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STUDY OF EFFECT OF DOXORUBICIN AND NATURAL ANTIOXIDANTS VITAMIN E, A SEPERATELY AND COMBINATION ON SELECTED ANTIOXIDANT ENZYME LEVELS OF ALBINO RAT – KIDNEY TISSUE

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ABSTRACT
The present study investigated the *in vivo* effects of 20 mg/kg wt of Doxorubicin and 80 mg/kg wt of Vitamin E, 40 IU/kg wt of Vitamin A separately and in combination over 10 weeks (weekly doses) on Kidney antioxidant enzyme activity levels in rats. After 10 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 the few parameters and few parameters increases are studied under Doxorubicin stress. Doxorubicin altered parameters were significantly reversed by vitamin E plus Doxorubicin, Vitamin A plus doxorubicin also significantly reversed the Doxorubicin altered parameters. From the results it is reported that Doxorubicin impairs 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, Kidney tissue.

List of abbreviations
Dox-Doxorubicin; VE - Vitamin E; VA - Vitamin A; LPO - Lipid peroxidation; SOD - Superoxide dismutase; CAT - Catalase; GR-Glutathione reducatese; GPx - Glutathione peroxidase; GST - Glutathione-s-tranferase.

MATERIALS AND METHODS
Materials
Experimental animals
Experiments were carried out with adult male Swiss albino rats, weighing 125 ± 10g. 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 6 rats act as control one's and the remaining each group contain 7 rats each. Second group rats were received 80mg per kg body weight of vitamin E (VE) for 10 weeks (weekly doses). Third Group rats were received 40 IU per kg weight of Vitamin A (VA) for 10 weeks (weekly doses). Fourth group administered with 20 mg/ kg weight of Doxorubicin (Dox) over 10 weeks (weekly doses).

Fifth Group rats were administered with 80 mg per kg weight of vitamin E followed by 20 mg per kg of Doxorubicin over 10 weeks (weekly doses). Sixth group rats were administered with 40 IU per kg weight of vitamin A followed by 20 mg per kg Doxorubicin over 10 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; Alam et al., 2018. Dose of vitamin E and A chosen based on the previous studies of B. Vijayudu et al., (2015) from our laboratory. (Animal Ethics Resolution Number: 24/2012-2013(i)/a/ CPCSEA/IAEC/SVU/KVK-BV)

Collection of kidney samples
Animals were killed by cervical dislocation/decapitation by using mild ether anesthesia. Kidney 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 -80C until analysis. A portion of the Kidney 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 40C to separate the nuclear debris. The homogenate centrifuged at 10,000 rpm for 10min in a cooling centrifuge at 40C, after removal of the cell debris, supernatant was used for the assay of antioxidant enzymes.

Chemicals

Dox hydrochrloride injection (ADRIM) was purchased from BDH Chemicals Co, India. Vitamin E (D-Alfa-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 Kidney tissue of rat. The lipid peroxide level was measured by the procedure of Ohkawa et al., (1979). Superoxide dismutase activity was determined by the method of Misra and Fridovich (1972). Catalase activity was determined according to the method of Beers and Sizer (1952). The Glutathione reductase activity was assayed by the method of Carlberg and Mannervik (1985). Assay of Glutathione peroxidase was carried out by using the method of Wendel, (1981). Glutathione-s-transferase activity was measured as per the method of Habig et al., (1974). Protein content in various samples was estimated by the method of Lowry et al., (1951).

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.

INTRODUCTION

The first two anthracyclines were isolated from the pigment-producing Streptomyces peucetius early in the 1960s and were named doxorubicin (DOX) and daunorubicin (DNR)[1] both drugs possessing aglyconic and sugar moieties. The aglycone consists of a tetracyclic ring with adjacent quinone-hydroquinone groups, a methoxy substituent and a short side chain with a carbonyl group. The sugar, called daunosamine, is attached by a glycosidic bond to one of the rings and consists of a 3-amino-2,3,6- trideoxyL-fucosyl moiety. The only difference between these two molecules is the fact that the side chain of DOX terminates with a primary alcohol, whereas that of DNR terminates with a methyl group.[1] Despite its widespread use, the cytotoxic effects of anthracyclines are multidirectional, cardiotoxicity being the most known side effect. In order to find a better anthracycline, about 2000 analogs were produced with several chemical modifications or substitutions and/or conjugations introduced in the tetracyclic ring, the side chain or the amino-sugar. For example, epirubicin (EPI) is a semisynthetic derivative of DOX obtained by an axial-to equatorial epimerization of the hydroxyl group in a daunosamine carbon. This positional change has little effect on the mode of action and spectrum of antineoplastic activity of EPI compared with DOX but it introduces pharmacokinetic and metabolic changes such as increased volume of distribution and consequent enhanced total body clearance or shorter terminal half-life.[2,3] Yet, DOX replacement by EPI does not eliminate the risk of developing chronic cardiotoxicity. Only two more anthracyclines have attained clinical approval; idarubicin and valrubicin. Idarubicin hydrochloride is an analogue of daunorubicin that also intercalates into DNA, having an inhibitory effect on nucleic acid synthesis, and interacts with topoisomerase II. The absence of a methoxy group in the anthracycline structure gives the compound a high lipophilicity which results in an increased rate of cellular uptake compared with other anthracyclines.[4] Valrubicin (N-trifluoroacetyladriamycin-14-valerate, Valstar) is a chemotherapeutic drug used to treat bladder cancer. It is a semisynthetic analog of DOX, and it is administered by direct infusion into the bladder. However, several side effects are associated with the use of valrubicin, including blood in urine, incontinence, painful or difficult urination [5]. Nevertheless, the studies focusing the activity and toxicity of the most commonly used anthracyclines suggest that a better anthracycline has yet to come, i.e., superior antineoplastic activity without cardiotoxicity. It is therefore not surprising that relatively old drugs like DOX and DNR remain the focus of clinical and preclinical research aimed at improving our appraisal of their mechanisms of activity and/or toxicity and identifying new strategies for a safer use in cancer patients.

Doxorubicin as a therapeutic agent

DOX is one of the most potent antineoplastic drugs prescribed alone or in combination with other agents, remaining the compound of its class that has the widest spectrum of activity. Indeed, DOX is used in the treatment of solid tumours and hematological malignancies, including breast, bile ducts, prostate, uterus, ovary, oesophagus, stomach and liver tumours, childhood solid tumors, osteosarcomas and soft tissue sarcomas, Kaposi's sarcoma, as well as acute myeloblastic and lymphoblastic leukaemia and Wilms Tumor.[3,6-10] Many studies have attributed the antitumor activity of DOX to its ability to intercalate into the DNA helix and/or bind covalently to proteins involved in DNA replication and transcription.[11] Such interactions result in inhibition of DNA, RNA, and protein synthesis, leading ultimately to cell death.[12,13] Recently, Ashley

and Poulton,[14] using a novel method utilising the fluorescent DNA dye PicoGreen, found that anthracyclines intercalated not only into nuclear DNA but also mitochondrial DNA (mtDNA). Several studies classified DOX as a topoisomerase II poison. The topoisomerase family of enzymes modify the topology of DNA without altering its structure and sequence and catalyze the unwinding of DNA for transcription and replication, involving the process of cleavage of one strand of DNA duplex and passing a second duplex through this transient cleavage. The intermediate that is formed is termed the ‘‘cleavable complex’’[15] DOX poisons the cleavable complex, inhibiting the re-ligation of the cleaved duplex, a lesion that results in a DNA double-strand break (DSB).[16,17] Failure to repair DNA DSB results in an apoptotic response.

In the last few years it has been suggested that the proteasome modulates anthracyclines activity.[1] It has been shown that DOX enters cancer cells by simple diffusion and binds with high affinity to the proteasome in cytoplasm. DOX then binds to the 20S proteasomal subunit, forming a DOX proteasome complex that translocates into the nucleus via nuclear pores in an ATP-dependent process facilitated by nuclear localization signals. Finally, DOX dissociates from the proteasome and binds to DNA due to its higher affinity for DNA than for the proteasome.[18]

Interestingly, metabolic activation of drugs can also occur inside tumour cells. It is known that intracellular NADPH cytochrome P450 reductase (CPR) expression can be modulated in cells by many internal factors such as oxygen deficiency, intracellular pH changes and by malignant transformation.[19,20] DOX can suffer a one-electron reduction by a range of cellular oxidoreductases, including NADH dehydrogenase, NADPH cytochrome P450 reductase (CPR), xanthine oxidase and nitric oxide synthase.[21-24] The process comprises the one-electron transfer from reduced nucleotides, which converts the anthracycline molecule to a semiquinone radical form. Subsequent nonenzymatic semiquinone radical re-oxidation by molecular oxygen (O_2) can form superoxide (O_2^-) and hydrogen peroxide (H_2O_2) that interact with various macromolecules.[25,26] Clinical use of DOX soon proved to be hampered by serious problems such as the development of resistance in tumor cells or toxicity in healthy tissues.

Besides removing waste products, the kidney also removes normal components of the blood that are present in greater-than-normal concentrations. When an excess of water and ions are present, the excess is quickly eliminated in the urine. On the other hand, if the levels of these substances are too low in the blood, the kidney as the ability to recover them back to the blood. Thus, the kidneys continuously regulate the chemical composition of the blood within narrow limits. For this reason, the kidneys are one of the major homeostatic devices of the body.[27,28] Since the regenerative capacity of kidneys is too low, they are very susceptible to damage. The epithelial degeneration seems to have a primary role in the deterioration of the renal glomerulus.[29] where the filtration of plasma occurs; in fact, the damage of the glomerulus is a hallmark of nephropathies and may lead to the development of glomerulosclerosis.

Several studies revealed that DOX interferes with glomerular podocytes leading to their injury and, consequently, nephropathy.[29-35] The most common event documented in all the studies is the presence of a severe proteinuria. Exposure of renal tissue to the local passage of leaked proteins may evoke structural changes in the nephron leading to focal glomerulosclerosis,[29,32,35,36] a glomerular disease characterized by marked proteinuria, steroid resistance, hypertension, and a high incidence of progression to renal failure.[37] Proteinuria is also related with focal fusion of podocytes foot processes and swelling, extensive glomerular vacuolization and quick and progressive renal failure.[29,30-32,34] Other important effects, although present in a smaller scale of intensity, are the presence of extensive glomerular lesions, tubular dilatation, interstitial fibrosis and inflammation,[29,36,38] an increase in plasma creatinine levels and hypoalbuminemia,[29,32,33] dyslipidemia,[39-42] hypercoagulability, increase in kidneys size with a granular pale color surface,[29,32] and glomerular capillary permeability.[43] The mechanisms by which DOX induces toxicity are not fully understood but some studies suggest that they are most likely mediated by the formation of an iron– anthracycline complex that generates free radicals, which in turn, causes oxidative lesions on critical cellular components.[57,58,44-48] Lebrecht and co-workers[35] suggested that DOX enters mitochondria leading to the production of ROS, which cause mtDNA damage causing mitochondrial dysfunction and, consequently, contributes to the fast progression of nephron damage. This hypothesis support the studies of Okuda and collaborators[29] showing that DOX induced glomerular injury was related with ROS formation. Indeed, the authors also demonstrated that DOX was responsible for a decrease in mitochondrial complexes I and IV activities, by an increase in citrate synthase activity and triglycerides, a common occurrence in situations characterized by the impairment of the respiratory chain[49] and an increase in O_2 - levels, which can react with NO and induce the apoptotic process[50]. It has also been reported that DOX increases mtDNA mutations, which could be a result of topoisomerases II inhibition and/or due to an increase in oxidative stress.[35]

Other studies suggest that lipid peroxidation and reduction in natural antioxidant levels (vitamin E and GSH) could be the reason for DOX-induced nephropathies[59] leading to Bowman’s capsule, presence of multifocal tubular casts and adhesion of the glomerular tuft to Bowman’s capsule.[60] According to Rook et al.,[33] the tissue angiotensin-converting enzyme (ACE) is also involved in renal tissue damage induced by

DOX treatment. The authors observed an increase in ACE activity, which is responsible for the interstitial damage due to pro-inflammatory and pre-fibrotic effects that interfere with the kidney's ability to autoregulate glomerular pressure and, consequently, glomerular filtration rate.[51] The same authors hypothesize that the susceptibility of the nephrons to DOX-induced toxicity could be genetic and that genetically-determined individual differences in ACE activity, could be the reason for the different susceptibilities to DOX therapy. Indeed, the cases of proteinuria and nephropathy in humans are rare and may represent a primary genetic susceptibility.[33,34,52] In this line, Zheng et al.[31] suggested that the susceptibility to DOX-induced nephropathy is a defect in a gene with recessive inheritance.

However, the bulk of the studies show that a direct exposure of cells to DOX is necessary to the development of nephropathy, independently of drug metabolism.[56,36] This was proved by the clipping of the renal artery during DOX injection, which prevented DOX-induced nephropathy, suggesting that initial exposure to this drug, rather than its metabolites, causes kidney damage.[27,31] It is also well established that DOX nephropathy has chronic and self perpetuating characteristics of human progressive and chronic renal disease.[53] DOX-induced nephropathy in rats represents a good animal model to study kidney diseases[29] since glomerulosclerosis is the most common progressive glomerular disease in children and is the second leading cause of end-stage renal disease in this age group; focal and segmental glomerulosclerosis accounts for 20 to 25% of idiopathic nephrotic syndrome in adults.[54,55]

Altogether these studies show that ROS production and mitochondrial dysfunction induced by DOX are major causes of nephrons' damage.

RESULTS

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

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

In Dox treated animals LPO levels were significantly increased by 65% over the control. Vitamin E and vitamin A along with Dox (Group V & VI) treated rat kidney showed decreased levels of LPO and were found to be nearer to their control values and the recovery appeared to be 77% and 72% respectively.

The percent of SOD and CAT in Dox treated rat was 78.94%, 74.98% respectively over the control values. Vitamin E + Dox (Group V) administration showed a significant decrease 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 93.46%, of CAT by 92.78% of Vitamin A + Dox administration (Group VI) showed a significant decrease in SOD activity but not CAT enzyme activity as compared with the Dox intoxicated model group. In the Group VI, Vitamin A + Dox challenge was observed reverse the Dox induced alterations of SOD by 88.23% and CAT 85.75%.

Glutathione peroxidase (GPx) levels were Increased by 68.75% in Dox treated group. Dox + vitamin E, Dox + vitamin A administered rat kidney tissue showed reverse trends of their GPx levels over Dox treated group and the changes were found to be statistically significant ($p<0.01$). Percent recovery over Dox inhibited rat Kidney GPx levels appeared to be 84.61% and 73.33% respectively.

Dox treated rat kidney showed decreased activities of GR and GST and changes were found to be statistically significant ($p<0.01$) over the control values. The percent decrease of GR and GST in Dox treated rat was 29.62% and 25.23% respectively over the control values. Vitamin E along with Dox administration (Group V) showed a significant increase in GR and GST enzyme activities as compared with the Dox treated model group. In the Group V administration of vitamin E along with Dox challenge wasobserved to reverse the Dox induced alterations of GR by -109.37%, and GST by -151.28%. Vitamin A along with Dox administration showed a significant decrease in GR and GST enzyme activities, but not GPx activities as compared with the Dox treated model group. In the Group administration of vitamin A along with Dox challenge was observed reverse the Dox induced alterations of GR by -102.94% and GST by -115.80%.

Table 1: Effect of Dox, Vitamin E and Vitamin A separately and in combination on the rat kidney tissue LPO, SOD, CAT, GPx, GR 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	210	180.27*	194.36*	320.25	270	290
		SD	± 8.02	± 9.04	± 8.22	± 12.05	± 14.05	± 11.02
		% Change		32.24 %	16.32 %	65%	77.07%	72.41%

2	SOD	Mean	75	59.08*	75.15*	95	80.24	85
		SD	± 4.04	± 8.12	± 9.14	± 3.24	± 3.37	± 3.05
		% Change		16.32%	22.13%	78.94%	93.46%	88.23%
3	CAT	Mean	90	120.36*	140.34*	120.03	97	105
		SD	± 2.44	± 10.22	± 11.32	± 2.58	± 2.75	± 2.92
		% Change		30.26 %	40.28%	74.98%	92.78%	85.71%
4	GPx	Mean	55	85.44*	95.36*	80	65	75
		SD	± 3.47	± 8.42	± 9.36	± 3.22	± 3.48	± 3.86
		% Change		35.24%	47.22%	68.75%	84.61%	73.33%
5	GR	Mean	45	60.36*	65.22*	35	32	34
		SD	± 0.36	± 7.10	± 9.41	± 1.35	± 1.26	± 1.27
		% Change		35.22 %	30.43 %	29.62%	-109.37%	-102.94%
6	GST	Mean	35	60.29*	66.33*	24.23	26.22	29.27
		SD	± 0.15	± 9.31	± 11.22	± 0.24	± 0.28	± 0.32
		% Change		50.41 %	65.22%	25.23%	-151.28%	-115.80%

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.

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

DISCUSSION

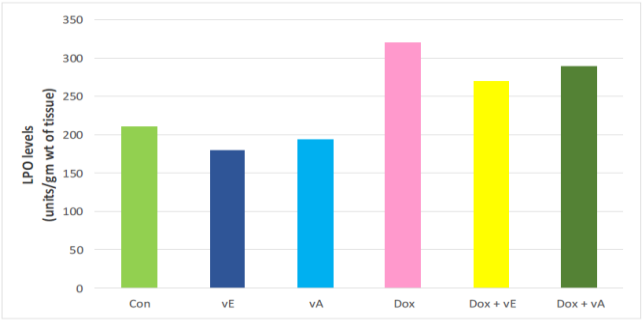

Fig. 1: Impact of Vitamin E & A and Dox Separately and In combination on Albino rat kidney tissue LPO average levels.

In the lipid peroxidase levels are decreased under Dox stress in the kidney tissue compared to control over due to renal disease and complication are to current global health concerns due to the vital roles of kidney in body homeostasis.[61-63] Acute kidney injury (AKI) is characterised by rapid renal function decline along with accompanying electrolytes abnormalities, fluid overload, severe acidosis for that reducing of above renal toxicities that are used Dox plus Vitamin E and Dox plus Vitamin A. Here Dox plus Vitamin E is more after Dox increased lipid peroxidase levels than Dox plus Vitamin A.

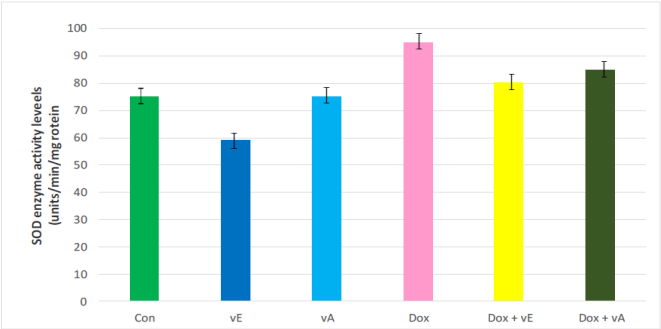

Fig. 2: Impact of Vitamin E & A and Dox Separately and In combination on Albino rat kidney tissue SOD average levels.

In this SOD levels are increased under Dox administration of compared to control ones. Once SOD levels are increased Dox activity also increased in turn if affect to propagated apoptotic process along with some kidney function are susceptible to certain inherited genetic diseases and aging process.[64,65] All different forms of renal injuries could ultimately progress into severe end stage renal disease with renal placement as the only therapeutic option. Therefore, therapeutic strategies are in dire need to prevent renal disease progression for reducing that kidney side effects. I was given Dox plus Vitamin E and Dox plus Vitamin A. Here Dox plus Vitamin E is more reduced than Dox plus Vitamin A.

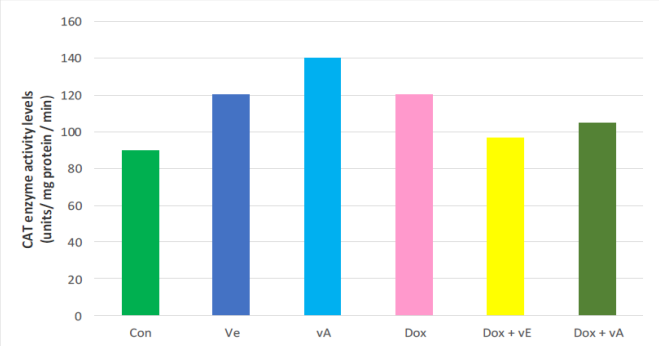

Fig. 3: Impact of Vitamin E & A and Dox separately and in combination on Albino rat kidney tissue CAT average levels.

Catalyse enzyme activity levels are increased under Dox stress in kidney compare to control ones and also gives kidney abnormalities such as indirectly effects mitochondrial abnormalities that maintain cellular redox and energy homeostasis and therefore a major source of intra cellular oxidase stress.[66] In addition to its roles in adenosine triphosphate (ATP) generation through oxidative phosphorylation, the mitochondria also plays an essential role in the metabolic signalling such as pyrimidine, heme biosynthesis, TCA cycle and fatty acid, β-oxidation pathways in calcium ions, homeostasis, thermogenesis proliferating and regulating intrinsic apoptotic pathway. In this way mitochondria functions are abnormal due to given of Dox. That's why we are applied Vitamin E plus Dox and Dox plus Vitamin A. Here Vitamin A plus Dox normalise the mitochondria functions.

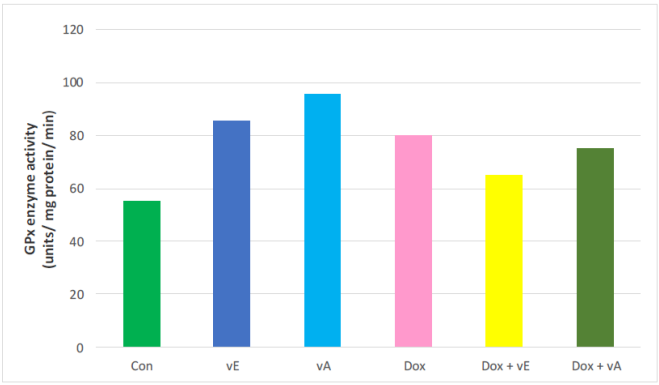

Fig. 4: Impact of Vitamin E & A and Dox separately and in combination on Albino rat kidney tissue GPx average levels.

Glutathione peroxidase levels are increased under Dox administration then compared to control kidney of rat. Because of Dox effect on kidney tissues and after the kidney function. That's why glutathione peroxidase levels are increased. Especially kidney consumes roughly 7% of the body's ATP energy expenditure.[67,68] Due to various energy demands, different nephron segments have different mitochondria densities and distributions. It is generally accepted that renal tubule cells are rich in mitochondria with the S1 segment containing the highest mitochondria density.[69] Several factors such as mitochondria biogenesis and turnover, bioenergetics, dynamics and autophagy regulate the condition of mitochondria. This is given contribute mitochondria function by Dox plus Vitamin E and Dox plus Vitamin A.

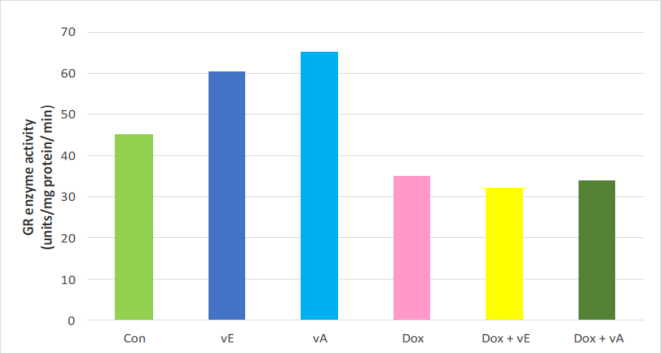

Fig. 5: Impact of Vitamin E & A and Dox separately and in combination on Albino rat kidney tissue GR average levels.

Reduced Glutathione levels are decreased under Dox administration in rat kidney tissues due to effect of renal diseases encompassing both acute and chronic condition of kidney injury by Dox with declined renal functions are current global health concern with tremendous medical burdens.[61,63] A common link between all forms of acute and chronic kidney injuries is to generation of toxic ROS and RNS when the disease manifests. This oxidative stress injury could be derived from ischemic reperfusion, energy shortage from impaired mitochondrial biogenesis or ATP energetics or defective clearance of damaged mitochondria. Vitamin E, A are best antioxidant, these two altered Dox changes kidney reduced glutathione levels.

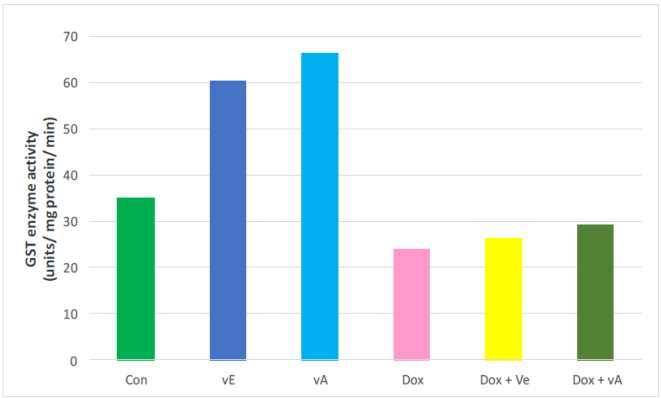

Fig. 6: Impact of Vitamin E & A and Dox separately and in combination on Albino rat kidney tissue GST levels.

Glutathione s transferase levels are decreased under Dox administered over control in kidney tissues. This is linked to exposure to nephrotoxins such as some chemotherapy drugs, medications, intra vascular contrast media, trace heavy metals, drug abuse or certain chemical drugs combinations could induce Acute kidney injuries especially in the elderly, young children's and high-risk patients.[70,71] Drug nephrotoxicity was found to be responsible for 19% of all AKI cases on critically ill patients. NSAIDs and renin-angiotensin system (RAS) antagonism, tubular epithelium direct cell injury from chemotherapy drugs like cisplatin, tubular obstruction of urinary flow due to precipitates of toxins or their metabolites, and angio pathogenesis from vascular injury.[72] For that I have given Vitamin E and Vitamin A combines with Dox for altered**.**

CONCLUSION

Doxorubicin administration brings about different harmful impacts, of which dose dependent nephrotoxicity is the commonest impact brings about nephropathy and Kidney failure. It has been believed to mediate by several mechanisms of which free radical generation is the predominant and principal 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 kidney tissue.

Rats were administered with a selective dose of doxorubicin 20 mg/kg and 80 mg/kg vitamin E and 40 IU / kg wt of vitamin A separately and in combination and the treatment period was 10 weeks (weekly doses). The control and experimental rat kidney tissue was subjected for biochemical analysis and data obtained and presented in the study. Dox treatment caused significant elevation in LPO, SOD, CAT, GPx and GR, GST

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.

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