [18]. Erythromycin has been proven to disrupt cardiac electrophysiology and metabolism, and experimental and clinical data show that it can induce arrhythmias, increase the QT interval, and modify the oxidative and inflammatory processes (Ray *et al.*, 2004; Svanström *et al.*, 2014) [17, 20, 11]. Long-term use can also impair lipid metabolism and improve oxidative stress, resulting in an increase in the number of reactive oxygen species (ROS) and endogenous antioxidants (Yang *et al.*, 2015) [12, 22]. Such changes, in their turn, lead to the injury of the myocardium and vascular dysfunction, which are usually indicated by high levels of serum biomarkers in the form of malondialdehyde (MDA) and creatine kinase (CK), C-reactive protein (CRP) and interleukin-6 (IL-6) (Aydin *et al.*, 2019; Ridker, 2007) [19, 16]. Thiamin (vitamin B1), is a water-soluble vitamin necessary to carbohydrate metabolism, energy production, and normal cellular functioning (Combs and McClung, 2017) [10]. It contributes to the functions of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, so that the tissues with high energy requirements, including the heart, do not lack adequate ATP (Lonsdale, 2017) [7].

Other than its metabolic effects, thiamin has antioxidant, anti-inflammatory and membrane-stabilizing effects, which could help reverse oxidative stress and tissue damage (Bettendorff & Wins, 2009) [14]. Other research studies have reported the thiamin deficiency as a risk factor to cardiovascular problems, such as heart failure, endothelial dysfunction, and predisposition to oxidative damage (Whitfield *et al*., 2018; Singleton and Martin, 2001) [13, 5]. Thiamin supplementation is known to stimulate cardiac performance and decreases lipid peroxidation and antioxidant defense systems (Gioda *et al*., 2010; Lakshmi and Sudhakar, 2010) [4, 2-3]. Although thiamin has been identified as having an important role in cardiovascular physiology there is still a dearth of information on the protective effect of thiamin against drug-induced cardiotoxicity, especially in connection with the use of erythromycin (Sayed-Ahmed *et al*., 2010) [15]. The potential clinical translation of studying this interaction is also present due to the fact that erythromycin is still used in clinical practice and thiamin supplementation is cheap, safe, and is readily available (Lonsdale, 2006) [7]. In this manner, the current research was aimed at determining the physiological impact of thiamin on the cardiovascular system of adult albino rats with erythromycin. Biochemical (MDA, glutathione), enzymatic (CK, AST) assessment of cardiac injury, lipid profile changes, inflammatory (CRP, IL-6), and histological analysis of cardiac and aortic tissue were involved in the assessment. This study will attempt to offer experimental data on the possibility of thiamin to counteract the cardiovascular side effects of erythromycin and to emphasize its value as a protective factor to ensure cardiac protective health.

Material and methods

Animals and Ethical Approval

Forty healthy adult male Swiss albino rats (weighing 180-220 g, aged 8-10 weeks) were used in this study. Animals were housed under controlled laboratory conditions (22±2 °C, 12-h light/dark cycle, and 50-60% relative humidity) with free access to a standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with the guidelines of Mustansiriyah University and were approved by the Mustansiriyah University College of Pharmacy Research Ethics Committee (Ref. No. 205) on January 5, 2025.

Experimental Design and Groups

Animals were randomly divided into four equal groups (n = 10 each):

- **Group 1 (Control):** Received normal saline orally and served as untreated control.
- **Group 2 (Thiamin):** Received thiamin at a dose of 400 µg/kg body weight.
- **Group 3 (Erythromycin):** Received erythromycin at a dose of 400 mg/kg body weight.
- **Group 4 (Thiamin + Erythromycin):** Received thiamin (2.4 µg/kg) in combination with erythromycin (400 mg/kg).

The treatments were administered once daily by oral gavage for a period of two months.

Drugs and Treatments

- **Thiamin (Vitamin B1):** Obtained in pure powder form and freshly dissolved in sterile normal saline before administration.
- **Erythromycin:** Supplied as an oral suspension, prepared at the required concentration for daily dosing.

Blood and Tissue Collection

At the end of the treatment period, animals were fasted overnight, anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), and blood samples were collected via cardiac puncture into plain and EDTA-coated tubes. Serum was separated by centrifugation at 3,000 rpm for 15 min at 4 °C and stored at −20 °C until analysis.

Following blood collection, the animals were euthanized by cervical dislocation under deep anesthesia. The heart and aorta were immediately excised, washed in ice-cold saline, and divided for biochemical and histological studies.

Biochemical Analysis

The parameters analyzed using the standard commercial assay kits and spectrophotometric methods in serum samples included: Reactive oxygen species: Malondialdehyde (MDA), Glutathione (GSH).

- Cardiac injury enzymes: Creatine kinase (CK), Aspartate aminotransferase (AST).
- Lipid profile: total cholesterol, triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein (HDL).
- Markers of inflammation: C-reactive protein (CRP) and interleukin-6 (IL-6). Each and every assay was done thrice to ascertain accuracy and reproducibility.

Histological Examination

Neutral-buffered formalin was used to fix and fix tissue of the heart and aortas and dried them in graded ethanol, then cleared them in xylene, and embedded them in paraffin wax. Thickness of 5 µm sections were cut and stained with hematocytin and eosin (H&E) to examine them under general histology. Microscopic examination was done under light microscope (Olympus BX51, Japan) and photomicrographs recorded to make a record.

Statistical Analysis

All data were in terms of mean and standard deviation (SD). One way analysis of variance (ANOVA) and the application of Tukey post hoc test were used to statistically compare groups. A p-value value below 0.05 was regarded as significant. The SPSS software version 25.0 (IBM Corp., Armonk, NY, USA) was used to conduct the statistical analyses.

Results

Biochemical Parameters

Oxidative Stress Markers

Serum malondialdehyde (MDA) levels were significantly elevated in the erythromycin-treated group (G3: 4.5±0.6 nmol/mL) compared with the control group (G1: 2.1±0.3 nmol/mL; $p<0.001$). Thiamin supplementation alone (G2: 2.0±0.4 nmol/mL) maintained MDA levels comparable to controls, while combined treatment (G4: 2.5±0.5 nmol/mL) significantly reduced MDA compared with erythromycin alone ($p<0.001$).

Glutathione (GSH) concentrations were markedly reduced in the erythromycin group (G3: 4.0±0.8 µmol/L) relative to controls (G1: 8.2±1.0 μmol/L; p<0.001). Thiamin supplementation restored GSH levels (G2: 9.0±1.2 μmol/L), while combined treatment (G4: 7.5±1.1 μmol/L) significantly improved antioxidant status compared with G3 (p<0.01).

Cardiac Enzymes

Creatine kinase (CK) activity was significantly increased in the erythromycin group (G3: 380±40 U/L) compared with controls (G1: 145±20 U/L; p<0.001). Combined treatment with thiamin (G4: 190±30 U/L) markedly reduced CK activity compared with G3 (p<0.001), though still higher than controls (p<0.05).

Aspartate aminotransferase (AST) levels were elevated in G3 (140±15 U/L) compared to G1 (60±8 U/L; $p < 0.001$). Thiamin alone showed no significant effect (G2: 62±7 U/L vs. G1; p>0.05), while G4 (75±10 U/L) demonstrated significant reduction compared with G3 ($p < 0.001$).

Lipid Profile: Erythromycin treatment resulted in dyslipidemia, with significantly increased total cholesterol (220±20 mg/dL), triglycerides (180±15 mg/dL), and LDL (120±12 mg/dL), and decreased HDL (30±4 mg/dL) compared with controls (145±12, 110±10, 65±8, and 52±6 mg/dL, respectively; p<0.001 for all). Thiamin supplementation improved lipid parameters (G2 similar to controls). Combined therapy (G4) significantly ameliorated erythromycin-induced dyslipidemia (total cholesterol 160±18, LDL 75±9, HDL 48±5; p<0.01 vs. G3).

Inflammatory Markers

CRP levels were significantly increased in G3 (4.8±0.6 mg/L) compared with G1 (1.2±0.2 mg/L; p<0.001). Thiamin supplementation (G2: 1.1±0.3 mg/L) maintained levels comparable to controls. Combined treatment (G4: 1.8±0.4 mg/L) significantly reduced CRP compared with G3 (p<0.001).

Similarly, IL-6 levels were significantly elevated in G3 (45±5 pg/mL) compared with G1 (18±3 pg/mL; $p < 0.001$). Thiamin alone (G2: 17±2 pg/mL) was not significantly different from controls. G4 (22±3 pg/mL) demonstrated significant reduction compared with G3 ($p < 0.001$).

Table 1: Biochemical parameters in control and experimental groups (mean±SD, n = 10).

Parameter	Control (G1)	Thiamin (G2)	Erythromycin (G3)	Thiamin + Erythromycin (G4)
MDA (nmol/mL)	2.1±0.3	2.0±0.4	4.5±0.6 *	2.5±0.5 †
GSH (μmol/L)	8.2±1.0	9.0±1.2	4.0±0.8 *	7.5±1.1 †
CK (U/L)	145±20	150±25	380±40 *	190±30 * †
AST (U/L)	60±8	62±7	140±15 *	75±10 †
Total Cholesterol (mg/dL)	145±12	140±15	220±20 *	160±18 †
Triglycerides (mg/dL)	110±10	105±12	180±15 *	125±13 †
LDL (mg/dL)	65±8	62±7	120±12 *	75±9 †
HDL (mg/dL)	52±6	55±5	30±4 *	48±5 †
CRP (mg/L)	1.2±0.2	1.1±0.3	4.8±0.6 *	1.8±0.4 †
IL-6 (pg/mL)	18±3	17±2	45±5 *	22±3 †

* indicate significant difference vs. control

† indicate significant difference vs. erythromycin group

Histopathological

Myocardial sections analysis through histological methods identified the specific structural changes in the experimental groups corroborating the biochemical results.

Group 1 (Control): Myocardial architecture in cardiac sections of control rats was normal, and cardiac fibers were well organized, centrally located nucleus, intact intercalated discs, and intact connective tissue were observed. To verify the structural integrity of the myocardium at the baseline, the absence of necrosis, inflammation, fibrosis or hemorrhage was established (Figure -1).

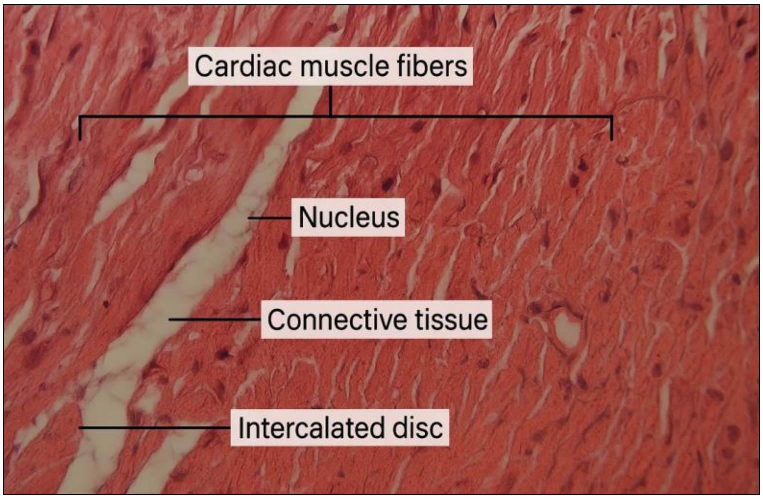

Fig 1: Myocardial section of Group 1 (Control) showing normal cardiac muscle fibers, nuclei, connective tissue, and intercalated discs without any pathological changes (H&E, ×400).

Group 2 (Thiamin)

The histological structure of rats treated with thiamin alone was normal, just like the control group. The myocardial fibers were seen to be intact and aligned, and the nuclei were clear without any inflammatory infiltration or fatty degeneration. These results indicate that thiamin supplementation maintained the myocardial structure and increased cardiac tissue resiliency (Figure-2).

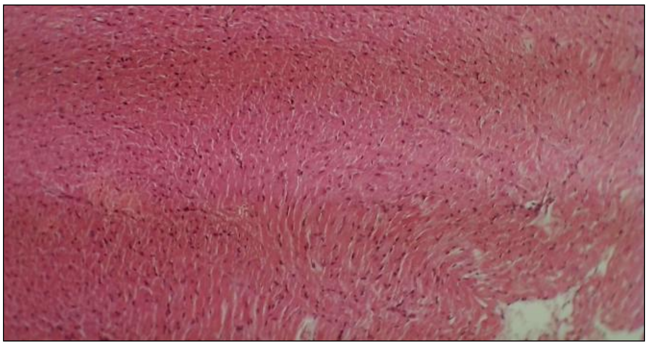

Fig 2: Myocardial section of Group 2 (Thiamin) showing preserved fibers and normal histology, with no evidence of necrosis or fibrosis (H&E, ×400).

Group 3 (Erythromycin): Slices of rats that were treated with erythromycin showed serious pathological alteration. There was intense coagulative necrosis and ghost cells and spaces showed marked evidence of early myocardial infarction. Incidence of inflammation cell infiltration areas were detected, and focal hemorrhages were seen in the area of destruction. Also, there was fatty infiltration next to the myocardial fibers that were a result of chronic degenerative changes (Figure 3&4).

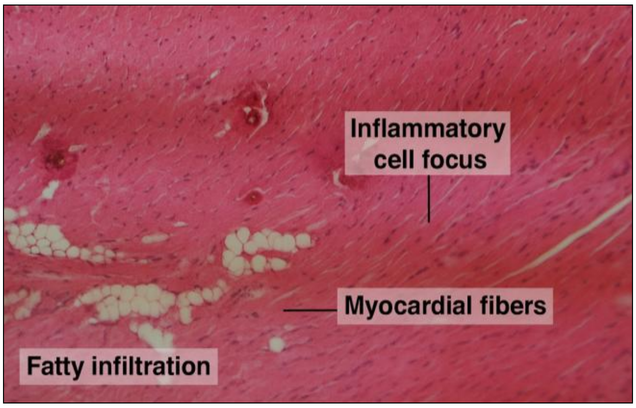

Fig 3: Myocardial section of Group 3 (Erythromycin) showing coagulative necrosis, ghost cells, early inflammatory response, and hemorrhage (H&E, ×200).

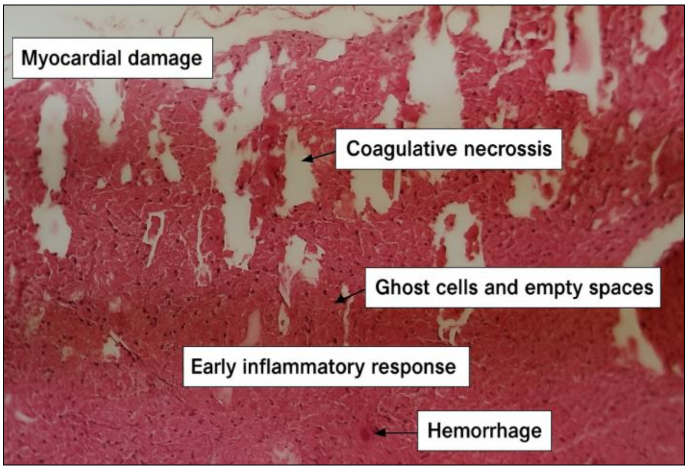

Fig 4: Myocardial section of Group 3 (Erythromycin) showing fatty infiltration and inflammatory cell focus adjacent to myocardial fibers (H&E, ×200).

Group 4 (Thiamin + Erythromycin)
Co-treatment with thiamin markedly improved cardiac histology compared with erythromycin alone. Myocardial fibers appeared regenerated and well-preserved, with restoration of striations and minimal inflammatory infiltration. Necrosis, fibrosis, and hemorrhage observed in G3 were absent. Overall, the myocardium demonstrated reversal of erythromycin-induced damage, suggesting a cardioprotective effect of thiamin (Figure-5).

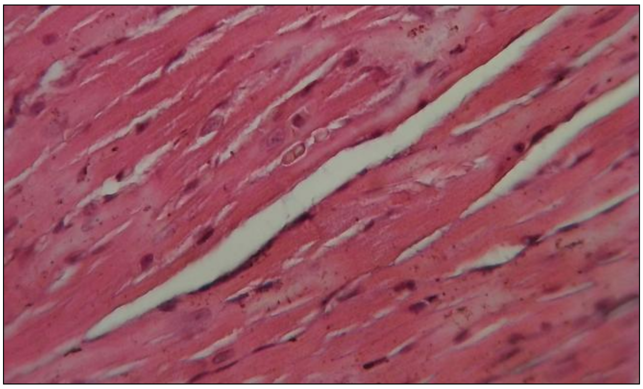

Fig 5: Myocardial section of Group 4 (Thiamin + Erythromycin) showing reversal of pathological changes with near-normal cardiac architecture and reduced inflammatory infiltration (H&E, ×400).

Discussion

Erythromycin had significant effects on raising the levels of serum MDA and lowering the level of GSH which implies increased lipid peroxidation and loss of antioxidant stores. The same results were documented in the cases where the macrolide antibiotics enhanced reactive oxygen species (ROS) and caused oxidative stress in cardiac and hepatic tissues (Yang *et al*., 2015; Ray *et al*., 2004) [12, 22, 17, 20]. Similarly, Aydin *et al*. (2019) [19] revealed that high levels of MDA and low ones of GSH are definite signs of myocardial oxidative stress. Nevertheless, a few studies indicated that there are drugs that do not have strong oxidative effects; Svanström *et al*. (2014) [11] revealed that azithromycin had less cardiotoxic potential than erythromycin. This variation could be attributed to the variation in pharmacokinetics and length of exposure. In our study, thiamin supplementation was a significant restorer of GSH and a decrease in MDA, which is in agreement with the other studies, which also found that thiamin prevented oxidative stress and enhanced antioxidant defenses in cardiac tissue (Gioda *et al*., 2010; Lakshmi and Sudhakar, 2010) [4, 2-3]. After the administration of erythromycin, CK and AST activities were high, which represents myocardial damage. This is consistent with the research that reported these enzymes as the sensitive biomarkers of cardiac damage (Mukherjee *et al*., 2016; Ridker, 2007) [1, 16]. Increased CK and AST following exposure to macrolide have been reported as well (Owens and Nolin, 2006) [18]. Conversely, other clinical reports focused on the electrophysiological effects (QT prolongation, arrhythmias) of erythromycin as opposed to biochemical ones (Roden, 2004) [6]. The difference can be due to the differences between species (rats and humans) or acute and chronic exposure models. Our study showed that co-treatment with thiamin lowered the leakage of CK and AST which has cardioprotective effects similar to those thiamin has shown in preventing drug-induced cardiotoxicity (Sayed-Ahmed *et al*., 2010) [15]. The treatment with erythromycin had a dyslipidemic effect of increased cholesterol, triglycerides, LDL, and decreased HDL. These findings correlate with the data that attributes mitochondrial dysfunction and oxidative stress caused by antibiotics to lipid abnormalities (Yang *et al*., 2015) [12, 22]. Similarly, Yang *et al*. (2015) [12, 22] have stated that lipid metabolism is compromised by oxidative stress. By contrast, other clinical trials failed to find significant lipid effects with short-term erythromycin (Owens & Nolin, 2006) [18] indicating that duration of treatment is critical in the effect manifested by the metabolic effects. In our research, the lipid profiles were enhanced by the thiamin supplementation, which is in line with Combs and McClung (2017) [10], who supported the importance of thiamin in the lipid metabolism and energy generation. Systemic inflammation was confirmed with a significant increase in CRP and IL-6 in treated rats by using erythromycin. The results are consistent with the ones found by Ridker (2007) [16], who noted CRP as the primary indicator of cardiovascular inflammation, and by Aydin *et al*. (2019) [19], who mentioned that IL-6 was one of the most sensitive indicators of myocardial injury. In comparison, Owens and Nolin (2006) [18] found no regular increase of CRP and antibiotic treatment. These differences can be caused by species difference and underlying inflammatory condition. Supplementation of thiamin highly attenuated CRP and IL-6 in agreement with Bettendorff and Wins (2009) [14], who emphasized the anti-inflammatory responses of thiamin by the modulation of cytokines secretion. Histological analysis showed that the structural damage in rat treated with erythromycin was severe with coagulative necrosis, fatty infiltration, hemorrhage, and inflammatory infiltration. These changes are similar to the pathological shifts in experimental and clinical research of erythromycin cardiotoxicity (Ray *et al*., 2004; Svanström *et al*., 2014) [17, 20, 11]. Nevertheless, Roden (2004) [6] suggested that the cardiotoxicity of macrolide is predominantly electrophysiological but not structural. We have shown that, in fact, structural myocardial injury can be caused by long-term exposure to erythromycin and thus fills a gap in existing knowledge. Notably, myocardial histoarchitecture was significantly restored with less necrosis and inflammation with thiamin co-treatment. These findings are consistent with the prior report of protective effect of thiamin against cardiotoxicity caused by doxorubicin (Gioda *et al*., 2010; Sayed-Ahmed *et al*., 2010) [4, 15].

Conclusion

Thiamin supplementation has a protective effect against cardiovascular toxicity caused by erythromycin by increasing antioxidant protection, biochemical markers, and histological integrity. These results have illuminated thiamin as a possible adjunct treatment in order to minimize cardiac issues that come along with prolonged intake of erythromycin.

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