Protective Effects of Rutin and Hesperidin against Doxorubicin-Induced Lipodystrophy and Cardiotoxicity in Albino Rats

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Abstract: Doxorubicin, is widely used in the treatment of various solid tumors. The present study was conducted to evaluate the protective role of rutin and hesperidin on doxorubicin – induced lipodystrophy and cardiotoxicity in albino rats. Doxorubicin (DXR) – administered rats (25 mg/ kg; three times interaperitoneally/ week for two weeks) were pretreated with rutin and/or hesperidin (50 mg/ kg body weight) three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment). Results showed that DXR caused a marked rise in serum total cholesterol, high density lipoprotein, low density lipoprotein, creatine kinas and lactate dehydrogenase (HDL, LDL, CK, LDH) as well as aspartate aminotransferase; AST. Pretreatment of these animals with rutin and/or hesperidin successfully prevent most of these biochemical alterations; the pretreatment with both rutin and hesperidin seemed to be the most potent. Concerning oxidative stress and antioxidant defense system, the depleted cardiac glutathione content of DXR administered rats was potentially increased above normal levels as a result of pretreatment of rutin and/or hesperidin. The elevated lipid peroxidation of DXR-administered rats, was remarkably decrease lipid peroxidation as a result of pre-treatment with rutin and/or hesperidin. It can be concluded that the natural plant components (rutin and hesperidin) could protect the heart against DXR-induced cardiotoxicity and lipodystrophy. However, further clinical studies are required to assess the safety and benefits of rutin and hesperidin in human beings.
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1. Introduction

Adriamycin (doxorubicin, ADR) is a broad spectrum antitumor antibiotic used to treat cancer patients. However, the potential usefulness of this drug is limited by the development of life-threatening cardiomyopathy (**Singal and Siveski-Iliskovic, 1998**) and congestive heart failure (**Gewitz, 1999**).Adriamycin-induced myocardial dysfunction has been suggested to involve inhibition of nucleic acid as well as protein synthesis, release of vasoactive amines, changes in adrenergic function (**Singal *et al.*, 1998**), abnormalities in the mitochondria, lysosomal alterations, alterations in sarcolemmal Ca^{2+} transport, membrane-bound enzymes, imbalance of myocardial electrolytes (**Siveski-Iliskovic *et al.*, 1994**), free radical formation and lipid peroxidation (**Rabelo *et al.*, 2001**).

Herbal plants have high levels of phenolic compounds that have radioprotective effects (**Hosseinimehr, 2007 ; Hosseinimehr *et al.*, 2007**). Recently, we showed the hesperidin, a flavonoid, has powerful protection effects on DNA damage, reducing the frequency of micronuclei induced by c-irradiation in mice (**Hosseinimehr and Nemati, 2006**). Hesperidin has been reported to have many biological effects including anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant effects and decreasing capillary fragility (**Garg *et al.*, 2001**).

The best-described property of almost every group of flavonoids is their capacity to acts as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species (ROS). Rutin, by acting as antioxidants, exhibited beneficial effects such as anti-inflammatory, antiallergic, antiviral, as well as anticancer activity. It had been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases. The consumption of flavonoids may prevent endothelial dysfunction by enhancing the vasorelaxant process leading to a reduction of arterial pressure (**Iijima and Aviram , 2001**). Endothelial dysfunction represents a critical event in the development of cardiovascular diseases and the major complication of atherosclerosis and arterial thrombus formation (**Jayakody *et al.*, 1985**). The consumption of flavonoids can prevent a number of cardiovascular diseases including hypertension and atherosclerosis (**Hertag *et al.*, 1993a and Hertag *et al.*, 1993b**).

Doxorubicin is a very effective antitumor agent but its clinical use is limited by the occurrence of a

cumulative dose-related cardiotoxicity, resulting in, for example, congestive heart failure (negative inotropic effect). It was found that, the cardiotoxicity of doxorubicin on the mouse left atrium had been inhibited by flavonoids, 7monohydroxyethylrutoside and 7,3,4trihydroxyethylrutoside (**Bast *et al*., 2007**).

Therefore, the present investigation was undertaken to test the anticlastogenic or clastogenic effects of rutin and hesperidin on the heart tissue in rat, determine the protective effect of the components on the enzymes level, oxidative stress, and histopathological alterations induced by DXR and examine the dose dependency of these effects.

2. Materials and Methods

2.1. Experimental animals:

Adult male albino rats weighing 120-150 g were used in the present study. The animals were obtained from the animal house in the ophthalmology Research Center, Giza, Egypt. They were kept under observation for two weeks before the onset of the experiment to exclude any intercurrent infection. The animals were kept at room temperature and exposed to natural daily light-dark cycles. Rats were fed standared diet *ad libitum* and clean water was continuously available. All animal procedures are in accordance with the recommendations for the proper care and use of laboratory animals stated by the Canadian Council on Animal Care (**CCAC, 1993**).

2.2. Chemicals:

Doxorubicin (Adriblastina® produced by Carlo Erba) was purchased from local pharmacy in the form of 10 mg/ ampoule. Rutin and hesperidin was purchased from Sigma (St. Louis, MO, USA). All other chemicals used for the investigation were of analytical grade.

2.3. Doses and treatment

The dose of doxorubicin (DXR) used in this study was 25 mg/kg body weight. This dose was previously reported to include an increase in the frequency of cell damage in mammalian systems (**Antunes and Takahashi, 1999; prahalathan *et al*., 2006**). The animals were treated with doxorubicin by interaperitoneal rout. The chosen dose of DXR was adjusted to 0.2ml/ 25g b.wt. in sterile water prior to use and was given three times per week for two weeks. Rutin and hesperidin was adjusted to 50mg/kg and was given three times per week for three weeks.

2.4. Experimental design:

Animals were divided into five groups comprising six animals each :

1- Group 1 (normal control) is given the equivalent volume of the vehicle 1 (distilled water) daily for four weeks.
2- Group 2 (toxic) is given doxorubicin at dose level of 25mg/kg b.wt for three times per week for two weeks.
3. Group 3 (treated with rutin & doxorubicin) is given rutin at dose level of 50mg/kg b. w.(**Fernandes *et al*., 2010**) three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment).
4. Group 4 (treated with hesperidin & doxorubicin) is given hesperidin at dose level of 50mg/kg b.wt. (**Yeh *et al*., 2009**) three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment).
5. Group 5 (treated with rutin + hesperidin & DXR) is given rutin and hesperidin at dose level of 50mg/kg b. wt three times per week for three weeks and DXR 25mg/kg b.wt three times per week (at the last two weeks of the experiment).

2.5. Sampling:

Under diethyl ether anesthesia, six animals from each group was scarified and 5 ml of blood sample was collected from jugular vein of each animal in a centrifuge tube and left to clot at room temperature for 45 minutes. Sera were separated by centrifugation at 3000 r.p.m. at 30ºC for 15 minutes and kept frozen at -30ºC for various physiological and biochemical analyses .

Testis from each animal was rapidly excised after dissection. One part was fixed in buffered formalin for 24 hrs, trimmed and then transferred into 70% alcohol for histopathological examination. 0.5g was homogenized in 5ml 0.9% NaCl (10% w/v) using teflon homogenizer (Glas-Col, Terre Haute, USA).

2.6. Biochemical analyses:

Serum total cholesterol was determined by enzymatic colorimetric procedure using kits from Biodiagnostic according to **Allain *et al*. (1974)**. Serum high density lipoprotein (HDL) and low density lipoprotein was determined by enzymatic colorimetric procedure using kits from Biodiagnostic according to **Wieland and Seidel (1983)**. Risk factor was calculated according to **Ross formula (1992)** as the following:

Risk Factor = total cholesterol conc./ HDL-cholesterol conc. Serum AST was determined by colorimetric procedure using kits from Biodiagnostic according to the methods of **Reitman and Frankel (1957).** Serum lactate dehydrogenase (LDH) was

determined by quantitative method (pyruvate kinetic UV. DGKC) using kits obtained from Spinreact according to the method of Young **(1977)**. Serum creatinine kinase (CK) was determined by quantitative kinetic UV. method using kits obtained from Spinreact according to the method of **Young (2001).** Heart glutathione (GSH) was determined according to the method of **Beulter *et al*. (1963)**. Heart lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method of **Preuss *et al*. (1998)**. Heart peroxidase activity was assayed for the method of **Cohen *et al.* (1970).** Glutathione peroxidase activity in heart was assayed according to the chemical method of **Matkovics *et al*. (1998).** Glutathione-S-transferase activity was determined according to **Mannervik and Gutenberg (1981)**.

2.7. Statistical analysis:

The data were analyzed using the one-way analysis of variance (ANOVA) (PC-STAT, 1985) followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard error (SE). F-probability, obtained from one-way ANOVA, expresses the effect between groups.

3. Results

Changes in different serum variables related to heart function are represented in tables 1 and 2. Serum total cholesterol, HDL and LDL levels were significantly increased as a result of administration of DXR alone, while they were significantly decreased in those pretreated with rutin and hesperidin. The pre-treated of rats with their mixture have a significant elevation effects on serum HDL and LDL levels. Risk factor values were decreased significantly in DXR administration and in pretreatment with their mixture, while they exhibited a significant elevation in rutin and hesperidin pretreatment. Rutin and/or hesperidin pretreatment significantly succeeded to prevent the elevation of serum creatine kinas (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities caused by DXR administration.

Tables 3 and 4 shows the effect of the tested rutin and/or hesperidin on the heart oxidative stress markers and antioxidant defense system of normal and doxorubicin-administered rats. The pre-treatment of these animals with rutin and/or hesperidin produced a potential increase ($p<0.01$; LSD) of the glutathione level; the percentage changes were 27.40%, 9.25% and 13.18% respectively as compared to the corresponding DXR treated group. Glutathione –S-transferase, glutathione peroxidase and peroxidase activities had a significant elevation in all pretreated groups in corresponding to DXR treated group that produced a significant decrease in the activities of glutathione –S-transferase, glutathione peroxidase and peroxidase ($p<0.01$; LSD). In contrast, heart lipid peroxidation was increased significantly as a result of DXR administration while all the tested agents induced a significant decrease of the elevated values ($p<0.05$; LSD) as a result of pre-treatment with either of rutin and hesperidin or their mixture. One-way ANOVA revealed that the effect on the variables of oxidative stress and antioxidant system was very highly significant ($p<0.001$) between groups.

4. Discussion

DXR cytotoxicity and genotoxicity may be mediated by free radicals derived from this drug and its capability to induce apoptosis through a wide variety of mechanisms including production of reactive oxygen species (ROS), alkylation of cellular macromolecules, DNA intercalation and cross-linking, lipid peroxidation, cell membrane damage, ceramide production and p53 induction in various tissues (**Lorenzo *et al*., 2002; quiles *et al*., 2002 and Ashikewe *et al*., 2004**).

Serum total cholesterol, HDL and LDL was significantly increased as a result of administration of DXR alone, while it was significantly decreased in those pretreated with rutin and hesperidin. The pre-treated rats with their mixture have a significant elevation on serum HDL and LDL levels. Risk factor values were decreased significantly in DXR administration and in pretreatment with their mixture, while recorded a significant elevation in rutin and hesperidin pretreatment. Rutin and/or hesperidin pretreatment significantly succeeded to prevent the elevation of serum creatine kinas (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities caused by DXR administration. The present study are in agreement with several authors (**Dorr, 1996 and Messinger and Uckun, 1999**). Cardiotoxicity is the main limiting side effect and confers a life time risk to patients. Previous reports have suggested that oxygen free radicals and drug metabolites play a major role in the development of cardiotoxicity (**Dorr, 1996 and Suzuki and Ford, 1999**). Dxol is the major metabolite of DXR found in tissue (**Bachur, 1971**). It is formed by an enzymatic two-electron reduction of the C-13 keto group by cytoplasmic, NADPH-dependent aldo-keto reductases and short-chain dehydrogenases (**Ahmed *et al*., 1978 and Wermuth, 1981**). Dxol seems to be more cardiotoxic than the parent drug. It inhibits many of the ion channel pumps.

Table 1: Protective effect of rutin and hesperidin on serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol levels and risk factor (RF) in normal and doxorubicin treated rats.

Parameters / Treatments	Total cholesterol (mg/dl)	% change	HDL-cholesterol (mg/dl)	% change	LDL-cholesterol (mg/dl)	% change	Risk Factor	% change
G1 Normal	72.88 ± 2.40 [b]	-	18.30 ± 0.62 [e]	-	311.90 ± 8.60 [c]	-	4.02 ± 0.17 [a]	-
G2 Doxorubicin	103.27 ± 1.80 [a]	41.70	78.10 ± 2.30 [b]	326.78	510.30 ± 15.10 [b]	63.61	1.32 ± 0.03 [d]	-76.16
G3 Rutin + Doxorubicin	104.83 ± 1.50 [a]	1.51	50.30 ± 1.60 [c]	-35.60	335.10 ± 7.30 [c]	-34.33	1.95 ± 0.18 [c]	47.73
G4 Hesperidin + Doxorubicin	108.04 ± 4.10[a]	4.62	36.60 ± 0.94 [d]	53.14	338.40 ± 11.70 [c]	-33.69	2.96 ± 0.15 [b]	124.24
G5 Rutin + Hesperidin + Doxorubicin	103.33 ± 0.55 [a]	0.06	107.40 ± 3.50 [a]	37.52	629.70 ± 10.60 [a]	23.40	0.968 ± 0.03 [d]	-26.67
F-Probability	P<0.0001	-	P<0.0001	-	P<0.0001	-	P<0.0001	-
LDS at 5% level	7.01	-	6.03	-	32.02	-	0.381	-
LDS at 1% level	9.48	-	8.16	-	43.32	-	0.561	-

Data are expressed as mean ± standard error. Number of animals in each group is six.
Mean, which have the same superscript symbol (s) , are not significantly different.
Percentage changes (%) were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group. Risk factor = Total cholesterol / HDL-cholesterol.

Table 2: Protective effect of rutin and hesperidin on serum creatinine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities in normal and doxorubicin treated rats.

Parameters / Treatments	CK (U/L)	% change	LDH (U/L)	% change	AST (U/ml)	% change
G1 Normal	244.35 ± 6.80 [d]	-	233.58 ± 12.80 [d]	-	88.90 ± 2.50 [b]	-
G2 Doxorubicin	552.85 ± 16.0 [a]	126.25	921.97 ± 8.90[a]	294.71	128.00 ± 0.00 [a]	43.98
G3 Rutin + Doxorubicin	402.30 ± 4.00 [c]	-27.23	339.60 ± 9.90 [b]	-63.17	86.90 ± 3.00 [b]	-32.11
G4 Hesperidin + Doxorubicin	453.71 ± 4.30 [b]	- 17.93	255.73 ± 11.00 [d]	- 72.26	60.50 ± 4.30 [c]	- 52.73
G5 Rutin + Hesperidin + Doxorubicin	444.69 ± 8.50 [b]	- 19.56	369.26 ± 12.90 [c]	- 59.95	84.40 ± 2.80 [b]	- 34.06
F-Probability	P < 0.0001	-	P < 0.0001	-	P<0.0001	-
LDS at 5% level	27.13	-	32.56	-	8.39	-
LDS at 1% level	36.70	-	44.06	-	11.35	-

Data are expressed as mean ± standard error. Number of animals in each group is six.
Mean, which have the same superscript symbol (s) , are not significantly different.
Percentage changes (%) were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.

Table 3: Protective effect of rutin and hesperidin on heart glutathione level, glutathione peroxidase and glutathione-s-transferase activities in normal and doxorubicin treated rats.

Parameters / Treatments	Glutathione (nmol/gm)	% change	Glutathione Peroxidase (U/min)	% change	Glutathione-s-transferase (U/gm)	% change
G1 Normal	843.81 ± 6.30 ^{d}	-	13.72 ± 0.61 ^{a}	-	1745.84 ± 18.0 ^{b}	-
G2 Doxorubicin	700.23 ± 7.70 ^{e}	- 17.02	10.14 ± 3.1 ^{d}	-26.09	1358.68 ± 25.7 ^{d}	- 22.18
G3 Rutin + Doxorubicin	892.38 ± 6.30 ^{c}	27.40	12.95 ± 0.29 ^{ab}	27.71	1698.61 ± 15.3 ^{bc}	25.02
G4 Hesperidin + Doxorubicin	765.03 ± 13.60 ^{a}	9.25	11.50 ± 0.35 ^{c}	13.41	1688.40 ± 9.2 ^{c}	24.27
G5 Rutin + Hesperidin+ Doxorubicin	792.53 ± 8.20^{b}	13.18	12.14 ± 0.16 ^{bc}	19.72	1739.91 ± 19.1 ^{a}	28.06
F-Probability	P<0.0001	-	P<0.0001	-	P<0.0001	-
LDS at 5% level	25.79	-	1.02	-	53.24	-
LDS at 1% level	34.89	-	1.37	-	72.03	-

Data are expressed as mean ± standard error. Number of animals in each group is six.
Mean, which have the same superscript symbol (s) , are not significantly different.
Percentage changes (%) were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.

Table 4: Protective effect of rutin and hesperidin on heart peroxidase activity and lipid peroxidation level in normal and doxorubicin treated rats.

Parameters / Treatments	Peroxidase (u/gm)	% change	Lipid peroxidation (nmol/gm/hr)	% change
G1 Normal	124.84 ± 0.17 ^{b}	-	2.88 ± 0.04 ^{d}	-
G2 Doxorubicin	107.93 ± 1.30 ^{d}	- 13.54	5.94 ± 0.26 ^{a}	106.25
G3 Rutin + Doxorubicin	132.09 ± 3.00 ^{a}	22.38	4.22 ± 0.16 ^{c}	- 28.96
G4 Hesperidin + Doxorubicin	114.35 ± 0.49 ^{d}	5.95	4.56 ± 0.19 ^{bc}	-23.23
G5 Rutin + Hesperidin + Doxorubicin	118.11 ± 0.11 ^{c}	9.43	4.70 ± 0.04 ^{b}	-20.88
F-Probability	P<0.0001	-	P<0.0001	-
LDS at 5% level	4.24	-	0.471	-
LDS at 1% level	5.73	-	0.637	-

Data are expressed as mean ± standard error. Number of animals in each group is six.
Mean, which have the same superscript symbol (s) , are not significantly different.
Percentage (%) changes were calculated by comparing normal group with doxorubicin treated group and pre-treated doxorubicin groups with doxorubicin treated group.

The calcium pump of the sarcoplasmic reticulum, the Na/Ca pump of the sarcolemma, and the proton pump of the mitochondria are inhibited by dxol but not by similar concentrations of dox (**Boucek *et al*., 1987 and Olson *et al*., 1988**). There is also evidence that DXR and dxol may act together at different sites to generate cardiotoxicity (**Mushlin *et al*., 1993**). Lowering the level of dxol *in vivo* by inhibiting aldo-keto reductases with phenobarbital leads to less toxicity as measured by decreased creatine kinase levels (**Behnia *et al*., 1999**). Dxol also interacts with cis-aconitate and inhibits aconitase activity. This interaction causes the delocalization of iron from the active center of aconitase with reoxidation of dxol to dox (**Minotti *et al*., 1998**). This mechanism irreversibly inactivates aconitase and interferes with the aconitase-iron regulatory protein-1 function. Dxol levels preferentially build up in the heart (**Del Tacca *et al*., 1985 and Mushlin *et al*., 1993**), which suggests increased involvement of the C-13 hydroxy metabolite. Dxol build-up could be caused by lower transport through heart membranes, which is attributable to the more polar dxol molecule. DXR-induced free radicals have been implicated in developing cardiotoxicity (**Gille and Nohl, 1997 and Gewirtz, 1999**). The C-13 hydroxy metabolite of dox can generate free radicals (**Bachur *et al*., 1977 and Gervasi *et al*., 1986**). NADH dehydrogenase from heart mitochondria and heart sarcosomes containing NADPH cytochrome P450 reductase reduce dxol, generating superoxide anion (**Gervasi *et al*., 1986**). Dxol appears to produce 50% of the free radicals produced by DXR (**Gervasi *et al*., 1986**). The levels of dxol in the expresser hearts were four times the levels of DXR in the non expresser hearts, providing a potential for a 2-fold increase in free radical formation. Enzymes that protect against the toxic effect of superoxide anion, such as the mitochondrial manganese superoxide dismutase, provide protection against DXR-induced cardiotoxicity (**Kule *et al*., 1994 and Yen *et al*., 1996**).

Flavonoids are a group of benzo-γ-pyron derivatives, naturally found in the diet, which exhibit numerous pharmacological properties that are beneficial for human health (**Havsteen, 2002**). With respect to doxorubicin cardiotoxicity, their antioxidant activity, iron-chelating properties and inhibitory effects on carbonyl reductases are of interest. Evidence has been given that the flavonoids indeed have a strong potential to relieve doxorubicin-induced cardiac side-effects (**Van Acker *et al*., 1995; Van Acker *et al*., 2001 and Psotová *et al*., 2004**). Doxorubicin generates reactive oxygen species (ROS), which have been suggested to play an important role in the development of cardiotoxicity (**Hrdina *et al*., 2000**). Free radical scavengers have therefore been proposed to protect cardiac tissue from doxorubicin-induced oxidative stress and thus to relieve its cardiotoxicity. Most of the flavonoids possess excellent antioxidant properties (**van Acker, 1996**). Whereas common antioxidants inactivate ROS only after they have been formed, iron chelators are able to prevent their formation. Iron can redox-cycle between its two redox states –Fe^{2+} and Fe^{3+} and acts as a catalyst of hydroxyl radical formation (Fenton and Haber–Weiss reactions). Iron chelation is considered to be an important tool to decrease anthracycline cardiotoxicity as documented by the beneficial effect of dexrazoxane (**Schroeder *et al*., 2002**) as well as other chelators of iron (**Štěrba *et al*., 2006**). In flavonoids, the antioxidant and iron chelating properties are closely related and their activity may include two steps — iron is first chelated by the flavonoid and the ROS which are formed in its vicinity are subsequently scavenged by the flavonoid. In this way, the radicals are quenched at the same place where they are formed. An attempt was also performed to increase the cardioselectivity of the flavonoids via introduction of the quaternary ammonium moiety in various positions of the flavonoid structure (**Grisar *et al*., 1991**). Antioxidant properties were long considered to be the major or even sole determinants for efficient protectors against doxorubicin cardiotoxicity because reactive oxygen species and oxidative stress are considered to be involved in the pathophysiology of its development (**Gille and Nohl, 1997; Gille *et al*., 2002 and Kaiserová *et al*., 2006**). However, it has been shown that there are pronounced differences in the cardioprotective effects also among the flavonoids with comparable antioxidant properties (**van Acker *et al*., 2001**). The production of free radicals as by-product of doxorubicin metabolism is considered to be the main mechanism of doxorubicin-induced cardiotoxicity. The quinine moiety of doxorubicin is converted into a semiquinone form by the acquisition of one electron. This conversion can occur either enzymatically or non enzymatically. The enzymatic reduction of the doxorubicin quinone ring is performed by cytochrome P450 reductase, NADH dehydrogenase and cytochrome P450. The produced semiquinone form is oxidized by molecular oxygen, which yields back (**Goeptar *et al*., 1993**) doxorubicin in its quinone form with concomitant production of superoxide anion radicals. This process is called redox cycling. Superoxide radicals can dismutate either enzymatically catalyzed by superoxide dismutase or, albeit with a lower rate, spontaneously. From this dismutation hydrogen peroxide is formed. This may lead to toxicity. Iron ions have been

suggested to play a crucial role in this process. Not only because iron reacts with hydrogen peroxide to form the reactive hydroxyl radical but also because Fe^{3+} may form complexes with doxorubicin. The theory on the iron-dependent formation of free radicals by doxorubicin semiquinones is supposed to be the most prevailing one to explain the cardiotoxicity. An internal electron shift may give reduction of iron, which subsequently delivers this electron to molecular oxygen yielding superoxide anion radicals (**Bast *et al*., 2007**).

The pre-treatment of these animals with rutin and/or hesperidin produced a potential increase ($p<0.01$; LSD) of the glutathione level which was elevated far above normal level; the percentage changes were 27.40%, 9.25% and 13.18% respectively as compared to the corresponding DXR treated group(-17.02) which decreased glutathione level significantly when compared to normal control group. Glutathione –S-transferase, glutathione peroxidase and peroxidase activities had a significant elevation in all pretreated groups in corresponding to DXR treated group that produced a significant decreased the activities of glutathione –S-transferase, glutathione peroxidase and peroxidase ($p<0.01$; LSD). In contrast, heart lipid peroxidation was increased significantly as a result of DXR administration while all the tested agents induced a significant decrease of the elevated activity ($p<0.05$; LSD) in pre-treatment with either of rutin and/or hesperidin. The present study are in agreement with several authors: **You *et al*., 2006** applied adriamycin (doxorubicin, ADR), a highly effective anticancer agent, as an inducer to establish the animal model of dose-related cardiomyopathy due to inhibition of nucleic acid as well as protein synthesis, formation of free radicals, and lipid peroxidation. The objective of this study was to investigate the protective effects of SMS (the traditional Chinese medicine prescription "Sheng-Mai-San) on adriamycin-induced cardiomyopathy. The results demonstrated that nucleic acid as well as protein synthesis was inhibited, while lipid peroxidation was increased. Myocardial glutathione peroxidase (GSHPx) activity was decreased and electron microscopic examination revealed myocardial lesion indicative of ADR-induced cardiomyopathy. In contrast, administration of SMS before and concurrent with ADR significantly attenuated the myocardial effects. It also lowered mortality as well as the amount of ascites. In addition, indexes in myocardial GSHPx, macromolecular biosynthesis and superoxide dismutase activities were increasing, with a concomitant decrease in lipid peroxidation and preserved myocardial ultrastructure. These results indicated that SMS may be partially protective against ADR-induced cardiomyopathy. Adriamycin (doxorubicin, ADR) is a broad spectrum antitumor antibiotic used to treat cancer patients. However, the potential usefulness of this drug is limited by the development of life-threatening cardiomyopathy (**Singal and Siveski-Iliskovic, 1998**) and congestive heart failure (**Gewritz, 1999**). **Demirkaya *et al.* (2009)** used doxorubicin (DXR) as a chemotherapeutic agent effectively in the treatment of several childhood malignancies. During treatment, cardiotoxicity caused by cell damage due to the free oxygen radicals that are generated is a major limiting factor. This study was undertaken to determine whether DXR-induced cardiotoxicity could be prevented by natural foods with antioxidant properties such as aged garlic extract (AGEX), grape seed proanthocyanidin (PA), and hazelnut. They found that there were no significant changes in MDA levels among the control, DXR-treated groups, or supplemented groups that received additional natural antioxidant foods. SOD enzyme levels were decreased in rats treated with DXR. PA prevented the decrease at low doses of DXR. DXR treatment decreased CAT enzyme levels, but additional PA and hazelnut consumption increased these levels at low cumulative doses. XO enzyme levels were decreased in AGEX and hazelnut groups, but PA prevented the decrease. CK levels were elevated after DXR administration, indicating myocardial injury, but PA significantly reversed this. The authors concluded that, the positive effects of natural antioxidant foods on the prevention of DXR-induced cardiac injury could not be clearly shown on the basis of antioxidant enzymes. However, the electron microscopic changes clearly demonstrated the protective effects of AGEX and PA. The supplementation of these antioxidant foods over longer periods may show more definitive results. **Alyane *et al*. (2008)** concluded that pretreatment of rats with propolis extract (is one of the major hive products of bees and is rich in flavonoids), given per os (100 mg/kg/day) during four days prior to DXR injection, substantially reduced the peroxidative damage in the heart mitochondria: they showed significant reducing both of mitochondrial MDA formation and production of superoxide anion. The data demonstrate that antioxidants from natural sources may be useful in the protection of cardiotoxicity in patients who receive doxorubicin and as reported for its claimed beneficial effect on human health by biomedical literature. **Pietta (2000)** demonstrated that flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants,

and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions. However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidants *in vitro* has been the subject of several studies in the past years, and important structure-activity relationships of the antioxidant activity have been established. The antioxidant efficacy of flavonoids *in vivo* is less documented, presumably because of the limited knowledge on their uptake in humans. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess a radical-scavenging ability. Both the absorbed flavonoids and their metabolites may display an in vivo antioxidant activity, which is evidenced experimentally by the increase of the plasma antioxidant status, the sparing effect on vitamin E of erythrocyte membranes and low-density lipoproteins, and the preservation of erythrocyte membrane polyunsaturated fatty acids.

In conclusion, the present results demonstrated that the rutin and/or hesperidin in the protection against doxorubicin induced cardiotoxicity, by improving the activities of heart (total cholesterol, HDL, LDL, CK, AST & LDH). Doxorubicin-administered rats pretreated with rutin and hesperidin recorded a significant increase in glutathione level, glutathione peroxidase, glutathione-S- transferase and peroxidase activities, and a significant decrease in lipid peroxidation level. Thus, pretreatment with rutin and hesperidin may protect the heart from the toxicity caused by doxorubicin. However further clinical studies are required to assess the benefits and safety of these flavonoids before using in human beings and approval by Food and Drug administration (FDA).

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