Research Article

Cardioprotective effect of ammonium glycyrrhizinate against doxorubicin-induced cardiomyopathy in experimental animals

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ABSTRACT

Objective: The objective of this study was to evaluate the cardioprotective effect of herbal bioactive compound ammonium glycyrrhizinate against doxorubicin-induced cardiomyopathy, in experimental animals.

Materials and Methods: Ammonium glycyrrhizinate (50, 100, 200 mg/kg, p.o.) was administered for four weeks in albino rats. Cardiomyopathy was induced with a dose of 2.5 mg/kg i.p. of doxorubicin on 1th, 7th, 14th, 21th, 28th day in the experimental animals. At the end of the experiment, on 29th day, serum and heart tissues were collected and hemodynamic, biochemical and histopathological studies were carried out.

Results: Administration of doxorubicin in normal rats showed significant ($P < 0.001$) changes in body weight, feed intake, urine output, hemodynamic parameters like (blood pressure, heart rate, cardiac output) and in lipid profile (cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein) indicating cardiomyopathy symptoms. Animals treated with ammonium glycyrrhizinate significantly ($P < 0.05$) decreased triglyceride, cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels. Moreover, high density lipoprotein (HDL) levels increased in rats treated with ammonium glycyrrhizinate as compared with the normal group.

Conclusion: Ammonium glycyrrhizinate is effective in controlling serum lipid profile and cardiac complications in experimentally induced cardiomyopathy in animals.

KEY WORDS: Ammonium glycyrrhizinate, cardiac failure, cardiomyopathy, doxorubicin

Introduction

Cardiomyopathy is a heterogeneous group of diseases of the myocardium associated with mechanical and/or electrical dysfunction that usually (but not invariably) exhibit unsuitable ventricular hypertrophy or dilatation and occurs due to varied causes that are generally genetic related. Cardiomyopathy either is confined to the heart or is part of generalized systemic disorders, which may lead to cardiovascular death or progressive heart failure-related disability.[1]

Doxorubicin, an anthracycline antibiotic, is indicated for the treatment of a wide variety of human solid tumors and leukemia patients. However, its clinical uses are limited due to high incidence of cardiotoxicity. An initial acute effect includes hypotension and transient electrocardiographic abnormalities. The chronic effects may occur several weeks or months after administration of cumulative doxorubicin.[2] Cardiomyopathy is associated with a reduction in ejection fraction thus indicating low cardiac output.[3] However, the mechanisms by which doxorubicin induces cardiac injury and dysfunction are incompletely understood. A number of doxorubicin biochemical changes have been identified that can damage cardiac reactive oxygen species (ROS),[4] production of reactive nitrogen species,[5] selective inhibition of cardiac muscle gene expression,[6] disturbance of myocardial adrenergic signaling[7] and induction of cardiac cell apoptosis.[8]

Since doxorubicin is reported to cause cardiac adverse effects by generating free radicals in cardiac cells, several natural or synthetic products with strong antioxidant potential are expected to prevent doxorubicin-induced cardimyopathy due to their free radical scavenging property. Liquorice (*Glycyrrhiza glabra*) is a tall shrub of the leguminosae family.[9] Pharmacological potential of *G. glabra* as hypoglycemic, hypocholesteremic, antiulcer, anti-inflammatory, renoprotective,

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and antiatherogenic have been demonstrated.[10] It is also used as a laxative, emmenagogue, contraceptive, galactagogue, antiasthmatic, antiviral agent antitussive, expectorant, anticoagulant, antiulcer, antimicrobial, antiviral, antioxidant, anti-inflammatory, antidiabetic and anticancer.[11] Since the ammonium glycyrrhizinate, which is an important constituent of *G. glabra* is reported to possess strong antioxidant activity,[12] it was thought worthwhile to screen this bioactive compound for its cardioprotective role in doxorubicin- induced cardiomyopathy in experimental animals.

Materials and Methods

Characterization of Ammonium Glycyrrhizinate

Ammonium glycyrrhizinate was procured from Yucca Enterprises, Mumbai and subjected for characterization to check the purity of this marker compound. The marker was thus tested for solubility, melting point, infrared (IR) and thin layer chromotography (TLC) pattern by using the standard protocol mentioned in the literature.

Experimental Animals

Swiss albino Wistar rats of either sex weighing between 100–150 g were obtained from animal house of Lala Lajpat Rai University of Veterinary and Animal Sciences (LLRUVAS), Hisar. The animals were kept in polypropylene cages (29 × 22 × 14 cm) at room temperature (25 ± 2°C) under natural dark-light cycle. Commercial pellet diet and water was provided *ad libitum*. The experimental protocol (Pharma Sc./485) was approved by the Institutional Animal Ethics Committee (Registration no. 134/99/CPCSEA) of Maharishi Dayanand University, Rohtak which is registered with committee for the purpose of control and supervision of experiments on animal (CPCSEA), India.

Drugs and Chemicals

Formaldehyde solution 37-41%w/v, eosin-stain solution, xylene (sulfur- free), paraffin wax 60°-62°C, hematoxylin (delafield's skin solution) was purchased from Rankam chemicals Private Limited. Glacial acetic acid, glycerin, thymol (crystals), picric acid was purchased from CDH Chemicals Private Limited. Distilled water was used for all biochemical assays. Commercially available diagnostic kits were used for the measurement of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) content (Robonik Pritest, Mumbai, India). All other chemicals used for the biochemical parameters estimation were of analytical grade.

Experimental Design

The animals were divided into six groups consisted of six rats in each group. The groups were designed as 1 normal, 3 tests, and 2 controls. Normal group received normal saline solution (0.9% w/v NaCl). The test groups received ammonium glycyrrhizinate with doxorubicin (2.5 mg/kg). Doxorubicin dose was administered by intra-peritoneal route and ammonium glycyrrhizinate was administered orally. All the oral doses were prepared in distilled water.

- Group 1: Normal saline (dose - 2 ml/kg)
- Group 2: Doxorubicin (2.5 mg/kg i.p)
- Group 3: Doxorubicin (2.5 mg/kg, i.p) + ammonium glycyrrhizinate (50 mg/kg, p.o)
- Group 4: Doxorubicin (2.5 mg/kg, i.p.) + ammonium glycyrrhizinate (100 mg/kg, p.o)
- Group 5: Doxorubicin (2.5 mg/kg, i.p) + ammonium glycyrrhizinate (200 mg/kg, p.o)
- Group 6: Ammonium glycyrrhizinate (100 mg/kg, p.o).

Each group comprised of six rats and dose of doxorubicin (2.5 mg/kg) were administered on 1th, 7th, 14th, 21th and 28th day by intra-peritoneal route and 0.5 ml of volume was injected for every 100 g of rat body weight in case of doxorubicin. Doxorubicin hydrochloride (ADRIM) vial of Fresenius Oncology, Kabi, India was purchased from the local market. Ammonium glycyrrhizinate (50, 100, 200 mg/kg) was administered by oral route. The selection of dose of doxorubicin to induce cardiomyopathy and the dose of ammonium glycyrrhizinate was administered as per previously conducted studies. Toxicity study of ammonium glycyrrhizinate has already been conducted as per The Organisation for Economic Co-operation and Development (OECD) guidelines, hence were not carried out in the present study.[9]

At the end of the experimental period (i.e. on the 29th day) the rats were sacrificed for biochemical estimation. After 36 h of the last treatment, orbital blood samples were collected from all groups. Serum samples were separated for the estimation of lipid profile such as cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) The hearts were isolated for histopathological investigations. Blood pressure, heart rate and cardiac output of the experimental animals were measured by non invasive B. P. Recording instrument (AD Instruments, India).

Histopathological Studies

At the end of the experiment, heart tissues from all groups were subjected to histopathological studies. The tissue were fixed in formalin (10%), routinely processed and embedded in paraffin wax. Paraffin sections (5μm) in size were cut in glass slides, and stained with hematoxylin and eosin after de-waxing and examined under a light microscope.

Statistical Analysis

The results obtained were expressed as Mean ± SD. The statistical comparison among the groups was performed with one-way analysis of variance (ANOVA) and the significant difference between normal and experimental groups was assessed by Duncan's multiple range test (DMRT). Statistical value of $P < 0.05$ was considered to be significant.

Results

Effect on Body Weight, Urine Output and Food Intake

Doxorubicin reduced the body weight, urine output and food intake in the normal group animals. However, administration of ammonium glycyrrhizinate was able to improve all the three parameters [Table 1].

Hemodynamic Studies

Doxorubicin caused significant changes in blood pressure, heart rate and cardiac output in the normal group animals. However, ammonium glycyrrhizinate was able to improve the blood pressure, heart rate and overall cardiac output [Table 2].

Serum Lipid Levels

Animals treated with doxorubicin produced a significant increase in the levels of cholesterol, triglycerides and LDL compared with the control group ($P < 0.01$). ammonium glycyrrhizinate (50 mg/kg) + doxorubicin, ammonium glycyrrhizinate (100, 200 mg/kg) + doxorubicin group showed

Table 1:

Effect of ammonium glycyrrhizinate on body weight, food intake and urine output in doxorubicin-induced cardiotoxicity in rats (*n*=6)

Treatment	Control	Doxorubicin (2.5mg. kg)	Ammonium glycyrrhizinate (50mg/kg) +doxorubicin	Ammonium glycyrrhizinate (100mg/kg) +doxorubicin	Ammonium glycyrrhizinate (200mg/kg) +doxorubicin	Ammonium glycyrrhizinate (200mg/kg)
Body weight	74.5±3.79	133.7±2.39[a]	83.5±1.55	98.75±2.25[b]	114.5±3.79	80.7±3.25[c]
Food intake	5.5±0.39	3.1±0.36[a]	4.3±0.16	4.6±0.36[b]	5.3±0.31[c]	5.0±0.32
Urine output	6.2±0.21	5.0±0.21[a]	4.3±0.26	5.1±0.19	6.0±0.18[b]	6.4±0.23

The values are expressed as mean±S.D for six animals. Values not sharing common superscript letters [(a-c)] differ significantly at $P<0.05$ (DMRT)

Table 2:

Effect of ammonium glycyrrhizinate on blood pressure, heart rate and cardiac output in doxorubicin-induced cardiotoxicity in rats

Treatment	Control	Doxorubicin (2.5 mg.kg)	Ammonium glycyrrhizinate (50 mg/kg) +doxorubicin	Ammonium glycyrrhizinate (100 mg/kg) +doxorubicin	Ammonium glycyrrhizinate (200 mg/kg) +doxorubicin	Ammonium glycyrrhizinate (200 mg/kg)
Blood pressure	116.5±1.44	106.5±2.02[a]	112.2±3.03	121.0±2.34	126.5±1.55[b]	120±2.08[c]
Heart rate	400±1.50	275.2±3.12[a]	313±3.61	329.6±3.55	369.8±3.90[b]	388.6±3.72
Cardiac output	100.10±1.03	115.8±1.39[a]	105.2±1.281	106.6±1.24[b]	108.6±0.519	96.8±1.158

The values are expressed as mean±S.D for six animals. Values not sharing common superscript letters [(a-c)] differ significantly at $P<0.05$ (DMRT)

a significant decrease in the level of cholesterol, triglycerides and LDL compared with the doxorubicin group, but significant increase ($P < 0.01$) in the level of HDL compared with the doxorubicin group [Table 3].

Histopathological Studies

Doxorubicin injected rats showed necrosis of muscle fibers, inflammatory cell infiltration and edema with fragmentation of muscle fibers as compared with the control group. Treatment with ammonium glycyrrhizinate in doxorubicin treated rats showed moderate degree of edema, necrosis and inflammatory cells compared with doxorubicin-injected rats [Figure 1].

Discussion

Cardiomyopathy refers to any disease of the cardiac muscle, which ultimately affects muscle function. Heart failure is a common manifestation of most cardiomyopathy and is one of the leading causes of human morbidity and mortality worldwide.[13] Various stimuli in the heart have been recognized, including oxidative stress, serum withdrawal, angiotensin II, hyperglycemia, pressure overload, mitochondrial dysfunction and loss of cardiomyocytes survival factors.[14] Efforts continue in the field of medicines find better medicines for the treatment of cardiovascular diseases. Experimental and clinical trials are aimed at reducing the mortality rate and prevention of cardiovascular diseases. Some of the drugs actually have the potential as antioxidants, which are thought to be beneficial for cardiovascular health.[15,16] An integrated approach is needed to manage cardiovascular diseases using the growing body of knowledge gained through scientific developments. Further, attempts were made to understand the molecular mechanism of cardio protection by the test drugs with reference to cardiovascular biomarkers, oxidative stress parameters and histopathological examination of the cardiac tissue. The present work was designed to investigate the cardioprotective effect of ammonium glycyrrhizinate against doxorubicin-induced cardiomyopathy in experimental

Table 3:

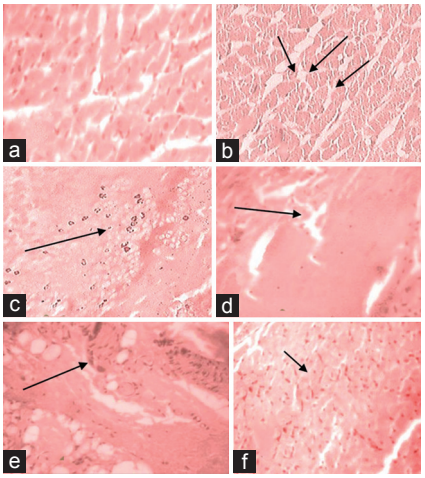
Effect of ammonium glycyrrhizinate on cardiac functions in doxorubicin-induced cardiotoxicity in rats

Groups	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Normal	71.7±0.21	63.3±0.79	25.3±0.34	33.02±0.56	12.8±0.24
Group 2	111.5±0.82[a]	112.12±4.6[a]	11.7±0.55[a]	77.5±0.78[a]	22.3±1.08[a]
Group 3	97.4±0.48	90.2±0.46	13.37±0.46	65.98±0.75	18.04±0.09
Group 4	103.2±0.54[b]	97.9±0.23[c]	16.9±0.37[b]	66.7±0.67[c]	19.5±0.05
Group 5	92.07±0.27	88.6±0.60	11.82±0.11	62.78±0.43	17.42±0.2
Group 6	79.1±1.21[c]	72.9±0.73[b]	25.4±1.42	39±0.96[b]	14.5±0.1[b]

The values are expressed as mean±S.D for six animals. Values not sharing common superscript letters (a-c) differ significantly at $P<0.05$ (DMRT). HDL=High density lipoprotein, LDL=Low density lipoprotein, VLDL=Very low density lipoprotein

animals. In this study, we took model of doxorubicin-induced cardiomyopathy to assess the therapeutic potential of the test drug i.e. ammonium glycyrrhizinate. In rats, 2.5 mg/kg of doxorubicin given subcutaneously once a week for 6 weeks produced heart failure.[17] Alternatively, doxorubicin in 2.5 mg/kg i.p on every alternate day for 2 weeks led to successful induction of cardiomyopathy and failure of the heart.[18] The major limitation of clinical use is the development of cardiotoxicity. The increased serum SGOT, SGPT, cholesterol, triglyceride, LDL, VLDL levels and decreased levels of HDL due to several mechanisms for doxorubicin-induced cardiomyopathy, which include inhibition of nucleic acid,[19] release of vasoactive amines,[20] alterations in membrane-bound enzymes,[21] abnormalities in mitochondria[22] and an imbalance of myocardial electrolytes[23] were noticed. Treatment with doxorubicin increased serum marker of SGOT and SGPT. The pre-treatment with attenuated this increase in SGOT and SGPT level. Serum level of SGOT and SGPT were significantly decreased by treatment with ammonium glycyrrhizinate solution. The increased levels of serum enzymes in myocardial toxicity may be due to the leakage of the enzymes

Figure 1: Effect of ammonium glycyrrhizinate for 29 days on histopathological alteration in normal and doxorubicin injected rats (a) Control rats (b) heart tissue administered with ammonium glycyrrhizinate (50 mg/kg) + doxorubicin showing mild focal vacoulation and loss of striations (c) heart tissue administered with ammonium glycyrrhizinate (100 mg/kg) + doxorubicin showing mild vascular congestion and hemorrhage and heat muscle show vacuolation with loss of cross striations with increased cyoplasmic eosinophilia (d) Doxorubicin induced and treated with ammonium glycyrrhizinate solution (200 mg/kg) showed focal hemorrhages and less loss of striations (e) Doxorubicin induced Cardiotoxicity heart showed more number of inflammatory cell, hemorrhage, edema and necrosis in cardiac cell (f) Ammonium glycyrrhizinate solution (100 mg/kg) showing no vacoulation, hemorrhages and loss of striations



into the blood.[24] Light micrograph of doxorubicin-injected rats showed necrosis of muscle fibers, inflammatory cell infiltration and edema with fragmentation of muscle fibers as compared with the control group. Treatment with ammonium glycyrrhizinate in doxorubicin-treated rats (ammonium glycyrrhizinate + doxorubicin) showed moderate degree of edema, necrosis and inflammatory cells compared with doxorubicin-injected rats. To conclude, the present result suggests that ammonium glycyrrhizinate prevented the doxorubicin-induced cardio-toxicity by boosting the endogenous antioxidant activity. It could be due to the antioxidant activity and restoration of myocardial biomarkers of lipid profile. Further studies are needed to understand the mechanism of action of ammonium glycyrrhizinate.

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References

1. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, *et al*.; American Heart Association; Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; Council on Epidemiology and Prevention. Contemporary definitions and classifications of the cardiomyopathies: An American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation 2006;113:1807-16.
2. El-Sayed M, El-azeem A, Afify A, Shabane H, Ahmed H. Cardioprotective effects of *Curcuma longa* L. extracts against doxorubicin-induced cardiotoxicity in rats. J Med Plants Res 2011;17:4049-58.
3. Balakumar P, Singh P, Singh M. Rodent models of heart failure. J Pharmacol Toxicol Methods 2007;56:1-10.
4. Santos RV, Batista ML, Caperuto EC, Costa Rosa LF. Chronic supplementation of creatine and vitamins C and E increases survival and improves biochemical parameters after doxorubicin treatment in rats. Clin Exp Pharmacol 2007;34:1294-9.
5. Pacher P, Liqudet L, Bai P, Mabley JG, Kaminski PM, Virag L, *et al*. Potent metalloporphyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin induced cardiac dysfunction. Circulation 2003;107:896-904.
6. Ito H, Torii M, Suzuki T. Decreased superoxide dismutase activity and increased superoxide anion production in cardiac hypertrophy of spontaneously hypertensive rats. Clin Exp Hypertension 1995;17:803-16.
7. Yoshikawa T, Handa S, Suzuki M, Nagami K. Abnormalities in sympathoneuronal regulation are localized to failing myocardium in rabbit heart. J Am Coll Cardiol 1994;24:210-5.
8. Dowd NP, Scully M, Adderley SR, Cunningham AJ, Fitzgerald DJ. Inhibition of cyclo-oxygenase-2 aggrevates doxorubicin mediated cardiac injury *in vivo*. J Clin Invest 2001;108:585-90.
9. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol 2006;46:167-92.
10. Ojha S, Golechha M, Kumari S, Bhatia J, Arya DS. Glycyrrhiza glabra protects from myocardial ischemia-reperfusion injury by improving hemodynamic, biochemical, histopathological and ventricular function. Exp Toxicol Pathol 2013;65:219-27.
11. Harwansh RK, Patra1 KC, Pareta1 SK, Singh J, Biswas R. Pharmacological studies of *Glycyrrhiza glabra*- a review. Pharmacologyonline 2011;2:1032-8.
12. Imai K, Takagi Y, Iwazaki A, Nakanishi K. Radical scavenging ability of glycyrrhizin. Free Radic Antioxid 2013;3:40-2.
13. Sheikh F, Chen JU. Mouse models for cardiomyopathy research. Prog Pediatr Cardiol 2007;24:27-34.
14. MacLellan WR, Schneider MD. Death by design. Programmed cell death in cardiovascular biology and disease. Circ Res 1997;81:137-44.
15. Kushi LH, Folsom AR, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N Engl J Med 1996;334:1156-62.
16. Miller LG. Herbal medicinal: Selected clinical considerations focusing on known or potential drug-herb interactions. Arch Intern Med 1998;158:2200-11.
17. Scenczi O, Kemecsei P, Hollthuijsen MF, Van Riel NA, Vander GJ, Pacher P, *et al*. Poly (ADP-ribose) polymerase regulates myocardial calcium handling in doxorubicin-induced heart failure. Biochem Pharmacol 2005;69:725-32.
18. Lou H, Danelisen I, Singal PK. Cytokines are not upregulated in doxorubicin-induced cardiomyopathy and heart failure. J Mol Cell Cardiol 2004;36:683-90.
19. Arena E, Bionda F, D'Alessndro N, Dusoncher L. Gebbia N, Gerbasi R. DNA, RNA protein synthesis in heart, liver and brain of mice treated with denaturation of adriamyin. Int Res Commun Syst Med Sci 1984;2:10543-1061.
20. Bristow MR, Mason JW, Billingham ME, Daniels JR. Dose-effect and structure-function relationships in doxorubicin cardiomyopathy. Am Heart J 1981;102:709-18.
21. Singal PK, Panagia V. Direct effect of Adriamycin on the rat heart sarcoplasma. Res Commun Chem Pathol Pharmacol 1984;43:67-77.
22. Singal PK, Segstro RJ, Singh RP, Kutryk MJ. Changes in lysosomal morphology and enzyme activities during the development of adriamycin induced cardiomyopathy. Can J Cardiol 1985;1:139-47.
23. Oslan HM, Younh DM, Prieur DJ, LeRoy AF, Reagan RL. Electrolyte and oxidation products of certain lipids. J Biol Chem 1974;174:257-64.
24. Bruce JIe. Plasma membrane calcium pump regulation by metabolic stress. World J Biol Chem 2010;1:221-8.

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