

Ameliorative Effect of Glycyrrhizic Acid against Doxorubicin-Induced Cardiotoxicity in Rats

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Abstract

Doxorubicin (DOX) is a potent anticancer drug whose application is hampered by its cardiotoxicity mediated by oxidative stress and inflammation.

Glycyrrhizic acid (glycyrrhizin) is a natural compound that exerts anti-inflammatory and anti-oxidant action.

The present study aimed to examine whether the administration of glycyrrhizic acid can produce protective effect against acute DOX cardiotoxicity.

Cardiotoxicity was induced in rats by intraperitoneal injection of DOX (15 mg/kg). Results revealed that DOX administration exhibited elevated serum level Lactate Dehydrogenase (LDH) myocardial TNF- α content, in addition to reduced myocardial Catalase (CAT) activity. Pretreatment with glycyrrhizic acid (100 mg/kg, orally) for nine days markedly ameliorated all these changes and substantially reduced the myocardium peroxidative damage.

The protective effects obtained by glycyrrhizic acid may be via its antioxidant and anti-inflammatory properties and suggest its possible use to prevent or reduce doxorubicin-induced cardiotoxicity.

Keywords: Glycyrrhizic Acid; Doxorubicin; Cardiotoxicity; Rats; Amelioration

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Introduction

Doxorubicin (DOX) is a potent, broad-spectrum cancer chemotherapeutic agent belonging to the class of anthracyclines. It is used in the treatment of hematologic malignancies and solid tumors; however, its use is limited by its acute and chronic cardio toxicity which can lead to progressive irreversible congestive heart failure [1]. Acute effects can occur immediately after treatment and characterized by transient arrhythmias, reversible hypotension and pericarditis [2].

There are several mechanisms hypothesized to explain DOX-induced cardio toxicity, including free radical production that can damage cells by lipid peroxidation, decreases in antioxidant enzyme levels, inducing mitochondrial apoptosis pathways and altered intracellular calcium homeostasis [3, 4].

Therefore, the search for an effective and safe drug against doxorubicin-induced heart failure remains a critical issue in both cardiology and oncology

In the last years, attention has been focused on the use of naturally occurring antioxidants for the prevention of oxidative stress mediated disorders such as diabetes, cancer, cardiac and liver toxicity [5].

Glycyrrhizic Acid (GA, glycyrrhizin, GL) is used as a flavoring agent in some candies, pharmaceuticals, and tobacco products and is known for its anti-inflammatory [6], anti-ulcer, antiallergic, anti-oxidant, anti-viral and anti-tumor activities [7]. In many parts of the world, GA is used to treat patients with acute and chronic hepatitis [8].

The current study was designed to investigate the possible protective effect of GA against DOX-induced acute cardiotoxicity in rats and the possible underlying mechanism was also studied.

Materials and Methods

Ethics Statement

Experimental design and animal handling procedures were performed in accordance with the guidelines of the animal ethics committee of the Faculty of Pharmacy, Zagazig University (ECAHZU).

Animals

Adult male Wistar rats weighing 130–150 g were obtained from the animal facility of Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. Rats were housed in clean cages and kept under controlled temperature ($25 \pm 3^\circ\text{C}$) and constant light cycle (12 h light/dark). Rats were allowed free access to a standard rodent chow diet and water ad libitum.

Chemicals and Drugs

DOX hydrochloride vial 10 mg was purchased from Pharmacia Italia, S.P.A., Italy,

Study Protocol

Rats were randomly divided into four groups. Group I (n = 8) - normal group: rats received 2% gum acacia suspension orally from 1st to 9th day. Group II (n = 10) - DOX: rats received single intra peritoneal (i.p.) injection of DOX (15 mg/kg; in saline) on 7th day of study. Group III (n = 10) - DOX + GA: rats received a daily dose of GA suspension in saline (100 mg/kg) orally from 1st day to 9th day of study and on 7th day a single i.p. injection of DOX (15 mg/kg; in saline). Group IV (n = 8) - GA: rats received a daily dose of GA suspension (100 mg/kg) orally from 1st day to 9th day of study. The dose of GA used was selected based on a previous study [9].

Serum and Tissue Sampling

At the end of the experiment, rats were sacrificed by decapitation. A blood sample of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at $8000\times g$ for 10 min. and used to determine Lactate Dehydrogenase (LDH) serum level.

The heart was removed, dissected, washed by ice-cold saline, then it was snap frozen in liquid nitrogen and kept at -80°C for determination of cardiac Catalase (CAT) content.

Measurement of Serum Lactate Dehydrogenase (LDH)

Serum activity of LDH was assayed enzymatically using a commercially available kit provided by Spinreact (Gerona, Spain).

Determination of Cardiac CAT Content

The level of CAT in cardiac tissue homogenate was determined by a colorimetric method using a commercially available kit supplied by Biodiagnostic (Giza, Egypt).

Determination of Cardiac TNF- α Content

Cardiac TNF- α content was assessed using Enzyme Linked Immunosorbent Assay (ELISA) using a microplate reader (Spectra III Classic, Tecan, Salzburg, Austria) as previously described [10].

Statistical Analysis

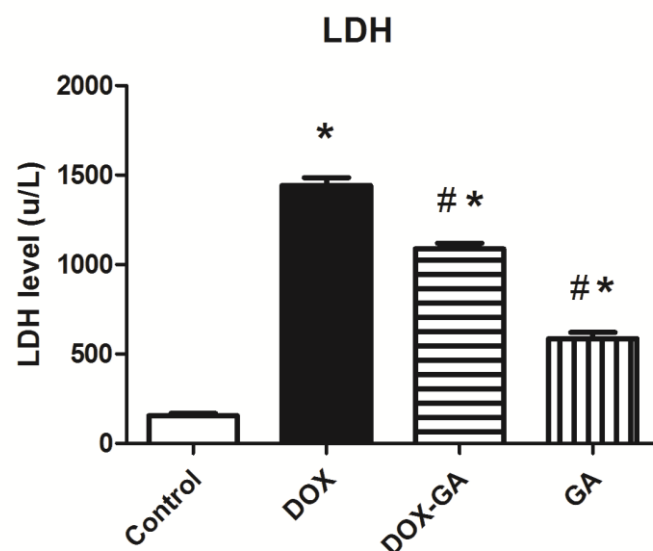
All data were expressed as mean $\pm$ standard error of mean (SEM). Statistical analysis was performed using Graph Pad Prism software v.5 (Graph Pad Software, Inc. La Jolla, CA, USA). The intergroup variation was measured by one way Analysis Of Variance (ANOVA) followed by Tukey's Post test. The minimal level of significance was identified at $p < 0.05$.

Results

Effects on serum LDH level

Serum total LDH level was increased significantly in DOX-treated rats as compared to control group (1442 vs 156 U/L) (Figure 1). On the other hand, GA pretreatment significantly reduced the serum LDH level as compared to DOX group (1088 vs 1442 U/L).

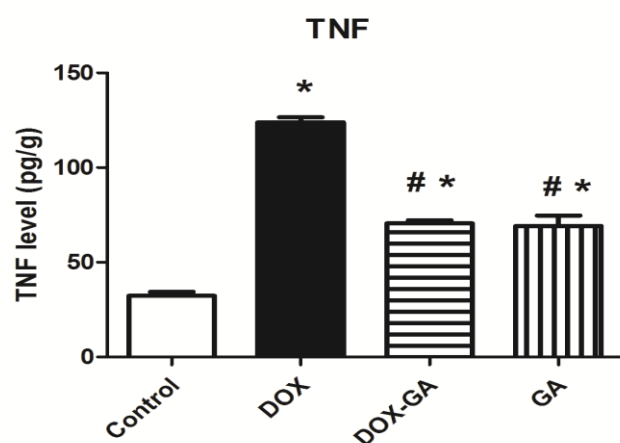
Figure 1: Effects of Doxorubicin (DOX) alone, Glycyrrhizic Acid (GA) alone or combined on serum LDH level. Data are expressed as mean $\pm$ S.E.M. * significantly different from control, # significantly different from DOX at $P < 0.05$ using ANOVA followed by Tukey's post- hoc test.



Effects on myocardial TNF- α Content

Serum TNF- α level was significantly increased in DOX-treated group compared to control group (124 vs 32 pg/g) as shown in Figure 2. GA administration resulted in a significant decrease in the serum TNF- α level as compared to DOX group (70 vs 124 pg/g).

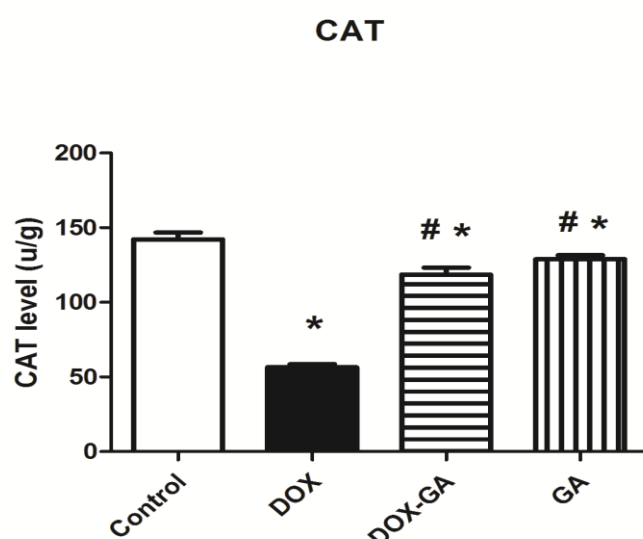
Figure 2: Effects of Doxorubicin (DOX) alone, Glycyrrhizic Acid (GA) alone or combined on myocardial TNF- α content. Data are expressed as mean $\pm$ S.E.M. * significantly different from control, # significantly different from DOX at $P < 0.05$ using ANOVA followed by Tukey's post- hoc test.



Effects on Myocardial CAT Content

Rats treated with DOX exhibited a marked decline in the myocardial antioxidant activity. This was evidenced by significant reduction in myocardial CAT content as compared with control group (56 vs 142 ug/g). Administration of GA restored CAT myocardial level compared with their respective DOX-treated rats (118 vs 56 ug/g) as shown in Figure 3.

Figure 3: Effects of Doxorubicin (DOX) alone, Glycyrrhizic Acid (GA) alone or combined on myocardial CAT content. Data are expressed as mean $\pm$ S.E.M. * significantly different from control, # significantly different from DOX at $P < 0.05$ using ANOVA followed by Tukey's post- hoc test.



Discussion

Glycyrrhizin is a natural compound derived from the root of licorice (*Glycyrrhiza glabra*) [11]. Glycyrrhizin has many biological activities, including anti-inflammatory [12, 13] and antioxidant [14, 15] effects.

In the current study, the administration of doxorubicin resulted in a significant increase in serum LDH level and myocardial TNF- α content compared to control group. This was accompanied by a marked decline in the myocardial antioxidant activity as evidenced by the reduction in myocardial CAT level.

These results demonstrate that doxorubicin has exerted a cytotoxic effect on myocardium as evidenced by the elevation in LDH. The cardiotoxic effect of doxorubicin is well documented [16].

The elevation in myocardial TNF- α induced by doxorubicin and the reduction in myocardial catalase content indicates a state of myocardial inflammation accompanied by oxidative stress which is in agreement with previous studies describing that oxidative stress mediates the activation of NF- κ B [17], which promotes the transcription of TNF- α [18].

Our results are in line with previous studies that demonstrated a significant increase in cardiac oxidative stress in DOX-treated animals [19]. Our results confirm the role of oxidative stress and inflammation in doxorubicin cardiotoxicity, [20].

On the other hand, pretreatment of rats with glycyrrhizin prior to doxorubicin significantly reduced the serum LDH level indicating that it ameliorated some of the toxic effects induced by doxorubicin.

GA administration resulted in a significant decrease in the TNF- α level and restored CAT level compared with their respective DOX-treated rats which confirms the previous reports showing that glycyrrhizin exerts anti-inflammatory effect [6, 21] and anti-oxidant action [15]. The mechanism of its effects include inhibition of prostaglandin E2 [22], and Reactive Oxygen Species (ROS) [14] production. In addition, it has been shown that glycyrrhizin down regulated all

components of the Toll-Like Receptor (TLR) pathway and reduced the level of the inflammatory cytokines TNF- α , IL-1 β , IL-6 and NF- κ B activity in ischemic mice [12].

In conclusion, the current investigation has confirmed the cardio toxic effect of doxorubicin in rats. In addition we have shown a protective potential for glycyrrhizin against DOX-induced cardio toxicity which might be attributed to its antioxidant and anti-inflammatory effects suggesting that glycyrrhizin might be used as an adjuvant therapy to abrogate toxicity of DOX.

Conflicts of Interest

The authors declare no conflicts of interest.

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