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ROLE OF GRAPE SEED AND SKIN EXTRACT IN PREVENTION OF DOXORUBICIN INDUCED CARDIOTOXICITY IN RAT MODEL

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ABSTRACT

Background: Doxorubicin is a potent chemotherapeutic drug The clinical usefulness of doxorubicin has been limited largely by the risk of cardiomyopathy and life-threatening heart failure. Cellular changes leading to this toxicity are suggested to be mediated through a drug-induced increase oxidative stress. Grapeseed Proanthocyanidin [GSP] seeds possessing a potent antioxidant properties **Aim:** So, the present study was conducted to investigate possible protective effects of Grape seed and skin extract in doxorubicin-induced cardiotoxicity in rat model. Method: The male Wister rats (n = 34) were randomly selected and divided into three groups. The control group (n = 12) received distilled water [1ml/kg body weight] orally for 15 days, second group (n = 10) rats were injected intra peritoneal with a single dose of Doxorubicin (10mg/kg) in normal saline and in third group Doxorubicin (10mg/kg) was administered intraperitoneally and pretreatment with Grape seed and skin extract extract (200mg/kg body weight) before one hour DOX treatment by oral gavage for 15 consecutive days. **Result:** It was revealed by elevated serum cardiac biomarkers in comparison of control and associated with increasing levels of myocardial malondialdehyde [MDA] with simultaneously increase in the level of Superoxide dismutase. On daily oral administration of aqueous suspension of GSP seeds extract in the dose of (200 mg\kg) for 15 days produced normalization in the serum levels of heart marker enzymes. **Conclusion:** The present study has demonstrated that Grape seed and its skin has got definite potency to ameliorate oxidative stress and Doxorubicin induced cardiotoxicity.

KEY WORDS: Grape seed and skin extract, Doxorubicin, Cardiotoxicity, Oxidative stress, Antioxidant.

INTRODUCTION

Doxorubicin is a potent chemotherapeutic drug from the anthracycline antibiotics [1] that is used extensively for the treatment of haematological malignancies and solid tumours.[2] However, despite therapeutic efficacy, its clinical usage is limited by the development of cumulative dose-dependent cardiomyopathy [3] which may occur many years after the cessation of doxorubicin treatment [4] and that precludes some patients from receiving a highly effective treatment. Development of cardiotoxic side effects is associated with a poor prognosis. [5]

The exact pathogenesis of doxorubicin-induced cardiotoxicity is still not entirely clear although a diverse set of mechanisms have been proposed, including oxidative stress,[6] intracellular calcium overload,[7] mitochondrial DNA damage, inhibition of protein synthesis, disturbance of myocardial adrenergic function, cytokine release, myofibrillar degeneration and cardiomyocyte apoptosis.[8] Among the multiple mechanisms, it is widely accepted that doxorubicin-induced cardiomyocyte apoptosis is primarily due to the generation of reactive oxygen species (ROS) in the myocardium which triggers intrinsic mitochondria-dependent apoptotic pathway in cardiomyocytes. [9,10]

Medicinal plants that have been found to have certain preventive measures in the treatment of ischemic heart disease (IHD). Grape seed proanthocyanidins [GSP] have pained considerable attention due to their wide range of biological and pharmacological properties.[11,12] Grape seed and skin extract is a nutritional supplement exhibiting beneficial health effects.[13] Grape seed and skin extract is a complex mixture of polyphenolics classified as flavonoid and non-flavonoid compounds.[14] Flavonoids, which are highly concentrated in grape seeds are mainly composed of monomeric catechins, proanthocyanidins and flavonols, such as quercetin.[15] Non flavonoids highly abundant in grape skin, contain stilbenes such as resveratrol, which is at the basis of the french paradox.[15] Resveratrol is multi-organ protective owing to its antioxidant [16] and anti-inflammatory properties.[17] Proanthocyanidins exert antineoplasic effects by inducing cytotoxicity towards some cancer cells,[18] by cell cycle arrest and induction of apoptosis.[19]

So, the present study was conducted to investigate possible protective effects of Grape seed and skin extract in doxorubicin-induced cardiotoxicity in rat model and accordingly, we carried out this study on experimental rat models assuming that the results would have more or less similar implications in humans also.

MATERIAL AND METHODS

Study area

The present study was an animal model based case control study undertaken between October 2011 and December 2013. in the departments of Biochemistry with the collaboration of the department of Pharmacology of Burdwan Medical College, Burdwan, West Bengal, India.

Animal

Male Wister strain albino rats (Rattus norvegicus albinus), between 1 to 2 months of age weighing 150 ± 12g, n = 34 were obtained from the appropriately maintained institutional animal house. The rats had free access to drinking water and rat food pellets. The light source in the animal room was regulated with 12 hr light period followed by 12 h dark schedule within a temperature of range of 22 to ± 2^{o}C at a relative humidity of 45 to 50 %. All rats were acclimatized for at least 7 days before induction of diabetes. All procedures involving animals were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda' and 'Guidelines for care and use of animals in scientific research' by the Indian National Science Academy (INSA), New Delhi, India. The study was approved and permitted by the institutional ethics committee for care and use of laboratory animals, and started after obtaining the written consent from the concerned ethics committee [Memo No.BMC/2179/1 (8)].

Preparation of grape seed and skin extract

The seeds of Grapeseed Proanthocyanidins were collected manually during month of October 2011. Seeds and skin were dried and ground separately with an electric mincer (FP3121 Moulinex) until a fine powder was obtained. Total phenolic content was determined by the folin-Ciocalteau colorimetric method,[20] flavonoids and condensed tannins according to Dewanto et al (2002) and Sun et al (1998) respectively.[21,22] Powder mixture containing grape seed (50%) and skin (50%) was dissolved in 1 ml of 10% ethanol in the dark, vigorously vortexed for 10 min, centrifuged at 10,000 g for 15 min at 4°C for debris elimination and the supernatant containing soluble polyphenols was used.

Chemicals

Doxorubicin was from Sigma-Aldrich, St Louis, MO, USA.

Study design

The rats were divided in to six groups of six rats each as follows:

GROUP I (n =12): Control rats received distilled water [1ml/kg body weight] orally for 15 days and animals were sacrificed on 16th day.

GROUP II (n =10): Rats were injected intra peritoneal with a single dose of Doxorubicin (10mg/kg) in normal saline and animals were sacrificed after 48hrs.

GROUP III (n =12): Rats were administered Doxorubicin (10mg/kg) intraperitoneally and pretreatment with Grape seed and skin extract extract (200mg/kg body weight) before one hour DOX treatment by oral gavage for 15 consecutive days and animals were sacrificed on 16th day.

Collection and processing of blood

Blood was withdrawn from tail of each rat under study for determination of serum concentrations of hs-cTnT (high sensitive cardiac troponin T) and NT-proBNP and allowed to coagulate at room temperature for 30–45 min, followed by centrifugation at 2500Xg for 15 min.

Preparation of heart extract

After collecting the blood samples all the animals were anesthetized and were sacrificed and isolated heart. For homogenisation, samples were first washed and minced with sharp surgical blade in small volumes of ice cold (not frozen) homogenisation buffer made of 0.1 M Tris–HCl (pH 7.35) and 100 μM ethylenediaminetetraacetic acid (EDTA). Immediately the samples were homogenised in 10 volumes of the ice cool buffer solution in a motor driven glass tissue homogenizer in presence of properly washed few particles of sand. During the whole homogenisation procedure the homogeniser was kept submerged in small ice particles to dissipate any heat. Thereafter the samples were centrifuged at 10,000 X g for 10 min in a refrigerated cold centrifuge machine at 4^{0}C. Supernatants from the homogenates were collected and were estimated for MDA, PC adducts, cytosolic superoxide dismutase (Cu^{2+}-Zn^{2+}-SOD) and tissue protein immediately.

Biochemical assay

(A) **Measurement of cardiotoxicity**

Levels of hs-cTnT was measured by an enzyme linked one-step sandwich immunoassay method (TOSOH A1A21 fluorescens Chemistry), and the lowest detectable level was 500 pg/ml.[23] Plasma concentrations of hs-cTnT (high sensitive cardiac troponin T) was

determined by electro-chemiluminescence immunoassay, sandwich technique using 4th generation Troponin T high sensitive STAT I kits, Elecsys 2010; Roche Diagnostics. The lower limit of detection was 10 pg/ml. The 99th percentile value of hs-cTnT for a normal reference population was 13.5ng/L, with a CV <10%.[24] Serum CK and CK-MB isoenzyme activities were determined using CK NAK liquiUV kit and immunoinhibition by monoclonal antibody to CK-M subunit, Human, Germany. [25] The cut-off values for individual markers were as follows: for CK-MB mass 4.94 µg/L. Levels of NT-pro-BNP were measured using electrochemiluminescent immunoassay (Roche Moduler Analytics E170, Elecys Module). The upper limit of normal (100ng/L for males,150 ng/L for females) is proper and can be used in clinical studies .[26] In the present study, values above the reference range based on a number of studies and recommended by the manufacturer were considered elevated and suggesting cardiac injury associated with the treatment.

(B) **Measurement of oxidative stress**

Blood was separated into a heparinised vial to obtain plasma and a plain vial to obtain serum. MDA, a marker of lipid peroxidation due to oxidative stress was measured by its reaction with thiobarbituric acid at 532 nm [27]. The brain tissue levels of MDA were calculated using a calibration curve derived from 1,1,3,3- tetraethoxypropane (Fluka, Germany) as the external calibration standard. The calibration curve was linear in range from 1.25 to 2.5 nmol/ml (r2=0.997). Oxidation induced changes in the tissue proteins were estimated by measuring the protein carbonyl products. The method is based on the reaction of carbonyl groups with 2,4- dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone reactive carbonyl derivate that was measured at 370 nm. [28].

Estimation of cytosolic superoxide dismutase (SOD) was done by the method of Kakkar et al. where one unit of SOD was defined as that amount of enzyme that inhibited the rate of electron transfer from NADH to nitroblue tetrazolium (NBT) by 50 % under specified conditions [29]. Tissue proteins were measured by the method of Lowry et al. [30] that involved reaction of proteins in tissue homogenates with alkaline copper sulphate followed by another reaction with Folin's phenol reagent (SRL, India) against a standard curve prepared form bovine serum albumin (Merck, Germany). All photometric measurements were performed in Dual beam spectrophotometer (UV 5704SS). Serum cardiac markers was assayed in pg/ml while other parameters were expressed in their corresponding units per mg of tissue protein.

Statistical analysis

Data obtained were analyzed for significance of differences of means between the control with Group II and Group III rats by independent t test. For all tests 'p' value was considered to be significant if it was less than 0.05 at a confidence level of 95 %. All statistical analyses were performed with the help of the statistical software package SPSS: version 11.5 for Windows.

RESULT

To compare the efficiency in reduction of oxidative stress by Grape seed and skin extract, independent sample t test was performed between Group I rats with Group II and Group III (Table 1), it was observed that MDA and PC product formation were significantly suppressed and SOD activity was improved in different areas of brain by Grape seed and skin extract administration ($P < 0.001$, Figure 2) in Group III rats at a mean serum NT-proBNP level of 108.9 (range 87.22 to 130.58) pg/ml and hs-cTnT concentration of 7.95 (range 4.81 to 11.09) than Doxorubicin group at a mean serum NT-proBNP level of 412.8 (range 322.32 to 503.28) pg/ml and hs-cTnT concentration of 43.6 (range 35.41 to 51.79) pg/ml (Figure 1). There was significant decrease of oxidative parameters and cardiac biomarkers of doxorubicin induced cardiac toxicity in Grape seed and skin extract treated group III rats than Doxorubicin administered group II experimental rats that was well proved by statistically non-significant difference of oxidative parameters and cardiac biomarkers.

Table 1: **Differences between the mean values of selected parameters in different area of brain of the three study groups of rats**

Parameters	Sources	Group I (Control) n = 12	Group II (Doxorubicin) n = 10	Group III (Doxorubicin + Grape seed and skin extract) n = 12	Group I vs Group III	Group I vs Group II	Group II vs Group III
NT-proBNP (pg/ml)	Serum	109.3 ± 23.49	412.8 ± 90.48	108.9 ± 21.68	p> 0.05	p<0.001	p<0.001
hs-cTnT (pg/ml)		6.38 ±3.27	43.6 ±8.19	7.95 ±3.14	p >0.05	p<0.001	p<0.001
Tissue MDA (nmol/mg of protein)	Heart	0.39 ± 0.08	1.**27** ± 0.25	0.47 ± 0.04	p> 0.05	p<0.001	p<0.001

Tissue PC (mM/mg of protein)	Heart	0.29 ± 0.07	0.49± 0.11	0.25 ± 0.04	p> 0.05	p<0.001	p<0.001
Cytosolic SOD (IU/mg of protein)	Heart	0.79 ± 0.12	1.38 ± 0.17	0.82 ± 0.20	p>0.05	p<0.001	p> 0.05

Values are mean ± SD; p < 0.05 consider statistically significant

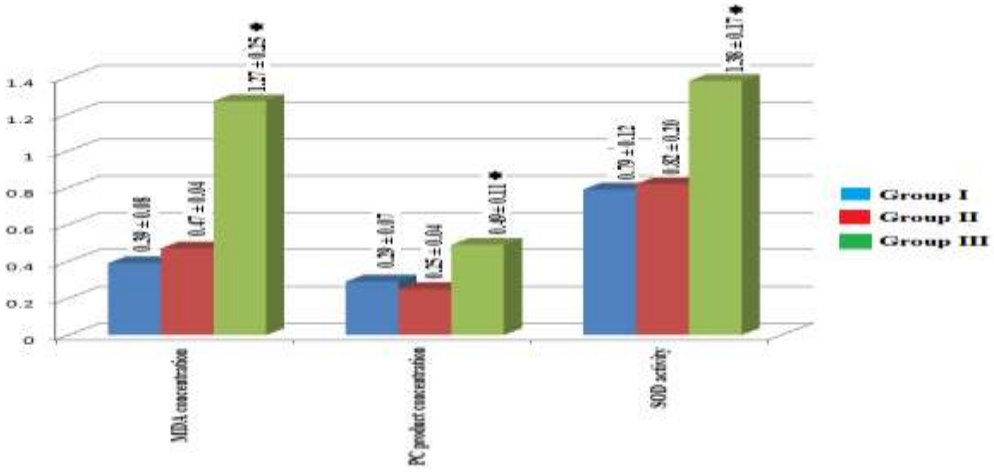

Figure 1: Histogram showing distribution of MDA, PC products and SOD of Group I (Control), Group III (Doxorubicin + Grape seed and skin extract) and Group II (Doxorubicin) rats. Asterisks indicate p<0.001.

DISCUSSION

The anthracycline antibiotic Doxorubicin is an important antineoplastic agent because of its high antitumor efficacy in hematological as well as in solid malignancies.[3] But its use is limited by the frequent induction of dose-dependent chronic cardiomyopathy. Oxidative stress has long been, and remains, the most studied and widely accepted cause of cardiotoxicity. Several therapeutic interventions have been attempted to reduce this toxicity as well as to improve the efficacy of this drug [31-34] and benefits of pharmaceutical factors to treat the disease aggressively early have been recommended, but medications may have unwanted side effects. Thus, there has been a growing interest in herbal remedies that can be introduced into the general population with the least side effects and the maximal preventive outcome.[35]

Grape seed and its skin contain polyphenol of members of the proanthocyanidins [36] and this compound show strong antioxidant activity.[37,38] So, the present study was conducted to investigate the potency of Grape seed and skin in amelioration of oxidative stress and therefore Doxorubicin induced cardiotoxicity.

At first, result of the present study clearly indicated that Doxorubicin induces oxidative stress and that leads to compromised antioxidant activity evidenced by increased serum MDA, PC product levels and plasma SOD activity. These results are consistent with studies in the animal studies.[39-41] The chemical structure of doxorubicin favours the generation of free radicals by reduction of the quinine moiety of doxorubicin to semiquinone, initiating a cascade of free radical formation that leads to many deleterious effects on cells, cell membranes and subcellular apparatuses.[42] Moreover, the compound can bind to iron and form complexes with DNA, inducing DNA damage.[43,44] Doxorubicin impair mitochondrial calcium homeostasis, causing loss of stability of the mitochondrial membrane and ultimately, cell death.[45] Cardiac cells are the most sensitive to the effects of Doxorubicin followed by the sarcoma and melanoma cells normal muscle fibroblast and normal skin fibroblast.[46] DOX produces acute injury to the myocardial membrane which causes significant elevation of marker enzyme [AST, ALT, LDH, CPK] activities in serum in the present study could be regarded as a sign of damage to the heart muscle membrane, which suggest the event of enhanced lipid peroxidation. Ultimately, these changes can lead to cell death and organ damage.

But when Grape seed and skin was used along with the Doxortubicin, it was observed that due to its own antioxidant activity as well as free radical scavenging effect, it alleviates oxidative stress as well as prevent the leakage of marker enzymes by scavenging lipid peroxides by protecting integrity of membrane. The antioxidant enzymes SOD, constituting the first line of defence mechanism to prevent and neutralize the reactive oxygen species (ROS) induced damage. Sharma G et al and Zhang XY et al showed their studies that grape seed proanthocyanidin synergize the efficacy of doxorubicin against human breast carcinoma cells and reverses drug resistance in doxorubicin-resistant K562/DOX cells thus the exact enhances doxorubicin-induced antitumor effect both in vitro and in vivo. [47-50] Zhang XY et al was found that proanthocyanidins strongly enhanced the anti-tumour effect of doxorubicin and completely eliminated myocardial oxidative stress and immunosuppression induced by doxorubicin in tumour-bearing mice.[50]

CONCLUSION

The present study has demonstrated that Grape seed and its skin has got definite potency to ameliorate oxidative stress and Doxorubicin induced cardiotoxicity rats. Further studies may be undertaken for consideration of use of Grape seed and its skin extract for the prevention of chemotherapy induced cardiotoxicity.

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CONFLICT OF INTEREST

We do not have any conflict of interest.

REFERENCES

1. Zhang J, Clark JR, Herman EH, Ferrans VJ. Dendritic cells in the hearts of spontaneously hypertensive rats treated with doxorubicin with or without ICRF-187. Am. J. Pathol. 1993;142: 1916–1926.
2. Smith LA, Cornelius VR, Plummer CJ, Levitt G, Verrill M, Canney P, Jones A. Cardiotoxicity of anthracycline agents for the treatment of cancer: systematic review and meta-analysis of randomised controlled trials. BMC Cancer 2010;10:337.
3. Chen Y, Wan Y, Zhang H, Jiao Z. Anticancer efficacy enhancement and attenuation of side effects of doxorubicin with titanium dioxide nanoparticles: International Journal of Nano medicine 2011;6 :2321–2326.
4. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity cardiotoxicity. Pharmacol Rev 2004; 56: 185-229.
5. Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis 2007; 49: 330 – 352
6. Raschi E, Vasina V, Ursino MG, Boriani G, Martoni A, de Ponti F. Anticancer drugs and cardiotoxicity: insights and perspectives inthe era of targeted therapy. Pharmacol Ther 2010; 125(2):196-218.
7. Raschi E, Vasina V, Ursino MG, Boriani G, Martoni A, de Ponti F. Anticancer drugs and cardiotoxicity: insights and perspectives indoxorubicin-induced inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase gene transcription. Circulation Research 2010;86(1):8-14.

8. Sawyer DB, Peng X, Chen B, Pentassuglia L, Lim CC. Mechanisms of anthracycline cardiac injury: can we identify strategies for cardioprotection? Prog Cardiovasc Dis 2010; 53(2): 105-113.
9. Simunek T, ˇSterba M, Popelová O, Adamcová M, Hrdina R, Gersi V. Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. Pharmacological Reports 2009;61(1):154-171.
10. Zhang YW, Shi J, Li YJ, Wei L. Cardiomyocyte death in doxorubicin-induced cardiotoxicity.Arch Immunol Ther Exp (Warsz) 2009;57(6):435-445.
11. Bors W. Fool LY. Hertkorn N. michel C. stetimaies k. chemical studies of proanthocyanidins and hydroobletams antioxidant redox signal 2001;3.995-1008.
12. Bagchi D. Bagchi M. Stohs SS. Ray SD, Sen CK prvess Hg leular protection with proanthocyanidins derived from GSP arm HG Acad pathol 1959; 1967 43-58.
13. Suwannaphet W, Meeprom A, Yibchok-Anun S, Adisakwattana S. Preventive effect of GSE against high fructose diet-induced insulin resistance and oxidative stress in rats. Food Chem Toxicol. 2010; 48: 1853-57.
14. Khanal RC, Howard LR, Prior RL. Procyanidin content of grape seed and pomace, and total anthocyanin content of grape pomace as affected by extrusion processing. J Food Sci. 2009; 74: H174-182.
15. Renaud S, De Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet. 1992; 339 (8808): 1523-26.
16. Kelen M, Tepe B. Screening of antioxidative properties and total phenolic compounds of various extracts of three different seed of grape varieties (Vitis vinifera L.) from Turkish flora. Pak J Biol Sci. 2007; 10: 403-08.
17. Kowalczyk MC, Kowalczyk P, Tolstykh O, Hanausek M, Walaszek Z, Slaga TJ. Synergistic effects of combined phytochemicals and skin cancer prevention in SENCAR mice. Cancer Prev Res (Phila). 2010; 3: 170-78.
18. Chatelain K, Phippen S, Mc Cabe J, Teeters CA, O'Malley S, Kingsley K. Cranberry and Grape Seed Extracts Inhibit the Proliferative Phenotype of Oral Squamous Cell Carcinomas. Evidence-Based Complementary and Alternative Medicine. 2011; ID 467691. doi:10.1093/ecam/nen047.
19. Kaur M, Mandair R, Agarwal R, Agarwal C. Grape seed extract induces cell cycle arrest and apoptosis in human colon carcinoma cells. Nutr Cancer. 2008; 60: 2-11.
20. Singleton VL, Rossi JA. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagent. Am J Enol Vitic. 1965; 16: 144-58.

21. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem. 2002; 50: 3010-14.
22. Sun BS, Ricardo-Da-Silva JM, Spranger MI. Critical factors of vanillin assay for catechins and proanthocyanidins. J Agric Food Chem. 1998; 46: 4267-74.
23. Murat Soker, Mehmet Kervancioglu. Plasma concentration of NT-pro-BNP and cardiac troponin I in relation to Doxorubicin induced cardiomyopathy and cardiac function in childhood malignancy Saudi Med J 2005;26 (8): 1197-1202.
24. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and Analytical Variability of a Novel High-Sensitivity Cardiac Troponin T Assay. Clin Chem. 2010;56(7):1086-90.
25. Wu AHB, Gornet TG, Bretaudiere JP, Panfili PR. Comparison of enzyme immunoassay and immunoinhibition for creatine kinase MB in diagnosis of acute myocardial infarction. Clin Chem. 1985;31:470-4.
26. Hess G, Runkel S, Zdunek D, et al. Reference interval determination for N-terminal-B-type natriuretic peptide (NT-proBNP): a study in blood donors. Clin Chem Acta 2005; 360: 187–93.
27. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 1962;98:253–261.
28. Levine RL, Garland D, Oliver CN et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–478.
29. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130–132.
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–275.
31. Hideg K, Kalai T. Novel antioxidants in anthracycline cardiotoxicity. *Cardiovasc Toxicol* 2007;**7**:160–164.
32. Kaiserova H, Simunek T, Sterba M, den-Hartog GJ, Schroterova L, Poelova O. New iron chelators in anthracyclin-induced cardiotoxicity. Cardiovasc Toxicol 2007;**7**:145–150.
33. Rajaprabhu D, Rajesh R, Jeykumar R, Buddhan S, Ganeshan B, Anandan R. Protective effect of *Picrorhiza Kurrora* on antioxidant status in adriamycin induced cardiomyopathy in rats. J Med Plant Res 2007;4:80–85.
34. Chvetzoff G, Bonnotte B, Chauffert B. Anticancer chemotheraphy: Prevention of toxicity. *Presse Med* 1998;**27**:2106–2112.

35. Matsui T, Ogunwande LA, Abesundara KJM, Matsumoto K: Antihyperglycemic potential of natural products. Mini-Rev Med Chem 2006, 6:109-120.
36. Rice-Evans CA, Miller NJ, Paganga G.Struture—antioxidant activity relationships of flavanoids and phenolic acid. Free Radical Biol Met 1996;20:933-56.
37. Bagchi D, Garg A, Krohn RL, Bagchi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Res Commun Mol Pathol Pharmacol 1997; 95:179-89.
38. Bagchi D, Garg A, Krohn RL, Bagchi M, Bagchi DJ, Balmoori J, et al. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA induced hepatic and brain lipid peroxidation and DNA fragmentation and peritoneal macrophage activation in mice. Gen Pharmacol 1998; 30:771-6.
39. Abd Elbaky NA, Ali AA, Ahmed RA. Cardioprotective effect of simvastatin on doxorubicin - induced oxidative cardiotoxicity in rats. Journal of Basic and Applied Sciences 2010; 6(1):29-38.
40. Xin Y, Zhang S, Gu L, Liu S, Gao H, You Z, Zhou G, Wen L, Yu J, Xuan Y. Electrocardiographic and biochemical evidence for the cardioprotective effect of antioxidants in acute doxorubicin-induced cardiotoxicity in the beagle dogs. Biol Pharm Bull 2011;34(10):1523-1526.
41. Al-Thamir SN, Oubaid EN. Protective Effect of Aminophylline on Doxorubicin-Induced Cardiotoxicity in Rats. Medical Journal of Babylon 2013;10(1):219-228.
42. Simunek T, ˇSterba M, Popelová O, Adamcová M, Hrdina R Gersi V. Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. Pharmacological Reports 2009; 61(1):154-171.
43. Eliot H, Gianni L, Myers C. Oxidative destruction of DNA by the adriamycin-iron complex. Biochemistry 1984; 23:928-36.
44. Ravi D, Das KC. Redox-cycling of anthracyclines by thioredoxin system: increased superoxide generation and DNA damage. Cancer Chemother Pharmacol 2004; 54:449-58.
45. Zhou S, Palmeira CM, Wallace KB. Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicol Lett 2001;121:151-157.
46. Abirami M, Kanagavalli U. Cardioprotective effect of grapeseed proanthocyanidin on Doxorubicin induced myocardial injury in rats. Int. J. of Pharm. & Life Sci. 2013;4(1):2288-2293.

47. Sharma G, Tyagi AK, Singh RP, Chan DC, Agarwal R. Synergistic anti-cancer effects of grape seed extract and conventional cytotoxic agent doxorubicin against human breast carcinoma cells. Breast Cancer Res Treat 2004; 85:1-12.
48. Zhang XY, Bai DC, Wu YJ, Li WG, Liu NF. Proanthocyanidin from grape seeds enhances antitumor effect of doxorubicinboth in vitro and in vivo. Pharmazie 2005; 60:533-8.
49. Zhang XY, Li WG, Wu YJ, Bai DC, Liu NF. Proanthocyanidin from grape seeds enhances doxorubicin- induced antitumoreffect and reverses drug resistance in doxorubicin-resistant K562/DOX cells. Can J Physiol Pharmacol 2005; 83:309-18.
50. Zhang XY, Li WG, Wu YJ, Gao MT. Amelioration of doxorubicin-induced myocardial oxidative stress and immunosuppression by grape seed proanthocyanidins in tumour-bearing mice. J Pharm Pharmacol 2005;57:1043-52.