Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 4, 2011

Research Article

POTENTIAL ACTION OF VITAMIN-E, MORIN, RUTIN AND QUERCETIN AGAINST THE DOXORUBICIN-INDUCED CARDIOMYOPATHY

[1,*]DR. RAJA KUMAR PARABATHINA, [2,]DR. E.MURALINATH, [3,]P. LAKSHMANA SWAMY, [4,]V.V.S.N. HARI KRISHNA

[1, 2]Department of Veterinary Biochemistry, NTR College of Veterinary Science, Gannavaram-521101, Andhra Pradesh, India, [3, 4]Department of Biotechnology, Acharya Nagarjuna University, Guntur. Andhra Pradesh, India. Email: kumar_rajaparabathina@yahoo.co.in

Received: 5 July 2011, Revised and Accepted: 13 Aug 2011

ABSTRACT

The authors evaluated the influence of DOX administration on the pre-treatment of flavonoids and vitamin-E. Thirty New-Zealand white rabbits aged between 5-6 months and averaging 2.5-3.0 Kgs in weight were divided into 5 groups of 6 each and used in one week feeding trail experiment. In the present study, the development of cardiomyopathy was prevented by reducing oxidative stress using natural antioxidant vitamin E (50 IU/kg body weight) and flavonoids morin, rutin and quercetin, which are generally available in the natural diet. The seven days treatment of flavonoids (20mg/kg body weight) were affectively influenced doxorubicin (10mg/kg body weight)-induced cardiomyopathy. The flavonoids affected the levels of serum enzymes SGOT, SGPT, ALP, oxidative markers catalase (CAT), lipid peroxidation, glutathione-s-transferase (GST), reduced glutathione (GSH) both in whole erythrocytes and tissues of liver, heart and kidneys. The study concludes that the flavonoids have a protective role in the doxorubicin-induced cardiomyopathy.

Keywords: Cardiomyopathy, Flavonoids, Oxidative stress, Doxorubicin, Tissue homogenate.

INTRODUCTION

Doxorubicin is an anthracycline antibiotic that has been used for human malignancies[1]. The clinical usefulness of DOX has been hampered by its detrimental cardiac toxicity [2, 3]. The dose related cardiomyopathy and congestive heart failure due to doxorubicin has limited the use of this drug. Cardiovascular diseases (coronary artery disease, hypertension, heart failure, and stroke) are the leading causes of death in human beings of modern days. Oxidative stress is the unifying mechanism for many cardiovascular risk factors (diabetes and obesity)[4]**.** Several mechanisms have been postulated to account for the effects of DOX, both in cancer treatment and cardiomyopathy. It is widely accepted that DOX – induced cardiomyopathy resides for the most part on oxidative stress and production of free radicals[3, 5]. DOX can generate free radicals through enzymatic and non enzymatic pathways[6]. Food is the major sources of antioxidants like vitamin C, vitamin E, selenium, and carotenoids that may help in providing protection against diseases by contributing, along with enzymes involved in scavenging of free radicals, to the total antioxidant defense system of the human body.

Flavonoids form a class of benzo-gamma pyrone derivatives that have high pharmacological potency. A great interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds[7]. Due to their radical-scavenging and iron-chelating properties[1], flavonoids can be considered possible potential protectors against DOX-induced cardiomyopathy. Morin, rutin and quercetin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, antiallergic, antiviral as well as an anticancer activity. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity. It is evident that the flavonoids play an important role in the various types of metabolic activities of life. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases[8]. According to their specificity in antioxidation function, certain selected flavonoids naturally available in diet (morin, rutin and quercetin) are used in the present study to investigate their affects on serum enzymes SGOT, SGPT, ALP, oxidative enzymes like catalase (CAT), lipid peroxidation, glutathione-s-transferase (GST), reduced glutathione (GSH) both in whole erythrocytes and tissues of liver, heart and kidneys were undertaken in this study as the oxidative markers of cardiomyopathy.

MATERIALS AND METHODS

Chemicals and reagent kits

The chemicals used in the present study were of analytical grade from E.Merck (India), SISCO Laboratories and Loba Chemicals and some chemicals were procured from Sd Fine Chemicals, Navi Mumbai, India. Vitamin E (Bio-E 400) was procured from the Dr.Reddy's Laboratories, Hyderabad and drug Doxorubicin (Doxopar-50) Parenteral drugs (India) limited, Indore, India. Some of the reagent kits were purchased from by the Laboratory of Ensure Biotech Pvt Limited, Hyderabad, and few reagent kits were purchased from the Transonic Bio-Medicals Limited, Solan (HP), was used for this study.

Experimental animals

Thirty apparently healthy, New-Zealand white rabbits weighing 2.5-3.0 Kgs in weight were obtained from Laboratory of small animal house, Department of Pharmacology, Dr.Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation, Chinnoutapalli, Gannavaram, Krishna District, Andhra Pradesh, India. The animals were housed in the cages of departmental laboratory animal shed. All animals were kept under uniform managerial and standard hygienic conditions through the experimental period. All the rabbits were randomly housed in cages and the cages were located in a well ventilated house and all the animals had free access to feed and water at all times. All animals were treated in accordance with the principles of laboratory animal care and the experimental protocol has been approved by the animal ethical committee of university.

Experimental Design

The thirty rabbits were randomly divided into five groups and six animals in each group. The group-I rabbits were fed on normal diet which considered as Controls for the present study. All the animals of group II-V were treated with a normal diet along with vitamin-E, morin, rutin and quercetin about seven days orally. On 8th day doxorubicin (10mg/ kg body weight) was administrated intra-venously for all groups. At the end of the week blood samples were collected and analyzed for serum enzymes and oxidative markers. After the treatment of doxorubicin again blood samples were collected and analyzed for serum enzymes and oxidative markers, then sacrificed the animals. The tissues of heart liver and kidney were collected and tissue homogenate was prepared by PBS solution in tissue homogenator.

Assessment of Serum Enzymes

The determination of SGOT activity is based on the tranamination of aspartic acid to α-ketoglutaric acid. One of the products of the reaction, namely, oxaloacetate is converted to pyruvate which is measured calorimetrically [9-13].

The SGPT activity is based on the tranamination of Alanine to α-ketoglutaric acid. One of the products of the reaction, namely, oxaloacetate is converted to pyruvate which is measured calorimetrically [9, 10, 14].

The substrate p-Nitrophenyl phosphate (pNPP) is hydrolyzed by Alkaline Phosphatse (ALP) present in the sample to p-Nitrophenol (pNP) and phosphate in alkaline medium in the presence of magnesium ions. pNP gives yellow colour. The rate of pNP formation is directly proportional to the ALP activity and is measured in terms of change in absorbance at 405 nm[15, 16].

Assessment Oxidative Markers in whole erythrocyte

Catalase (EC 1.11.1.6)

Catalase was estimated in erythrocytes by the spectrophotometric method as described by Bergmeyer (1983)[17]. Oxygen liberated by the action of the enzyme on hydrogen peroxide is measured either spectrophotometrically at 240 nm.

Lipid Peroxidation

Membrane peroxidative damage in the erythrocyte was determined in terms of malonialdehyde (MDA) production by the modified method of Stock and Dormandy (1971)[18] as described by Jain (1988)[19].

Reduced Glutathione (GSH)

Reduced glutathione was estimated by DTNB method of Beutler et al., (1983)[20].

Glutathione-S-Transferase (GST)

Glutathione-S-Transferase was estimated in erythrocytes by following the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate as described by Habig et al., (1974) [21].

Assessment Oxidative Markers in tissue homogenate (Liver, Heart and kidney)

Lipid Peroxidation

The extent of lipid peroxidation was evaluated in terms of MDA (Malonyl Dialdehyde) production determined by the thiobarbituric acid (TBA) method[22]. One ml of tissue homogenate in saline was mixed with 1 ml of TBA reagent (stock TBA reagent: 7% $HClO_4$:2.1). (Stock TBA (0.8%) was prepared by dissolving in small volume of 1N NaOH and then neutralized with 7% $HClO_4$).

Reduced glutathione (GSH)

Reduced glutathione was estimated by estimating free SH groups using DTNB method of Sedlak, et al., (1968)[23].

Glutathione-S-Transferase (GST, EC. 2.5.1.18)

In the procedure employed[21] the reaction mixture of 3 ml containing 1 mm GSH (reduced Glutathione), 1 mm 10Chloro-2, 4-dinitro-benzene (CDNB) in ethanol, and 100 mm potassium phosphate buffer pH 6.5 and requisite amount of enzyme (10% homogenate). The rate of increase in optical density at 340 nm at 25^0C was determined due to formation of CDNB-conjugate of glutathione. The substrates were prepared fresh, immediately before use. The increase in optical density was monitored for 3 min at 30 seconds interval. The enzyme activity was calculated by employing the extinction coefficient of the CDNB-GSH conjugate 9.6 / Mm / cm.

Statistical Analysis

Graph Pad Instat Demo (Dataset1.ISD) software was used for the statistical analysis presented in the experiment. The experimental data were statistically analyzed using one-way analysis of variance (ANOVA), followed by Dunnett test for multiple comparisons versus control. Data were expressed as Mean±S.E.M. Differences were considered significant at P value of less than 0.01 and 0.05.

RESULTS

Serum Enzymes

An attempt was made to evaluate the effects of flavonoids against doxorubicin in rabbit model. Treatment of the flavonoids and vitamin-E for consecutive seven days had maintained the normal SGOT levels, but the quercetin treated group had the lower SGOT levels throughout the study. So the treatment of flavonoids and vitamin-E had no significant effect but the single dose of DOX affected the SGOT levels in all the groups. The levels of SGOT decreased up to 10 IU/L in all the groups including the control group. SGPT levels were studied on the treatment of the flavonoids and vitamin-E for consecutive seven days had maintained the normal SGPT levels; again the quercetin treated group had the lower SGPT levels throughout the study. So the treatment of flavonoids and vitamin-E had no significant effect, but the DOX affected the SGPT levels in all the groups. The levels of SGPT decreased an average of 10-20 IU/L in all the groups except the control group, which had very little decreased value of SGPT.

Prior treatment of the flavonoids and vitamin-E for consecutive seven days had maintained the normal ALP levels with a little difference. As per the data of ALP in the Table: 1 the treatment of flavonoids and vitamin-E had no specific effect, but the DOX affected the ALP levels in all the groups. The levels of ALP decreased an average of 10-15 IU/L in all the vitamin-E and Morin treated groups, but increased up to 3-8 IU/L in remaining groups including the control group.

Oxidative Markers

The effects of flavonoids, vitamin-E and DOX on the enzyme activities of catalase, lipid peroxidation, GSH, GST in New Zealand white rabbits considered as oxidative markers are compiled in Table: 2. Pretreatment of flavonoids and Vitamin-E for seven days had maintained the catalase levels at optimum, but on the treatment of DOX shown a specific affect on catalase levels in all the groups i.e. the levels are significantly decreased about to 50%.

Administration of flavonoids and Vitamin-E for seven days had maintained the lipid peroxidation in whole erythrocytes and in tissue homogenates at normal level, but on administration of DOX shown a specific affect on lipid peroxidation levels in control group and quercetin group but the levels are normally maintained in the remaining groups. In the tissue homogenates of liver, heart and kidney the lipid peroxidation levels are significantly increased in quercetin group and little changes are shown in remaining groups (Table: 3).

Treatment of flavonoids and Vitamin-E for seven days had maintained the GSH in whole erythrocytes and in tissue homogenates at normal level, on the treatment two doses of DOX shown a significant effect on GSH levels in control group and quercetin group but the levels are increased in the remaining groups. In the tissue homogenates of liver, heart and kidney the GSH levels are significantly increased in all the groups whereas vitamin-E liver homogenate had lower GSH value (Table: 4).

Treatment of flavonoids and Vitamin-E for seven days had shown variations in the GST in whole erythrocytes and in tissue homogenates at normal level. On the treatment two doses of DOX shown a significant effect on GST levels. In control group the levels are increased but the remaining all the groups shown a specific decreased levels of GST. In the tissue homogenates of liver, heart and kidney the GST levels are normally maintained in all the groups (Table: 5).

Table 1: Rabbits-Serum Enzymes (Mean ± S.E.M) group's I-V on treatment of drugs

Groups	SGOT		SGPT		ALP	
	On 7days treatment	On DOX treatment	On 7days treatment	On DOX treatment	On 7days treatment	On DOX treatment
Control	102.71±2.14	91.80±3.65	75.62±2.85	72.54±2.93	67.60±1.82	70.21±1.35
Vitamin-E	106.86±2.17^{ab}	75.35±2.61**^{ab}	80.56±1.16	61.68±1.74*^{ab}	72.85±2.12^{a}	57.56±1.37**^{a}
Morin	77.85±2.34**^{a}	60.43±2.06**^{ab}	79.31±3.03	66.35±2.44^{ab}	83.59±1.74**	69.39±1.64^{a}
Rutin	102.28±3.21	79.20±2.32*^{ab}	83.81±1.65*	55.77±2.88**^{ab}	76.71±2.40*	82.86±2.26**
Quercetin	44.13±2.53**	30.41±3.15**	41.85±1.19**	31.25±1.68**^{ab}	55.36±1.06**	64.78±1.54

*In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment). ^{a} In a column differ significantly at P<0.05 (Between weeks within treatment). ^{ab} In a column differ significantly at P<0.01 (Between weeks within treatment).

Table 2: Rabbits- Oxidative Markers (Mean ± S.E.M) group's I-V on treatment of drugs

Groups	Catalase in K/g Hb		Lipid Peroxidation		Reduced Glutathione		GST	
	On 7days treatment	On DOX treatment	On 7days treatment	On DOX treatment	On 7days treatment	On DOX treatment	On 7days treatment	On DOX treatment
Control	132.10±1.86	76.04±1.57	2.88±1.12	1.96±0.15	100.00±1.58	91.66±1.79	4.91±0.28	6.41±1.26
Vitamin-E	138.93±1.17*	61.71±2.45*	3.07±1.31	3.88±1.37	118.33±2.07*	119.16±1.95**	9.04±0.21	3.35±1.48
Morin	125.53±1.75	66.45±2.54	3.31±1.14	3.89±1.54	111.66±1.93*	116.66±2.62**	5.92±0.83	4.46±1.60
Rutin	122.61±1.54	68.79±2.25	1.50±0.15	1.59±0.22	96.66±1.83	126.66±1.35**	4.58±0.40	3.83±1.61
Quercetin	93.17±2.27**	64.79±2.72	1.87±0.14	1.40±0.08	105.00±2.32	75.00±2.09**	3.48±0.59	2.95±0.31

*In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment).

Table 3: Tissue Homogenate-Oxidative Marker: Mean ± SE of Lipid Peroxidation in Units/mg Protein in Rabbits on Flavonoids and Doxorubicin treatment

Group	Liver	Heart	Kidney
Control	0.271±0.01	0.331±0.01^{ab}	0.254±0.01
Vitamin-E	0.212±0.009	0.179±0.007**	0.205±0.006*
Morin	0.211±0.001	0.159±0.004**	0.197±0.001*
Rutin	0.339±0.002	0.186±0.002**	0.217±0.002
Quercetin	1.123±0.053**	0.826±0.014**	0.956±0.024**

*In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment). ^{a} In a column differ significantly at P<0.05 (Between weeks within treatment). ^{ab} In a column differ significantly at P<0.01 (Between weeks within treatment).

Table 4: Tissue Homogenate-Oxidative Marker: Mean ± SE of Reduced Glutathione in Mm/g Tissue of Rabbits on Flavonoids and Doxorubicin treatment

Group	Liver	Heart	Kidney
Control	7.53±0.23	11.46±0.23	10.78±0.21
Vitamin-E	6.79±0.17	14.seven±0.09**	13.40±0.21*
Morin	13.17±0.053**	7.96±0.07**	13.40±0.21*
Rutin	11.70±0.06**	14.31±0.07**	8.seven±0.30*
Quercetin	22.30±0.70**	19.35±0.65**	16.19±0.60**

*In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment).

Table 5: Tissue Homogenate-Oxidative Marker: Mean ± SE of Glutathione-S-Transferase in U/mg Protein in rabbits groups I-V on flavonoids and doxorubicin treatment

Group	Liver	Heart	Kidney
Control	1.19±0.03	1.08±0.02	1.20±0.02
Vitamin-E	1.30±0.02	1.33±0.07*	1.19±0.03
Morin	1.01±0.03	1.22±0.07	1.49±0.02
Rutin	1.62±0.07*	1.13±0.05	1.37±0.02
Quercetin	2.58±0.17**	1.81±0.06**	1.87±0.37

*In a row differ significantly at P<0.05 (Between weeks within treatment). **In a row differ significantly at P<0.01 (Between weeks within treatment).

DISCUSSION

Serum Enzymes

Serum enzymes SGOT, SGPT and ALP were very important in the smooth regulation of the metabolism in all animals including human being. SGOT levels were increased in the necrosis and inflammation of heart, where as SGPT levels were increased in the liver damage. ALP levels were increased in malabsorption syndromes and hyper thyroidism and parathyroidism[24]. The normal range of the serum enzymes SGOT, SGPT and ALP levels were 10 - 98 IU/L, 25 - 65 IU/L and 10 - 70 IU/L respectively[25]. But the present study showed the levels as 44 to 107 IU/L, 42 to 84 IU/L and 55 to 83 IU/L. SGOT, in control group after the treatment of doxorubicin, decreased the SGOT from 102 to 91 IU/L. Vitamin E, morin, rutin and quercetin treated groups were also decreased the SGOT concentration after the treatment of doxorubicin.

SGPT, in controls after the treatment of doxorubicin at the end of one-week on normal diet, doxorubicin decreased the SGPT from 75 to 72 IU/L. Vitamin E, morin, rutin and quercetin treated groups were also decreased the concentration of SGPT after the treatment of doxorubicin.

ALP in control group after the treatment of doxorubicin increased from 67 to 70 IU/L. Vitamin E, and morin treated groups were decreased the ALP, where as rutin and quercetin treated group were increased the ALP concentration even after the treatment of doxorubicin. The decreased levels of SGOT and SGPT had no significant affect in the metabolism; where as increased levels may lead to severe liver disorders like necrosis and myocardial infarction, which are indicators of poor quality protein in diets fed[26]. ALP levels were also maintained in normal range that indicates no significant affect on the rabbits. Eventhough the values were decreased in Serum enzymes by doxorubicin treatment had no specific affects, but the flavonoids were protected at optimum levels to maintain the normal range.

Oxidative Markers

DOX administration induced oxidative stress in tissues as manifested by the alterations observed in oxidative markers like catalase (CAT), reduced glutathione (GSH), glutathione-s-transferase (GST) and lipid peroxidation were determined in the blood and GSH, GST and lipid peroxidation were determined in tissues of heart, liver and kidney. Though the exact mechanism(s) whereby DOX would be inducing cardiac toxicity is not fully explored, the principle mechanism could possibly be through free radical generation by the "redox-cycling" of anthracycline molecule and/or by the formation of anthracycline-iron complexes[33]. This concept of oxidative damage has been well documented in a plethora of previous reports[34-38]. Pretreatment of flavonoids significantly ameliorated all the biochemical parameters altered by DOX suggesting anti-oxidant role for cardiomyopathy.

Catalase: DOX decreased the catalase concentration from 132 to 76 K/g Hb in control group. Vitamin E and flavonoids treated groups were shown decreased in the concentration of catalase on the administration of doxorubicin indicating that either vitamin E or flavonoids could not alter the catalase reduced by the DOX. It is reported that DOX causes the decrease in catalase concentration[27].

Lipid Peroxidation: The raise in MDA concentration in the blood or tissues was the indication of lipid peroxidation by oxidative stress by free radicals. The MDA levels observed in all groups on the administration of DOX showed no indication of lipid peroxidation in the whole erythrocyte. However, significant decreases in the MDA levels are noticed in the tissues of heart, liver and kidney. These results indicative of protection of heart and kidney from oxidative damage by DOX. The flavonoid quercetin appears to be causing pro-oxidant effect in the liver, heart and kidney. It was reported that the quercetin may enhance certain effects of DOX[28]. Reactive oxygen species primarily responsible for causing lipid peroxidation. Enhanced levels of MDA were indicative of lipid peroxidation by oxidative damage[29]. The low levels of MDA were observed in flavonoids (morin & rutin) and vitamin E treated groups after DOX administration when compared with control group. Flavonoids were reported to be good antioxidant to overcome oxidative stress[30].

Glutathione is one of the essential compounds for regulation of variety of cell functions. It has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulfides. The depletion of GSH seems to be a prime factor that permits lipid peroxidation[28]. Glutathione-S-Transferase is GSH dependent antioxidant enzyme which catalyzes the conjugation of reduced glutathione via the sulfhydral group, to electrophilic centers on a wide variety of substrates[28]. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids, as well as the metabolism of xenobiotics[28]. In the present study, the control group decreased non significantly the reduced glutathione (GSH) concentration in whole erythrocytes and increased non significantly the GST concentration. Whereas vitamin E, morin treated groups there was no significant increase in reduced glutathione concentration, but the rutin treated group shown a significant increase in the GSH concentration. This is an evidence of antioxidant effect by the vitamin E, morin and rutin. Quercetin shown a significantly decreased the GSH concentration, that shows the pro-oxidant effect of quercetin[28].

Tissue Homogenate -Oxidative Markers: Lipid peroxidation and glutathione parameters were studied in the tissues of liver, heart and kidneys. An increased lipid peroxidation levels were observed in the tissues. On the vitamin E, flavonoids morin, and rutin treated groups were significantly but the decreased the lipid peroxidation levels observed in quercetin treated group. GSH levels were significantly increased in the tissues after the treatment of DOX. The vitamin E, flavonoids were also increased the GSH concentration which gives a clear idea about the protective affect from the DOX-induced oxidative stress. GST levels were maintained constantly in the tissues of control group, where as quercetin increased the GST compared with the remaining flavonoids and vitamin E treated groups. In the present study, the suppression of lipid peroxidation, GSH and GST signifies the free radical oxidative stress is increased due to DOX treatment[31]. The observed decline in the level of GSH indicating in enhanced lipid peroxidation, and excessive lipid peroxidation caused increased GSH consumption[28]. The reduced activity of GST in DOX treated group might be also due to decreased availability of its substrate, the reduced GSH. The present observation concurs with earlier reports[28,32] which showed that myocardial antioxidant defense system was operating at a lower rate despite higher level of oxidative stress in DOX-induced cardiomyopathy condition. The DOX-induced generation of free radicals in the myocardium might have exceeded the ability of the free radicals, resulting in myocyte lesions and reduction of scavengers, as evident from the present study. The flavonoids administration significantly counteracted the DOX-induced cardiomyopathy by maintaing the lipid peroxidation and increased the GSH and GST levels. Considering GSH levels due to oxidative stress by DOX, the group's vitamin E, morin, rutin and quercetin showed significant increase in the GSH levels in the tissues of heart, liver and kidney. These results indicative of protection of heart, liver and kidney by flavonoids used in the present study from oxidative damage by DOX. It reports that flavonoids were able to consider as potential protectors on DOX induced cardiomyopathy due to oxidative stress [31].

CONCLUSION

The present study concludes that pre-treatment of the flavonoids ahead DOX challenge to rabbits ameliorate all the serum and tissue oxidative marker parameters altered by the cytotoxic agent. Apart from the regulatory role of flavonoids and vitamin-E on tissues observed in the current work, the cardio protective effects of the flavonoids could possibly reside for the most part on its anti-radical effects. Thus morin, rutin and quercetin could improve the therapeutic benefits of DOX.

REFERENCES

1. Quiles JL, Huertas JR, Battino M, Mataix J, Ramerez- Tortosa MC. Antioxidant nutrients and adriamycin toxicity. 2002; 180: 79-95.
2. Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut, Irmak MK. Erdosteine doxorubicin-induced cardiotoxicity in rabbits. Pharmacol Res, 2003; 48: 377-82.
3. Chularojmontri L, Wattanapitayakul SK, Herunsalee A, Charuchongkolwongse S, Niumasakul S, Srichairat S. Antioxidative and cardioprotective effects of Phyllanthus urinaria L, on doxorubicin-induced cardiotoxicity. Biol Pharm Bull. 2005; 28(7):1165-71.
4. Leila Bettina Seres. Oxidative stress in cardiovascular diseases and in experimental models., Ph.D thesis: 2006; 74.
5. Singal PK, Li T, Kumar D, Danelisen I, Iliskovic N. Adriamycin-induced heart-failure: mechanism and modulation. Mol Cell Biochem. 2000; 207: 77-85.
6. De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabecule after acute and chronic treatment: a review. Eur J Pharmacol. 2001; 415: 1-11.

7. Diplock AT, Charleux JL, Crfozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Functional food science and defence against reactive oxygen species. Br J Nutr. 1998; 80: 77-112.
8. Raja Kumar Parabathina,P. Lashmana Swamy, V. V. S. N. Harikrishna,G. SrinivasaRao and Kaza Somasekhara Rao. Vitamin-E, Morin, Rutin, Quercetin prevents tissue biochemical changes induced by Doxorubicin in oxidative stress conditions: Effect on heart, liver and kidney homogenates. J. Chem. Pharm. Res. 2010; 2(4):826-834.
9. Bradley D.W., Maynard J.E., Emery G, and Webster H. 1972. Clin. Chem, 18;1442.
10. Wolf P.L., Williams D., Coplon N, and Coulson A.S. *Clin. Chem,* 1972; 18 (567).
11. Rej R., Fasce C.F. and Vanderlinde R.E. *Clin. Chem,* 1973; 19 (92).
12. Clinica Chimica Acta, 1980; 105: 145-175 F.
13. Tietz N.W. Fundamentals of Clinical chemistry, (W.B.Saunders eds, Philadelphia PA) 1986.
14. Wroblewski F, and La Due J.S. *Proc. Soc. Exper, Biol, and Med*. 1956; 91 (569).
15. Z.Klin. Chem, *U.kin. Biochem.* 1970 and 1972; 8, 658: 10, 182.
16. Rick W. Klinische Chemie and Mikrskopie, p: 242, 5th ed, springer vertag, Berlin. 1977.
17. Bergmeyer HU. "U.V. method of catalase assay. In methods of enzymatic analysis", Vol-III 3rd ed. Weinheim. Deerfield Beacn, Florida, Basal. 1983; pp.273.
18. Stock, T. and Dormandy, T.L. The autooxidation of human red cell lipids induced by hydrogen peroxide. *British J. Haematol*. 1971; 20: 95-111.
19. Jain SK. Evidence for membrane lipid peroxidation during the *in vivo* aging of human erythrocytes. *Biochemica Biophysica Acta*., 1988; 937: 205-210.
20. Beutler, E., Duran, O. and Kelly, B.M. "Improved method for the determination of blood glutathione", *J. Lab. Med*., 1963; 61: 882-888.
21. Habig, W.H., Michael, J.P. and Jacoby, W.B. "Glutathione-S-Transferase, the first enzymatic step in mercapturic acid formation", *J.Biol. Chem*., 1974; 249: 7130-7139.
22. Placer, Z.A., Cushman, L.L. and Johnson B. Estimation of product of lipid peroxidation (Malonyl dialdehyde) in biochemical systems. *Anal Biochem*., 1966; 16: 359-364.
23. Sedlak, J. and Raymonds, H. "Estimation of total protein bound NPSH groups in tissues with Ellman's reagent", *Biochemica Biophysica Acta*., 1968; 25: 192-205.
24. Pattabiraman TN. "Laboratory manual in biochemistry", 2001; 3rd Eds, pp:72.
25. Mitruka B.M. Ranwnley H.M. Clinical Biochemical and hematological reference values in normal and experimental animals. Masson publishing USA, 1977; Inc.pp 83,134- 5.
26. Facina, O.E., A.D. Ologhobo, C.O. Ayoade, G.A. Adeniran, and O.A. Adeyemi. "Nutritional and toxicological assessment of various amyadalin tears in nutrition of broiler chicks: affection performance of heamotological and biochemical indices", *Proc. Anim. Sci. Assoc. Nig.,* 1999; 4: 19-22.
27. Takacs I.E. Matkovics B. Varga S.I. Homolay P. Feer G & Seres T. "Study of the myocardial antioxident defence in various species", *Pharmacological research.* 1992; 25: 177.
28. Raja Kumar P. "Studies on amelioration of oxidative stress by vitamin-E, flavonoids (Morin, rutin and quercetin) in drug (doxorubicin) induced cardiomyopathy in rabbits" Ph. D thesis, 2010.
29. B C Koti, AHM Vishwanathswamy, Jyothi Wagawade and A H M Thippeswamy. "Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in New-Zealand rabbits", *Indian Journal of Experimental Biology*, 2009; Vol: 47, 41-46.
30. Li T.& Singhal P.K. "Doxirubicine induced early changes in myocardial antioxident enzymes and their modulation by probucol", *Circulation*. 2000;102: 2105.
31. Naiyra A. Abd Elbaky, Azza A. Ali and Raeesa A. Ahmed. "Cardioprotective effect of Simvastatin on Doxorubicin-induced oxidative cardiotoxicity in rabbits", *Journal of Basic and Applied Sciences*, 2010; Vol. 6 (1), 29-38.
32. Gnanaprakasam A, Ebenezar KK, Sathish V, Govindaraju P, Devaki T. "Protective effect of *Centella asiatica on anti oxidant tissue diffence system against ardiamycine induced cardiomyothy in rabbits*", Life Sci. 2004; 76: 585-597.
33. Hrdina R, Gersl V, Klimtova I, Simunek T, Mach J, Adamcova M. Anthracycline-induced cardio toxicity. Acta Medica, 2000; 43 (3): 75-82.
34. Morishima I, Okumura K, Matsui H, Kaneko S, Numaguchi Y, Kawakami K. Zinc accumulation in adriamycin-induced cardiomyopathy in rabbits: effects of melatonin, a cardioprotective antioxidant. Pineal Res, 1999; 26 (4): 204-10.
35. Li T, Danelisen I, Bello-Klein A, Singal PK. Effects of probucol on changes of antioxidant enzymes in adriamycin-induced cardiomyopathy in rabbits. Cardio Vascular Res, 2000; 46 (3): 523-30.
36. Abd El-Aziz MA, Othman AI, Amer M, Elmissiri M. Potential protective role of angiotensin converting enzyme inhibitors captopril and enalapril against adriamycin-induced acute cardiac and hepatic toxicity in rabbits. J Appl Toxicol, 2001; 21 (6): 469-73.
37. Deepa PR, Varalakshmi P. Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced acute cardiac and hepatic toxicities. Chem Biol Interact, 2003;146 (2): 201-10.
38. Buyukokuroglu ME, Taysi S, Bukavci M, Baka E. Prevention of acute adriamycin- cardiotoxicity bydantrolene in rabbits. Hum Exp Toxicol, 2004; 23 (5): 251-6.