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To Evaluate the Levels of Antioxidants Enzymes in Albino Rat Liver Tissue by Injecting Doxorubicin and Antioxidants Separately and in Combination

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Abstract: ***Doxorubicin is a anticancer drug, it is used in treatment of cancer through chaemotherapy, its effect mainly on cancer cells and also this produce some side effects. for reducing the adverse effects i was used some antioxidants with Dox combination and individually. In this study i was used in 20 mg/kg wt of Doxorubicin and 80 mg/kg wt of Vitamin E, 40 IU/kg wt of Vitamin A individually and in combination over 10weeks (weekly-Doses) on Albino rat Liver. Some antioxidant enzymes such as Superoxide Dismutase, Catalyse, Glutathione Peroxidase, Glutathione Reducatase, Glutathione-S-Transferase levels were estimated in the liver tissue of albino rat. The data of these enzymes I will show in result table from this result. It is reported that the antioxidants like Vitamin E, K reduce the Doxorubicin altered enzyme levels. It is used for cancer studies for better understanding.***

Keywords: Liver Tissue, Doxorubicin, Antioxidant Enzymes, Vitamin E, and Vitamin A

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

1. Introduction

The liver is the principal organ that metabolizes xenobiotics and endogenous molecules to sustain metabolic homeostasis in the organism. Therefore, the liver is a objective of many insults that bring about dysregulated hepatic homeostasis and lead to hepatic diseases [1, 2]. The liver is comprised of the following cells types: hepatocytes, Kupffer cells, liver sinusoidal endothelial cells, pit cells, and hepatic stellate cells (HSC) [3]. Cirrhosis is triggered by liver injury from a variety of etiological factors and is the fianl phase of progressive fibrosis [4]. Oxidative stress plays an important role in the establishment of fibrosis and subsequently in cirrhosis [5]. As a result, the use of molecules with antioxidant properties has been proposed as a treatment for fibrosis and cirrhosis caused by oxidative stress.

Doxorubicin is an antibiotic obtained from the Streptomyces peucetius bacterium. It has extensive use as a chemotherapeutic agent since the 1960s. Doxorubicin is part of the anthracycline group of chemotherapeutic agents. Doxorubicin may be used to cure soft tissue also bone sarcomas and cancers of the breast, ovary, bladder, and thyroid. Additionally it is used to treat acute lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung cancer. This activity will emphasize the mechanism of action, adverse event profile, pharmacology, monitoring, and relevant interactions of doxorubicin, pertinent for interprofessional team members in the treatment of cancer cases for which it is indicated. [6] [7] [8] [9]

DOX is a certain one most potent antineoplastic drugs ordained alone or in blend with other agents, remaining the compound of its class that has the broad spectrum of activity. Indeed, DOXis 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 tissues arcomas, Kaposi's sarcoma, as well as acute myeloblastic and lymphoblasticleukaemia and Wilms Tumor [10, 11, 12, 13]. Numerous 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. [14] Suchinteractions lead to inhibition of DNA, RNA, and protein synthesis, leading ultimately to cell death. [15, 16] Recently, Ashley and Poulton, using a novel method utilising the fluorescent DNA dye PicoGreen, found thatanthracyclines intercalated in addition to nnuclear DNA also mitochondrial DNA (mtDNA). Several studies classified DOX as a topoisomerase II poison. The topoisomerase family of enzymes modifies the topology of DNA without varying in its structure and sequence and catalyze the unwinding of DNA for transcription and replication, concerning 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 as The "cleavable complex" [17]. DOXpoisons the cleavable complex, inhibiting the re-ligation of the cleaved duplex, a lesion that

results in a DNAdouble-strand break (DSB) [18, 19]. Failure to repair DNA DSB results in an apoptotic response.

The body's trillion more or cells face formidable threats, from deficiency of food to infection with a virus. Another constant risk comes from chemicals called free radicals. In extremely high levels, they are capable of damaging cells and genetic material. The body produces free radicals as the inevitable derivatives of turning food into energy. Free radicals are also developed after exercising or exposure to cigarette smoke, air pollution, and sunlight. [20]

Free radicals come in many shapes, sizes, and chemical configurations. What they all share is a ravenouss appetite for electrons, thieving them from any nearby substances that will yield them. This electron theft can radically amend the "loser's" structure or function. Free radical damage can alter the instructions coded in a strand of DNA. It can make a circulating low-density lipoprotein (LDL, sometimes called bad cholesterol) molecule more possibly to get trapped in an artery wall. Or it can bring alterations in a cell's membrane, changing the flow of what inscribes the cell and what leaves it. An immoderate chronic amount of free radicals inthe body causes a condition called oxidative stress, which may injure the cells and lead to chronic diseases. [21]

Vitamin E is a fat-soluble vitamin with diverse forms, but alpha-tocopherol is one of a kind used by the human body. Its main role is to act as an antioxidant, scavenging loose electrons—so-called "free radicals"—that can damage cells. [22] It also strengthens immune function and prevents clots from forming in heart arteries. Antioxidant vitamins, including vitamin E, came to public consideration in the 1980s when scientists began to understand that free radical damage was involved in the early stages of artery-clogging atherosclerosis, and might also contribute to cancer, vision loss, and a host of other chronic conditions. Vitamin E has the ability to protect cells from free radical damage as well as reduce the production of free radicals in certain situations. However, conflicting study results have dimmed certain of promise of using high dose vitamin E to prevent chronic diseases.

Vitamin A is a fat-soluble life-crucial set of compounds both of animal and vegetal origin distinguished by an unsaturated isoprenoid chain structure. entire vitamin A forms share a same structure and the similar physiological functions in an organism. These compounds can also be classified as retinoids, including compounds with a common structure of four isoprenoid units being of either a natural or synthetic origin. Few synthetic derivatives don't resemble the natural isoprenoids from vitamin A class at first sight. However, the basic vitamin A string is hidden in their structures, and they are similar to other retinoids in their interaction with retinoid receptors. All these compounds are liposoluble and, unlike water-soluble vitamins, are easily accumulated in the body, especially in the liver and adipose tissue. This represents, on one hand, an advantage since temporal deprivation of vitamin A intake is not associated with clinical symptoms, but on the other hand, accumulation with subsequent toxicity can appear.

Vitamin A possess pleiotropic functions in the body, muchobliged to its several biologically active forms. Althoughretinol, which is also accountable for some processes, is the most copius form in the body, ATRA is the primary active form of vitamin A [23, 24, 25, 26, 27, 28]. To a smaller extent, other metabolites of this vitamin, 9-cis-retinoic acid and 13-cis-retinol, are also biologically active. Each form of the vitamin shows pecularity for different tissues and processes in which they are involved. However, they share identical common properties. Retinol functions as a cofactor in multiple enzymatic processes, 11-cis-retinal is concerned in vision, and ATRA exerts different functions by binding to nuclear receptors with the subsequent regulation of genetic expression.

2. Materials and Methods

Materials Experimental Animals

In this Experiment Male Pathogenic free wistar albino rats were taken by weighing about 120± 10gm. The Animals are homed to a polypropylene cage and supplied with food and water adds libitum. The Animals were sustained under standard condition of temperature and Humidity with an altering 12 Hours light/dark cycleand maintained in correspondence with the guidelines of the National Institute of Nutrition and Indian Council of Medical Research Hyderabad, India**.**

Treatment of Animals

The animals randomly divided in to 6 groups. First Group having 6 rats act as control one's and The remaining5 Groups also ha ving 6 rats, these 5 groups are experimental. First Group Rats considered as Control One's and Second group rats were received 80mg per kg body weight of vitamin E (VE) for 10 weeks (weekly doses). Third crowd rats were received 40 IU per kg weight of Vitamin A (VA) for 10 weeks (weekly doses). Fourth group rats were administered with 20 mg/ kg weight of Doxorubicin (Dox) over 10 weeks (weekly doses) and Fifth Group rats were administered with 20 mg per kg of Doxorubicin Followed by 80 mg per kg weight of vitamin E over 10 Week Period (weekly doses). Sixth group rats were accompanied by 20 mg per kg wt of Doxorubicin followed by 40 IU per kg weight of vitamin A over 10 weeks period (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 my previous studies, (2015) from our laboratory. (Animal Ethics Resolution Number: 24/2012-2013 (i)/a/ CPCSEA/IAEC/SVU/KVK-BV)

Collection of Liver samples

Animals were killed by cervical dislocation/decapitation by using mild ether anesthesia. Liver were amputated, cropped of connective tissue, flushed with ice-cold saline to eradicate blood contamination, dried by blotting with filter and weighed. The tissues then held in freezer at-80C until analysis. A section of the Liver was weighed, perfused with saline and homogenate (10%) was prepared in ice cold PBS (50 mM, pH 7) using a homogenizer. The homogenate

centrifuged at 10, 000 rpm for 10min in a cooling centrifuge at 40C, after expulsion 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 Liver tissue of Rat, Such as Superoxide dismutase activity was resolved the method of Misra and Fridovich (1972). Catalase activity was determined according to the method of Beers and Sizer (1952). Assay of Glutathione peroxidase was carried out by using the method of Wendel, (1981). The Glutathione reductase activity was examined by the method of Carlberg and Mannervik (1985) Glutathione-s-transferase activity was assessed as per the method of Habig et al., (1974).

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 numerous comparison test by using statistical software package.

Table 1: Levels of Antioxidant Enzymes like SOD, CAT, GPx, GR and GST in Albino Rat Liver Tissueby Injecting Doxorubicin, Vitamin E, Vitamin A Separately and in Combination

Sl. No	Parameter		Group I (Con)	Group II (vE)	Group III (vA)	Group IV (Dox)	Group V (Dox + vE)	Group VI (Dox + vA)
1	SOD	Mean	78.4	77.8*	79.18*	60.24	68.30	70.30
		SD	± 4.9	± 5.03	± 3.03	+4.4	±3.5	± 4.5
2	CAT	Mean	80.2	85.12	87.30	70.4	75.3	78.00
		SD	± 7.7	± 4.2	± 0.5	± 0.6	± 7.01	± 4.0
3	GPx	Mean	40.2	48.4	52.3*	35.6	40.7	41.4
		SD	± 11.2	± 2.2	± 6.3	± 3.2	± 4.21	± 12.6
4	GR	Mean	45.4	50.6*	55.8*	40.4	41.4	43.8
		SD	± 6.2	± 5.3	± 2.2	± 4.2	± 9.12	± 4.2
5	GST	Mean	50.1	56.6*	62.8*	45.4	46.6	48.9
		SD	± 6.1	± 8.2	± 11.4	± 12.2	± 10.2	± 11.3

Values are expressed as Mean ± SD of six rats in each group. Data was analysed by one way ANOVA followed by Tukeys HSD test. Units: SOD - mg protein/Min; CAT-micromoles of H2O2 decomposed /mg protein /min; ; GPx-micromoles of oxidized /mg /min; GR - micromoles of NADPH oxidized /mg /min; GST-mg proteins/Min

3. Results

In the Dox treatment SOD levels were decreased compared to control ones, When Dox plus Vitamin E combination SOD levels were increased than Dox treated ones and reduced compared to Dox plus Vitamin A combination.

In the Dox treatment CAT levels were decreased compared to control ones when Dox plus Vitamin E combination levels increased then compared to dox treated ones and reduced compared Dox plus Vitamin A combination.

In the Dox treatment Glutathione peroxidase levels were decreased compared to control ones and Dox plus Vitamin E combination Gpx levels were increased when compared to Dox treated ones and Dox plus Vitamin A combination Gpx levels were increased than compared to Dox plus Vitamin E combination.

In the Dox treatment Glutathione reductase levels were lowered when equated to control ones and when Dox plus Vitamin E combination Glutathione reductase levels were increased than compared to Dox treated ones and when Dox plus Vitamin A combination Glutathione reductase levels were increased Than Dox treated ones and Dox plus Vitamin E treated ones.

In the Dox treated Glutathione-S-Transferase levels were decreased than compares to control ones and if Doxplus Vitamin E combination Glutathione transferase levels were increased compared to Dox treated ones and if Dox plus Vitamin A combination Glutathione transferase levels were increased compared to Dox Treated ones and Dox plus Vitamin E combination oneS

Finally Dox treatment SOD, CAT, GPx, GR, and GST Levels are Decreased than compared to control ones and Dox plus Vitamin E combination above enzyme levels are high than compared to Dox treated ones and decreased than compared to Dox Plus Vitamin A combination

4. Discussion

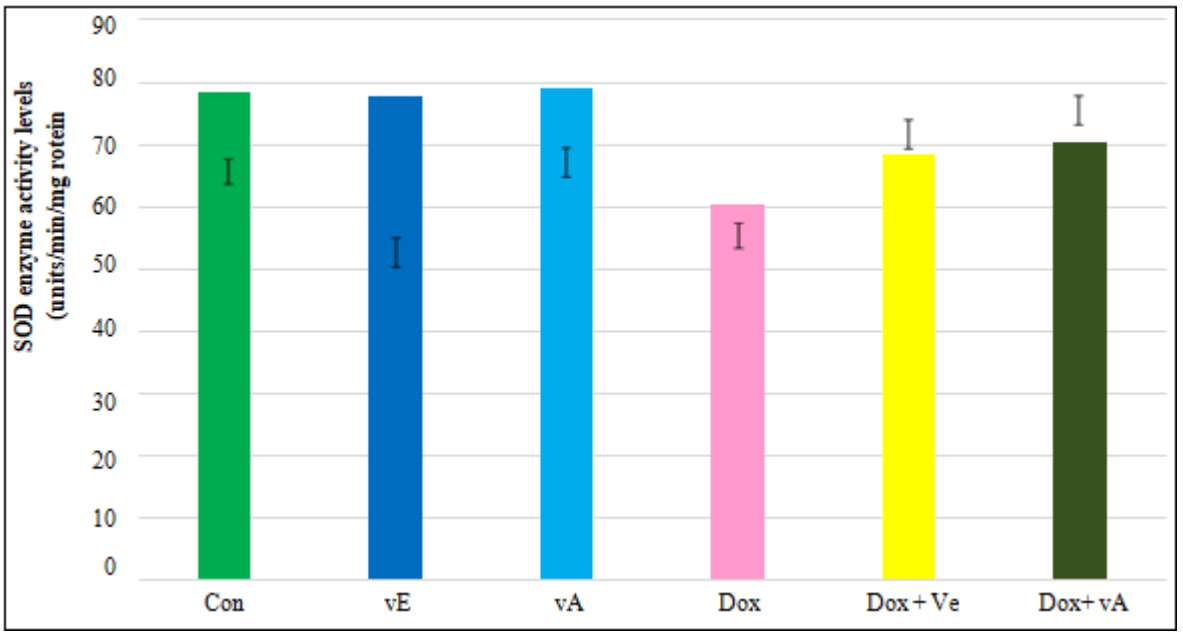

Figure 1: Shows Effect of Vitamin E & A and Dox separately and in combination on Albino rat Liver tissueSOD average levels

In this SOD levels were decreased under Dox administration when compared to control ones because of High levels of intracellul ar as well as extracellular ROS (Superoxide and H202) are indispensable in many biochemical processes including intracellular signaling and cell functions (29, 30).

Once SOD levels are increased Dox activity also increased in turn if affect to propagated apoptotic process along with some Liver function are responsive to specific inherited genetic diseases and aging process. All various forms of liver injuries could ultimately develop into severe end stage liver disease with liver placement as the only curative option. That's the reason I chose Dox plus Vitamin E and Dox plus Vitamin A. Here Dox plus Vitamin A is more reduced Dox altered one than Dox plus Vitamin A.

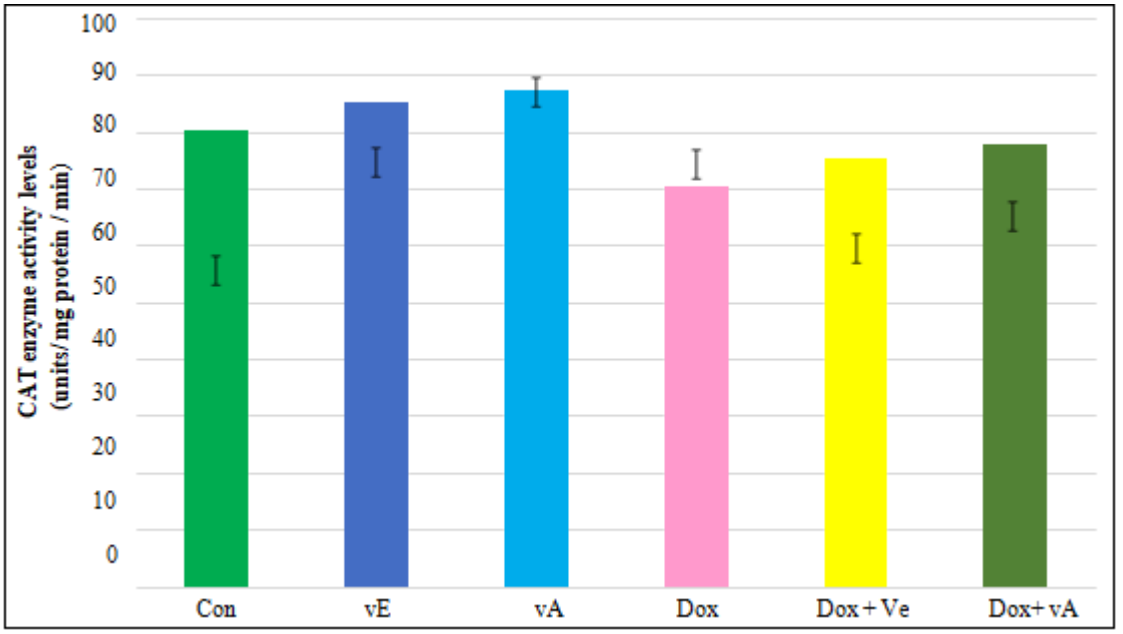

Figure 2: Shows Effect of Vitamin E & A and Dox separately and in combination on Albino rat Liver tissue CAT average levels

Catalyse enzyme activity levels were decreased under Dox stress in Liver compare to control ones due to Catalyse breakdown in to the Hydrogen Peroxidase molecules and one Molecule of oxygen and two molecules of water in a two level reaction. Several studies have reviewed CAT polymorphism and its intimacy in the various diseases as well as its role as thermostat in the CAT gene expression. The first that's why we are applied Vitamin E plus Dox and Dox plus Vitamin A. Here Vitamin A plus Dox normalise the Dox altered one's than compared to Dox plus Vitamin E. (31)

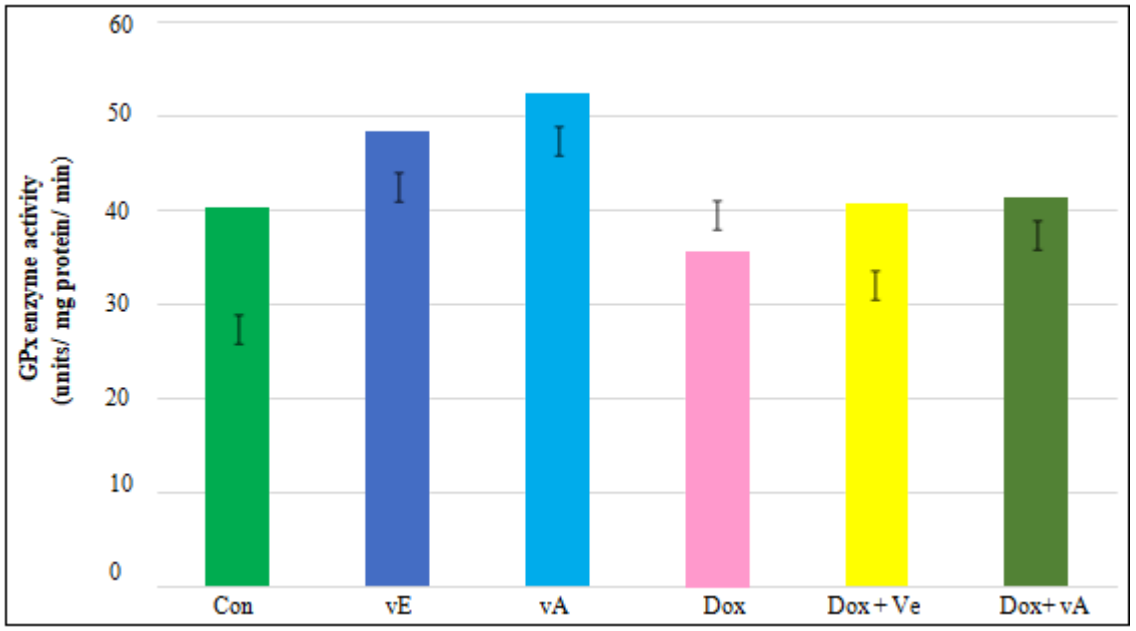

Figure 3: Shows Effect of Vitamin E & A and Dox separately and in combination on Albino rat Liver tissue GPx average levels

The Glutathione Peroxidase Levels are decreased under Dox administration when compared to the control ones due to The activity of the enzyme has been reported to be decreased whereas in case of copper deficiency in the liver and plasma. Glutathione peroxidase has a mitochondrial isoform that intervenes the apoptic response to oxidative stress and has a peroxidase independent structural sperm maturation. (32) In this was given Dox plus Vitamin E and Dox plus Vitamin A as a blend. Here Dox plus Vitamin A combination was more altered than Dox plus Vitamin E combination.

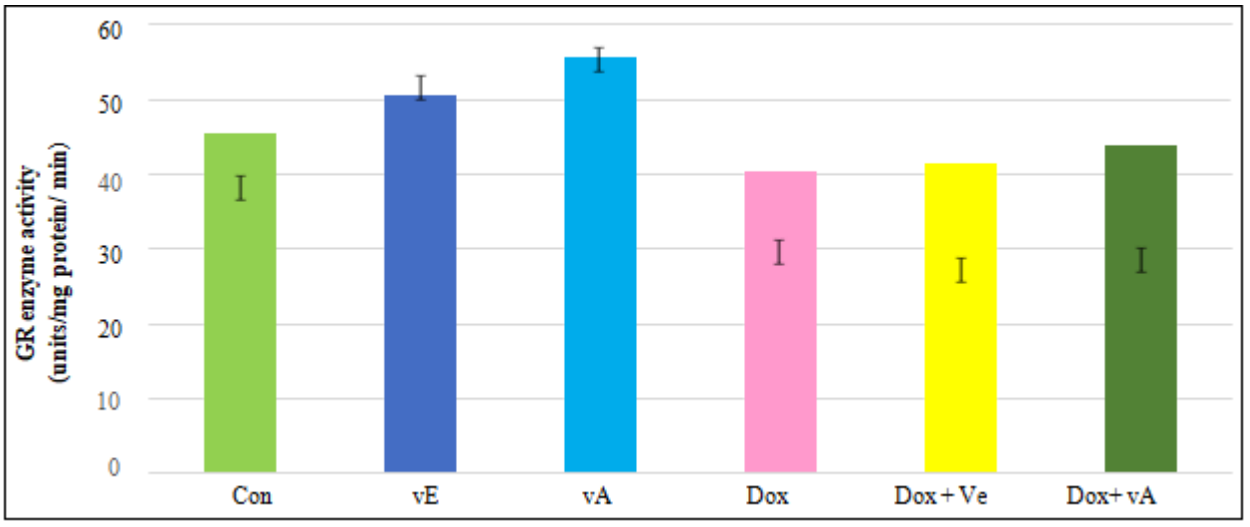

Figure 4: Shows Effect of Vitamin E & A and Dox separately and in combination on Albino rat Liver tissue GR average levels

Figure 5 Impact of Vitamin E & A and Dox separately and in combination on Albino rat kidney tissue GR levels

Glutathione Reductase levels were decreased under Dox Stress than compared to the control ones because of Glutathione reductase Catalyses the reduction of oxidised Glutathione to reduced glutathione NADPH as the reducing co-factor end this by maintains a constant GSH Levels in the system. The addition of minute amount of the FAD was found to activate the Glutathione reductase. So deficiency of FAD and irreversible inhibition by Dox through Covalent bond formation between 2AAPA and Cysteine reduces at the active site of the enzyme. Dox altered ones controlled by Dox plus vitamin A combination is more than Compared to Dox plus Vitamin E combination. (33)

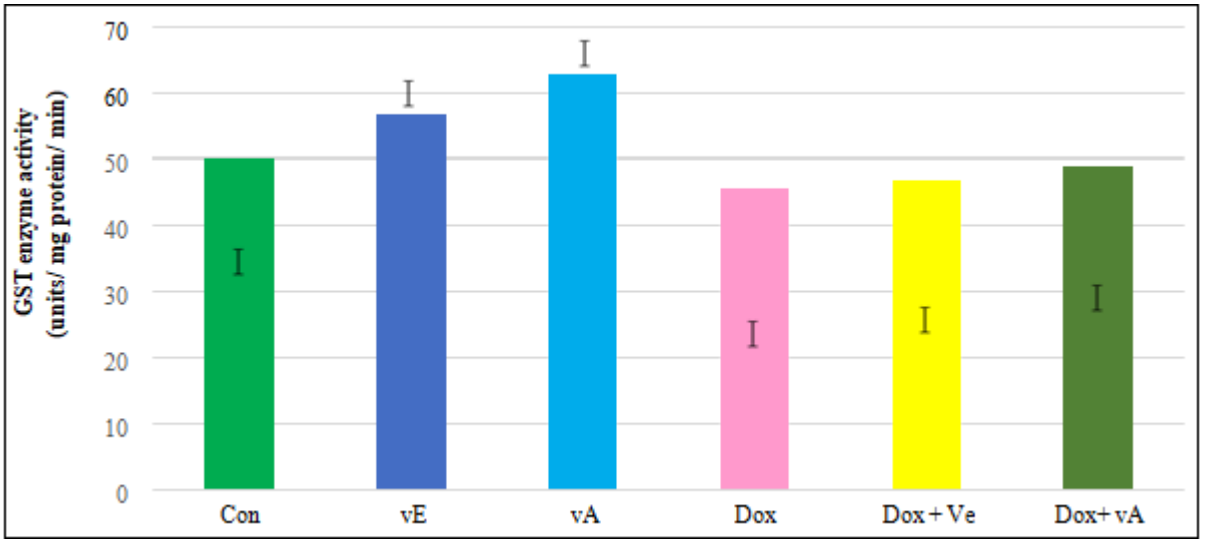

Figure 5: Shows Effect of Vitamin E & A and Dox separately and in combination on Albino rat Liver tissue GST average values

Glutathione s transferase levels are decreased under Dox administered over control in liver tissues.

Glutathione s transferase levels found decreased under Dox administered over control in case of liver tissues. Due to Glutathione-S-Transferase has been concerned in the development of resistance toward chemotherapy agents. It is possible that GST assists two diverse roles in the development of drug resistance across direct detoxification in addition to acting as an inhibitor of the map kinase pathway (34). That's why I was used Dox plus Vitamin E plus and Dox plus Vitamin A combination, Here Dox plus Vitamin A combination more altered the Dox treated ones than compared to Dox plus Vitamin E combination.

5. Conclusion

Considering the toxic adverse effects induced by the cause of Doxorubicin and the defensive role as offered by vitamin E and A in experimental animals and also in humans, the current review carried out with the role of investigating the in vivo effect of Doxorubicin and vitamin E & A apart and in combination on selected enzymatic antioxidants in albino rat liver tissue.

Rats were administered with a selective proportion of doxorubicin 20 mg/kg and 80 mg/kg vitamin E and 40 IU / kg wt of vitamin A apart and in combination and the treatment duration was 10 weeks (weekly doses). The control and experimental rat liver tissue was subjected for biochemical analysis and data obtained and presented in the study decrease in all the antioxidant parameters SOD, CAT, GPx, GR, and GST by Dox Treatment. Dox plus Vitamin A Combination more alter the Dox treated antioxidant enzymes than compare to Dox plus Vitamin E combination. Hence in chemotherapy treatment along with Dox Vitamin A also useful for reducing the side effects of Dox.

The main focus of the research was to reduce Dox produce side effects by injecting vitamin E, A separately and in combination with Dox. Here In this study i was concluded Vitamin E plus Dox Combination alter small level to Dox reduce enzyme levels but Vitamin A plus Dox Combination altered high level to Dox reduced enzymes levels. Hence this concept will useful for Cancer Chemotherapy Treatment.

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