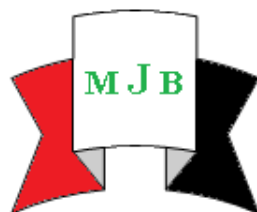


Rosemary Leaves Aqueous Extract for Protection against Acute Doxorubicin-Induced Cardiotoxicity in Mice

Entisar Jawad Al Mukhtar* Zena Hasan Sahib* Afraa M. Jawad* Hamid Naji*
College of Medicine, Babylon University
E-mail: ejhas28@yahoo.com, drzinahasan@yahoo.com



Received 18 January 2015

Accepted 18 February 2015

Abstract

Doxorubicin (DOX) is a cancer chemotherapy widely used to treat a many types of human malignancies, DOX is potent anthracycline antibiotic. As a complicating effect of DOX cardiotoxicity has long been recognized; To explain this cardiotoxicity there are several hypotheses and the most thoroughly investigated one is free radical hypothesis. Our study was designed to investigate if the aqueous extract of rosemary leaves has a protective effect against cardiotoxicity induced by DOX in mice.

Twenty eight male Swiss Albino mice were randomly divided into four groups including group1 (negative control), treated with distill water (D.W), group 2 (positive control), treated with 15 mg/kg DOX as a single intraperitoneal (i.p) injection, groups 3 and 4 received 15mg/kg and 30mg/kg respectively of the aqueous extract of *Rosmarinus officinalis* leaves (ROE) orally (p.o), once daily for 2 weeks, then injected i.p with 15 mg/kg DOX. Two days after DOX or D.W (in control group) injection, animals in all groups were scarified and the levels of the cardiac biomarkers including serum creatine kinase (CK-MB) and serum lactate dehydrogenase (LDH) were measured. Also the cardiac histopathological sections were prepared, stained by hematoxylin and eosin stains and examind under light microscope.

The administration of 15mg/kg DOX caused cardiomyopathy which was manifested by extremely significant elevation ($p<0.001$) in serum CK-MB and LDH levels. In addition, cardiac histopathological sections showed moderate cytoplasm vacuolization and inflammatory cells infiltrate with vascular congestion. Oral administration of 30mg/kg ROE for 2 weeks prior to DOX provided significant protection which was evidenced by extremely significant reduction ($p<0.001$) in the levels of CK-MB and LDH. Moreover, histopathological sections revealed only mild cytoplasm vacuolization, infiltration of inflammatory cells and vascular congestion in comparison to DOX positive control group ($p<0.01$). Whereas oral administration of 15mg/kg ROE for 2 weeks prior to DOX showed no significant protection neither in CK-MB and LDH levels, nor in the histopathological sections.

Administration of 30mg/kg ROE protect against DOX-induced cardiotoxicity. This might serve as novel adjuvant therapy with DOX.

Key words: Doxorubicin, *Rosmarinus officinalis*, cardiotoxicity, mice

الخلاصة

إن عقار الدوكسوروبيسين هو مضاد حيوي فعال من مجموعة الانثراسايكلين يستعمل لمعالجة عدة أنواع من الأورام السرطانية. إن التأثير السمي على القلب هو من أهم الآثار الجانبية لهذا العقار. لقد أجريت هذه الدراسة لمعرفة فاعلية نبات إكليل الجبل للحماية ضد التأثير السمي للدوكسوروبيسين على القلب في ذكور الفئران.

لقد تم استخدام ٢٨ ذكرا من الفئران البالغة لإجراء هذه الدراسة حيث قسمت عشوائيا إلى أربعة مجاميع. أعطيت المجموعة الأولى الماء المقطر عن طريق الفم (بواسطة الأنبوب المعدني) لمدة أسبوعين، أما المجموعة الثانية فقد تم حقنها (داخل البريتون) بعقار الدوكسوروبيسين (١٥ ملغم/كغم) كجرعة مفردة، إما المجموعة الثالثة والرابعة فقد عولجت بجرعتين (١٥ و ٣٠ ملغم/كغم بالترتيب) من المستخلص المائي لأوراق نبات إكليل الجبل عن طريق الفم مرة واحدة يوميا لمدة أسبوعين ومن ثم تم حقنها (داخل البريتون) بعقار الدوكسوروبيسين (١٥ ملغم/كغم) كجرعة مفردة. تم سحب الدم بعد يومين من حقن الماء المقطر (للمجموعة الأولى) او الدوكسوروبيسين (لبقية المجاميع) حيث تم قياس مستوى

إنزيمي اللاكتيت ديهيدروجينيز (lactate dehydrogenase) والكرياتين كينيز (creatine kinase MB) في مصل الدم. كما تم تقطيع قلوب الفئران بعد قتلها وتحضير المقاطع النسيجية وصبغها بصبغة الهيماتوكسيلين والايوسين حيث فحصت تحت المجهر الضوئي للكشف عن أي تغيرات نسيجية محتملة.

لقد بينت نتائج هذه الدراسة حصول انخفاض معنوي ($p < 0.001$) في إنزيمي الاكتيت ديهيدروجينيز والكرياتين كينيز-MB في مصل الدم للمجموعة التي عولجت بالمستخلص المائي لأوراق نبات إكليل الجبل بجرعة ٣٠ ملغم/كغم لمدة أسبوعين قبل حقنها بالدوكسوروبيسين مقارنة بالمجموعة التي حقنت بالدوكسوروبيسين فقط، كما أكد هذا بنتائج الفحص النسيجي حيث حصل انخفاض معنوي ($p < 0.01$) في نسبة التغيرات النسيجية المرضية في العضلة القلبية لهذه المجموعة. أن المستخلص المائي لأوراق نبات إكليل الجبل بجرعة ١٥ ملغم/كغم المعطى لمدة أسبوعين قبل حقن الدوكسوروبيسين لم يوفر الوقاية الكافية لمنع التأثير السمي للدوكسوروبيسين على القلب كما ظهر ذلك في مستوى إنزيمي الاكتيت ديهيدروجينيز والكرياتين كينيز-MB وفي المقاطع النسيجية. إن نبات إكليل الجبل المعطى بجرعة ٣٠ ملغم/كغم لمدة اسبوعين قبل حقن الدوكسوروبيسين كان له دور في حماية القلب من التأثير السمي لعقار الدوكسوروبيسين.

الكلمات المفتاحية: الدوكسوروبيسين، إكليل الجبل، سمية القلب، الفئران.

Introduction

Chemotherapy-induced cardiotoxicity

Several antineoplastic agents can cause cardiovascular toxicity [1]. Anthracyclines is a group of such antineoplastic and biologic agents which are recognized to cause cardiotoxicity [2]. The potential cardiovascular toxicities associated with the use of anticancer drugs range from myocardial injury (which can lead to myocardial infarction) to disturbances in conduction with cardiac arrhythmia, pericarditis and/or myocarditis, cardiomyopathy, hypertension and congestive heart failure (CHF), also abnormality in the electrocardiography can occur [3, 4]. Doxorubicin cardiovascular toxicities are either acute, subacute or chronic, the acute toxicity occurs during or just after DOX infusion, while subacute toxicity occurs after days or weeks of DOX administration. Whereas chronic toxicity appeared after months (or may be years) of the last course of treatment with DOX. [5].

Doxorubicin (DOX):

Doxorubicin (also called adriamycin) is a potent anthracycline antibiotic that is largely used as a chemotherapeutic agent to treat many sort of malignancies.[6,7] Including solid and hematopoietic tumors. [8,9] DOX is an important component included in the chemotherapeutic regimens of small-cell lung or breast cancers. DOX may be the best obtainable chemotherapeutic agent used to treat metastatic thyroid cancer, in addition it is

an important component for the treatment of Hodgkin's or non-Hodgkin's lymphomas and many other cancers [10,11].

Mechanisms of action of doxorubicin:

The exact mechanism of action of DOX as anticancer agent is not fully understood, it was attributed to the intercalation into DNA, leading to inhibition of macromolecular synthesis, thus it prevents the cancer cells which are growing rapidly from replication [6,7,12].

Today, it is thought that topoisomerase II- α inhibition (induced by DOX) is the main mechanism for DOX actions.[9,12,13,14] Thus, DOX stabilizes the intermediate reaction by which DNA strands are broken and bound to tyrosine residues (in the topoisomerase II) covalently, preventing the following DNA resealing. Thus, relax the supercoiled DNA will prevent duplication and transcription of the DNA. Furthermore, apoptosis of cancer cells may be triggered by the breaks of the DNA strand, through the p53-dependent pathway.[15] Other mechanism of action include metal ion chelation and free radical generation, leading to DNA damage or lipid peroxidation,[6,16,17] it also may induce apoptosis.[18] DOX also can reduce the viability of cancer cells via RNA damage and inhibit the synthesis of DNA, RNA and proteins.[7,19]

Doxorubicin-induced cardiotoxicity:

Although DOX has been shown to be effective, its clinical usage is limited by the occurrence of cardiomyopathy which is

cumulative dose-dependent which may appear several years after the removal DOX treatment.[14] Three characteristic types of cardiotoxicity induced by DOX which are the main important harms associated with the clinical use of DOX. First, acute cardiac toxicity which appeared as hypotension and myocardial injury occurs immediately after a single dose of DOX most likely as a form of cardiac arrhythmia.[20,21] Second, chronic cardiotoxicity which is a cumulative dose-dependent cardiomyopathy resulting in cardiomyopathy which appeared as cardiac dysfunction that can lead to CHF, which is a common and an important type of cardiac damage.[20,22] Third, arrhythmia and ventricular dysfunction represent the late-onset cardiomyopathy which appeared after years or decades of DOX treatment removal [23,24].

DOX is given as an intravenous infusion (40–75 mg/m²) that can be repeated at intervals of three to four weeks, but it should not pass the cumulative dose (450–550 mg/m²).[20] The clinical usefulness of DOX therapy with the above doses is limited by the myocardium toxicity induced by DOX which represent a serious CHF. However many tachyarrhythmias can occur with the acute use of a single high dose of DOX, which is a progressive worsening of cardiac contractile function that can limits DOX clinical usefulness. With the use of the cumulative doses of DOX greater than 500 mg/m², the percentage of cardiac failure is high as 20% in patients.[20,22] Dysfunction of the myocardium may appeared within the latter phases of therapy or may be late to be latent for months or even for more than 20 years after the last administration of the DOX which is principally represent a challenging problem when DOX is used to treat cancers in childhood.[23,24]

Mechanisms of doxorubicin cardiotoxicity:

Several mechanisms have been implicated in the progression of DOX cardiotoxicity, including gene expression reduction, nucleic acid and protein

synthesis inhibition, impaired mitochondrial respiratory chain function, vasoactive amines release, adrenergic function alteration, calcium handling aberration and myocardial energy metabolism alteration. [25,26,27] Besides, DOX is metabolized into a more toxic metabolite doxorubicinol (DOXol) which accumulates in cardiac tissues and extensively contributes to the development of DOX- induced chronic cumulative cardiac toxicity.[28] Myocyte cell death by both apoptosis and necrosis has also been suggested.[18,29] The formation of reactive oxygen species (ROS) that is mediated by iron and the following encouragement of the myocardial oxidative stress is still the most accepted probable mechanism.[9,30]

Herbal protection against chemotherapeutic toxicity:

Anciently herbal therapy play an important role as a traditional and alternative medicine that is used to treat various diseases.[31] Large number of naturally occurring compounds are present in the plants which are included in our diet, such constituents may have a protecting favorable effect for the body. In damaged systems one or more of the following actions: (1) free radicals scavenging (2) cellular antioxidants elevation (3) promotion for the recovery of bone marrow (4) regeneration of extraheamatological tissue, which are induced by plants or herbs may represent the suggested principal mechanisms of protection.[32] Rosmary is one of the plants whose constituents reputed to having antioxidant properties.

Rosmarinus Officinalis (RO):

Rosmarinus Officinalis (also called Rosemary) a perpetual evergreen shrub. It is one of the herb spices of Labiatae family. It was cultivated in Mediterranean first, then transplanted to China in Dynasty, but cultivated in all of the world now.[33] It's leaves are needle-like.[34] Traditionally rosemary is one the herbs which have a wide range of uses in folk medicine or cosmetics, in addition it is used as a foods flavor.[35] Medicinally the essential oil of RO has a powerful antibacterial,

antiphlogistic, cytotoxic, antimutagenic and chemopreventive properties. [36,37] *R. Officinalis* leaves have a wide range of bioactivities; such as antioxidant[38,39], antitumor,[40,41] antibacterial [34,37] and anti-inflammatory actions. [42] These bioactivities of rosemary leaves extract are comparable with known antioxidants constituents, such as carnosic acid, carnosol, rosmarinic acid, ursolic acid, butylated hydroxytoluene and butylated hydroxyanisole, with no associate risk to carcinogenicity or cytotoxicity of the synthetic antioxidants. [43,44]

This study aimed to investigate the possible protective effect of *Rosmarinus officinalis* leave extract (ROE) on cardiac tissue against DOX-induced cardiotoxicity.

Materials and Methods:

1. Plant Extract Preparation:

Rosemary's leaves were purchased locally from the market of Hilla city and then identified by a specialist of botany in the science collage for girls, at Babylon university, Iraq. After a careful washing of the leaves they were dried by air in the shadow at room temperature, then leaves were grinded into fine powder. Extract of the plant was prepared by adding 40 gm of the powder to 80 ml of distill water and by refluxing with sohxcilate instrument for thirty six hours at 50-60 °C. By evaporating the liquid contents of the mixture through the use of an incubator, pellets' extract were obtained. By dissolving the pellets with distilled water the desired amount of the extract was prepared , and by using the stomach tube the extract was administered at a doses of 15 mg/kg and 30 mg/kg body weight daily for 14 consecutive days. [45]

Animals:

Twenty eight male Swiss Albino mice (weighting 25 – 30 g) were used in this study. The mice kept in the animal house of the college of medicine / Babylon university for two weeks previous to and during the employment of the experiment under constant surrounding temperature (22 ± 2) °C and light cycle (12:12hr daylight: gloomy). Animals were maintained on a

standard commercial mice chow and tap water were presented *ad-libitum*.

2. Experimental design:

The mice were divided randomly into 4 groups (7 mice in each group) as follows:

Group 1 (negative control): received 0.3ml D.W, orally (p.o) by using stomach tube once daily for two weeks, then intraperitoneally (i.p) injected with D.W

Group 2 (positive control): received 0.3ml D.W, orally (p.o) by using stomach tube once daily for two weeks, then injected i.p with 15mg/kg DOX (EBEWE Pharma Ges.m.b.H. Nfg.KG, AUSTRIA). [46,47,48]

Group 3: received 15 mg/kg of ROE, p.o once daily for two weeks, then i.p injected with 15 mg/kg DOX.

Group 4: received 30 mg/kg of ROE, p.o once daily for two weeks, then i.p injected with 15 mg/kg DOX.

After 2 days of DOX or D.W (in negative control group) injection, animals in all groups were scarified [47] under light anesthesia with diethyl ether. Blood was collected by cardiac puncture to measure the levels of creatine kinase (CK-MB) and lactate dehydrogenase (LDH) in the serum. [49] Then the hearts were extracted and fixed in 10% formalin to investigate the probable histopathological changes.

Blood samples preparation:

The blood was aspirated from the heart of mice in all groups 2 days after D.W. or DOX injection.[50]The blood was directly collected through intracardiac puncture by disposable plastic syringes and immediately transferred into plastic test tubes without anticoagulant and left for 15 - 20 minutes at room temperature to promote blood coagulation. Serum was obtained after centrifugation at 3000 rpm for 10 minutes and preserved at -20 °C until the determination of serum CK-MB, LDH.[51]

Measurement of serum creatine kinase (CK-MB isozyem) activity:

Stein method According to the National Committee for Clinical Laboratory Standards was used to measure CK level

and by using the diagnostic kits Biolabo sa maizy France .[52]

Measurement of serum lactate dehydrogenase (LDH) activity:

Henry method (according to SFBC recommendations) was used to measure LDH level and by using a Biolabo sa maizy France kit.[53]

Histopathological slides preparation:

The hearts of mice were sectioned, and by using the haematoxiline and eosin stain they were stained and prepared for the examination by using light microscope to detect the histopathological changes.

Statistical Analysis

The SPSS version 17.0 was used for the statistical analysis, one - way ANOVA test was used in this study for serum CK-MB and LDH, while chi-square test was used for histopathological changes. The mean $\pm$ SD was used to express our data, statistically less than 0.05, 0.01 and 0.001 P-values were considered either significant, high significant and extremely significant respectively.[54]

Results

1. Biochemical (Table 1):

In group 2 (received 15mg/kg DOX only) haematological examination showed extreme significant ($p < 0.001$) raise in the levels of both serum CK-MB and serum LDH activities, in comparison to mice in group 1 (received D.W). In group 4 (received 30mg/kg ROE 2 weeks before DOX injection) ROE treatment resulted in an extreme significant reduction ($p < 0.001$) in the levels of both serum CK-MB and serum LDH activities, when compared to the mice in group 2, while in the group 3 (received 15mg/kg ROE 2 weeks before

DOX injection) ROE treatment showed no significant changes ($p > 0.05$) in the levels of both serum CK-MB and serum LDH activities in comparison to mice in group 2 and 4.

2. Histopathological (Table 2) :

1- Cardiac histopathological sections for all mice in group 1 (negative control) (100%) showed normal cardiac histology (Fig. 1)

2- Cardiac histopathological sections for mice in group 2 (positive control) which injected with DOX (15mg/kg) only, showed moderate cytoplasm vacuolization and inflammatory cells infiltrate in 6(85.72%) mice with mild cytoplasm vacuolization with inflammatory cells infiltrate in 1(14.28%) mouse, that was highly significantly different from negative control group ($P < 0.01$), (Fig. 2).

3- Cardiac histopathological sections for mice in group 3 which received ROE 15mg/kg for 2 weeks before DOX (15mg/kg) injection showed moderate cytoplasm vacuolization and inflammatory cells infiltrate with vascular congestion in 5 (71.43%) mice with mild cytoplasm vacuolization, inflammatory cells infiltrate and vascular congestion in 2(28.57%) mice, which showed no significant difference from DOX positive control group ($p > 0.05$), (Fig. 3).

4- Cardiac histopathological sections for mice in group 4 which received ROE 30mg/kg for 2 weeks before DOX (15mg/kg) injection showed mild cytoplasm vacuolization with inflammatory cells infiltrate in 6(85.72%) and moderate cytoplasm vacuolization with inflammatory cell infiltrate in 1(14.28%) mouse only which was highly significantly different from DOX positive control group ($P < 0.01$), (Fig. 4).

Table 1: Serum CK-MB and serum LHD changes in the experimental groups (results are expressed as mean $\pm$ SD)

Groups	Serum CK-MB isozyem (U/L) Mean $\pm$ SD	Serum LHD (U/L) Mean $\pm$ SD
Negative control	552.85 $\pm$ 46.08	277.85 $\pm$ 28.84
Positive control	776.42 $\pm$ 30.91 ^a	582.14 $\pm$ 35.33 ^a
DOX + ROE 15mg/kg 2 weeks before DOX	774.28 $\pm$ 26.36 ^a	570.71 $\pm$ 31.14 ^a
DOX + ROE 30mg/kg 2 weeks before DOX	616.42 $\pm$ 40.48 ^{a,b}	464.28 $\pm$ 29.78 ^{a,b}

a=P< 0.001 against negative control; b=P< 0.001 against DOX positive control.

Table 2: Histopathological changes in the experimental groups(results are expressed as mean $\pm$ SD)

Experimental groups	Normal cardiac histology	Mild cytoplasm vacuolization + inflammation+ vascular congestion	Moderate cytoplasm vacuolization + inflammation + vascular congestion	Total
Negative control	7 (100%)	0	0	7 (25%)
Positive control	0	1 (14.28%)	6 (85,72%) ^a	7 (25%)
DOX + ROE 15mg/kg 2 weeks before DOX	0	2 (28.57%)	5 (71.43%) ^a	7 (25%)
DOX + ROE 30mg/kg 2 weeks before DOX	0	6 (85.72%) ^{a,b}	1 (14.28%) ^b	7 (25%)
Total	7 (25%)	9 (32.14%)	12 (42.86%)	28 (100%)

a= P< 0.01 against negative control; b= P< 0.01 against DOX positive control group.

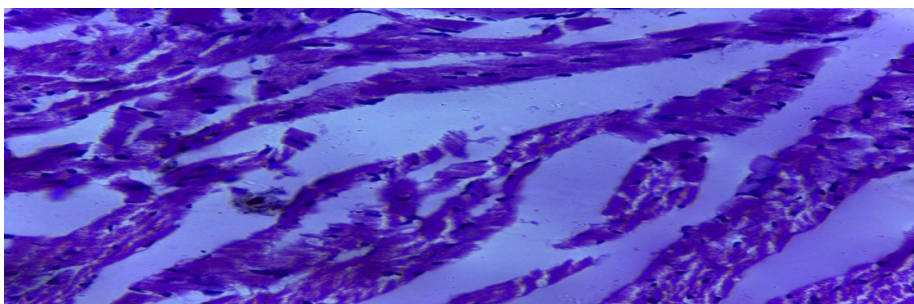


Figure 1: Cardiac section show the normal histology in group1 (magnification x10).

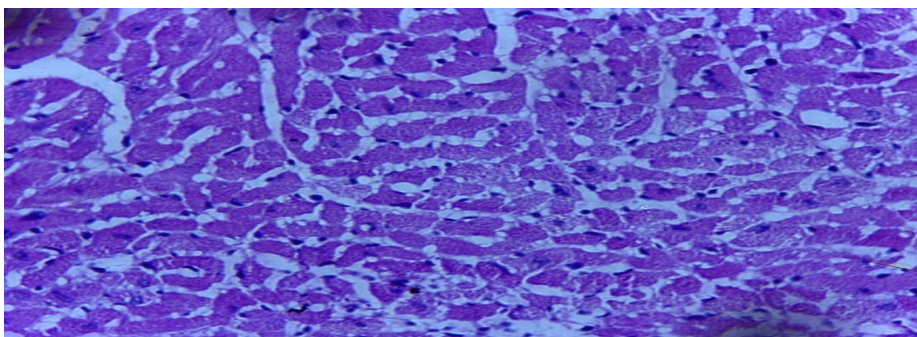


Figure 2: Cardiac histopathological section show the mild cytoplasm vacuolization, inflammatory cells infiltrate and vascular congestion in group 2 (magnification x10).

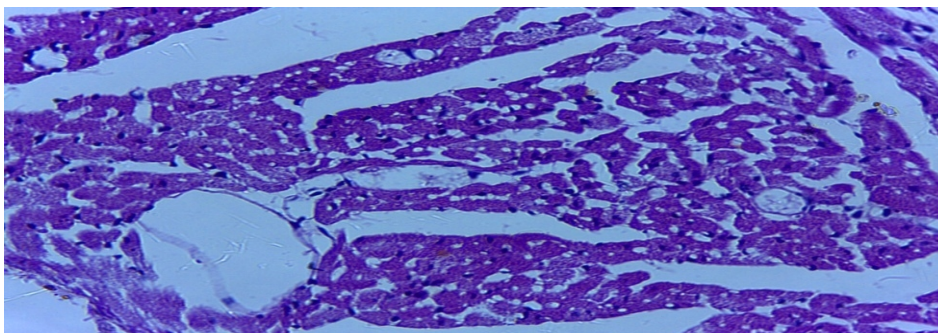


Figure 3: Cardiac histopathological section show the moderate cytoplasm vacuolization, inflammatory cells infiltrate and vascular congestion in group 3 (magnification x10).

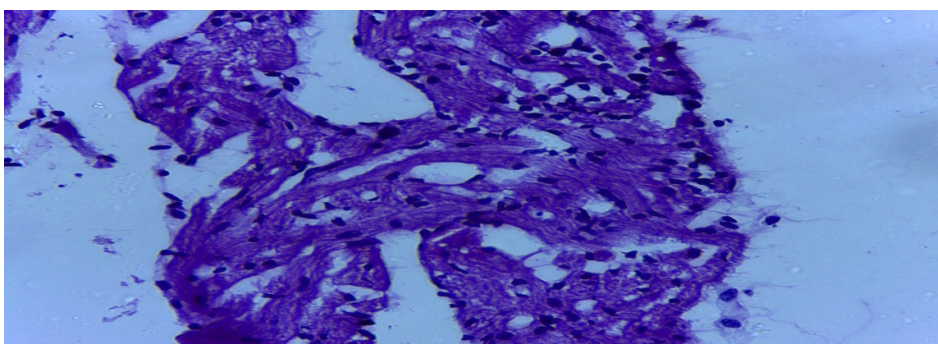


Figure 4: Cardiac histopathological section show the mild cytoplasm vacuolization, inflammatory cells infiltrate and vascular congestion in group 4 (magnification x10).

Discussion

Doxorubicin-derived free radicals may be responsible for the cytotoxic and or genotoxic effects of this drug and its ability to provoke apoptosis which may be mediated by different mechanisms such as ROS production, cellular macromolecules alkylation, intercalation of DNA and cross-linking, peroxidation of lipid, damage of cell membrane, production of ceramide and induction of p53 in a variety of

tissues.[16,55] DOXol and aglycones can cause damage of cardiac cell either by perturbing iron homeostasis or by oxidative stress induction, respectively.[56] Also, the DOX-iron (Fe^{2+}) complex formation that also have the ability to produce free radicals (interact with hydrogen peroxide to generate the radical of hydroxyl ($\text{OH}^{\bullet}$)) may mediate the cardiac cellular damage caused by DOX, through the interaction with protein, lipids and other constituents

of the cell, the ROS can cause damage to the cell membranes of the cardiac muscle cells and mitochondria.[57] However, more recent studies have shown that the oxidative stress is not an important contributor to the DOX anticancer effect.[14]

It has been shown that the antioxidants have a beneficial property against cardiotoxicity induced by DOX in mice and rats[58] as they protect cells and tissues from free radicals induced oxidative damage and injury.[59] Thus when the antioxidant related defense mechanisms are found in a lesser quantity, cardiac tissue is particularly more susceptible to be injured with ROS induced by anthracycline.[60] The reduced endogenous antioxidants, with subsequent increase in oxidative stress lead to loss of myofibrils and vacuolization of myocardial cells.[61] Removal of the excessive ROS and reactive nitrogen species by antioxidants can prevent or mitigate DOX-induced cardiotoxicity. [14,62]

The antioxidant treatment provide protection against DOX-induced cardiac contractile dysfunction in both experimental and clinical settings, suggesting that increased ROS are important in the development of this condition.[63] Results of our study indicate that 15 mg/kg of DOX caused an oxidative stress in the tissue of the heart which was distinct by the elevated levels of LDH and CK-MB in the serum, these enzymes represent a markers indicate the early and delayed injuries to which cardiac tissue can exposed particularly throughout the follow-up to DOX treatment clinically.[62] These results are agree with Abd-Allah *et al* (2002)and with Ganesh and Tiyyagur(2014)[47,48], and with other numerous former studies which confirmed similar augmentation in the activity of cardiac enzymes in rats subsequent to a challenge by DOX single and/or cumulative doses. [64,65,66] Also the histopathological changes found by our study in DOX- only treated group are in agreement with other studies which found a powerful relationship between oxidative stress and the inflammatory response of the cardiac tissue

including release of cytokine release following DOX administration. [67,68] In that regard, DOX is known to induce NFK-B activation and COX-2 expression in cardiomyocytes.[46] Also, it was reported that DOX induced changes in the properties of membrane bound ATPases of cardiac cells affect cardiac function and may be a relevant mechanism leading to lethal myocardial cellular injury. [66] In addition to that, DOX mediated vascular congestion may contribute to this pathogenesis. [69]

It has been found that the extract of RO leaves contains potent antioxidants like flavonoids, phenols, volatile oil and terpenoids. [43] Also Moreno *et al* (2006) reported that the extracts of rosemary having high scavenging competence against diverse type of ROS and species of nitrogen, it has been thought that the ability for free radicals scavenging is one of the major mechanisms by which the phenolic phytochemicals act as antioxidant. [70] Amongst antioxidant components found in the leaves of rosemary, about 90% of the antioxidant action could be related to the presence of carnosic acid and carnosol. [71] In another study the supplementation of chicken with rosemary extract result in an efficient sluggish in lipid peroxidation. [72]

In the present study oral administration of 30mg/kg ROE 2 weeks prior to DOX produced a reduction in CK-MB and LDH levels, in addition to the histopathological sections that show only mild cytoplasmic vacuolization, inflammatory cells infiltration and vascular congestion in comparison to DOX positive control group. Whereas oral administration of 15mg/kg ROE 2 weeks prior to DOX showed no significant protection neither in CK-MB and LDH levels, nor in the histopathological sections. The favorable action of RO could be ascribed to antioxidative effect of any of it's constituents [38,39], that may reduce cardiac toxicity due to DOX-induced oxidative stress. [70]

Conclusions

Our study revealed that aqueous extract of rosemary reduce cardiac toxicity induced by DOX. in mice.

References

1. **Ewer**, M. S. and Ewer, S. M. (2010). Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. *Nat Rev Cardiol*;7(10): 564-575.
2. **Meinardi**, M.T.; Gietema, J.A.; Veldhuisen, D.J. van; Graaf, W.T. van der; Vries, E.G.E. and Sleijer, D.T. (2000). Long-term chemotherapy-related cardiovascular morbidity. *Cancer Treat Rev*;26:429-447.
3. **Wojtacki**, J.; Lewicka-Nowack, E. and Lesniewski-Kmak, K. (2000): Anthracycline-induced cardiotoxicity: clinical course, risk factors, pathogenesis, detection and prevention - review of the Literature. *Med Sci* ;6:411-420.
4. **Eschenhagen**, T.; Force, T.; Ewer, M.S.; de Keulenaer, G.W.; Suter, T.M.; Anker, S.D.; Avkiran, M.; de Azambuja, E.; Balligand, J.L.; Brutsaert, D.L.; Condorelli, G.; Hansen A.; Heymans, S.; Hill, J.A.; Hirsch, E.; Hilfiker-Kleiner, D.; Janssens, S.; de Jong, S.; Neubauer, G.; Pieske, B.; Ponikowski, P.; Pirmohamed, M.; Rauchhaus, M.; Sawyer, D.; Sugden, P.H.; Wojta, J.; Zannad, F. and Shah, A.M. (2011). Cardiovascular side effects of cancer therapies: a position statement from the Heart Failure Association of the European Society of Cardiology. *European Journal of Heart Failure*; 13(1):1-10.
5. **Chanan-Khan**, A.; Srinivasan, S. and Czuczman, M.S. (2004): Prevention and Management of Cardiotoxicity From Antineoplastic Therapy: supportive oncology; 2(3) :251-266.
6. **Sauter**, K.A.D.; Magun, E.A.; Iordanov, M.S. and Magun, B.E. (2010). ZAK is required for DOX, a novel ribotoxic stressor, to induce SAPK activation and apoptosis in HaCaT cells *Cancer Biology & Therapy*, 10 (3): 258-266.
7. **Shi**, Y.; Moon, M.; Dawood, S.; McManus, B. and Liu, P. (2011). Mechanisms and management of DOX cardiotoxicity. *Herz*, 36 (4): 296-305.
8. **Lothstein**, L.; Israel, M.; Sweatman, T. W. (2001). Anthracycline drug targeting: cytoplasmic versus nuclear- a fork in the road. *Drug Resist. Updat.* 4:169-177.
9. **Simunek**, T.; Stirba, M.; Popelova, O.; Adamcova, M.; Hrdina, R. and Gersl, V. (2009). Anthracycline-induced cardiotoxicity: Overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacological Reports*, 61 (1): 154-171.
10. **Kufe**, D.W.; Holland, J.F. and Frei E. (2003). American Cancer Society: Cancer medicine 6., BC Decker, Hamilton.
11. **Smith**, L.A.; Cornelius, V.R.; Plummer, C.J.; Levitt, G.; Verrill, M.; Canney, P. and Jones, A. (2010): Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. *BMC Cancer*; 10:337.
12. **Hynek**, D.; Krejcova, L.; Zitka, O.; Adam, V.; Trnkova, L. and Sochor, J. (2012). Electrochemical Study of DOX Interaction with Different Sequences of Double Stranded Oligonucleotides, Part II, *Int. J. Electrochem. Sci.*, 7: 34-49.
13. **Horenstein**, M.S.; Vander Heide, R.S. and L'Ecuyer, T.J. (2000): Molecular basis of anthracycline-induced cardiotoxicity and its prevention. *Mol. Genet. Metab.*; 71(1-2):436-444.
14. **Minotti**, G.; Menna, P.; Salvatorelli, E.; Cairo, G. and Gianni, L. (2004): Anthracyclines: molecular advances and pharmacologic developments in antitumor activity cardiotoxicity. *Pharmacol Rev*;56: 185-229.
15. **Ruiz-Ruiz**, C., Robledo, G., Cano, E., Redondo, J. M., and Lopez-Rivas, A. (2003). Characterization of p53-mediated up-regulation of CD95 gene expression upon genotoxic treatment in human breast tumor cells. *J. Biol. Chem.* 4,4.

16. **Sparreboom**, A.; Nooter, K. and Verweij, J. (2002). Mechanisms of Action of Antitumour Antibiotics. Rotterdam Cancer Institute, Netherlands. pp. 4.
17. **Xu**, X.; Persson, H.L. and Richardson, D.R. (2005). Molecular pharmacology of the interaction of anthracyclines with iron. *Mol. Pharmacol.*, 68: 261-271.
18. **Takemura**, G. and Fujiwara, H. (2007). DOX-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Prog Cardiovasc Dis*, 49: 330-352.
19. **Fimognari**, C.; Sestili, P.; Lenzi, M.; Cantelli-Forti, G. and Hrelia, P. (2009). Protective effect of creatine against RNA damage. *Mutat Res*; 670: 59-67.
20. **Koka**, S. and Kukreja, R.C. (2010). Attenuation of doxorubicin-induced cardiotoxicity by Tadalafil : A Long Acting Phosphodiesterase-5 Inhibitor. *Mol Cell Pharmacol* ;2(5):173-178.
21. **Miyata**, S.; Takemura, G.; Kosai, K.; Takahashi, T.; Esaki, M.; Li, L.; Kanamori, H.; Maruyama, R.; Goto, K.; Tsujimoto, A.; Takeyama, T.; Kawaguchi, T.; Ohno, T.; Nishigaki, K.; Fujiwara, T.; Fujiwara, H. and Minatoguchi, S. (2010). Anti-fas gene therapy prevents doxorubicin- induced acute cardiotoxicity through mechanisms independent of apoptosis. *American Journal of Pathology*.176:687-698.
22. **Von Hoff**, D. D., Layard, M. W., Basa, P., Davis, H. L., Jr., Von Hoff, A. L., Rozenzweig, M., and Muggia, F. M. (1979). Risk factors for doxorubicin-induced congestive heart failure. *Ann. Intern. Med.*, 91: 710-717.
23. **Lipshultz**, S. E., Colan, S. D., Gelber, R. D., Perez-Atayde, A. R., Sallan, S. E., and Sanders, S. P. (1991). Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N. Engl. J. Med.*, 324: 808-815.
24. **Steinherz**, L. J., Steinherz, P. G., Tan, C. T., Heller, G., and Murphy, M. L. (1991). Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *J. Am. Med. Assoc.*, 266: 1672-1677.
25. **Migrino**, R. Q.; Aggarwal, D.; Konorev, E.; Brahmabhatt, M. and Bright, M. (2008). Detection of doxorubicin cardiomyopathy using 2-dimensional strain echocardiography. *Ultrasound in Medicine and Biology*, 34 (2): 208-214.
26. **Sawyer**, D.B.; Peng, X.; Chen, B.; Pentassuglia, L. and Lim, C.C. (2010). Mechanisms of Anthracycline Cardiac Injury: Can we identify strategies for cardio-protection? *Prog Cardiovasc Dis.*; 53 (2): 105-113.
27. **Al-Biltagi**, M.; Tolba, O.A.; El-Shanshory, M.R.; El-Shitany, N.A. and El-Hawary, E.E. (2012). Strain Echocardiography in Detection of DOX-Induced Left Ventricular Dysfunction in Children with Acute Lymphoblastic Leukemia, *ISRN Pediatrics*, 1-9.
28. **Novotna**, R.; Wsol, V.; Xiong, G. and Maser, E. (2008). Inactivation of the anticancer drug doxorubicin and oracin by aldo-keto reductase 1C3. *Toxicol Lett.*; 181 (1): 1-6.
29. **Sayed-Ahmed**, M.M.; Al-Shabanah, O.A.; Hafez, M.M.; Aleisa A.M. and Al-Rejaie S.S. (2010). Inhibition of gene expression of heart fatty acid binding protein and carnitine in DOX cardiomyopathic rat model. *Eur J Pharmacol*; 640 (1-3): 143-149.
30. **Alshabanah**, O.A.; Aleisa, A.M.; Hafez, M.M.; Al-Yahya, A.A.; Bakheet, S.A.; Al Rejaie, S.S.; Al-Harbi, M.M.; and Sayed-Ahmed, M.M. (2012). Desferrioxamine Attenuates DOX-Induced Acute Cardiotoxicity through TFG- β /Smad p53 Pathway in Rat Model. *Oxidative Medicine and Cellular Longevity*, 1-7.
31. **Nabavizadeh**, S, H.; Majid, O. and Anushiravani, A. (2009). Direct ex vivo effects of herbal extracts on serum bilirubin in neonatal blood samples. *African Journal of Biochemistry Research*;3(5):226-228.
32. **Joshi**, Y.M.; Jadhav, T.A.; Kadam, V.J. (2010). A pharmacological intervention for protection against ionizing radiations: A review. *The Internet Journal of Internal Medicine*. 8 (2): 1-20.
33. **Hui-Hur**, Z., T.U. Peng-Fei, Z. Kan, W. Hui, W. Bao- Huai and L.U. Jing-

- Feng (2001). Antioxidant properties of phenolic diterpenes from *Rosmarinus Officinalis*. *Acta. Pharmacol. Sin.*, 22(12): 1094-1098.
34. **Bousbia**, N.; Vian, M.A.; Ferhat, M.A.; Petitcolas, E.; Meklati, B.Y.; Chemat, F. (2008). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food Chem.* 14,355-362.
35. **Pintore**, G.; Usai, M.; Bradesi, P.; Juliano, C.; Boatto, G.; Tomi, F.; Chessa, M.; Cerri, R.; Casanova, J. (2002). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. *Flav. Frag. J.* 17, 15-19.
36. **Ohno**, T.; Kita, M.; Yamaoka, Y.; Imamura, S.; Yamamoto, T.; Mitsufuji, S.; Kodama, T.; Kashima, K.; Imanishi, J. (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. *Helicobacter* 8, 207-215.
37. **Celiktas**, O.Y.; Kocabas, E.E.H.; Bedir, E.; Sukan, F.V.; Ozek, T.; Baser, K.H.C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem.* 100,553-559.
38. **Bozin**, B.; Mimica-Dukic, N.; Samojlik, I.; Jovin, E. (2007). Antimicrobial and antioxidant properties of rosmarin and sage (*Rosmarinus officinalis* and *Salvia officinalis*, Lamiaceae) essential oils. *J. Agric. Food Chem.* 55, 7879-7885.
39. **Wang**, W.; Wu, N.; Zu, Y.G.; Fu, Y.J. (2008). Antioxidant activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food Chem.* 108, 1019-1022.
40. **Singletary** K, MacDonald C, Wallig M. (1996). Inhibition by rosemary and carnosol of 7, 12 dimethylbenz [a] anthracene (DMBA)-induced rat mammary tumorigenesis and in vivo DMBA-DNA adduct formation. *Cancer Lett.*; 104: 43-48.
41. **Abdullah** Ijaz Hussain; Farooq Anwar; Shahzad Ali Shahid Chatha; Abdul Jabbar; Shahid Mahboob; Poonam Singh Nigam (2010). *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. *Braz. J. Microbiol.* vol.41 no.4 São Paulo Oct./Dec. 42. **Altinier** G, Sosa S, Aquino RP, Mencherini T, Della Loggia R, Tubaro A. (2007). Characterization of topical anti-inflammatory compounds in *Rosmarinus officinalis* L. *J. Agric. Food Chem.*; 55: 1718-1723.
43. **Almela**, L., B. Sanchez-Munoz, J.A. Fernandez- Lopez, M.J. Roca and V. Rabe (2006). Liquid chromatographic-mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. *J. Chromatogr.*, 1120: 221-229.
44. **Ramirez** P, Garcia-Risco M, Santoyo S, Seiorins F, Ibítez E Reglero, G. (2006). Isolation of functional ingredients from rosemary by preparative-supercritical fluid chromatography (Prep-SFC). *J. Pharm. Biomed. Anal.*; 41(5):1606-13.
45. **Jindal**, A., D. Soyal, I. Sihgh and R. Reszka (2006). Modification of radiation-induced damage in mice by *Rosmarinus officinalis* extracts (ROE). *Pharmacology Line.*, 2: 63-75.
46. **Li**, L.; Takemura, G.; Miyata, S.; Esaki, M.; Okada, H.; Ogino, A.; Fujiwara, H. et al. (2006). Preventive Effect of Erythropoietin on Cardiac Dysfunction in Doxorubicin-Induced Cardiomyopathy. *Circulation*; 113: 535-543.
47. **Abd-Allah AR**, Al-Majed AA, Mostafa AM, Al-Shabanah OA, Din AG, Nagi MN. (2002). Protective effect of arabic gum against cardiotoxicity induced by doxorubicin in mice: a possible mechanism of protection. *J Biochem Mol Toxicol.*; 16(5):254-9.
48. **Ganesh Chandra Jagetia**, Tiyyagura Koti Reddy (2014). The grape fruit flavonone naringin protects mice against doxorubicin-induced cardiotoxicity) Vol 3, No 1.
49. **Enass Najem Oubaid**, 2012. Thesis in Evaluation of the Protective Properties of Different Phosphodiesterase Inhibitors (Aminophylline, Sildenafil and

- Diazepam) on Doxorubicin-Induced Cardiotoxicity in Rats.
50. **Pacher P, Liaudet L**, Bai P, et al. Potent metallopor-phyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin-induced cardiac dysfunction. *Circulation* 2003;107:896-904.
51. **Thippeswamy**, A.H.M.; Shirodkar, A.; Koti, B.C.; Sadiq, A.J. ; Praveen, D.M.; Viswanatha Swamy, A.H.M. and Patil, M. (2011): Protective role of phyllanthus niruri extract in doxorubicin-induced myocardial toxicity in rats. *Indian J Pharmacol.*; 43(1): 31–35.
52. **Stein**, W. (1981): CK-MB methods and clinical significance; Proceedings of the CKMB symposium, Philadelphia ; 61-74.
53. **Henry**, R.J.; Chiamori, N., Golub, O.J.; and Berkman, S. (1974): Evaluation and utilization of a kinetic enzyme direct measuring photometer. *Clin. Path.* ; 28(7): 587–591.
54. **Daniel**, W.W. (1999). Probability and distribution. Biostatistics. A foundation for analysis in the health sciences .7th ed.; 83-123.
55. **Ashikawa K**, Shishodia S, Fokt I, Priebe W, Aggarwal B B. (2004). Evidence that activation of nuclear factor- κ B is essential for the cytotoxic effects of doxorubicin and its analogues. *Biochemical Pharmacology*. 67(2): 353-364.
56. **Sabrina** Licata , Antonella Saponiero , Alvaro Mordente , and Giorgio Minotti (2000). Doxorubicin Metabolism and Toxicity in Human Myocardium: Role of Cytoplasmic Deglycosidation and Carbonyl Reduction. *Chem. Res. Toxicol.*, , 13 (5), pp 414–420.
57. **El-Sayed**, E.M.; Abd El-azeem, A.S.; Afify, A.A.; Shabana, M.H.; and Ahmed, H.H. (2011). Cardioprotective effects of Curcuma longa extracts against DOX-induced cardiotoxicity in rats. *Journal of Medicinal Plants Research*, 5 (17): 4049-4058.
58. **Liu X**, Chen Z, Chua CC, Ma Y, Youngberg GA, Hamdy R and Chua BHL. (2002). Melatonin as an effective protector against Doxorubicin induced cardiotoxicity. *Americ J Physiol Heart and Circulatory Physiol.*, 283, H254-H263.
59. **Nakagawa** and Yokozawa T.(2002). Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem. Toxicol.*, 40, 1745-50.
60. **Abou-El-Hassan M A**, Rabelink M J, van der Vijgh W J, Bast A and Hoeben RC.(2003), A comparative study between catalase gene therapy and the cardioprotector monohydroxyethylrutoside (MonoHER) in protecting against doxorubicin-induced cardiotoxicity in vitro. *Br J Cancer.*, 89, 2140-6.
61. **Rajasekaran**, M. and Kalaimagal, C. (2012). Cardioprotective Effect of a Medicinal Mushroom, Ganoderma lucidum Against Adriamycin Induced Toxicity. *International Journal of Pharmacology*, 8 (4): 252-258.
62. **Fogli S**, Nieri P, Breschi MC. (2004). The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage. *FASEB J*. 18: 664–75.
63. **Grieve**, D.J. (2011). Potential Therapeutic Targeting of NADPH Oxidases for Prevention of doxorubicin Cardiotoxicity. *British Heart F. &Med Res Counc*, 6 (4): 136-138.
64. **Yagmurca M**, Fadillioglu E, Erdogan H, M, Ucar Sogut S and Irmak M K. (2003). Erdosteine prevents doxorubicin induced cardiotoxicity in rats. *Pharmacol Res.*, 48, 377-382.
65. **Vijay T**, Rajan M S D, Sarumathy K, Palani S and Sakthivel K.(2011). Cardioprotective, antioxidant activities and phytochemical analysis by GC-MS of Gmelina arborea (GA) in doxorubicin-induced myocardial necrosis in Albino rats. *J Appl Pharm Sci*. 01 (05), 198-204.
66. **Ragavendran**, P.; Sophia, D.; Arulraj, C. and Gopalakrishnan, V.K. (2012). Cardioprotective effect of aqueous, ethanol and aqueous ethanol extract of Aerva lanata (Linn.) against doxorubicin induced cardiomyopathy in rats. *Asian Pacific Journal of Tropical Biomedicine*, 212-218.

67. **Bien**, S.; Riad, A.; Ritter, C.A.; Gratz, M.; Grube, M.; Krieg, T.; Felix, S.B.; Staudt, A.; and Kroemer, H.K. (2007). The endothelin receptor blocker bosentan inhibits doxorubicin-induced cardiomyopathy. *Cancer Res.* 67: 10428-10435.
68. **Rasha**, A.R and Abdella, E.M. (2010). Modulatory Effects of Rosemary Leaves Aqueous Extract on Doxorubicin-Induced Histological Lesions, Apoptosis and Oxidative Stress in Mice. *Iranian Journal of Cancer Prevention*, 3 (1): 1-22.
69. **Singal**, P.K. and Iliskovic, N. (1998). DOX-induced cardiomyopathy. *The New England Journal of Medicine*, 339: 900–905.
70. **Moreno**, S., Scheyer T., Romano C.S., Vojnov A.A. (2006): Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research*, 40 (2): 223-231.
71. **Lo** A, Liang Y, Lin-Shiau S, Ho C, Lin J. (2002). Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor- B in mouse macrophages *Carcinogenesis*. 23 (6): 983- 991.
72. **Serdaroglu** M, Yildiz-Turp G. (2004). The effects of ascorbic acid, rosemary extract and α -tocopherol/ascorbic acid on some quality characteristics of frozen chicken patties. *Food Sci. Technol.* 7(1): 1-5