

IMPACT OF DOXORUBICIN AND VITAMIN E, A SEPARATELY AND IN COMBINATION ON SELECTED ANTIOXIDANT ENZYME ACTIVITIES OF RAT MYOCARDIAL TISSUE

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Article Received on
24 April 2020,

Revised on 14 May 2020,
Accepted on 04 June 2020

DOI: 10.20959/wjpr20207-17785

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ABSTRACT

The present study investigated the *in vivo* effects of 15 mg/kg wt of Doxorubicin and 100 mg/kg wt of Vitamin E, 50 IU/kg wt of Vitamin A separately and in combination over 8 weeks (weekly doses) on myocardial antioxidant enzyme activity levels in rats. After 8 weeks the activities of Superoxide dismutase, Catalase, Glutathione reductase, Glutathione-S-transferase, Glutathione peroxidase and the levels of Lipid peroxides and reduced Glutathione were presented. The data indicated a significant decrease in all the parameters studied under Doxorubicin stress. Doxorubicin altered parameters were significantly reversed or recovered by vitamin E plus Doxorubicin. Vitamin A plus

doxorubicin also significantly recovered the Doxorubicin altered parameters except Catalase and Glutathione peroxidase activities. From the results it is reported that Doxorubicin impair overall antioxidant enzyme activities and the antioxidants like vitamin E and vitamin A by their antioxidant mechanism might be neutralizing the Doxorubicin altered antioxidants *in vivo*.

KEYWORDS: Doxorubicin, Vitamin E, Vitamin A, Antioxidant enzymes, Cardiac tissue.

List of abbreviations

Dox-Doxorubicin; vE - Vitamin E; vA - Vitamin A; MDA - Malondialdehyde; LPO - Lipid peroxidation; GSH - Reduced Glutathione, SOD - Superoxide dismutase; CAT - Catalase; GR-Glutathione reductase; GPx - Glutathione peroxidase; GST - Glutathione-s-transferase

INTRODUCTION

Anthracyclines regarded as essential and approved anticancer drugs. Doxorubicin (trade name: Adriamycin) also known as hydroxydaunorubicin, is classified as an anthracycline antibiotic drug produced from *Streptomyces peucetius* as a secondary metabolite. Dox has been using widely, frequently and effectively as an anticancer chemotherapy drug in the therapy of various cancers.^[1] Dox exerts its anticancer effects or cytotoxic effects by intercalating DNA or irreversibly damaging the tumour cell DNA. Dox binds with DNA and the topoisomerase II forming a covalent topoisomerase-Dox-DNA ternary complex, which causes double stranded DNA breaks and eventually induces programmed cell death (Apoptosis) in proliferating cancer cells.^[2] In quiescent cell, including cardiomyocytes Topoisomerase II β isoenzyme is target of Dox induced toxicity, finally results in death of cardiac cell.^[3]

Dox administration brings about different harmful impacts, of which dose dependent cardiotoxicity is the commonest impact brings about cardiomyopathy and cardiac failure. Dox induces cardiotoxicity has been believed to mediated by several mechanisms. Highly reactive free radical generation is the predominant and principal mechanism as acknowledged by majority of the researchers.^[4,5] These free radicals not only damage the cardiomyocytes but also alter the cardiac output function. As the dose of Dox is higher, congestive heart failure become more noteworthy. Other events also contributing the pathogenesis of Dox includes impaired calcium homeostasis, generation of iron complexes or iron accumulation, impairment of mitochondrial metabolism and biogenesis, activation of matrix metallo proteinases and eventually triggered cardiomyocytes death.^[6-8]

Myocardial protection during Dox treatment out to stay an objective to enhance the beneficial effects of the drug as well as to evacuate the danger of short and long-haul cardiovascular issues. Several therapeutic strategies, designed to intensify endogenous defence system as antioxidants have recognized as promising way to combat against Dox toxicity. Recently, co-therapy or combination therapy with Dox has increased more consideration. In co-therapy, Dox toxicity made to prevent by administering antioxidant agents along with Dox like dietary antioxidants or redox modulators, or iron chelators or phyto-products etc.^[9-10] Therefore, co-therapy has proven to be a useful strategy to lessen the side effects associated with Dox while remaining its restorative capacity. In view of this, endeavor has been made to check or study the effect of doxorubicin, vitamin E and A

independently and together on the levels of certain antioxidant enzymatic activities in Myocardial tissues of rats.

MATERIALS AND METHODS

Materials

Experimental Animals

Experiments were carried out with adult male Swiss albino rats, weighing 150 ± 10 g. They were maintained in standard laboratory conditions, with a 12-hour light/dark cycle and with free access to feed and water ad libitum. They were allowed to acclimate for laboratory conditions for at least ten days after arrival before use.

Treatment of Animals

The animals randomly divided in to 6 groups. First Group having 8 rats act as control one's and the remaining each group contain 12 rats each. Second group rats were received 100 mg per kg body weight of vitamin E (vE) for 8 weeks (weekly doses). Third Group rats were received 50 IU per kg weight of Vitamin A (vA) for 8 weeks (weekly doses). Fourth group administered with 15 mg/ kg weight of Doxorubicin (Dox) over 8 weeks (weekly doses).

Fifth Group rats were administered with 100 mg per kg weight of vitamin E followed by Doxorubicin over 8 weeks (weekly doses). Sixth group rats were administered with 50 IU per kg weight of vitamin A followed by 15 mg per kg Doxorubicin over 8 weeks (weekly doses).

Doxorubicin hydrochloride dissolved in Saline (0.9% normal saline or Sodium chloride solution) and treatment given through tail vein. Group I animals were given saline only. Vitamin E and A dissolved in olive oil and treatment given through gavage. Dox dose selected by the previous studies of Nimbal and Koti, 2016^[11]; Alam *et al.*, 2018.^[12] Dose of vitamin E and A chosen based on the previous studies of Vijayudu *et al.*, (2015)^[13] from our laboratory. (Animal Ethics Resolution Number: 24/2012-2013(i)/a/CPCSEA/IAEC/SVU/KVK-BV)

Collection of Cardiac samples

Animals were killed by cervical dislocation/decapitation by using mild ether anesthesia. Hearts were excised, trimmed of connective tissue, rinsed with ice-cold saline to eliminate blood contamination, dried by blotting with filter and weighed. The tissues then kept in freezer at -80°C until analysis. A portion of the Heart was weighed, perfused with saline and

homogenate (10%) was prepared in ice cold PBS (50 mM, pH 7) using a homogenizer. The homogenates centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The homogenate centrifuged at 10,000 rpm for 10min in a cooling centrifuge at 4°C, after removal of the cell debris, supernatant was used for the assay of antioxidant enzymes.

Chemicals

Dox hydrochloride injection (ADRIUM) was purchased from BDH Chemicals Co, India. Vitamin E (D-Alpha-tocopheryl acetate) and Vitamin A (Retinyl Palmitate) were purchased from Sigma chemicals Co. India. All other chemicals and reagents used were of analytical grade.

Methods

The following parameters were assayed in the cardiac tissue of rat. The lipid peroxide level was measured by the procedure of Ohkawa *et al.*, (1979).^[14] Superoxide dismutase activity was determined by the method of Misra and Fridovich (1972).^[15] Catalase activity was determined according to the method of Beers and Sizer (1952).^[16] Tissue Glutathione content was determined by the method of Theodorus *et al.*, (1981).^[17] The Glutathione reductase activity was assayed by the method of Carlberg and Mannervik (1985).^[18] Assay of Glutathione peroxidase was carried out by using the method of Wendel, (1981).^[19] Glutathione-s-transferase activity was measured as per the method of Habig *et al.*, (1974).^[20] Protein content in various samples was estimated by the method of Lowry *et al.*, (1951).^[21]

Statistical analysis

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into consideration. Statistical analyses were conducted by a one-way Analysis of Variance (ANOVA) followed by Tukey's HSD multiple comparison test by using statistical software package. P values <0.01 were considered as significant.

RESULTS

The results shown in the table 1, Lipid peroxidation, Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Glutathione Reductase (GR), Glutathione Peroxidase (GPx) and Glutathione-s-transferase (GST) activity or levels in the control, Dox and vitamin E, A separately and in combination treated rat cardiac tissue. Dox treatment (Group IV) elevated the LPO levels and decreases the GSH levels as well as SOD, CAT, GR, GPx and GST enzymes significantly. These changes were found to be statistically significant (P<0.01).

None of the parameter studied in the cardiac tissue affected or altered by vitamin E and A alone treatments (Group II & III).

In Dox treated animals MDA levels were significantly increased by 85.9% over the control. Vitamin E and vitamin A along with Dox (Group V & VI) treated rat heart showed decreased levels of MDA and were found to be nearer to their control values and the recovery appeared to be 70.5% and 50% respectively.

The percent decrease of SOD and CAT in Dox treated rat was 44.94%, 39.71% respectively over the control values. Vitamin E + Dox (Group V) administration showed a significant increase in SOD and CAT enzyme activities as compared with the Dox intoxicated model group. Vitamin E + Dox challenge was observed reverse the Dox induced alterations of SOD by 65.85%, of CAT by 37.75%. Vitamin A + Dox administration (Group VI) showed a significant increase in SOD activity but not CAT enzyme activity as compared with the Dox intoxicated model group. In the Group V, Vitamin A + Dox challenge was observed reverse the Dox induced alterations of SOD by 37.75% only but not affected the CAT.

Reduced Glutathione (GSH) levels were decreased by 38.9% in Dox treated group. Dox + vitamin E, Dox + vitamin A administered rat cardiac tissue showed reverse trends of their GSH levels over Dox treated group and the changes were found to be statistically significant ($p < 0.01$). Percent recovery over Dox inhibited rat cardiac GSH levels appeared to be 55.24% and 35.50% respectively.

Dox treated rat heart showed decreased activities of GPx, GR and GST and changes were found to be statistically significant ($p < 0.01$) over the control values. The percent decrease of GR, GPx and GST in Dox treated rat was 40.36%, 44.04% and 45.14% respectively over the control values. Vitamin E along with Dox administration (Group V) showed a significant increase in GR, GPx and GST enzyme activities as compared with the Dox treated model group (Table 1). In the Group V administration of vitamin E along with Dox challenge was observed to reverse the Dox induced alterations of GR by 53.67%, GPx by 60.02% and GST by 68.72%. Vitamin A along with Dox administration (Group VI) showed a significant increase in GR and GST enzyme activities, but not GPx activities as compared with the Dox treated model group (Table 1). In the Group VI administration of vitamin A along with Dox challenge was observed reverse the Dox induced alterations of GR by 35.85% and GST by

47.02%. But no significant change observed in the activity of GPx in vitamin A + Dox treated rats.

Table 1: Effect of Dox, Vitamin E and Vitamin A alone and in combination on the rat cardiac tissue LPO, GSH levels, SOD, CAT, GR, GPx and GST enzymatic activity levels.

Sl. No	Parameter		Group I (Con)	Group II (vE)	Group III (vA)	Group IV (Dox)	Group V (Dox + vE)	Group VI (Dox + vA)
1	LPO	Mean	249.5	242.57*	245.3*	463.66	326.66	356.3
		SD	± 10.04	± 11.4	± 10.4	± 15.29	± 12.97	± 11.96
		% Change		-2.80%	-1.60%	85.90%	25.29%	42.97%
		% Recovery					70.50%	50%
2	SOD	Mean	36.42	36.8*	34.7*	20.05	27.86	27.19
		SD	± 2.10	± 1.9	± 1.8	± 1.35	± 1.48	± 0.9
		% Change		1.03%	-4.72%	-44.94%	-15.34%	-27.91%
		% Recovery					65.85%	37.75%
3	CAT	Mean	61.44	62.31*	63.52*	37.04	48.24	38.32 [#]
		SD	± 1.89	± 2.07	± 1.55	± 2.21	± 2.03	± 1.92
		% Change		1.41%	3.38%	-39.71%	-16.60%	-36.65%
		% Recovery					58.20%	4.42%
4	GSH	Mean	8.31	8.67*	8.42*	5.07	6.86	6.22
		SD	± 0.38	± 0.42	± 0.49	± 0.29	± 0.36	± 0.29
		% Change		4.15%	1%	-38.90%	-17.44%	-25.15%
		% Recovery					55.24%	35.50%
5	GR	Mean	10.295	10.07*	9.87*	6.14	8.37	7.63
		SD	± 0.37	± 0.270	± 0.19	± 0.26	± 0.15	± 0.32
		% Change		-2.18%	-4.11%	-40.36%	-18.69%	-25.88%
		% Recovery					53.67%	35.85%
6	GPx	Mean	45.32	46.44*	47.02*	25.36	37.34	26.24 [#]
		SD	± 2.06	± 3.02	± 2.17	± 2.10	± 1.45	± 1.09
		% Change		2.47%	3.75%	-44.04%	-17.38%	-42.10%
		% Recovery					60.02%	4.40%
7	GST	Mean	1.041	1.058*	1.038*	0.571	0.894	0.792
		SD	± 0.04	± 0.06	± 0.05	± 0.04	± 0.03	± 0.04
		% Change		1.53%	-0.20%	-45.14%	-14.12%	-23.91%
		% Recovery					68.72%	47.02%

Values are expressed as Mean ± SD of six rats in each group. Data was analysed by oneway ANOVA followed by Tukeys HSD test.

* Not Significant; P<0.01 when compared to control; [#] Not significant; P<0.01 when compared to Dox treated group. % change over the Controls; % Recovery over the Dox group.

Units: LPO – nanomoles of MDA / gr tissue ; GSH – micromoles of reduced GSH /gr tissue; SOD – Units /min /mg protein; CAT- micromoles of H₂O₂ decomposed /mg protein /min; GR – micromoles of NADPH oxidized /mg /min; GPx- micromoles of GSH oxidized /mg /min; GST- Units /min /mg proteins

DISCUSSION

Dox triggers the massive generation and accumulation of ROS and RNS by enzymatic as well as non-enzymatic pathways.^[4] Cardiac muscles are rich in mitochondria where Dox accumulates and binds with cardiolipin and metabolically converted into Dox-Semiquinone radical in the presence of NADH dependent dehydrogenase complex I enzymes of mitochondrial e^- transport chain then reacts with oxygen, generating superoxide anion radicals. Superoxide anion radical spontaneously or catalyzed by SOD converted to form H_2O_2 , which can further produces OH^* radicals. Dox (semiquinone form) non-enzymatically in the presence of iron (Fe^{3+}) generates highly reactive, toxic hydroxyl radicals.^[22] Dox also binds to endothelial nitric oxide synthase reductase domain, generates Superoxide anion radicals. NO produced from the iNOS reacts with superoxide radicals generates peroxynitrite radicals.^[4]

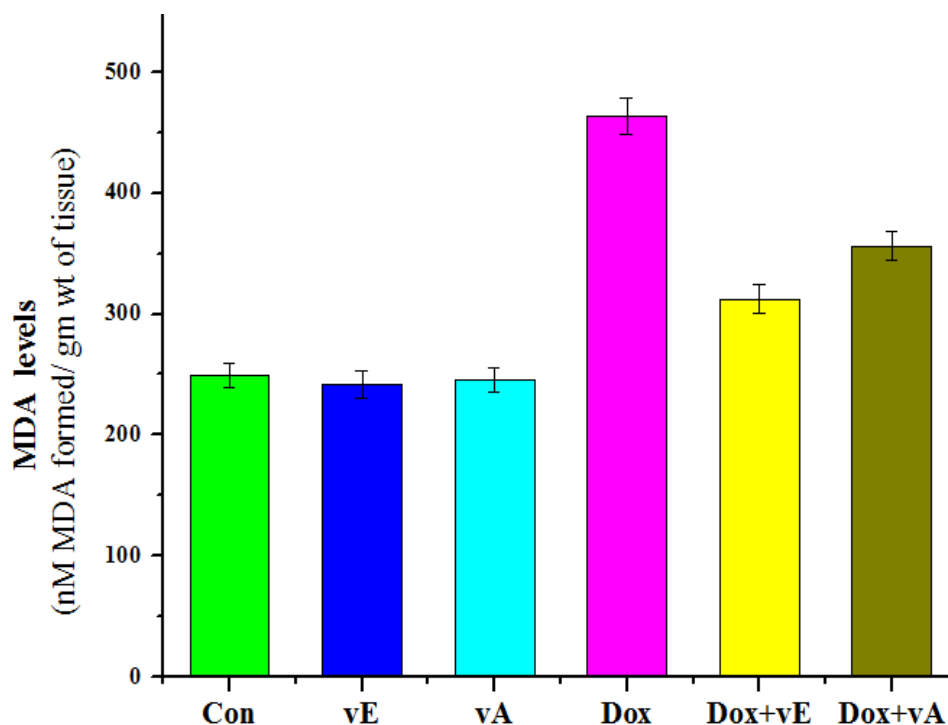


Fig 1: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue LPO (MDA) levels

Rats treated with Dox showed significantly increased level of MDA of the Cardiac tissue compared to control groups (Fig. 1). LPO usually initiated by the interaction of Reactive Oxygen Species or the free radicals generated by Dox with PUFA, leads to the formation of lipid radicals and lipid peroxy radicals, which intern interacts with other lipid molecule and forms lipid hydroperoxides. The breakdown products of Lipid hydroperoxides such as MDA,

are biologically active, capable of diffusion and attack other areas of the cell. MDA is widely used as marker of Lipid peroxidation.^[23] Enhanced Lipid peroxides as observed in the present study might result from increase production of free radicals i.e., Oxidative stress due to LPO induced by Dox. This findings is in agreement with the investigators that reported Dox as a strong inducer of oxygen free radicals in turn the oxidative stress is a central feature of Dox induced Cardiotoxicity.^[12,24]

Rats treated with Vitamin E & A (Group V & VI) along with Dox found to reduce the levels of LPO. It indicates the antioxidant roles of vitamin E & A in reducing the lipid peroxidation. Vitamin E prevents the free radicals to abstract a hydrogen atom from PUFA but allows to abstract from the antioxidant molecules, thus prevents the free radical chain reactions.^[6,9] Moreover, vitamin E also acts as direct scavenger of hydroxyl and superoxide radical.^[25] As per the reports of Fayez and Zaafan (2018)^[26], regarding LPO, vitamin E has offered good results in several tissues of rat like kidney and heart after Dox administration. Ciaccio *et al.*, (1993)^[27] also described a protective effect exerted by vitamin A against LPO toxicity against Dox. Tesoriere *et al.*, (1994)^[28] have also reported that rats treated with vitamin A, reduced the oxidative damage to the cardiac tissue proteins and lipids significantly against the Dox induced toxicity.

In the present study, rats treated with Dox have shown significantly decreased cardiac tissue SOD and CAT enzyme activities when compared to the control groups (Fig. 2 & 3).

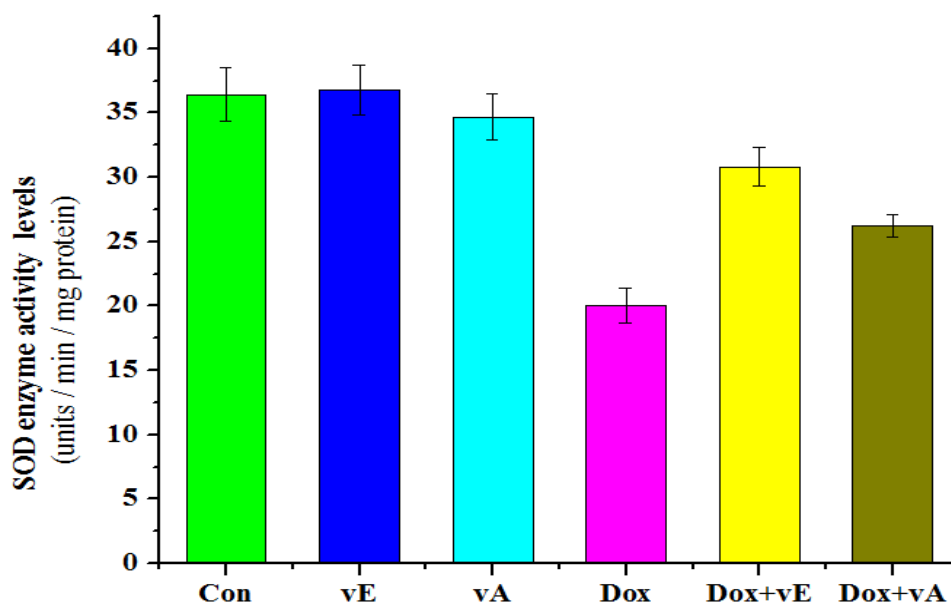


Fig 2: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue SOD enzyme activity levels

SOD and CAT are the most important first line defence antioxidants enzymes, both function in sequence. SOD catalyzes the dismutation of the superoxide anion free radical to H_2O_2 and molecular oxygen. Catalase rapidly decompose the Hydrogen peroxide into water molecules and gaseous oxygen. It could be reasonable to assume that the decrease in the activities of SOD and CAT may be due to over production of free radicals and LPO end products alters or inactivates and /or inhibiting the enzyme protein biosynthesis. Superoxide anion radical able to alter or inhibited the SOD and CAT action.^[29] Decreased activity of SOD and CAT could even be because of exhaustion or consumption of enzymes due to increased lipid peroxidation and other free radicals generated because of the Dox.^[12] Similar decrease in the activity of SOD and CAT in Dox treated rat heart also reported by earlier investigators.^[30,31]

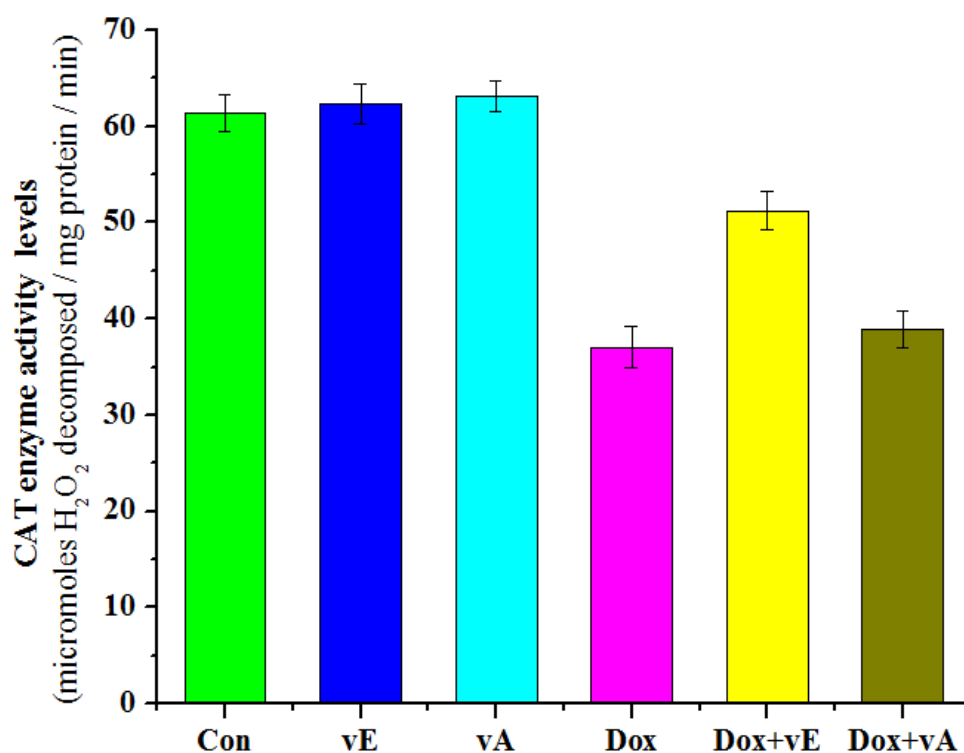


Fig 3: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue CAT enzyme activity levels

By Co-administration of vitamin E accompanied with Dox, cardiac tissue SOD and CAT activities were significantly restored when compared to Dox alone intoxication, suggested that it could be restored enzymes / or activates enzymatic activities in Dox damaged tissue. Vitamin E may directly scavenges the free radicals like hydroxyl and superoxide anions^[25] and prevents the subsequent formation of other reactive free radicals. Vitamin A + Dox (group VI) restored the SOD activity only, but not affected the CAT levels. Recent reports

demonstrated that vitamin A supplementation increased the SOD levels but no change in the CAT levels in rat Liver.^[32] The present result as observed in the study showed that treatment of vitamin E and A + Dox caused significant decrease in the levels of MDA and an increase in GSH levels, resulting in an increase in SOD, thereby preventing the detrimental effects of superoxide anion radicals. The presence of vitamin E with Dox alleviates its harmful effects on SOD, CAT activity levels. These results are in good accordance with the works of earlier authors, found that vitamin E maintained the levels of activities of SOD and CAT antioxidant enzymes near normal levels, thus emphasizing their effects as antioxidant.^[9,11]

In the present study, GSH level significantly decreased in the Dox treated cardiac tissue (Fig. 4). GSH is the major thiol, metabolic, and endogenous antioxidant present in the mammalian cells. GSH protects the cells from the effects of free radicals (R^*) and other reactive oxygen species (hydroxyl radical, lipid peroxyl radical, peroxynitrite and H_2O_2) by directly reacting with them. It also detoxifies the products of free radical species promoted lipid peroxidation products such as MDA and HNE and other products of free radical species interaction with cellular components. GSH extensively used as a cofactor or co-substrate by various antioxidant enzymes like GPx, GST etc.^[33]

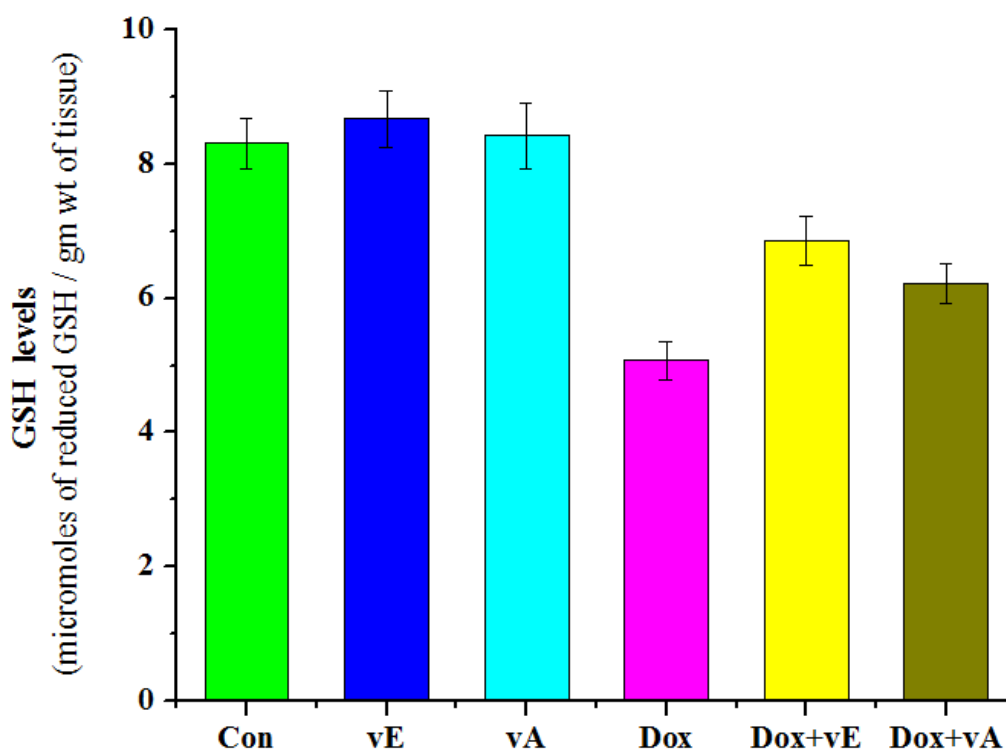


Fig 4: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue GSH levels

GSH is a critical determinant of tissue susceptibility to oxidative damage^[34] and therefore the depletion of GSH has been shown to be associated with an enhanced oxidative damage due to Dox toxicity. The decreased level of GSH in the present study might result from the Dox induced free radicals, which reacts with sulfhydryl groups^[35] or due to its increased utilization by the cardiomyocytes, as an endeavor to counteract the increased formation of lipid peroxides^[36] or large consumption by the GSH-related enzymes.^[37] The decreased GSH levels may also be attributed as due to the functional impairment of cardiac glutathione reductase activity, as observed in the present study. Similar reduction of GSH in the Dox treated cardiac tissue of rats is consistent with the reports of earlier authors.^[12, 29]

In the vitamin E and A along Dox treated rats showing the increased levels of cardiac tissue GSH content when compared to the Dox alone treated rats. Vitamin E has a role in glutathione sparing effect^[38] as well as in the maintenance of reduced state of SH groups, and simultaneously maintains GSH stability.^[39] Likewise, mechanisms appear to be responsible for the recovery of GSH levels by vitamin E. In agreement with the present study, it has been found that cardiac tissue glutathione level was significantly greater in rats treated with vitamin E.^[26] Vitamin A treatment with Dox also recovered the GSH levels to certain extent and but the recovery is less when compared to vitamin E treated rats. In support of this Cha *et al.*, (2016)^[32] reported that vitamin A significantly elevated the GSH concentration in the liver tissue of the male rats. In another study vitamin A deficiency caused decrease in GSH concentration and GSH/GSSG ratio in mitochondria of rats and those alterations recovered by vitamin A administration.^[40]

In the present study rats treated with Dox have shown significantly decreased cardiac tissue Glutathione reductase (GR), Glutathione peroxidase (GPx), and Glutathione-s-transferase (GST) enzyme activities when compared to the control groups (Fig. 5, 6 & 7).

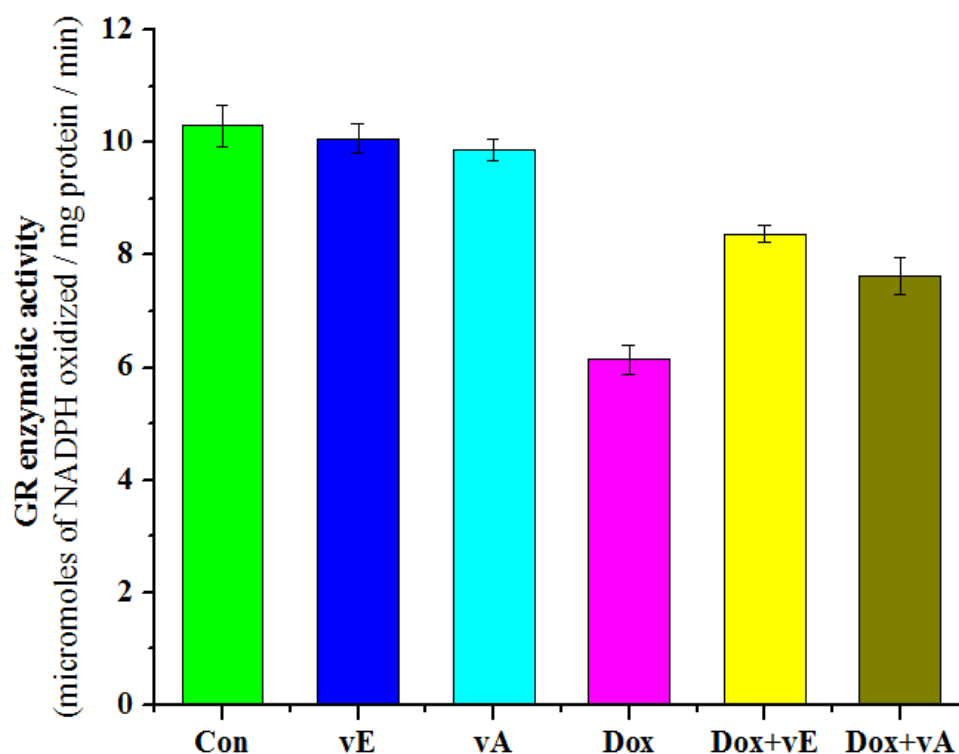


Fig 5: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue GR enzyme activity levels

GR reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH. This mechanism helps in maintaining the GSH level and successively maintains the GPx and GR catalytic functions.^[33] A significant decrease in the Cardiac tissue GR activity in the present study after Dox treatment is indicative of impaired reduction of GSSG to GSH. The decrease in GR activity and GSH level after Dox treatment reflecting the same, which is an indication of lower GR activity. GR contains sulfhydryl groups for its activity and are vulnerable to free radical mediated inactivation^[35] or the decrease in enzyme activity due to high efflux of GSSG out of the cardiac cells under high oxidative stress conditions or GSSG reacts with protein thiols to produce mixed disulfides or protein disulfides.^[41] Similar reasons for decreased activity of GR also reported by previous works.^[12]

GPx converts the reduction of H_2O_2 and lipid hydroperoxides to non-toxic products and prevents the decomposition of those peroxides into free radicals that reinitiate peroxidation. GST catalyzes the GS-conjugants formation and reduction of hydroperoxides.^[33] GST efficiently diminishes the hydroperoxides of PUFA, phospholipids, mononucleotides and DNA^[42] before damaging the membrane or cell. GPx and GST uses GSH as a co-substrate for their activities.^[33] It could be reasonable to assume that the decrease in the GSH content as

observed in the present study might result in decreased GPx and GST activities.^[36, 43] Enhanced condition of oxidative stress and free radicals (ROS) especially $O_2^{\cdot -}$ free radicals might inactivates or inhibits the function of GPx and GST enzymes through oxidation^[44] or the decrease might also be due alteration in their protein structure by ROS^[45] or inhibition of enzyme protein synthesis or inhibition of enzyme by Dox metabolites or some LPO products by oxidizing active sites or by forming protein cross links.^[46] Similar trend of decreased activity of GPx in Dox treated rats was also reported by several earlier researchers.^[12,30]

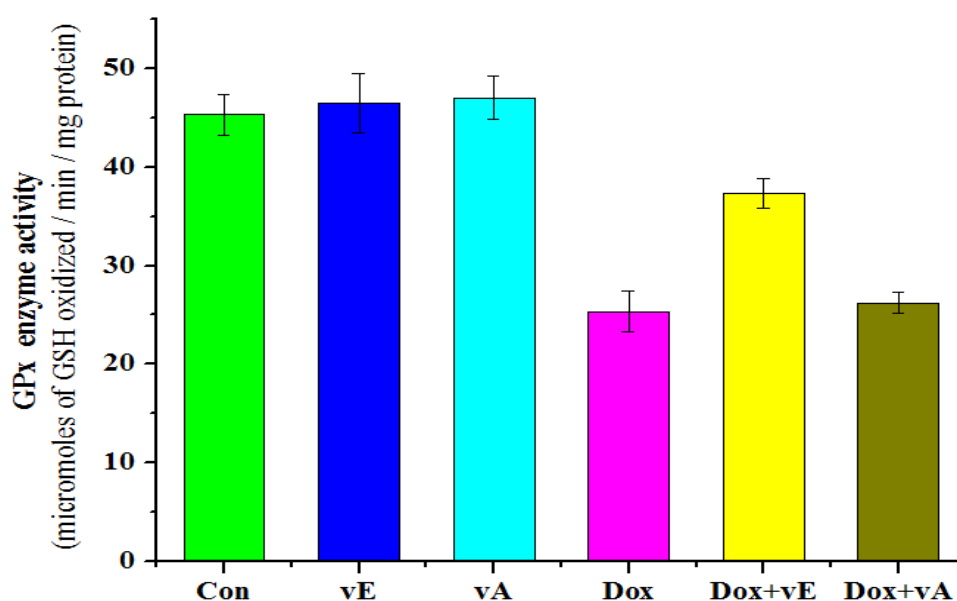


Fig 6: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue GPx enzyme activity levels

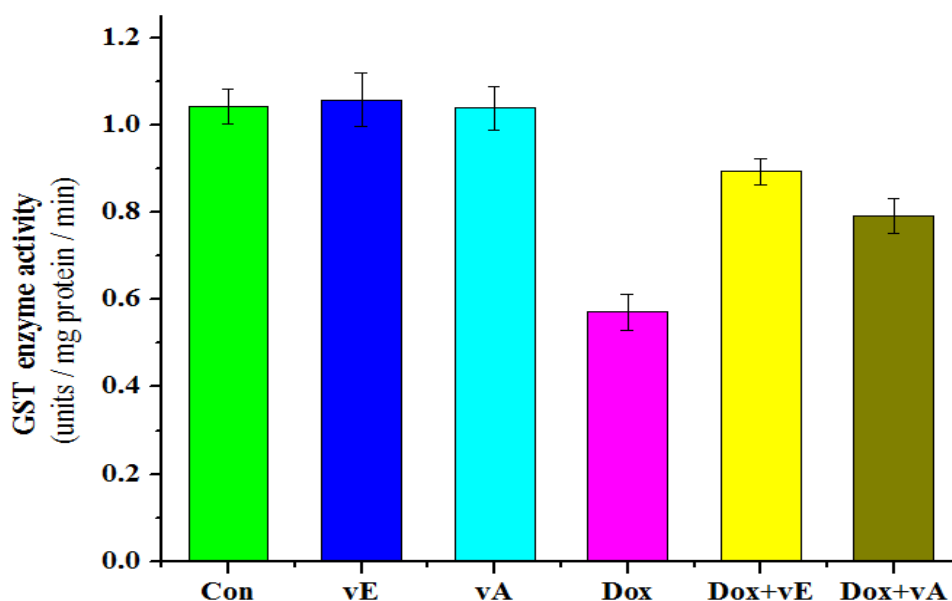


Fig 7: Effect of vitamin E & A and Dox separately and in combination on the rat cardiac tissue GST enzyme activity levels

Administration of Vitamin E with Dox was found to increase the decreased levels of GR, GPx and GST caused by Dox stress. Vitamin E considered as first line of defence against LPO and free oxygen radicals. Because of its action and sharing of some functions of these enzymes, their levels were increased. Vitamin A with Dox was also found to increase the decreased activities of GR and GST caused by Dox stress. GPx activity not affected by the vitamin A plus Dox administrations. The exact mechanism how the vitamin A modulates the antioxidant enzyme levels is not known due to scares or less availability of literature. Decrease in LPO, increase in GSH levels by vitamin E and A along with Dox (Group V & VI) as observed in the present study might result in enhanced activities of these GSH-related enzymes, indicates the vitamins role as antioxidant. Vitamin E treatments with anthrocyclines significantly increased the activities of GPx^[26, 46] as well as GR and GST.^[47] Studies of Xia *et al.*, (1996)^[48] presented that vitamin A upregulates the several major scavenger enzymes like GST by modulating the genes at mRNA level. Regarding the role of vitamin A in relation to antioxidant metabolism, further investigation is necessary to establish the exact mechanism.

CONCLUSION

Doxorubicin administration brings about different harmful impacts, of which dose dependent cardiotoxicity is the commonest impact brings about cardiomyopathy and cardiac failure. It has been believed to mediate by several mechanisms of which free radical generation is the predominant and principle mechanism. Several therapeutic strategies, designed to intensify endogenous defence system as antioxidants have recognized as promising way to combat against Doxorubicin toxicity. Keeping in mind the toxic side effects induced by the cause of Doxorubicin and the protective role as offered by vitamin E and A in experimental animals and also in humans, the current study carried out with the objective of investigating the in vivo effect of Doxorubicin and vitamin E & A separately and in combination on selected enzymatic and non-enzymatic antioxidants in albino rat cardiac tissue.

Rats were administered with a selective dose of doxorubicin 15 mg/kg and 100 mg/kg and 50 IU / kg wt of vitamin A separately and in combination and the treatment period was 8 weeks (weekly doses). The control and experimental rat Myocardial tissue was subjected for biochemical analysis and data obtained and presented in the study. Dox treatment caused significant elevation in LPO and decrease in all the antioxidant parameters studied. These results indicate enhanced free radical generation and depression of antioxidant metabolism. As expected vitamin E and A appeared to reverse the Doxorubicin altered antioxidant

parameters here. Vitamin E plus Dox appears to more reversed Dox altered antioxidant enzymes than compared to vitamin A plus Dox in antioxidant metabolism in the present study.

ACKNOWLEDGEMENT

I am also very grateful to Principal Dr D Nagalinga Reddy for creating research environment in the form of facilities and permissions to visit various universities in the form of fieldwork.

FUNDING

My sincere thanks to UGC – SERO, Hyderabad for releasing Grant-in-aid for this Minor Research Project (MRP) in Ref No. F. MRP-7086/16 (SERO-UGC), Feb 2018.

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