International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2018; 6(6): 35-39
Received: 09-09-2018
Accepted: 10-10-2018

Pritam Saha Podder
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Pijus Madhu
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

Rajib Das
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Md. Abdul Awoal
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Nusrat Jahan
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Md. Shah Jinat Ullah
Department of Medicine, BIRDEM General Hospital, Shahbag, Dhaka, Bangladesh

Correspondence
Pritam Saha Podder
Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh

Preclinical study on cardioprotective effect of ethanolic extract of *Eucalyptus globulus* against doxorubicin induced cardiotoxicity

Pritam Saha Podder, Pijus Madhu, Rajib Das, Md. Abdul Awoal, Nusrat Jahan and Md. Shah Jinat Ullah

Abstract
Doxorubicin is an effective broad-spectrum anthracycline chemotherapeutic agent successfully used in the treatment of a wide range of malignancies. However, the effectiveness of this drug has become limited by the occurrence of dose related cardiotoxicity and heart failure. The aim of the present study was to investigate the potential cardioprotective effect of "*Eucalyptus globulus*" against doxorubicin induced cardiotoxicity in the Wister albino rats. Animals received either Doxorubicin (3 mg/kg, i.p.) every other day or combination of *E. globulus* (100mg/kg and 200mg/kg;p.o.) with Doxorubicin or *E. globulus* (200mg/kg;p.o.) extract alone for 2 weeks. Cardiotoxicity was assessed by recording changes in ECG (increased QT interval), measuring the levels of cardiac marker enzymes such as lactic acid dehydrogenase (LDH), creatinin kinase (CK-MB) at the end of treatment schedule. Treatment with *E. globulus* (100mg/kg and 200mg/kg) significantly ($p<0.05$) decreased the levels of cardiac marker enzymes, reversed the changes in ECG and prevented the decrease in heart weight in DOX-treated group. These results suggest that *E. globulus* has the potential in preventing the cardiotoxic effects induced by Doxorubicin.

Keywords: Doxorubicin, *Eucalyptus globulus*, ECG, LDH, CK-MB and antioxidant enzymes

1. Introduction
Doxorubicin, an anthracic line glycoside anti-tumor drug, is widely used for chemotherapy of a broad spectrum of solid tumors and hematological malignancies [1].
Doxorubicin has also been shown to induce ECG abnormalities such as QT interval prolongation and QT dispersion, during chemotherapy [2] apart from its well-known long term dose-dependent cardio toxicity [3].The treatment of cancer patients with doxorubicin may be complicated by acute and chronic side effects. Among the acute side effects nausea, vomiting, myelo suppression, and arrhythmias are most common [4]. The chronic side effects represented by the progression of cardiomyopathy and congestive heart failure. Cardiotoxicity induced by Dox is normally generated through lipid peroxidation long fatty acid oxidation in cardiac tissues [5, 6, 7]. It has been suggested that DOX-induced myocardial dysfunction involves the inhibition of nucleic acid as well as protein synthesis [8], release of vasoactive amines [9], changes in adrenergic function [10]. An increase in free radicals and lipid peroxidation as well as a decrease in antioxidants, plays an important role in the pathogenesis of DOX-induced cardio toxicity [11].
Eucalyptus globulus (*E. globulus*) is an over green tree which belongs to the family of Myrtaceae. Another name of the tree is fever tree. From ancient period of time, the use of medicinal plants in subcontinent is very common issue. The leaves of *E. globulus* have been used as a natural remedies for the treatment of several diseases such as influenza [12], fungal infections [13] pulmonary tuberculosis [14] and diabetes [15, 16]. The antioxidant properties of the *Eucalyptus globulus* bark has been reported by scientist [17]. In view of this, the since DOX induced cardiomyopathy is linked to oxidative stress, I have attempted to investigate the possible cardio protective effect of the *E. globulus* against DOX-induced cardio toxicity.

2. Material and Methods
2.1 Plant Materials and Extract Preparation
E. globulus barks were collected from the few *E. globulus* trees behind the faculty of life science, Jahangirnagar University, Savar, Dhaka. The barks were air dried and milled into powder. About 500 gram of the powder was extracted with was extracted with 1:1 of acetone-ethanol for 72 h. The extract on removal of solvent (16.2 g) was allowed to cool.

Appropriate concentration of the extracts was made in 0.2% acacia in distilled water. The phyto-constituents present in the crude extract are flavonoids and alkaloids.

2.2 Animals and Chemicals

Twenty five male Wister albino rats with an average weight of 140±10g used for this study were obtained from the small Animal Holding Unit of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. They were fed with normal animal feed and given water ad libitum throughout the experimental period. Animal husbandry and experimentation were consistent with the guiding principles in the use of animals in toxicology. The rats were maintained under standard laboratory conditions of 25 ± 1°C with 12 h light dark cycle. Care was taken to avoid any kind of stressful condition. All experimental procedures were performed between 9 and 11 am. Doxorubicin (Xorubin, Beacon Pharmaceutical Limited, Dhaka, Bangladesh.) was purchased from local market. All the reagents used for this study were of analytical grade and were prepared in all glass distilled water.

2.3 Experimental Protocol

The experimental protocol applied for evaluation of cardioprotective effect of *Eucalyptus globulus* against DOX induced cardiotoxicity is as follows. The rats were randomly assigned into five groups.

Group 1: Vehicle-treated group,

Group 2: DOX (3mg/kg, i.p.) every other day for two weeks, with a total cumulative dose of 18 mg/kg DOX,

Group 3: *E. globulus* (100 mg/kg, p.o.) daily + DOX (3 mg/kg, i.p.) every other day for two weeks, with a total cumulative dose of 18 mg/kg DOX,

Group 4: *E. globulus* (200 mg/kg, p.o.) daily + DOX (3 mg/kg, i.p.) every other day for two weeks, with a total cumulative dose of 18 mg/kg DOX,

Group 5: *E. globulus* (200mg/kg, p.o.) daily for two weeks.

At the end of two weeks, the animals were anaesthetized with ketamine, ECG recorded and then sacrificed by a high dose of ketamine. Blood samples were collected immediately for enzyme assays. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min [18] and collected by pipetting The heart of the rats were rapidly dissected and washed in isotonic saline and homogenized quickly with ice cold 0.1 M Tris HCl buffer (pH-7.5).

2.4 Electrocardiography (ECG)

ECG was recorded before and after the treatment schedule. The ECG was recorded in ketamine anesthetized rats using Edan ECG recorder. The needle electrodes were inserted under the skin for the limb lead at position II.

2.5 Cardiac biochemical markers

Blood samples were collected from the animals and serum obtained by centrifugation. The LDH, AST and CK-MB activities were determined from serum according to standard methods using standard kits Bio Systems, S.A. (Barcelona, Spain).

2.6 Determination of Lipid peroxide levels

Lipid peroxide level was estimated by thiobarbituric acid (TBA) reaction with Malondialdehyde (MDA), a product formed due to the peroxidation of lipide membranes [19] in brief, heart tissues were homgenized in 0.15 M KCl using Teflon pestle followed by centrifugation. After centrifugation 1 ml of suspension medium was taken from the supernatant and 0.5 ml of 30% trichloroacetic acid (TCA) followed by 0.5 ml 0.8% TBA was added to it. This were centrifuged for 10 min at 1000 rpm after 30 min incubation. The absorbance of clear supernatant was measured at 540 nm against an appropriate blank. Protein was estimated by lowry method [20].

2.7 Determination of Catalase (CAT) activity

The CAT activity of cardiac tissue was determined according to the method described by Aebi [21]. The method based on determination of the H_2O_2 decomposition rate at 240 nm. The values are expressed as U/mg protein.

2.8 Determination of Glutathione (GSH) content

Reduced Glutathione was estimated by the method of Ellma [22]. About 1.0 ml of 10% TCA was added to 1.0 ml of homogenate and centrifuged. After centrifugation 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 1.0% sodium citrate] and 3 ml of phosphate buffer (pH-8.0). The color developed was measured at 412 nm.

2.9 Determination of Superoxide dismutase (SOD) activity

The SOD was determined by the ability of the enzyme to inhibit the oxidation of adrenaline to adrenochrome [23]. The 0.05 ml supernatant was added to 2.0 ml of carbonate buffer as well as 0.5 ml of 0.01mMEDTA solution. 0.5 ml of epinephrine was added for the initiation of reaction. The auto-oxidation of adrenaline to adreno chrome was measured by following change in OD at 480 nm. The results are expressed at U/mg protein.

2.10 Statistical Analysis

The Collected data were expressed as the mean ± SEM. For statistical analysis of the data, group were compared by ANOVA (one way analysis of variance), followed by Dunnett's test. The p values<0.05 was considered significant.

Table 1: The effect of *E. globulus* treatment on Superoxide dismutase, Lipid Peroxidase, Catalase and Glutathione enzyme levels. Values are mean±SE, ANOVA followed by Dunnetts test,*p<0.05, compared to vehicle, #p<0.05, compared to DOX.

Groups	SOD	LPO	CAT	GSH
Control	33.460±1.576	1.308±0.155	8.122±0.218	4.480±0.195
Dox	14.380±2.448*	7.880±0.278*	3.030±0.310*	1.920±0.259*
E. globulus	35.800±1.933	1.258±0.040	8.294±0.168	4.680±0.198
E. globulus (100mg/kg)+DOX	21.840±2.065#	3.620±0.226#	5.780±0.290#	2.510±0.198#
E. globulus (200mg/kg)+DOX	29.760±1.465#	2.500±0.1897#	8.140±0.372#	4.110±0.248#

Table 2: The effect of *E. globulus* treatment on various serum biological marker enzymes. Values are mean±SEM, ANOVA followed by Dunnetts test, **p*<0.05, compared to vehicle, #*p*<0.05, compared to DOX.

Group	LDH	CK-MB	AST
Control	122.200±1.157	92.600±5.325	50.800±2.596
Dox	223.800±1.933*	208.800±5.200*	207.000±7.516*
E. globulus	125.400±3.472	88.560±1.490	49.600±2.839
E. globulus (100mg/kg)+DOX	185.400±8.059#	150.200±3.773#	168.600±2.204#
E. globulus (200mg/kg)+DOX	131.800±2.634#	98.720±3.040#	67.800±1.562#

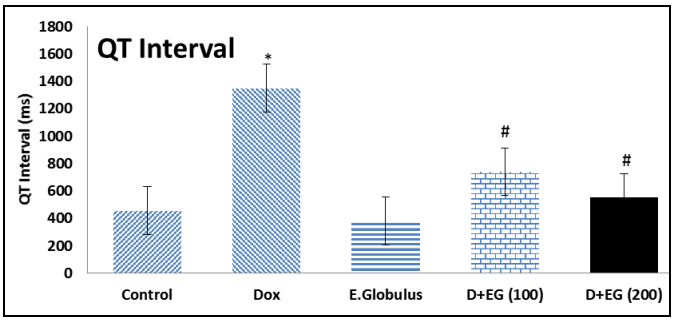

Fig 1: The Effect of *E globulus* extract on doxorubicin-induced alteration in QT interval. Values are mean± SEM, ANOVA followed by Dunnetts test,**p*<0.05, compared to vehicle, #*p*<0.05, compared to DOX.

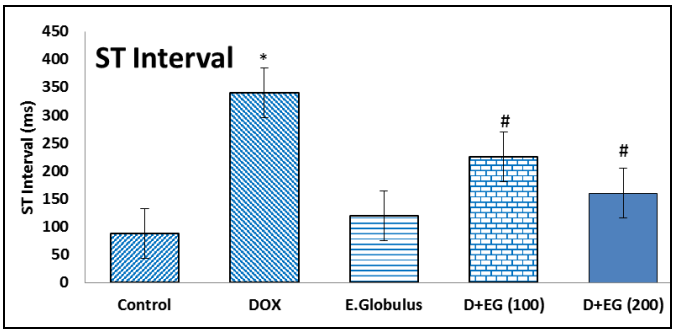

Fig 2: The Effect of *E. globulus* extract on doxorubicin-induced alteration in QT interval. Values are mean± SEM, ANOVA followed by Dunnetts test,**p*<0.05, compared to vehicle, #*p*<0.05, compared to DOX**.**

3. Results& Discussion

3.1 Cardiac marker enzymes

The serum LDH, AST and CK-MB are the important biomarkers of the heart tissue damage. As a cardiotoxic agent DOX significantly increased the level of LDH, CK-MB and AST in the treated animals (*p*<0.05, compared with control group), which indicated the occurrence of cardiac damage. Compared with the DOX treated groups, however the rats treated with various dosage of *E. globulus* (100mg/kg and 200 mg/kg) were found to have an significant reduction in AST (*p*<0.05, compared with DOX group), CK-MB and LDH (*p*<0.05, compared with DOX group). (Table-2).

3.2 ECG and heart weight

The animals with DOX injections were manifested with and widened QRS complex and elevated heart rates with prolongation of QT interval and ST interval (Graph 1-2), which indicated that DOX-treated rats suffered from cardiomyopathy. *E. globulus* extract (100 and 200 mg/kg) significantly (*p*<0.05) decreased the prolongation of QT and ST intervals in animals treated with DOX as compared to DOX-treated group. Treatment with *E. globulus* extract (200 mg/kg) alone significantly (*p*<0.05) decreased the prolongation of QT and ST interval as compared to vehicle- and DOX-treated groups. The heart weight of DOX treated animal significantly decreased which was prevented by *E. globulus* (100 and 200 mg/kg) extract treatment for two weeks.

3.3 Antioxidant enzymes

SOD, CAT and GSH are important antioxidant biomolecules in the tissue against oxidant stress, especially in cardiac tissue. After chronic treatment with DOX, the SOD, GSH, and CAT in mouse heart tissue were significantly decreased (*P*<0.05, compared with the control group) but the LPO level increased significantly (Table-1). Animals treated with *E. globulus* extract (200 mg/kg) alone have significantly decreased the cardiac marker enzymes as compared to DOX treated group.

4. Discussion

The Clinical use of Doxorubicin is limited due to the development and progression of life- threatening dose dependent cardiotoxicity. It has been suggested that Adriamycin-induced myocardial dysfunction involves nucleic acid as well as protein synthesis [8] release of vasoactive amines [24] alterations in membrane-bound enzymes [25], alterations in sarcolemmal Ca++ transportt [26] imbalance in myocardial electrolytes [27], free radical formation [28] and lipid peroxidation [26]. Cardiac muscle is particularly susceptibleto free-radical injury, because it contains low levels of antioxidant enzymes like superoxide dismutase, GSH and catalase [29]. The present study was designed to investigate the potential protective effects of the ethanolic extract of *E. globulus* against DOX-induced cardio toxicity in rats. In this study rats, DOX administration was accompanied by a high mortality as compared to control animals as well as accumulation of ascites [30], and significantly decreased heart weight compared to the control group. Live animals showed excessive amount of peritoneal, pericardial and pleural effusion. The existence of mortality and effusions are explained on the basis of the development of cardiomyopathy. The ability of *E. globulus* to protect against DOX-induced high mortality was considered as an early sign of cardio protective agent.

Since oxidative stress is a cornerstone in DOX-induced Cardiotoxicity [31], it was essential to investigate the oxidant/antioxidant status of the rats. In addition to antioxidant properties, *E. globulus* may also act as a stimulator for the activity of antioxidant enzymes. The current data showed that the cardiac enzyme levels of CAT, GSH and SOD were significantly reduced whereas the LPO level was increased in the DOX treated group compared to the vehicle treated group. The data clearly indicate the elevation of oxidative stress due to the formation of free radicals. It has been suggested that DOX semi Quinone play a major role in its cardiotoxic effects [32] by increasing oxygen free radical activity [33]. Lipid peroxidation is known to cause cellular damage and is primarily responsible for ROS-induced organ damage [34]. Antioxidants [Vitamin-E] provide protection from cardiac cell damage with the attenuation in lipid

peroxidation [35]. Another mechanism implicates that, DOX free radicals are produced by a non-enzymatic system that involves reaction with iron. Iron-DOX complex can reduce oxygen to H2O2 and other active oxygen species. Thus, *E. globulus* improves endogenous antioxidant reserve and may improve cardiac structure and function. The mechanisms of AD Rinduced decrease in GSH and *E. globulus*-induced increase in antioxidants are still not clear. This study demonstrates that *E. globulus* may be providing protection by acting as an antioxidant and by promoting endogenous antioxidants.

DOX administration to rats significantly elevated serum LDHactivity, CK-MB and AST levels; which are released from damaged myocytes and sensitive indicators of cardiac injury [36]. CKMB, an enzyme that is found primarily in the myocardium, is used to evaluate the existence and extent of cardiomyocyte injury. *E. globulus* was found to inhibit the Dox-induced CKMB release in the serum of rats. It was reported that, Dox-induced free-radical generation triggers membrane peroxidation and disruption of cardiac myocytes, which can lead to increased release of CK-MB in the serum [37]. From the present study, complete inhibition of CKMB release in Dox-treated animals at the highest concentration of *E. globulus* has been evaluated. Lactate dehydrogenase (LDH) is of medical significance because it is found extensively in heart muscle. Because it is released during tissue damage, it is a marker of cardiac injuries and disease [38].AST is widely distributed in cardiac and hepatic tissues. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. From the current study, both the increased level of LDH and CK-MB was attenuated by the *E. globulus* extract ($p<0.05$).

It has been reported that the severity of ECG alterations, including the widening of QRS complex, Q-T prolongation and ST interval prolongation by doxorubicin induced cardiotoxicity in patients. The QRS-intervals are directly related to cell depolarization whereas the QT interval is an expression of the late repolarization phase. Anthracycline prolong the repolarization phase by disturbing the ion flux across the cellular membrane. QRS prolongation was demonstrated in doxorubicin-treated mice in the present study and was significantly increased compared with control mice. The QRS prolongation induced by doxorubicin, however, was attenuated by combination treatment with. *E. globulus*. The study shows that, *E. globulus* (100mg/kg and 200mmg/kg) extract significantly decreased the prolongation of QT and ST intervals.

5. Conclusion

In summary, the present study reported the protective effects of *E. globulus* against doxorubicin-induced cardio toxicity in an *in vivo* mouse model. Pretreatment with *E. globulus* significantly inhibited doxorubicin-induced prolongation of QR Sduration, QT interval and ST interval, attenuated myocardial injury and reduced LDH, CK-MB and AST activity. This suggests that *E. globulus* may have a role in the attenuation and prevention of the serious cardiac complications of doxorubicin. The combination of *E. globulus extract* with doxorubicin is a novel strategy that has the potential for protecting against doxorubicin-induced cardiotoxicity. In conclusion the above data suggests that *E. globulus* has the potential antioxidant activity in preventing the cardiotoxic effects induced by Doxorubicin.

6. References

1. Carter SK, Blum RH. New chemotherapeutic agents… bleomycin and adriamycin. CA: a cancer journal for clinicians. 1974; 24(6):322-31.
2. Nousiainen T, Vanninen E, Rantala A, Jantunen E, Hartikainen J. QT dispersion and late potentials during doxorubicin therapy for non-Hodgkin's lymphoma. Journal of internal medicine. 1999; 245(4):359-64.
3. Saltiel E, McGuire W. Doxorubicin (Adriamycin) cardiomyopathy—a critical review. Western Journal of Medicine. 1983; 139(3):332.
4. Lefrak EA, Piťha J, Rosenheim S, Gottlieb JA. A clinic pathologic analysis of Adriamycin cardiotoxicity. Cancer. 1973; 32(2):302-14.
5. Nohl H, Gille L, Staniek K. The exogenous NADH dehydrogenase of heart mitochondria is the key enzyme responsible for selective cardiotoxicity of anthracycline. Zeitschrift für Naturforschung C. 1998; 53(3-4):279-85.
6. Doroshow JH. Anthracyclines and anthracenediones. Cancer Chemotherapy and Biotherapy: Principles and Practice 2nd ed Philadelphia, Pa: Lippincott-Raven, 1996, 409-34.
7. Abdel-aleem S, El-Merzabani MM, Sayed-Ahmed M, Taylor DA, Lowe JE. Acute and chronic effects of Adriamycin on fatty acid oxidation in isolated cardiac myocytes. Journal of molecular and cellular cardiology. 1997; 29(2):789-97.
8. Monti E, Prosperi E, Supino R, Bottiroli G. Free radical-dependent DNA lesions are involved in the delayed cardiotoxicity induced by adriamycin in the rat. Anticancer research. 1995; 15(1):193-7.
9. Jackson J, Reeves J, Muntz K, Kruk D, Prough R, Willerson J, *et al*. Evaluation of free radical effects and catecholamine alterations in adriamycin cardiotoxicity. The American journal of pathology. 1984; 117(1):140.
10. Tong J, Ganguly P, Singal P. Myocardial adrenergic changes at two stages of heart failure due to adriamycin treatment in rats. American Journal of Physiology-Heart and Circulatory Physiology. 1991; 260(3):H909-H16.
11. Singal PK, Khaper N, Palace V, Kumar D. The role of oxidative stress in the genesis of heart disease. Cardiovascular research. 1998; 40(3):426-32.
12. Hasegawa T, Takano F, Takata T, Niiyama M, Ohta T. Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of Eucalyptus globulus. Phyto chemistry. 2008; 69(3):747-53.
13. Takahashi T, Kokubo R, Sakaino M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from Eucalyptus maculata. Letters in applied microbiology. 2004; 39(1):60-4.
14. Sherry E, Warnke P. Successful use of an inhalational phytochemical to treat pulmonary tuberculosis: A case report. Phyto medicine. 2004; 11(2/3):95.
15. Gallagher A, Flatt P, Duffy G, Abdel-Wahab Y. The effects of traditional antidiabetic plants on *in vitro* glucose diffusion. Nutrition research. 2003; 23(3):413-24.
16. Jouad H, Maghrani M, Hassani RAE, Eddouks M. Hypoglycemic activity of aqueous extract of Eucalyptus globulus in normal and streptozotocin-induced diabetic rats. Journal of herbs, spices & medicinal plants. 2004; 10(4):19-28.
17. Boulekbache-Makhlouf L, Slimani S, Madani K. Total phenolic content, antioxidant and antibacterial activities of fruits of Eucalyptus globulus cultivated in Algeria.

Industrial crops and products. 2013; 41:85-9.
18. Ogbu S, Okechukwu E. The effect of storage temperature prior to separation on plasma and serum potassium. J Med Lab Sci. 2001; 10:1-4.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979; 95(2):351-8.
20. Ulmer VerlagLowry O, Rosebrough N, Farr A, Randall R. Protein measurement with Folin-phenol reagent. J Biol Chem. 1951; 193:265-275.
21. Aebi H. Catalase *in vitro*. Methods in enzymology. 105: Elsevier, 1984, 121-6.
22. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959; 82(1):70-7.
23. Saggu H, Cooksey J, Dexter D, Wells F, Lees A, Jenner P, *et al*. A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. Journal of neurochemistry. 1989; 53(3):692-7.
24. Bristow MR, Sageman WS, Scott RH, Billingham ME, Bowden RE, Kernoff RS, *et al*. Acute and chronic cardiovascular effects of doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. Journal of cardiovascular pharmacology. 1980; 2(5):487-515.
25. Singal P, Panagia V. Direct effects of Adriamycin on the rat heart sarcolemma. Research communications in chemical pathology and pharmacology. 1984; 43(1):67-77.
26. Singal PK, Pierce GN. Adriamycin stimulates low-affinity Ca2+ binding and lipid peroxidation but depresses myocardial function. American Journal of Physiology-Heart and Circulatory Physiology. 1986; 250(3):H419-H25.
27. Van Acker SA, Kramer K, Voest EE, Grimbergen JA, Zhang J, van der Vijgh WJ, *et al*. Doxorubicin-induced cardiotoxicity monitored by ECG in freely moving mice A new model to test potential protectors. Cancer chemotherapy and pharmacology. 1996; 38(1):95-101.
28. Gewirtz D. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics Adriamycin and daunorubicin. Biochemical pharmacology. 1999; 57(7):727-41.
29. Takś I, Matkovics B, Varga SI, Homolay P, Fehér G, Seres T. Study of the myocardial antioxidant defence in various species. Pharmacological research. 1992; 25(SUPPL. 2):177-8.
30. Kim C, Kim N, Joo H, Youm JB, Park WS, Van Cuong D, *et al*. Modulation by melatonin of the cardiotoxic and antitumor activities of adriamycin. Journal of cardiovascular pharmacology. 2005; 46(2):200-10.
31. Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy: from the cardiotoxic mechanisms to management. Progress in cardiovascular diseases. 2007; 49(5):330-52.
32. Bachur NR, Gordon SL, Gee MV, Kon H. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. Proceedings of the National Academy of Sciences. 1979; 76(2):954-7.
33. Lee V, Randhawa AK, Singal PK. Adriamycin-induced myocardial dysfunction *in vitro* is mediated by free radicals. American Journal of Physiology-Heart and Circulatory Physiology. 1991; 261(4):H989-H95.
34. Prasad MS. Effect of Organophosphate Compound Acephate on Different Regions of Brain in Albino Mice, 2013.
35. Machlin L. Vitamin E, Handbook of Vitamins. Marcel Dekker Inc New York, 1991, 99-147.
36. Herman E, Mhatre R, Lee I, Vick J, Waravdekar V. A comparison of the cardiovascular actions of daunomycin, Adriamycin and N-acetyldaunomycin in hamsters and monkeys. Pharmacology. 1971; 6(4):230-41.
37. Liu X, Chen Z, Chua CC, Ma Y-S, Youngberg GA, Hamdy R, *et al*. Melatonin as an effective protector against doxorubicin-induced cardiotoxicity. American journal of physiology-heart and circulatory physiology. 2002; 283(1):H254-H63.
38. Bernstein LH, Everse J, Shioura N, Russell PJ. Detection of cardiac damage using a steady state assay for lactate dehydrogenase isoenzymes in serum. Journal of molecular and cellular cardiology. 1974; 6(4):297-315.