The Beneficial Effect of Erdosteine Against Choloroquine-Induced Cardiotoxicity in Adult Male Albino Rats: A Biochemical and Histopathological Short-Term Study

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ABSTRACT

Chloroquine (CQ) is a widely used drug to treat malaria as well as other autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus for several decades. Its toxic effects include retinopathy, myopathy, and neuropathy. In addition, serious cardiac complications may occur during long-term CQ therapy. Erdosteine (ES), a mucolytic drug used in chronic pulmonary diseases, is rich in sulphhydryl thiol group, which accounts for free radical scavenging and antioxidant activity of the drug and plays a pivotal role in protecting cells against oxidative damage. Hence, the present study was undertaken to determine the protective role of ES against CQ-induced cardiotoxicity in rats through evaluation of some serum cardiac enzymatic parameters, various oxidants and antioxidants enzymatic activities in cardiac tissues, as well as histopathological changes of the myocardium. Forty adult male albino rats were divided into 4 equal groups (10 rats each) and each fasted group received its corresponding substance at a single dose per day orally after being dissolved in 1 ml of distilled water for 30 consecutive days as following: Group 1 (Control group; C-gp): Each rat had received 1 ml of distilled water; Group 2 (Erdosteine treated group; ES-gp): Each rat had received ES at 50 mg/kg; Group 3 (chloroquine treated group; CQ-gp): Each rat had received CQ at 15 mg/kg; and Group 4 (erdosteine + chloroquine treated group; ES-CQ-gp): Each rat had received ED at 50 mg/kg two hours prior to administration of 15 mg/kg CQ. The obtained data from ES-gp, as compared to C-gp, showed non significant changes in rats’ final body weights, absolute and relative heart weights, and cardiac troponin T (cTnT) levels, however, a significant decreases in serum total lactate dehydrogenase (LDH), total creatine phosphokinase (CPK), malondialdehyde (MDA), and myeloperoxidase (MPO) levels that accompanied with significant increases in reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) values were noticed. On the contrary, the results achieved from CQ-gp demonstrated several toxicological consequences in experimental animals. These findings were significant when compared with control parameters and included reduction in final body weights, gain in absolute and relative heart weights, biochemical elevations of serum LDH, CPK, and cTnT levels. Additionally, significant disturbance between oxidants/antioxidants balance measured in cardiac tissues were observed as increased MDA and MPO levels, accompanied with decreased GSH, SOD, and CAT values. Moreover, marked histopathological changes of myocardium in the form of waviness and disarrangement of the muscle fibers, variable degrees of cardiomyocytes degeneration, cellular enlargement with cytoplasmic vacuolization, and intense interstitial inflammatory cells infiltration were noticed. On the other side, administration of ES before CQ showed improvement of these alterations in biochemical indices and histopathologic changes that induced by CQ alone. This protective effects of ER against CQ associated different metamorphoses is possibly achieved by preventing free radical damaging cascades, oxidant radical release, and proinflammatory processes. In conclusion, the results of this experimental study suggest that ES has cardioprotective effects against CQ induced cardiac injury in adult male albino rats. Thus, patients on long term CQ administration making them prone to cardiac injury and hence co-administration of erdosteine may have beneficial effects in reducing the occurrence of this incidence.

INTRODUCTION

Chloroquine (CQ) [7-chloro-4-(4-diethylamino -1-methylbutylamino) quinoline] is a widely used antimalarial agent. It has been used for decades in the treatment of rheumatic diseases, including systemic lupus erythematosus (SLE), discoid lupus erythematosus, rheumatoid arthritis (RA),...
Sjögren syndrome, scleroderma, and psoriasis. The popularity of CQ for treatment of several diseases in many Third World countries emanates from it being cheap, widely available, relatively well tolerated, and having a rapid onset of action.

The oral route is mainly used for administration of the drug. In animal models and in healthy volunteers, the researchers found high bioavailability of CQ when given orally as a tablet or solution. After a single dose of 300 mg CQ, the peak plasma concentration was high and the time to peak was between 1 and 6 hours. Thus, CQ is well absorbed from the gastrointestinal tract and shows only a small first-pass effect. CQ is metabolized in liver through the N-dealkylation pathway, to desethylchloroquine (DCQ) and bisdesethylchloroquine (BDCQ), by cytochrome P-450. The same findings were noticed in albino rats after single oral administration of radioactive ¹⁴C-chloroquine at a dose of 20 mg/kg. Extensive uptake of radioactive drug into tissues was signified by higher concentrations in most tissues compared with serum indicating high tissue distribution. Approximately 70% of the tested dose recovered in urine and stool up to 144 hours after dosing indicating cumulative effect with very long terminal elimination half-life.

The mode of action of CQ and other quinoline antimalarials is not fully understood. However, these drugs are diprotonic weak bases and are concentrated in the acidic organelles of the intraerythrocytic stages of the parasite. In addition, potential of CQ action include interrelated anti-inflammatory, immunosuppressive effects, photoprotection, lysosomal stabilization, suppression of antigen presentation and inhibition of prostanandin and cytokines synthesis. There is growing evidence that CQ inhibits toll-like receptor-mediated immune activation that induces proinflammatory cytokine production.

Chloroquine has a low margin of safety and there is a wide range of possible side effects associated with its use, including cardiac and neurological disorders, retinopathy, skeletal myopathy, and otoxicity. The overall mortality rate resulting from an overdose of CQ is in the range of 2.5% to 25%. Death usually occurs within 1–3 hours after ingestion and is related to the direct action on the myocardium.

Cardiotoxicity following accidental or suicidal CQ overdose has been reported. Ironically, such effects have been associated with usual doses of CQ administered for short or long term therapy. Cardiac complications may manifest as congestive heart failure, myocardial hypertrophy, restrictive or congestive cardiomyopathy, and conduction disorders, such as bundle branch block or complete heart block. The acute toxic effects on the heart consist of hypotension and conduction disturbances, sometimes with fatal arrhythmias, and have been observed after accidental overdose.

The same acute reactions have been reproduced in experimental animals by administration of large amounts of CQ. In addition, rats chronically administered CQ (100 up to 1000 mg/kg) in food for up to 2 years demonstrated myocardial and other muscle damage, centrilobular liver necrosis, and testicular damage.

The exact mechanism of CQ-induced cardiotoxicity remains unclear. The cardiovascular toxicity of CQ is related to transient high blood levels present early in the distribution phase, resulting from incomplete distribution of the central compartment. CQ exerts its toxicity on the myocardium by a quinidine-like mechanism, negative inotropism, inhibition of spontaneous diastolic depolarization, slowing conduction, increase of effective refractory period, prolongation of QT segment and QRS interval, Torsades de pointes, and multiple ventricular ectopic beats. Refractory hypotension progressing into cardiogenic shock and respiratory depression progressing to respiratory arrest are secondary to cardiac depression.

Furthermore, CQ may act directly or indirectly and alters antioxidant status that makes certain organs, more susceptible to oxidative stresses. The oxidative stress caused by CQ leads to excessive formation of free radicals with subsequent cellular imbalance between oxidants and antioxidants, and this is assumed to
play a key role in the pathogenesis of miscellaneous diseases resulting in organ injury.\textsuperscript{[22]}

However, this potential cardiac toxicity seems to be under-appreciated in the literature. Lists of drug-induced cardiomyopathies named in numerous publications and Physicians Desk Reference make no or very short mention of myocardial changes caused by chronic use of the antimalarial drugs CQ or hydroxychloroquine (HCQ).\textsuperscript{[23]} Additionally, by contrast with what is proposed for ophthalmologic assessment, there is no recommendation concerning electrocardiographic screening and survey of patients with prolonged treatment with antimalarials.\textsuperscript{[24]}

Erdosteine (ES) [N-(carboxymethylthioacetyl) - homocysteine thiocarbonate] is mucolytic agent for chronic pulmonary diseases that contains two blocked sulphydryl groups which became free only after hepatic metabolization and opening of the thiocarbonate ring. The free sulphydryl group breaks the disulphide bridges of the high molecular-weight mucus glycoproteins, resulting in reduced sputum physical properties.\textsuperscript{[25]} Thiols play a pivotal role in protecting cells against oxidative damage. The reducing potential of these sulphydryl groups accounts for free radical scavenging and antioxidant activity of ES\textsuperscript{[26]} Experimental evidences in animals support the protective effect of ES against various types of tissue injuries induced by a variety of pharmacological or noxious agents, mediated by products of oxidative stress.\textsuperscript{[27]} In addition, ES has been reported to protect the cardiac tissue from several toxicants, including anticancer agent doxorubicin,\textsuperscript{[28,29]} immunosuppressive agent cyclosporine A,\textsuperscript{[30]} and nicotine.\textsuperscript{[31]}

The following study was designed to test whether a clinically used drug like erdosteine could prevent chloroquine - induced cardiotoxicity in adult male albino rats, through evaluation of some serum cardiac enzymatic parameters, various antioxidants enzymatic activity levels, as well as morphological changes of the myocardial tissue.

**MATERIAL and METHODS**

**Drugs**


Erdosteine (ES): Each commercially available hard gelatin capsule contains 300-mg Erdosteine (MUCOTEC, GLOBAL NAPI Pharmaceuticals, 6th of October, Egypt).

**Animals**

Forty adult male albino rats weighting between 150-180 gm were used in the present study. They were divided into 4 equal groups (10 rats each) and housed under constant environmental conditions, and were kept for 10 days of acclimatization before treatment with food and water were freely allowed. The experiments were performed in accordance with "Guide for the Care and Use of Laboratory Animals", mentioned by Cuschieri and Backer.\textsuperscript{[32]} Animals were subjected to treatment regimen as follows:

- **Group 1** (Control group; C-gp): Each rat had received 1-ml of distilled water.
- **Group 2** (ES treated group; ES-gp): Each rat had received erdosteine at 50 mg/kg. The dose was chosen according to Ozyurt et al.\textsuperscript{[33]}
- **Group 3** (CQ treated group; CQ-gp): Each rat had received chloroquine at 15 mg/kg according to the study done by Karawya.\textsuperscript{[34]}
- **Group 4** (ES + CQ treated group; ES-CQ-gp): Each rat had received ES at 50 mg/kg two hours prior to administration of 15 mg/kg CQ.

Both ES and CQ dosages were dissolved in 1-ml distilled water and gavaged as single dose per day to each fasted animal for 30 consecutive days.

Twenty-four hours after the last dose, the animals were anesthetized with ether, thoracotomy was performed and blood withdrawn by syringe from descending aorta into clean test tubes and centrifuged. Then animals were sacrificed by cervical dislocation. Finally hearts were harvested rapidly and washed with distilled water to avoid clot formation, weighed, and cut into two parts. One part was fixed in neutral formalin solution (10%) for histological examination and the other parts of heart tissue were homogenized for analysis of designated biomarkers of cardiac antioxidants enzymatic activities.
Methods
I- Biochemical analysis:-

1- Determination of serum cardiac enzymes:

A) Serum total lactate dehydrogenase (LDH) and total creatine phosphokinase (CPK) activities: were assessed spectrophotometrically according to the method of Buhl and Jakson\textsuperscript{38} and Swanson and Wilkinson\textsuperscript{36} respectively, and values were calculated as units per liter (U/L).

B) Serum concentrations of cardiac troponin T (cTnT) were determined by immunoassay and values were given as nanograms per milliliter (ng/mL).\textsuperscript{37}

2- Determination of Cardiac Anti-Oxidants:

The heart homogenate, supernatant, and extracted samples were prepared as described by Irmak et al.\textsuperscript{38} One gram of tissue was homogenized by sonication in ice-cold phosphate buffer at a concentration 30% and centrifuged at 3000 r.p.m. for 15 minutes. Aliquots were prepared and used for the assessment of different cardiac oxidative and antioxidants enzymes levels. The supernatant obtained was used for spectrophotometrical estimation of the followings:

A) Malondialdehyde (MDA): It was determined using a spectrophotometric method of Uchiyama and Mihara\textsuperscript{39} Cardiac MDA content was expressed as nmol/g wet tissue.

B) Myeloperoxidase (MPO): It was determined according to Wei and Frenkel.\textsuperscript{40} Data are presented as mU/g protein.

C) Reduced glutathione (GSH): It was determined according to the method described by Ellman\textsuperscript{41} and expressed as µmol/g protein.

D) Superoxide dismutase (SOD): It was measured by using Marklund\textsuperscript{42} method and expressed as U/g wet tissue.

E) Catalase (CAT): The enzyme activity was measured as described by Bock et al.\textsuperscript{43} with values articulated as U/g wet tissue.

II-Histopathological examination:-

For histopathological evaluation, formalin-fixed specimens were processed, embedded in paraffin wax and sliced at 4-6 µm thickness by a microtome. Then, sections were deparaffined, rehydrated and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT) staining procedures Drury and Wallington\textsuperscript{44}.

Statistical analysis

Data were analyzed by using a commercially available statistics software package (SPSS for Windows, version 16.0, Chicago, USA). One-way analysis of variance (ANOVA) test performed and Post Hoc multiple comparisons were done with least significant difference (LSD). Results were presented as mean±SEM and p values < 0.05 was regarded as statistically significant.

RESULTS

All treated animals survived to the end of experimental period.

The data obtained from the mean body weights are given in Table (1). A statistical comparison of the mean values of final body weights from ES-gp (changes percentage; 0.59%) and ES-CQ-gp (changes percentage; 2.63%) showed non-significant changes, while, in CQ-gp (changes percentage; 8.23%), rats exhibited significant decrease in their final body weights as compared to C-gp. Furthermore, the mean values of absolute and relative heart weights of ES-gp (changes percentage; 1.29% and 0.87%, respectively) depicted statistically non significant changes when compared with those of control animals. On the other hand, a significant increases in the mean values of absolute and relative heart weights from CQ-gp animals (changes percentage; 51.95% and 64.63%, respectively) and ES-CQ-gp (changes percentage; 19.71 and 15.36% %, respectively) were noticed as compared to those of C-gp.

Nevertheless, a significant increase and significant decreases in animals' final body weights (changes percentage; 11.83%) as well as in both absolute (changes percentage; 21.21%) and relative (changes percentage; 29.93%) heart weights figures, respectively, were recorded in ES-CQ-gp animals when compared with those of CQ-gp.

Biochemical analysis of cardiac function tests are shown in Table (2). The results of data analysis demonstrated significant decreases in the mean serum levels of LDH (changes percentage; 7.16%), CPK (changes percentage;
7.35%), and non significant depression of cTnT (changes percentage; 18.18%) when ES-gp was compared with C-gp animals.

Nonetheless, the mean LDH, CPK, and cTnT values were significantly elevated in the CQ-gp (changes percentage; 96.56%, 171.10%, and 2986.36%, respectively) and ES-CQ-gp (changes percentage; 11.15%, 11.90%, and 550%, respectively) when compared to the C-gp.

Yet, the mean figures of LDH, CPK, and cTnT (changes percentage; 43.54%, 58.72%, and 78.94%, respectively) delineated a statistically significantly lower levels in ES-CQ-gp as compared to those of CQ-gp.

The results of oxidative and antioxidative enzymatic activities in cardiac tissues are summarized in Table (3). In ES-gp, both MDA (changes percentage; 20.56%) and MPO (changes percentage; 24.62%) mean levels in the heart tissues were significantly depleted, while, the mean values of GSH (changes percentage; 39.82%), SOD (changes percentage; 22.99%), and CAT (changes percentage; 36.61%) expressed high activities in cardiac tissues when compared with those of C-gp. However, in CQ-gp, analysis of the heart tissues revealed statistically significant elevation of MDA (changes percentage; 81.74%) and MPO (changes percentage; 171.22) accompanied with significant depression of GSH (50.44%), SOD (58.96%), and CAT (27.68%) activities in comparison with C-gp. Similarly, in ES-CQ-gp, MDA (changes percentage; 17.98%) and MPO (changes percentage; 29.23%) mean levels were elevated, but, associated with non significant decreases in GSH (changes percentage; 3.54%), SOD (changes percentage; 8.55%), and CAT (changes percentage; 8.04%) values as compared to C-gp.

In contrast, comparison between ES-CQ-gp and CQ-gp mean values of cardiac tissues MDA (changes percentage; 35.08%) and MPO (changes percentage; 52.35%) as well as GSH (changes percentage; 94.64%), SOD (changes percentage; 122.82%), and CAT (changes percentage; 27.16%) depicted significant decreases and significant increases, respectively, in the former group.

Histopathological examination of H & E stained myocardial sections from the C-gp (group 1; Figures 1-2) as well as ES-gp (group 2) revealed the classical architecture of the cardiac muscle fibers. They appeared typically as slender, branching and anastomosing fibers ramifying in different directions with narrow interstitial clefts in between. The sarcoplasm was uniformly pale acidophilic. Each cardiomocyte exhibited a single centrally located vesicular nucleus.

The cardiac sections of CQ-gp (Figures 3-10) depicted prominent myocardial alterations in the form of waviness and disarrangement of the muscle fibers. Variable degrees of cardiocytic degeneration were also noticed. They included cellular enlargement associated with perinuclear cytoplasmic vacuolization within some myocytes as well as patchy hypereosinophilic areas seen among the other faintly stained muscle fibers. Focal areas of myocytolysis and complete interruption of the muscle fibers were frequently encountered together with hemorrhagic area with exudates. Many transversely cut muscle fibers exhibited hazy granular sarcoplasm with peripherally located dark nuclei, dilated blood vessel with thick wall, and wide interstitial space. Furthermore, wide areas of intense interstitial inflammatory cells infiltration with hypertrophied muscle fibers were observed.

On the other hand, administration of ES greatly protected the cardiac muscle against CQ-induced myocardial lesions as most of the muscle fibers examined from ES-CQ-gp (Figures 11-14) evidently retained nearly to normal appearance. Nevertheless, some areas still showed wavy myocardial fibers with slight foci of hypereosinophilic myocytes, deeply stained nuclei, and mild inflammatory cell infiltrates. Additionally, scattered foci of swollen muscle fibers with pale minimally vacuolated sarcoplasm were evidenced. Also, blood vessel appeared undilated with normal wall thickness that surrounded by normal myocytes but with slight interstitial space widening and few degenerated granular cells.

Masson’s trichrome (Figures 15-20) stained cardiac sections from control as well as ES treated groups showed scanty collagen fibers in between the cardiac myocytes. However, the stain showed increase in the amount of collagen fibers in the endomysium surrounding the cardiac myocytes as well as fibrous tissue invasion of blood vessels with complete atherosclerotic area in cardiac sections from CQ-
Conversely, administration of ES plus CQ revealed minimally stained collagen fibers and normal connective tissue surrounding blood vessels.

**Table (1):** The effects of Erdosteine, Chloroquine, and Erdosteine-Chloroquine combination on animals’ body weights as well as absolute and relative heart weights when compared with control group by using ANOVA test and Post Hoc with least significant difference.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Absolute Heart Weight (g)</th>
<th>Relative Heart Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-gp</td>
<td>Mean 174.4 ± 1.63</td>
<td>201.7 ± 3.34</td>
<td>0.695 ± 0.006</td>
<td>0.345 ± 0.003</td>
</tr>
<tr>
<td>ES-gp</td>
<td>Mean 172.6 ± 2.54</td>
<td>200.5 ± 2.57</td>
<td>0.686 ± 0.004</td>
<td>0.342 ± 0.003</td>
</tr>
<tr>
<td>CQ-gp</td>
<td>Mean 169.3 ± 2.67</td>
<td>185.1 ± 2.29</td>
<td>1.056 ± 0.067</td>
<td>0.568 ± 0.029</td>
</tr>
<tr>
<td>ES-CQ-gp</td>
<td>Mean 170.3 ± 2.55</td>
<td>207 ± 2.89</td>
<td>0.832 ± 0.034</td>
<td>0.398 ± 0.017</td>
</tr>
<tr>
<td>ES-CQ-gp Vs CQ-gp</td>
<td>Sig. NS</td>
<td>NS</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Changes % 2.35% ↓</td>
<td>2.63% ↑</td>
<td>19.71% ↑</td>
<td>15.36% ↑</td>
</tr>
</tbody>
</table>
| Values are given as mean±SEM for group of 10 animals each; NS = non-significant difference compared to control; * = significant difference compared to control; changes % compared to control.
Table (2): The effects of Erdosteine, Chloroquine, and Erdosteine-Chloroquine combination on animals' lactate dehydrogenase (LDH), total creatine phosphokinase (CPK), and cardiac troponin T (cTnT) levels when compared with control group by using ANOVA test and Post Hoc with least significant difference.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (U/L)</th>
<th>CPK (U/L)</th>
<th>cTnT (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-gp</td>
<td>Mean</td>
<td>255.6</td>
<td>164.7</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>2.14</td>
<td>2.81</td>
</tr>
<tr>
<td>ES-gp</td>
<td>Mean</td>
<td>237.3</td>
<td>152.6</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>2.86</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Changes %</td>
<td>7.16% ↓</td>
<td>7.35% ↓</td>
</tr>
<tr>
<td>CQ-gp</td>
<td>Mean</td>
<td>502.4</td>
<td>446.5</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>8.58</td>
<td>5.97</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Changes %</td>
<td>96.56% ↑</td>
<td>171.10% ↑</td>
</tr>
<tr>
<td>ES-CQ-gp</td>
<td>Mean</td>
<td>284.1</td>
<td>184.3</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>7.27</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Changes %</td>
<td>11.15% ↑</td>
<td>11.90% ↑</td>
</tr>
<tr>
<td>ES-CQ-gp Vs CQ-gp</td>
<td>Sig.</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Changes %</td>
<td>43.54% ↓</td>
<td>58.72% ↓</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM for group of 10 animals each; NS = non-significant difference compared to control; * = significant difference compared to control; changes % compared to control.
Table (3): The effects of Erdosteine, Chloroquine, and Erdosteine-Chloroquine combination on animals’ hearts oxidative malondialdehyde (MDA) and myeloperoxidase (MPO) and anti-oxidative enzymes reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) levels when compared with control group by using ANOVA test and Post Hoc with least significant difference.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/g wet tissue)</th>
<th>MPO (mU/g protein)</th>
<th>GSH (µmol/g protein)</th>
<th>SOD (U/g wet tissue)</th>
<th>CAT (U/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-gp</td>
<td>Mean</td>
<td>53.89</td>
<td>1.324</td>
<td>1.13</td>
<td>56.58</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>1.98</td>
<td>0.013</td>
<td>0.04</td>
<td>1.57</td>
</tr>
<tr>
<td>ES-gp</td>
<td>Mean</td>
<td>42.81</td>
<td>0.998</td>
<td>1.58</td>
<td>69.59</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>1.71</td>
<td>0.044</td>
<td>0.09</td>
<td>3.13</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Changes %</td>
<td>20.56% ↓</td>
<td>24.62% ↓</td>
<td>39.82% ↑</td>
<td>22.99% ↑</td>
<td>36.61% ↑</td>
</tr>
<tr>
<td>CQ-gp</td>
<td>Mean</td>
<td>97.94</td>
<td>3.591</td>
<td>0.56</td>
<td>23.22</td>
</tr>
<tr>
<td></td>
<td>±SEM</td>
<td>3.89</td>
<td>0.060</td>
<td>0.04</td>
<td>2.29</td>
</tr>
<tr>
<td>Sig.</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>Changes %</td>
<td>81.74% ↑</td>
<td>171.22% ↑</td>
<td>50.44% ↓</td>
<td>58.96% ↓</td>
<td>27.68% ↓</td>
</tr>
<tr>
<td>ES-CQ-gp</td>
<td>Mean</td>
<td>63.58</td>
<td>1.711</td>
<td>1.09</td>
<td>51.74</td>
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<td></td>
<td>±SEM</td>
<td>1.57</td>
<td>0.136</td>
<td>0.09</td>
<td>2.25</td>
</tr>
<tr>
<td>Sig.</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Changes %</td>
<td>17.98% ↑</td>
<td>29.23% ↑</td>
<td>3.54% ↓</td>
<td>8.55% ↓</td>
<td>8.04% ↓</td>
</tr>
<tr>
<td>ES-CQ-gp Vs</td>
<td>Sig.</td>
<td>***</td>
<td>***</td>
<td>***</td>
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</tr>
<tr>
<td>CQ-gp</td>
<td>Changes %</td>
<td>35.08% ↓</td>
<td>52.35% ↓</td>
<td>94.64% ↑</td>
<td>122.82% ↑</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM for group of 10 animals each; NS = non-significant difference compared to control; * = significant difference compared to control; changes % compared to control.
Figures (1 and 2): Photomicrographs of control rat myocardium (group 1) showing 1) Longitudinally sectioned (LS), slender branching and anastomosing fibers (dashed arrows) with centrally located nuclei (solid arrows). 2) Transversely sectioned (TS) muscle fibers, many of which exhibit central nuclei (solid arrows). H&E stain; Original magnification (OM) X 200.

Figure (3): Photomicrograph of CQ-treated rat myocardium (group 3; LS) illustrating wavy muscle fibers (W) with deeply stained nuclei. Some fibers exhibit swollen, hypereosinophilic homogenous sarcoplasm (H). H&E; OM x 200.

Figure (4): Photomicrograph of CQ-treated rat myocardium (group 3; LS) showing inflammatory cells infiltrate (IC), fibers hypertrophy (F), and cytoplasmic vacuolization (V). H&E; OM x 200.

Figure (5): Photomicrograph from CQ-treated rat myocardium (group 3; TS) depicting dilated blood vessel (BV) with thick wall (T), mild congestion (C), wide interstitial space (IS), edema (E), and hypertrophied myocytes (H).

Figure (6): Photomicrograph from CQ-treated rat myocardium (group 3; TS and LS) illustrating hemorrhagic (HA) area with exudates (EX) and degenerative myofibril (D) area surrounded by disorganized muscle fibers. H&E; OM
Figure (7): Photomicrograph of CQ-treated rat myocardium (group 3; LS) expressing localized areas of myocytic degeneration (D) up to complete interruption of the muscle fibers. H&E; OM x 200.

Figure (8): Photomicrograph of CQ-treated rat myocardium (group 3; TS) demonstrating inflammatory cell infiltrates (IC) and wide interstitial space (IS). H&E; OM x 200.

Figure (9): Photomicrograph of CQ-treated rat myocardium (group 3; TS) implying extensive vacuolization of cardiac cells (V) intermingling with intact myocytes (M). H&E; OM x 200.

Figure (10): Photomicrograph of CQ-treated rat myocardium (group 3; TS) indicating swollen transversely sectioned muscle fibers (S) with hazy turbid granular sarcoplasm and dark nuclei. H&E; OM x 200.

Figure (11): Photomicrograph from ES-CQ-treated rat myocardium (group 4; TS) describing apparently normal

Figure (12): Photomicrograph of ES-CQ-treated rat myocardium (group 4; LS) implying the presence of mild
undilated blood vessel (BV) without thickening of the wall and few degenerated (D) cardiac cells with granularity surrounded by normal myocytes but with slight interstitial space widening (IS). H&E; OM x 200.

Figure (13): Photomicrograph of ES-CQ-treated rat myocardium (group 4; LS) announcing the presence of mild inflammatory cell infiltrates (IC) with some wavy changes (W) in muscle fibers and mild hypereosinophilic homogenous sarcoplasm (H). H&E; OM x 400.

Figure (14): Photomicrograph of ES-CQ-treated rat myocardium (group 4; LS) evidencing the presence of less swollen (S) muscle fibers with minimally vacuolated sarcoplasm and dark nuclei as well as areas of hypereosinophilic myocytes (H). H&E; OM x 400.

Figure (15): Photomicrographs of control rat myocardium (group 1; LS) showing normal appearance of connective tissue fibers (arrows) surrounding myocytes (Masson Trichrome Stain). OM x 200.

Figure (16): Photomicrographs of control rat myocardium (group 1; LS) showing normal appearance of connective tissue fibers (arrow) surrounding blood vessel (BV) (Masson Trichrome Stain). OM x 200.
Figure (17): Photomicrograph of CQ-treated rat myocardium (group 3; LS) showing extensive fibrous tissue formation (arrows) in the endomysium surrounding the cardiac myocytes (Masson Trichrome Stain). OM x 200.

Figure (18): Photomicrograph of CQ-treated rat myocardium (group 3; TS) showing blood vessel (BV) with thick wall and luminal congestion (C) surrounded by extensive fibrous tissue (F) with invasive (I) area denoting atherosclerosis formation (Masson Trichrome Stain). OM x 100. A small window shows fibrous tissue completely invading the wall of BV. OM x 400.

Figure (19): Photomicrographs from ES-CQ-treated rat myocardium (group 4; LS) showing slight fibrous tissue formation (F) nearly similar to control group surrounding myocytes (Masson Trichrome Stain). OM x 200.

Figure (20): Photomicrographs from ESCQ-treated rat myocardium (group 4; LS) showing apparently normal fibrous tissue formation surrounding blood vessel (BV) (Masson Trichrome Stain). OM x 200.
DISCUSSION

Chloroquine is used widely around the world to combat malaria. It also has immunomodulatory properties and hence used in the treatment of autoimmune disorders.\textsuperscript{[45]} However, cardiac complications such as cardiomyopathy and conduction system disturbances may occur following acute and chronic administration of CQ.\textsuperscript{[66]} Hence, the present study was carried out to investigate the protective effect of ES on cardiotoxic effect of prolonged use of CQ in adult male albino rats.

Results of the present study demonstrated several toxicological consequences in experimental animals secondary to prolonged exposure to CQ. These findings included reduction in body weights, gain in absolute and relative heart weights, biochemical elevations of LDH, CPK, and cTnT levels, and disturbance in oxidants/antioxidants balance (increased MDA and MPO levels accompanied with decreased GSH, SOD, and CAT values) as well as marked histopathological changes of myocardium. However, administration of ES before CQ induced protective effects against these abnormalities as evidenced by marked correction of previously mentioned metamorphosis.

After three days of oral exposure to CQ at 200 mg/kg, maternal and fetal Sprague-Dawley rats exhibited signs of general toxicity, including reduced weight gain when compared to controls.\textsuperscript{[47]} Also, animals administered CQ orally (20 μg/kg) lost weight throughout the 4-week period with a concomitant reduction in food consumed.\textsuperscript{[48]} Additionally, adult male Sprague-Dawley rats fed low protein diet with CQ (10 mg/kg, intramuscularly) for 40 days showed loss of body weight gain.\textsuperscript{[49]} Several clinical reports also demonstrated weight loss in patients taken CQ as a side effect.\textsuperscript{[50-51]} These findings are consistent with the result of reduced weight in the present study. However, chronic administration of CQ at low doses and for short duration to adult wistar rats caused significant increase in body weight as mentioned by Adjene and Adenowo,\textsuperscript{[52]} which disagree with the present work. This loss in body weight gain may be due to protein deficiency secondary to suppression of appetite and low caloric intake as mentioned by Mbajiroug et al.\textsuperscript{[49]}

In the present study, CQ induced myocardial hypertrophy with increase in heart weight which is in agreement with Baguet et al.;\textsuperscript{[53]} Soong et al.;\textsuperscript{[54]} Fragasso et al.\textsuperscript{[55]} As a matter of fact, an increase or decrease in relative or absolute weight of an organ after administering a chemical or drug indicating the toxic effect of that chemical.\textsuperscript{[56]}

Moreover, oxidants/antioxidants imbalance, also reported in this study, due to excessive generation of reactive oxygen species (ROS) have been implicated in the pathophysiology of a large number of diseases.\textsuperscript{[57]} Evidence from experimental as well as clinical studies suggests the role of oxidative stress in the pathogenesis of heart dysfunction.\textsuperscript{[58]} This increase in ROS triggers cardiomyocyte expression of the proto-oncogene c-fos, one of the first indicators of hypertrophy.\textsuperscript{[59]} Also, ROS activate members of the Mitogen-Activated Protein Kinase family, protein kinase C, phosphatidyl inositol 3-kinase, and calcineurin, ultimately leading to increased cardiomyocyte protein synthesis, hypertrophic gene expression and increased cardiomyocyte volume.\textsuperscript{[60]}

Measurements of the isozymes of CPK and LDH have been used for the detection of myocardial injury. These are enzymes of intermediary metabolism found in both cardiac and skeletal muscle, although in different forms reflecting the different metabolic and functional properties of these tissues.\textsuperscript{[61]} Thus, these two cardiac markers are not conclusive enzymes, but, only suggestive markers of the possibility of cardiac muscle injury.

Changes in biochemical enzymes assay of this study was also noticed in other several works. The long-term chronic CQ administration (5 and 10 mg/kg b.w. 6 days a week for 6 months) caused an increase in serum LDH level as documented by Gaafar et al.\textsuperscript{[62]} Also, oral administration of CQ to rats (5 mg/kg) three times per week for 8 weeks was associated with double increase in LDH level in liver tissues.\textsuperscript{[63]} Many patients on chronic use of CQ and HCQ for treatment of rheumatic disease depicted enzyme disturbance in the form of increases in their serum LDH levels.\textsuperscript{[64]} Also, the study of Bolaños-Meade et al.\textsuperscript{[65]} showed that patients on long-term therapy with CQ and HCQ were shown to have elevated serum CPK levels. These findings synchronize with the CPK result of the current study.
There is an overwhelming weight of evidence that certifies cardiac TnT as the preferred, translational, safety biomarker for detection of minimal myocardial damage. Furthermore, several preclinical published studies have directly confirmed its effectiveness as well as its high sensitivity and nearly absolute specificity in laboratory animals for assessment of cardiotoxicity. No other biomarker of myocardial injury comes close to cTnT in effectiveness including LDH and CPK. Elevation of cTnT levels reported in this study is in agreement with Reffelmann et al. who documented elevated levels of serum cTnT in clinical cases with cardiomyopathy and cardiotoxicity as a result of taking CQ for prolonged time. Hence, this rise in cTnT levels is highly suggestive of occurrence of cardiac injury in tested animals of the current work. Cardiac troponins are not detectable in the blood of healthy persons. Release of these enzymes can occur when myocytes are damaged by a variety of conditions such as inflammation, trauma, exposure to toxins, and necrosis. There is also a direct proportional relationship between the occurrence of cardiac oxidative stress and cTnT level as indicated by Scott et al. as well as in the existing work.

In CQ-gp of this work, myocardial oxidative stress was evidenced by elevation of oxidants (MDA and MPO) and suppression of antioxidants enzymes activities (GSH, SOD, and CAT), as measured in cardiac tissues homogenates. Similarly, CQ has been shown to impose oxidative stress with subsequent development of oxidants/antioxidants imbalance in experimental animals. The authors reported marked increase in MDA level that accompanied with major decreases in enzymic GSH and non-enzymic (SOD and CAT) antioxidants levels. The same findings were also reported in patients treated with CQ. These toxic effects of CQ may be attributed to the action of ROS arising from its metabolism in the cell as mentioned by Farombi. Collectively, these data demonstrates excessive production of free radicals and/or ROS in cardiac muscle cells by CQ.

Myeloperoxidase (an index of granulocyte infiltration or accumulation of activated leukocytes) is an enzyme found in the azurophilic granules of neutrophils and in the lysosomes of monocytes. When neutrophils become activated, which can happen in conjunction with phagocytosis, they undergo a process referred to as a respiratory burst leading to production of superoxide, hydrogen peroxide, and other reactive oxygen derivatives, which are all toxic by-products. These granule contents are released into the phagolysosomes and outside the cell and come into contact with the surrounding tissues. Myeloperoxidase catalyzes the conversion of hydrogen peroxide and chloride ions into hypochlorous acid, which is more potent than hydrogen peroxide. Organ response to inflammatory condition involves an excessive stimulation of the polymorphonuclear neutrophils releasing ROS and MPO. In this study, elevated MPO levels in cardiac tissues homogenates was related to the presence of inflammatory cells infiltrations as shown histopathologically.

Oxygen free radicals generation can damage the myocardium through the release of lysosomal enzymes. CQ acts by inhibiting the enzymes of the antioxidant defense mechanism and of mitochondrial respiratory activity, cytochrome oxidase and several dehydrogenases. The drug accumulates in lysosomes and mitochondria and the lysosomal concentration of CQ is reported to be about 1000 times higher than in plasma. Experimental studies have shown that CQ impedes mitochondrial oxidative metabolism and is noxious to myocardial function in acute intoxication. Chronic treatment can lead to alteration of intracytoplasmic membranes that are stored in lysosomes and protected against enzyme attack. The intralysosomal phospholipases are in fact inactivated by the increase in pH induced by CQ.

Additional CQ effects included activation of cathepsin D and other lysosomal hydrolases. The increased activities of cathepsin D and glycohydrolases in heart tissue indicate the possible infiltration of inflammatory cells at the site of injury. When myocardial cell death and degeneration occurs, proteolysis of necrotic myocardium occurs with a concomitant influx of inflammatory cells. Histologically, cardiac muscle is a type of involuntary striated muscle similar in structure to that of skeletal muscle. HCQ and CQ toxicity have been reported to cause cellular
pathologic features that are shared between cardiomyopathy, skeletal myopathy, and neuropathy.\[^{54}\] These typical myocardial lesions are very similar to muscles changes encountered in antimalarial myopathy as reported by Piette et al.\[^{80}\] Long term CQ treatment can produce cardiac complications, such as cardiomyopathy (restrictive and hypertrophic) and auricular-ventricular blocks or other conduction disorders. These can be produced by the structural alteration of the interventricular septum, rather than by biochemical alterations in pacemaker cells. This toxicity seems to be restricted to patients receiving high doses or long term treatment.\[^{81}\] Histopathological results of this study had confirmed that a cumulative dose of CQ induces cardiac damage in rats as manifested by different alterations in cardiomyocytes architectures. Several other studies had documented similar histopathological changes in myocardium after CQ ingestion. Light and electron microscopic examination of cardiac tissues revealed dark, hypertrophic, vacuolated myocytes surrounded by a delicate network of interstitial sclerosis and interstitial fibrosis of smaller intramural coronary arteries, marked cytoplasmic accumulations of partly dissolved lamellar and concentric lipids and membrane-enclosed aggregates which resembled curvilinear bodies in distended lysosomes.\[^{23}\]

After long-term administration of CQ to rats\[^{82}\] and rabbits\[^{83}\] various morphological changes were found in the myocardium, especially focal necrosis and fibrosis. Also, CQ when administered orally (30-40 mg/kg/day) or subcutaneously (20 mg/kg/day) for two to four weeks, produced marked degenerative changes in the mitochondria and myofibrils as well as marked ultrastructure impairment of the rats' myocardium.\[^{84}\]

Histopathological examinations of endomyocardial biopsies showed hypertrophy of cardiomyocytes with heavily vacuolated cytoplasm and disorganization of the myofibrillar architecture, in addition to focal interstitial fibrosis.\[^{53};^{55}\] CQ accumulates inside the cells by the action of lysosomes, and intracellular CQ increases the intralysosomal pH, which results in dysfunction of the lysosomal enzymes by direct binding. It also causes acute interference with mitochondrial oxidative metabolism leading to the accumulation of glycogen and phospholipids with subsequent formation of myelinoid (lamellar) and curvilinear bodies that cause dysfunction of myocytes.\[^{53}\] Moreover, the cytotoxic effect of the drug appeared to be related to total dose, direct action on the heart, and slow elimination of the drug and its metabolites leading to latent overdose and toxic effects.\[^{85}\]

Accumulation of ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals along with a compromised antioxidant capacity contribute to excessive damage to cellular carbohydrates, proteins, lipids and nucleic acids.\[^{86}\] The oxidative stress caused by CQ leads to excessive formation of free radicals and enhanced lipid peroxidation resulting in tissue injury. Thiobarbituric acid reactive substances produced by lipid peroxidation can cause cross-linking and polymerization of membrane components. This can alter the intrinsic membrane properties such as deformability, ion transport, enzyme activity and the aggregation state of cell surface determinant.\[^{87}\]

In the present study, administration of ES prior to CQ (ES-CQ-gp) showed protective effects on cardiac tissues against CQ induced cardiotoxicity when compared with CQ-gp. Analogous to these findings, experimental studies showed that ES was capable of protecting the myocardium against doxorubicin,\[^{28};^{29}\] nicotine,\[^{31}\] and cyclosporine A\[^{30}\] induced cardiotoxicity through preventing oxidative tissue injury and increases antioxidants enzymatic levels. The authors also found that ES succeeded to preserve the normal myocardial structure and markedly reduced degenerative, necrotic and inflammatory changes with little amount of interstitial fibrosis and disorganization of myocardial fibers.

Erdosteine owing to the presence of two sulphydryl groups in its metabolites can act as a free oxygen radical scavenger and this component of the mechanism of action is likely to be involved in the cardioprotection afforded towards cardiac injuries induced by oxidative stress.\[^{30}\] In animal studies, ES works via its scavenger activity that prevents the accumulation of free oxygen radicals when their production is accelerated and increases antioxidant cellular protective mechanisms. The final result is a protective effect on tissues which reduces lipid peroxidation, neutrophil infiltration
and cell apoptosis mediated by noxious agents.\textsuperscript{[88]}

The antioxidant action of ES could be related with its inhibitory effect on neutrophil recruitment and subsequent release of proinflammatory mediators. Also, it inhibits cytokine synthesis and subsequently reduces increased MPO and lipid peroxidation activities.\textsuperscript{[89]}

On the other hand, Sener et al.\textsuperscript{[89]} and Yesildağ et al.\textsuperscript{[27]} observed significant protective effect of ES on GSH levels in colonic and liver injuries in rats, respectively, because the metabolization of mucoactive drug ES produces an active metabolite with a reducing SH group that acts as precursor for GSH synthesis.

Selcoki et al.\textsuperscript{[30]} showed that ES increases the reduced SOD, CAT, glutathione-peroxidase activities and reduces the enhanced nitric oxide (by inhibiting inducible nitric oxide synthase), MDA, and protein carbonyl levels as measured in cardiac homogenates. In addition, it decreased inflammatory cell accumulation and prevented inflammation-induced cardiac tissue injury. Moreover, ES inhibited protein carbonyl formation (a phenomenon of protein oxidation. and a useful marker to evaluate oxidative stress in vivo).

In conclusion, long-term administration of CQ to rats induced cardiotoxicity as manifested by reduction in animals' body weights as well as gain in absolute and relative heart weights. Additionally, CQ produced biochemical elevations of serum LDH, CPK, and cTnT levels associated with unbalance between oxidants/antioxidants enzymatic levels measured in cardiac tissues homogenates. Thus, it can be speculated that consequence of oxidative stress caused the CQ induced cardiac damage. These effects were confirmed via alterations in cardiac histopathological architectures. On the other hand, administration of ES prior to CQ showed unlikely occurrence of these alterations in biochemical indices and histopathologic changes induced by CQ alone. These results may suggest that ES has cardioprotective effects against CQ induced cardiac injury in CQ-treated patients possibly via its radical scavenging and antioxidant activities that preventing free radical damaging cascades and oxidant radical release.

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التأثير المفيد للإردوستين ضد السمية القلبية المحدثة بالكلوروكين في ذكور الجرذان البضاء البالغة: دراسة قصيرة مدى بيوكيميائية وهستوتوتولوجية

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المتخصوص العربي

يستخدم عقار الكلوروكين على نطاق واسع لعلاج الملاريا، فضلاً عن غيرها من الأمراض ذاتية المناعة مثل التهاب المفاصل الروماتيزمي، заболевания الحمراء لعدة قرون، ومن أثارها الشائعة إعتلال الشبكية، والإعتلال العضلي والعصبي. بالإضافة إلى ذلك، قد تسجل مضاعفات خطرة على القلب أثناء العلاج على مدى الطويل بالكلوروكين. الإردوستين. عقار يمكن استخدامه في الأمراض الروتوية المزمنة، وفي مجموعة السفالياترافيك بالكلوروكين. من إعدادات الفحوصات النقالة للأكيدة، وليبيا، وإيجابية في حاليا. هناك الكثير من التأكديات. أجريت هذه الدراسة لتحديد الدور الوظيفي للأدبيات للكلوروكين في الجرذان، من خلال قياس بعض الاعراض مثل نفسية القلب، وغذاء الأكل، والكبدة. يمكن استخدام عقار الكلوروكين على نطاق واسع لعلاج الملاريا.

عند انتقال باكستاني، وأعمال نفسية القلب. يتم استخدام عقار الكلوروكين على نطاق واسع لعلاج الملاريا، به تركيزاتية. هذه الدراسة تشير إلى التأثير الوظيفي للأدبيات للكلوروكين في ذكور الجرذان البضاء البالغة. وكذلك إعطاء الكلوروكين للمرضى على مدى الطويل يجعلهم عرضة للإصابة القلبية، ومن ثم التناول المشترك مع الإردوستين قد يكون له آثار مفيدة في الحال من حدوث مثل هذه الإصابات.